Chittaranjan Kole *Editor*

Genomic Designing of Climate-Smart Oilseed Crops

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Previously Professor and Bascom Chair in Agronomy in the University of Wisconsin at Madison,
Where I worked with him on the Brassicaceae system and learnt how to develop novel concepts of plant molecular genetics and side-by-side generate genetic resources for crop improvement.

Preface

The last 120 years have witnessed a remarkable evolution in the science and art of plant breeding culminating in quite a revolution in the second decade of the twenty-first century! A number of novel concepts, strategies, techniques, and tools have emerged from time to time over this period, and some of them deserve to be termed as milestones. Traditional plant breeding, immediately following the rediscovery of the laws of inheritance, has been playing a spectacular role in the development of innumerable varieties in almost all crops during this entire period. Mention must be made on the corn hybrids, rust-resistant wheat, and obviously the high-yielding varieties in wheat and rice that ushered the so-called green revolution. However, the methods of selection, hybridization, mutation, and polyploidy employed in traditional breeding during this period relied solely on the perceivable phenotypic characters. But most, if not all, of the economic characters in crops are governed by polygenes which are highly influenced by environment fluctuations, and hence, phenotype-based breeding for these traits has hardly been effective.

Historical discovery of DNA structure and replication in 1953 was followed by a series of discoveries in the 1960s and 1970s that paved the way for recombinant DNA technology in 1973 facilitating the detection of a number of DNA markers in 1980 onwards and their utilization in construction of genetic linkage maps and mapping of genes governing the simply inherited traits and quantitative trait loci controlling the polygenic characters in a series of crop plants starting with tomato, maize, and rice. Thus, new crop improvement technique called as molecular breeding started in later part of the twentieth century. On the other hand, genetic engineering made modification of crops for target traits by transferring alien genes, for example, the *Bt* gene from the bacteria *Bacillus thuringiensis*. A large number of genetically modified crop varieties have thus been developed starting with the commercialization of "flavr Savr" tomato in 1994.

Meantime, the manual DNA sequencing methodology of 1977 was being improved with regard to speed, cost-effectiveness, and automation. The first-generation sequencing technology led to the whole genome sequencing of Arabidopsis in 2000 and followed by rice in 2002. The next-generation sequencing technologies were available over time and used for sequencing of genomes of many

other model and crop plants. Genomes, both nuclear and organellar, of more than 100 plants have already been sequenced by now, and the information thus generated are available in public database for most of them. It must be mentioned here that bioinformatics played a remarkable role in handling the enormous data being produced in each and every minute. It can be safely told that the "genomics" era started in the beginning of the twenty-first century itself accompanying also proteomics, metabolomics, transcriptomics, and several other "omics" technologies.

Structural genomics has thus facilitated annotation of genes, enumeration of gene families and repetitive elements, and comparative genomics studies across taxa. On the other hand, functional genomics paved the way for deciphering the precise biochemistry of gene function through transcription and translation pathways. Today, genotyping-by-sequencing of primary, secondary, and even tertiary gene pools; genome-wide association studies; and genomics-aided breeding are almost routine techniques for crop improvement. Genomic selection in crops is another reality today. Elucidation of the chemical nature of crop chromosomes has now opened up a new frontier for genome editing that is expected to lead the crop improvement approaches in near future.

At the same time, we will look forward to replacement of genetically modified crops by cisgenic crops through transfer of useful plant genes and atomically modified crops by employing nanotechnology that will hopefully be universally accepted for commercialization owing to their human-friendly and environment-friendly nature.

I wish to emphatically mention here that none of the technologies and tools of plant breeding are too obsolete or too independent. They will always remain pertinent individually or as complementary to each other, and will be employed depending on the evolutionary status of the crop genomes, the genetic resources and genomics resources available, and above all the cost-benefit ratios for adopting one or more technologies or tools. In brief, utilization of these crop improvement techniques would vary over time, space, and economy scales! However, as we stand today, we have all the concepts, strategies, techniques, and tools in our arsenal to practice 'genome designing', as I would prefer to term it, of crop plants not just genetic improvement to address simultaneously 'food, nutrition, energy, and environment security, briefly the FNEE security', as I introduced the concept in 2013 and have been talking about for the last 5 years at different platforms.

Addressing FNEE security has become more relevant today in the changing scenario of climate change and global warming. Climate change will lead to greenhouse gas emissions and extreme temperatures leading to different abiotic stresses including drought or waterlogging, on the one hand, and severe winter and freezing, on the other hand. It will also severely affect uptake and bioavailability of water and plant nutrients and will adversely cause damage to physical, chemical, and biological properties of soil and water in cropping fields and around. It is also highly likely that there will be emergence of new insects and their biotypes and of new plant pathogens and their pathotypes. The most serious concerns are, however, the unpredictable crop growth conditions and the unexpected complex interactions among all the above stress factors leading to drastic reduction in crop yield and

quality in an adverse ecosystem and environment. Climate change is predicted to significantly reduce productivity in almost all crops. For example, in cereal crops, the decline of yield is projected at 12–15%. On the other hand, crop production has to be increased at least by 70% to feed the alarmingly growing world population, projected at over 9.0 billion by 2050 by even a moderate estimate.

Hence, the unpredictability of crop growing conditions and thereby the complexity of biotic and abiotic stresses warrant completely different strategies of crop production from those practiced over a century aiming mostly at one or the few breeding objectives at a time such as yield, quality, resistance to biotic tresses due to disease–pests, tolerance to abiotic stresses due to drought, heat, cold, flood, salinity, acidity, improved water and nutrient use efficiency, etc. In the changing scenario of climate change, for sustainable crop production, precise prediction of the above limiting factors by long-term survey and timely sensing through biotic agents and engineering devices and regular soil and water remediation will play a big role in agriculture. We have been discussing on "mitigation" and "adaptation" strategies for the last few years to reduce the chances of reduction of crop productivity and improve the genome plasticity of crop plants that could thrive and perform considerably well in a wide range of growing conditions over time and space. This is the precise reason for adopting genomic designing of crop plants to improve their adaptability by developing climate-smart or climate-resilient genotypes.

Keeping all these in mind, I planned to present deliberations on the problems, priorities, potentials, and prospects of genome designing for development of climate-smart crops in about 50 chapters, each devoted to a major crop or a crop group, allocated under five volumes on cereal, oilseed, pulse, fruit, and vegetable crops. These chapters have been authored by more than 250 of eminent scientists from over 30 countries including Argentina, Australia, Bangladesh, Belgium, Brazil, Canada, China, Egypt, Ethiopia, France, Germany, Greece, India, Ireland, Japan, Malaysia, Mexico, New Zealand, Kenya, Pakistan, Philippines, Portugal, Puerto Rico, Serbia, Spain, Sri Lanka, Sweden, Taiwan, Tanzania, Tunisia, Uganda, UK, USA, and Zimbabwe.

There are a huge number of books and reviews on traditional breeding, molecular breeding, genetic engineering, nanotechnology, genomics-aided breeding, and gene editing with crop-wise and trait-wise deliberations on crop genetic improvement including over 100 books edited by me since 2006. However, I believe the present five book volumes will hopefully provide a comprehensive enumeration on the requirement, achievements, and future prospects of genome designing for climate-smart crops and will be useful to students, teaching faculties, and scientists in the academia and also to the related industries. Besides, public and private funding agencies, policy-making bodies, and the social activists will also get a clear idea on the road traveled so far and the future roadmap of crop improvement.

I must confess that it has been quite a difficult task for me to study critically the different concepts, strategies, techniques, and tools of plant breeding practiced over the last 12 decades that also on a diverse crop plants to gain confidence to edit the chapters authored by the scientists with expertise on the particular crops or crop groups and present them in a lucid manner with more or less uniform outline of contents and formats. However, my experience gained over the last 7 years in the capacity of the Founding Principal Coordinator of the International Climate Resilient Crop Genomics Consortium (ICRCGC) was highly useful while editing these books. I have the opportunity to interact with a number of leading scientists from all over the world almost on regular basis. Organizing and chairing the annual workshops of ICRCGC since 2012 and representing ICRCGC in many other scientific meetings on climate change agriculture offered me a scope to learn from a large number of people from different backgrounds including academia, industries, policy-making bodies, funding agencies, and social workers. I must acknowledge here the assistance I received from all of them to keep me as a sincere student of agriculture specifically plant breeding.

This volume entitled Genomic Designing of Climate-Smart Oilseed Crops includes eight major crops including Soybean, Oilseed Rape, Groundnut, Sunflower, Flax, Rape and Mustard, Sesame, and Castor Bean. These chapters have been authored by 54 scientists from six countries including Australia, Canada, China, India, Serbia, and USA. I place on record my thanks for these scientists for their contributions and cooperation.

My own working experience on oilseed crops dates back to early 90s in the laboratory of Prof. Thomas C. Osborn in the Department of Agronomy of the University of Wisconsin-Madison. I must confess that this period of about 4 years through working on the Brassicaceae system in his lab and other two labs of his collaborating faculties including Prof. Paul H. Williams in the Department of Plant Pathology and Prof. Jiwan P. Palta in the Department of Horticulture had tailored my mind-set and enriched my expertise and helped me to grow as a science worker. Hence, I have dedicated this book to Prof. Osborn as a token of my respect, thanks, and gratitude.

New Delhi, India Chittaranjan Kole

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Abbreviations

^{13}C	Isotope carbon 13
$\Delta^{13}C$	Carbon isotope discrimination
ABA	Abscisic acid
AC	Ash content
ADH	Alcohol dehydrogenase
AFLP	Amplified fragment length polymorphism
ALA	α-linolenic acid
ALS	Acetolactate synthase
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
AQP	Aquaporin
ARF	Auxin-response factor
ATP	Adenosine triphosphate
ATR	Atrazine
ATT	Acquired thermotolerance
BABA	β-Aminobutyric acid
BAC	Bacterial artificial chromosome
Bar	Bialaphos resistance gene
BC	Back cross
BH	Branching habit
BIM	Bayesian interval mapping
BL	Bayesian LASSO
BLAST	Basic local alignment search tool
BLUP	Best linear unbiased prediction
BPMV	Bean pod mottle virus
BRR	Bayesian ridge regression
BSA	Bulked segregant analysis
Bt	*Bacillus thuringiensis*
C1O	C+1 alleles with capsule open at tip

CaMV	Cauliflower mosaic virus
Cas9	CRISPR-associated 9 protein
CAT	Catalase
CC	Climate change
CDS	Coding DNA sequence
CEG	Core eukaryotic genes
CGMCP	Centre for Genetic Manipulation of Crop Plants
CGRIS	Chinese Genetic Resources Information System
CID	Carbon isotope discrimination
CIM	Common Information Model
CIM	Composite interval mapping
CL	Capsule length
cM	CentiMorgan
CNV	Copy number variant
CO_2	Carbon dioxide
CMS	Cytoplasmic male sterility
CN	Capsule number per plant
CNN	Capsule node number
CNPA	Centro Nacional de Pesquisa de Algodao
CNS	Capsule number per stem
CNV	Copy number variant
CR	Clubroot resistance gene
CRISPR	Clustered regularly interspaced short palindromic repeats
CRP	Coordinated research project
CRR	Charcoal rot resistance
cry1AcF	Delta-endotoxin of *Bacillus thuringiensis* gene (1AcF)
cry1EC	Delta-endotoxin of *Bacillus thuringiensis* gene (1EC)
CS	Climate smart
CTD	Canopy temperature depression
CWR	Crop wild relative
CZL	Capsule zone length
DAG	Diacylglycerols
DALP	Direct amplification of length polymorphism
DAP	Days after planting
DArT	Diversity arrays technology
DAS	Days after sowing
DDBJ	DNA Databank of Japan
DEG	Differentially expressed gene
DGAT	Diacylglycerol acetyltransferase
dgatA	Acyl-CoA:diacylglycerol acyltransferase A
DH	Doubled haploid
DI	Disease index
DMO	Dicamba monooxygenase
DREB	Dehydration responsive element binding (protein)
DREB2A	Drought responsive element binding protein 2A

DS	Determinate sesame
DSB	Double-stranded break
DSF	Days from sowing to flowering
dsRNA	Double-stranded RNA
Dt	Determinate
DTF	Days to flowering
Dw	Dwarf
Dwf	Dwarfing gene
ECP/GR	European Cooperative Programme for Crop Genetic Resources Network
EDB	European Brassica Database
ELS	Early leaf spot
EMF	Embryonic flower
EMS	Ethyl methanesulphonate
EPA	Eicosapentaenoic acid
EPA	Environmental Protection Agency (USA)
EPSP	5-Enolpyruvylshikimate-3-phosphate
ESCORENA	European Co-operative Research Network on Flax and other Bast Plants
ESPS	5-Enolpyruvylshikimate-3-phosphate synthase
EST	Expressed sequence tag
ETI	Effector-triggered immunity
F_2	Second filial generation
FA	Fatty acid
FA	Flowers per leaf axil
FAO	Food and Agriculture Organization
FC	Fiber content
FCL	Length of the lateral capsule
FCT	Thickness of the lateral capsule
FCW	Width of the lateral capsule
FDA	Food and Drug Administration (USA)
FLC	Flowering Locus C
FNEE	Food, nutrition, energy and environment
FNI	Fast neutron irradiation
FOS	*Fusarium oxysporum* f.sp. *sesami*
FSD	Fresh seed dormancy
FT	Flowering locus T
G × E	Genotype × environment
GAB	Genomics-assisted breeding
GBS	Genotyping-by-sequencing
GCA	General combining ability
GE	Genetically engineered
GEAC	Genetic Engineering Appraisal Committee (India)
GEBV	Genome-estimated breeding value
GFF	General feature format

GFP	Green fluorescent protein
GM	Genetically modified
GMHRA	Glyphosate acetyltransferase and modified soybean acetolactate synthase
GN	Grain number per capsule
GO	Gene ontology
GP	Gene pool
GR	Glyphosate resistant
GRD	Groundnut rosette disease
GRDC	Grains Research and Development Corporation
GRIN	Germplasm Resources Information Network (USA)
GRU	Germplasm Resources Unit
GS	Genomic selection
GSO	Seamless capsule open at tip
GSS	Genome survey sequence
GUS	β-glucuronidase gene
GWAS	Genome-wide association study
H	Index of genetic diversity
HAAS	Henan Academy of Agricultural Sciences
HDR	Homology-directed repair
He	Average expected heterozygosity per locus
HFC	Height to the first capsule
Hi-C	Chromosome conformation capture
HOA	High OA
HPPD	4-Hydroxyphenylpyruvate dioxygenase
HR	Highly resistant
HS	Highly susceptible
Hsfs	Heat shock transcription factors
HSP	Heat shock protein
HSRC	Henan Sesame Research Center
I	Shannon's information index
IAEA	International Atomic Energy Agency
IBC	Institute of Biodiversity Conservation
IBPGR	International Bureau of Plant Genetic Resources
ICGR-CAAS	Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ID	Indehiscent
IFDB	International Flax Database
IFVCNS	Institute of Field and Vegetable Crops
IL	Internode length
ILs	Interspecific lines
IND	Improved nondehiscent
InDel	Insertion/Deletion
INTA	Instituto Nacional de Tecnología Agropecuaria

IOD	Iodine value
IP	Intellectual property
IPCC	Intergovernmental Panel on Climate Change
ISSR	Inter-simple sequence repeat
ITPGRFA	The International Treaty for Plant Genetic Resources for Food and Agriculture
KASP	Kompetitive allele-specific polymerase chain reaction
KEGG	Kyoto Encyclopedia of Genes and Genomes
LD	Linkage disequilibrium
LD	Long day
LEA	Late embryogenesis abundant
LG	Linkage group
LIN	Linolenic acid
LIO	Linoleic acid
LIS-1	Linum insertion sequence 1
LLS	Late leaf spot
LN	Leaf number per plant
LncRNA	Long noncoding RNA
LOA	Low OA
LOD	Logarithm of odds
LRR	Leucine-rich repeat
MABC	Marker-assisted backcrossing
MAGIC	Multiparent advanced generation intercross
MAPKK	Mitogen-activated protein kinase kinase
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MBB	Mexican bean beetle
MBPG	Multinational Brassica Genome Sequencing Project
MCL	Length of the central capsule
MCT	Thickness of the central capsule
MCW	Width of the central capsule
MDA	Malondialdehyde
MG	Maturity group
MIM	Multiple interval mapping
MLS	Multilateral System
MoEF&CC	Ministry of Environment, Forest and Climate Change (India)
MR	Moderately resistant
MSD	Main stem diameter
MSIL	Length of main stem internode
MSNN	Node number of main stem
MTA	Marker-trait association
MTA	Material Transfer Agreement
Na	Number of alleles
NAM	Nested association mapping
NARS	National Agricultural Research System (India)

NBPGR	National Bureau of Plant Genetic Resources (India)
NBS	Nucleotide-binding site
NCBI	National Center for Biotechnology Information
NDVI	Normalized difference vegetation index
Ne	Effective number of alleles
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
NIL	Near-isogenic lines
NN	Node number
*npt*II	Neomycin phosphotransferase II gene
NTG	N-methyl-N′-nitro-N-nitrosoguanidine
NUE	Nutrient use efficiency
OA	Osmotic adjustment
OA	Oxalic acid
OC	Oil content
OLE	Oleic acid
OLP	Osmotin-like protein gene
PAGE	Parametric analysis of gene set enrichment
PAL	Palmitic acid
PAT	Phosphinothricin acetyltransferase
PAV	Presence/absence variants
PCR	Polymerase chain reaction
PE	Paired end
PEG	Polyethylene glycol
PGR	Pod growth rate
PH	Plant height
PHB	Polyhydroxybutyrate
PIABS	Photosynthetic efficacy index
PIC	Polymorphic information content
PiHS	Population-based integrated haplotype score
Pl	Downy mildew resistance gene
PLCP	Papain-like cysteine protease
PLH	Potato leafhopper
PO	Protein content
POX	Peroxidase
PPO	Polyphenol oxidase
PR	Pathogenesis-related
PRH	Bearing height of primar raceme
PUFA	Polyunsaturated fatty acids
PVE	Phenotypic variation explained
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
R	Resistance gene
RAD	Restriction site-associated DNA
RAPD	Random amplified polymorphic DNA

RCA	*R. communis* agglutinin
RcPAL	*Ricinus communis* phenylalanine ammonialyase gene
RcPEPC	*Ricinus communis* phosphoenolpyruvate carboxylase gene
Rf	Fertility restoration gene
RFLP	Restriction fragment length polymorphism
RGB	Red, green and blue
RGC	Resistance gene candidate
RHL	Residual heterozygous line
RIL	Recombinant inbred line
RNAi	RNA interference
ROD	Reduction of density
RR-BLUP	Ridge regression best linear unbiased prediction
RRGS	Reduced-representation genome sequencing
RRS	Reduced-representation sequencing
RSA	Root system architecture
RSAMPL	Random selective amplification of microsatellite polymorphic locus
RSLs	Recombinant substitution lines
RT-PCR	Real-time PCR
RT-PCR	Reverse transcription PCR
RXBS	Rongxian black sesame
SAM	Sequence alignment map
SAT	Semi-arid tropics
SBA	Soybean aphid
SbDV	Soybean dwarf virus
SBL	Soybean looper
SbNHX1	*Salicornia brachiata* reverse transporter protein gene
SC	Sesamin content
SCAR	Sequence-characterized amplified region
scFv	Single-chain variable fragment
SCMR	SPAD chlorophyll meter reading
SCoT	Start codon targeted polymorphism
SD	Short day
SDS	Sudden death syndrome
SEA	Singular enrichment analysis
SFW	Sesame Fusarium wilt
SG	Selective genotyping
SGMD	Soybean Genomics and Microarray Database
SGP	The Sesame Genome Project
SGWG	The Sesame Genome Working Group
SHA	Shattering
SIM	Simple interval mapping
SiNPs	Silicon nanoparticles
SLA	Specific leaf area
SLAF	Specific length amplified fragment

SLAF-seq	Specific length amplified fragment sequencing
SMA	Single marker analysis
SMG	Suppressor with morphogenetic effects on genitalia
SMV	Soybean mosaic virus
SN	Seed number per plant
SNC	Seed number per capsule
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SoyGD	Soybean Genome Database
SR	Shatter resistant
SRAP	Sequence-related amplified polymorphism
SSCP	Single-strand conformational polymorphism
SSH	Semi-shattering
SSR	Simple sequence repeat
STE	Stearic acid
STF	Days from sowing to flowering
STS	Sequence tagged site
SUS	Super-shattering
TAG	Triacylglycerols
TALEN	Transcription activator-like effector nuclease
TE	Transpiration efficiency
TFL-like	Terminal flower-like
TIGR	The Institute for Genomic Research
TILLING	Targeting induced local lesions in genomes
TIR	Temperature induction response
TL	Tip length without the capsule
TP	Training population
TRAP	Target region amplification polymorphism
TRAP	Tartrate-resistant acid phosphatase
TSS	Total soluble sugars
TSW	Thousand seed weight
TSWV	Tomato spotted wilt virus
TT	Triazine tolerant
TUFGEN	Total Utilization Flax Genomics
UGM	Ungrouped matches
UPM	The Universidad Politécnica de Madrid
UPOV	International Union for the Protection of New Varieties of Plants
USDA	United States Department of Agriculture
UTRs	Untranslated regions
VBC	Velvet bean caterpillar
VIR	Vavilov Institute of Plant Industry
VNIIMK	All-Russia Research Institute of Oil Crops
VPD	Vapor pressure deficit
WGR	Whole genome re-sequencing
WGS	Whole genome shotgun

WSC	Water soluble carbohydrates
WUE	Water use efficiency
ZFN	Zinc-finger nucleases

Chapter 1
Approaches, Applicability, and Challenges for Development of Climate-Smart Soybean

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Abstract Soybean (*Glycine max* L.) is an economically important crop providing a great source for vegetable oil and protein. Yield losses of soybean under current climate change keep increasing, despite the progressive increase in yield through breeding and management practices since the 1960s. Conventional breeding facilitated the development of high-quality soybeans with enhanced tolerance to severe environmental fluctuations such as drought, flooding, heat, and salinity. However, conventional approaches are laborious, time consuming, and looks inefficient to fulfill the increasing demands of the growing world population. The advances in marker-assisted and genomics-assisted breeding, sequencing technologies, and bioinformatics tools have enabled the soybean improvement at a faster pace. The rapidly accumulating genomic resources have enabled the development of molecular markers associated with many important quantitative trait loci, provided a clear picture of genomic variations in soybean germplasm, and identified key genes for genetic engineering. This knowledge is being utilized to facilitate the development of climate-smart soybeans. In this chapter, we discuss and summarize the advances

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in soybean improvement through conventional and genomics-assisted breeding, genetic engineering approaches, and available bioinformatics tools for soybean. This chapter also highlights soybean genetic resources, diversity analysis, association mapping, as well as recent strategies such as gene editing and nanotechnology application in soybean breeding programs. This information could facilitate the incorporation of climatic-smart traits in breeding for more stable soybean production with the changing climate.

Keywords Soybean · Climatic change · Genetic diversity · Marker-assisted breeding · Genetics · Genomics · Stress resistance · Gene editing

1.1 Introduction

Soybean is a self-pollinated plant that belongs to the family Fabaceae and *Glycine* genus. The *Glycine* genus is subsequently divided into subgenera, *Glycine* and *Soja*. The subgenus *Soja* has two highly recognized species including the cultivated soybean *Glycine max* and the wild soybean *Glycine soja*. Soybean is an economically important legume crop that is rich in seed protein (40%) and oil (20%), which provides sources of starch, dietary fiber, protein, lipids, and essential minerals for human as well as for livestock (Chaudhary et al. 2015). It is widely grown as a grain legume and oilseed crop in the world including the countries United States, Brazil, Argentina, China, and India. The US is the leading producer with 35% (119.5 Million Metric Tons) of the global production (340.9 Million Metric Tons) of soybean (SoyStats 2018 www.soystats.com).

Soybean is in high demand not only for food and feed consumption, but also it can potentially serve as a future fuel feedstock and biodegradable plastics (Candeia et al. 2009; Song et al. 2011). Furthermore, soybean is also used in industrial and pharmaceutical applications as well as in the production of biodiesel (Goldberg 2008). Due to diverse uses, soybean has become a highly desirable crop and its demand is rapidly increasing. However, the increasing global population will need doubled food production by the year 2050 and we can only achieve $\sim$55% of the required production at the current rate of yield improvement. It is expected to be more difficult to produce sufficient yield with the changing climate (Ray et al. 2013; Deshmukh et al. 2014). Climate change and extreme weather conditions have a negative impact on crop yield, because solar radiation, temperature, and precipitation are the main drivers of crop growth. Additionally, climate change influences the plant diseases and pest infestations, as well as the supply of and demand for irrigation water (Rosenzweig et al. 2001). Therefore, the emphasis must be given toward the production of high-yielding soybeans with high nutritional value, which are environmentally stable and resistant to extreme weather conditions.

Plant breeding has undoubtedly improved soybean yield and resistance to biotic and abiotic stresses to achieve the current level of demand, but the main challenge is to continue to increase the production under the current scenario of climate

change. In general, breeding for a complex trait is challenging due to their control by multiple genes and they are also greatly influenced by the environment. The conventional breeding procedures such as backcrossing, single pod descent, pedigree breeding, and bulk population breeding are used in order to develop improved varieties of soybean (Poehlman et al. 1995). To facilitate breeding advances, it is necessary to employ modern breeding techniques such as marker-assisted breeding, recombinant DNA technology, genome editing and "omics" (genomics, transcriptomics, proteomics, metabolomics, ionomics) to improve the soybean quality and yield. In addition, the concerns about environmental stress due to climate change and demand of ample supply have instilled a new urgency into accelerating the rates of genetic gain in breeding programs. Therefore, regardless of the conventional breeding efforts, it is essential to integrate the next-generation molecular and omics approaches for the production of high-yielding soybeans with enhanced adaptation to various environmental stresses.

1.2 Prioritizing Climate-Smart Traits

1.2.1 Flowering Time and Maturity

Plants can perceive various environmental signals, such as photoperiod, temperature, and stresses, to flower, and thus control seed production. In soybeans, flowering time and maturity are important agronomic traits, which are useful for developing soybean cultivars with a wider geographical adaptation. Soybean is a short-day plant. Short days induce flowering while long-day conditions delay flowering. Photoperiod and in-season temperature are the primary factors that dictate the region where a soybean variety is adapted. Soybean can grow in a wide range of latitudes, from 50°N to 35°S (Watanabe et al. 2012). The adaptability of soybean in a wide latitude is caused by natural variations of many genes controlling flowering time and maturity. The study on the flowering and maturity controlling mechanism of soybean can provide a theoretical basis and genetic materials for soybean breeding, especially under the climate change scenario. Understanding the regulatory mechanisms of flowering time and maturity allows us to modify the growth cycles of soybean to overcome or avoid different stresses by manipulation of the two traits.

1.2.1.1 Overview of Flowering and Maturity Regulating Genes in Soybean

Flowering time (days to R1) and maturity (days to R8) in soybean have been reported to be highly correlated traits (Mansur et al. 1996). Photoperiod insensitivity, flowering time, and maturity were found to be controlled by the same genes or by tightly clustered genes in the same chromosomal region (Tasma et al. 2001).

In 1927, a major gene locus was detected to control maturity (Owen 1927). Subsequent research work found that the *E1* locus is largely responsible for the variation in flowering time among cultivars (Bernard 1971; Abe et al. 2003). To date, ten genes related to flowering and maturity have been reported including nine *E* genes (*E1*–*E9*) and one *J* gene (Bernard 1971; Buzzell and Voldeng 1980; Bonato and Vello 1999; Cober et al. 2010; Kong et al. 2014). Six *E* genes, *E1*, *E3*, *E4*, *E7*, *E8,* and *E9*, can specifically participate in photoperiod response (Cober et al. 1996; Cober and Voldeng 2001; Cober et al. 2010), with *E1*, *E3*, *E4*, *E7*, and *E8* as recessive loci (Watanabe et al. 2012; Kong et al. 2014). Introgression of these early flowering alleles results in earlier flowering under long day and improved adaptation to short summers at high latitudes. The *J* locus was identified in the progeny of crosses between standard and late flowering cultivars with a long-juvenile habit, whose recessive allele causes late flowering under short days (Ray et al. 1995). In general, the trait of "delayed juvenile" is useful for adaptation to low latitudes and spring sowings at the lower latitudes (Tomkins and Shipe 1997).

1.2.1.2 Cloning Genes Underlying the Flowering and Maturity Traits

Efforts were made to clone the underlying genes of the loci to understand the mechanisms of flowering and maturity in soybean. The *E1* gene was map-based cloned to encode a B3-like protein, which is belonging to a family of plant-specific transcription factors. *E1* from soybean shows high similarity to other legumes, such as *Medicago truncatula* and *Lotus corniculatus*. However, the *E1* gene does not exist in the model plants Arabidopsis and rice. The *E2* gene was identified as the homolog of *GIGANTEA* (*GI*), the unique plant-specific nuclear clock-associated protein, which contributes to the maintenance of circadian period length and amplitude, and regulates flowering time and hypocotyl growth in response to day length (Watanabe et al. 2011). *E2* can enhance the photoperiod response of soybean, and it is closely related to the early flowering phenotype of soybean and the light adaptability. *E3* and *E4* encode the phytochrome (phy) family of photoreceptors PHYA3 and PHYA2, respectively (Liu et al. 2008; Tsubokura et al. 2013). Soybean contains four *PHYA* genes that consist of two pairs of homologs. *E3* and *E4* represent in different homolog pairs. The homolog of *E4*, *PHYA1*, is apparently functional, whereas the homolog of *E3* carries a deletion and is probably a pseudogene (Watanabe et al. 2009). *E9* was identified as *FT2a*, an ortholog of Arabidopsis *FLOWERING LOCUS T*, through fine-mapping, sequencing, and expression analysis. Recessive allele of *E9* delays flowering because of lower transcript abundance that is caused by allele-specific transcriptional repression.

1.2.1.3 Application of Classification of Maturity Group (MG) in Soybean

Understanding the mechanism of soybean flowering time and maturity diversity and adaptation is very important for breeding for high productivity in diverse latitudes. Many soybean cultivars were bred with different maturity to adapt various ecological environments. For the convenience of breeding layout, 13 MGs from 000, 00, 0, I, II, to X were classified based on photoperiod and yield trial in North America (Zhang et al. 2007). Maturity group zones represent defined areas, where a cultivar is best adapted. But the classification of maturity group is still not internationally unified. Based on the knowledge mentioned above, flowering and maturity were highly controlled by major genes in soybean. Therefore, flowering time and maturity can be adjusted by soybean genetic change through breeding efforts and genetic engineering. Although photoperiod remains constant, climatic conditions, management practices, and soybean genetics have changed during the past decades. Maturity group adaptation zones need to be understood, applied and adjusted for the breeding benefit (Mourtzinis and Conley 2017).

1.2.2 Seed Composition

Soybean is a major crop for oil and protein resources, accounts for 56% of total oilseed production in the world (Wilson 2008). The seed quality is determined by the seeds' composition, including protein, oil, sugars, and minerals. Soybean seeds contain 40% protein, 20% oil, 15% soluble carbohydrate, and 15% fiber on a dry weight basis. Protein and oil are the most abundant and valuable compositions in soybean.

1.2.2.1 Oil

Soybean seed contains about up to 230 g kg^{-1} of oil on a dry weight basis and the oil contents are constituted by 16% saturated, 23% monounsaturated, and 58% polyunsaturated fatty acids (Bellaloui et al. 2015). The major unsaturated fatty acids in soybean are the polyunsaturated alpha-linolenic acid (7–10%), linoleic acid (51%), and the monounsaturated oleic acid (23%) (Poth 2000; Ivanov et al. 2010). This makes soybean oil valuable in terms of human healthy diets. However, soybean oil has approximately 24% monounsaturated fatty acids (C18:1), which are significantly less than competing oils such as canola (61%) and olive (40%) (Terés et al. 2008). Oleic Acid (C18:1), a monounsaturated omega-9 fatty acid typically makes up 55–83% of total oil content in olive. Monounsaturated fats are resistant to high heat, making extra virgin olive oil a healthy choice for cooking.

Genetic Regulation of Seed Oil Production

The oil concentration in soybean seeds is a quantitative trait governed by a number of genes mostly with small effects and under influence of the environment. A negative relationship between seed oil and protein was well documented, which makes it difficult for breeders to develop high-oil soybean genotypes while retaining a high level of protein (Wilcox and Shibles 2001; Hyten et al. 2004b). There are >130 quantitative trait loci (QTLs) reported to be associated with oil content in soybean (Qi et al. 2011), since the first documented report to detect oil QTL (Diers et al. 1992). Among these oil QTLs, only a few have been detected in multiple genetic backgrounds or environments, and none have been widely used in marker-assisted selection (MAS) for high oil in soybean breeding programs. This could be due to several factors affecting the usefulness of QTL, including large confidence intervals, QTL × environment, and QTL × genetic background interactions, which all impede the use of QTL in breeding programs (Qi et al. 2011).

Except QTLs, some transcription factors have been reported to modify the seed oil content in soybean, such as transcription factors, *LEC1*, *LEC2*, *ABI3*, and *FUS3*, which are master regulators of seed development, and thus regulate oil content (Mendoza et al. 2005). Besides, overexpression of *GmDOF4* and *GmDOF1*1 increased lipid content in seeds of transgenic Arabidopsis plants via direct activation of lipid biosynthesis genes and the repression of storage protein genes (Wang et al. 2007). Transcription factor *GmbZIP123*, also elevated lipid contents in seeds of transgenic Arabidopsis plants by activating Suc-transporter genes and cell-wall-invertase genes for sugar translocation and sugar breakdown, respectively (Song et al. 2013).

Metabolic Engineering of Fatty Acid Composition

Most domesticated oilseed crops have been successfully modified through either breeding or genetic engineering approaches to optimize the ratio of endogenous fatty acids in the storage oil for specific end uses (Drexler et al. 2003). For example, suppression of the oleate D12-desaturase gene in soybean, sunflower, cotton, and canola has resulted in the production of oils with a high C18:1 fatty acids, which have a greater oxidative stability and improved performance in high-temperature cooking applications. Oils with a high C18:1 ratio are also desired by the chemical industry, as C18:1 can be used in a variety of applications including detergents, soaps, lubricants, cosmetics, and emulsifying agents, and as a source of C9 monomers for plastics (Metzger and Bornscheuer 2006). Buhr et al. (2002) described the development of transgenic soybean events in which the expression of *FAD2-1* and *FatB* was simultaneously downregulated in a seed-specific fashion, thereby generating soybean oil with a reduced content of C16:0 (<5%) and significantly increased C18:1 content (>85%) (Buhr et al. 2002). Recently, naturally occurred mutant alleles of *FAD2-1A* and *FAD2-1B* in soybean plant introduction (PI) collections were identified (Pham et al. 2011). The traditionally bred soybean

lines carrying both homozygous mutant *FAD2-1A* and *FAD2-1B* alleles were developed by marker-assisted backcrossing and the C18:1 contents of these lines have increased from 20% to an average of 82–86%. On the other hand, upregulating the endoplasmic reticulum oleoyl and linoleoyl desaturases in oilseeds could lead to a substantial increase in polyunsaturated fatty acids (PUFAs) in the oil. For example, the overexpression of a fungal bifunctional Δ12 and Δ15 desaturase in soybean resulted in over 70% of C18:3, compared to 19% in the wild type, in somatic embryo oils (Damude et al. 2006).

1.2.2.2 Protein

The total content of protein in soybean seed is very important, approximately 60% of the value comes from soybean meals (Pettersson and Pontoppidan 2013). Poultry and livestock need a minimum of 47.5% protein content in soybean meal for their proper growth and development. Soybean seed composition, especially seed storage protein, is also a complex trait controlled by a network of genes, and interaction with the environment. Besides, increasing seed storage protein is difficult due to its strong negative correlation with oil content and seed yield (Bandillo et al. 2015; Chaudhary et al. 2015).

Seed Protein Composition

Soybean, like many other seeds, has two major storage proteins, glycinin (11S legumin type) and conglycinin (7S vicilin type), which dominate the proteome (Herman and Larkins 1999). The soybean seed proteome also includes many moderately abundant proteins that are bioactive and allergenic, such as the Kunitz and Bowman–Birk trypsin inhibitors, lectin, P34 allergen, sucrose-binding protein, urease, and oleosins, together with several thousand low abundance proteins (Herman and Burks 2011). The specific mix of proteins and each protein's abundance within the proteome determine this protein amino acid composition trait (Herman 2014). The development of soybean cultivars with enhanced protein and amino acid content would further increase the economic value of the crop and will help to enrich the entire value chain from farmers to processors to end users.

Genetic Regulation of Protein Content

Over the past decades, considerable resources, including genomic, transcript, single nucleotide polymorphism (SNP), simple sequence repeat (SSR) maps, and proteomics, have been helped to elucidate the genetic regulation of soybean protein content. With the advancement of genetic map construction, the availability of a well-annotated reference genome (Schmutz et al. 2010), resources for association mapping (Song et al. 2013), and whole-genome resequencing (WGR) data (Zhou

et al. 2015; Valliyodan et al. 2016) a large number of QTLs for seed protein content have been identified.

Currently, >160 QTLs have been identified to be associated with seed protein content in soybean. Among these, a major QTL for seed protein and oil content has been consistently mapped on Chr. 20 and remarkable attention has been given to this QTL due to its high additive effect and stability (Diers et al. 1992; Hwang et al. 2014). Due to the large environmental effects, only two QTLs, one on Chr. 15 (cqPro-15) and the other one on Chr. 20 (cqPro-20), are designated as officially confirmed QTL based on error rate (lower than 0.01) (http://soybase.org/). However, the presence of QTL for higher protein on Chr. 20 was negatively correlated with seed yield (Nichols et al. 2006), which suggests that it is a tradeoff when we try to increase protein content. Introgression of this QTL into elite backgrounds would increase the value of soybean to compensate the yield drag.

1.2.3 Abiotic Stress Tolerance

Plants face a constant threat from various abiotic stresses, including drought, waterlogging, heat, cold, nutrient deficiency, and so on. Climate changes increase the occurrence of extreme weather patterns including irregular precipitation and extreme temperatures in the global agricultural areas, which cause a significant reduction in crop production and threaten food security (Lesk et al. 2016). Yield losses of major crops under irregular weather patterns keep increasing, despite the progressive increase in yield through breeding and management practices since the 1960s (Boyer et al. 2013; Lobell and Tebaldi 2014). To achieve sustainability in agriculture, it is crucial to develop crops with tolerance to abiotic stresses. During the evolution, plants have been developing tolerance traits to overcome these stresses. Incorporation of these tolerance traits into current elite germplasm is a key to maintain sustainable crop production.

1.2.3.1 Drought Tolerance

Drought is the major abiotic stress that threatens crop production. Climate changes are anticipated to intensify the occurrence of irregular precipitation patterns worldwide, which will further negatively affect crop production and food security. Soybean as one of the most important crops with multiple consumable purposes, also suffers from drought stress. In crops, drought resistance is translated to traits enhancing yield stability rather than that increasing survivability under drought (Blum 2009; Passioura 2010; Sinclair 2011; Passioura 2012; Valliyodan et al. 2016). These translated traits are correlated with yield under drought and have no yield penalty under nonstress conditions. The success of soybean improvement under drought and heat stress depends on the discovery and utilization of genetic variations present in the germplasm. Identification of genetic diversities for traits

related to drought and heat tolerance has helped identify genetic resources in soybean. In this section, advance in drought tolerance in soybean is summarized by highlighting the traits contributing to drought tolerance, including root system architecture (RSA), water use efficiency, canopy wilting, and sustained N-fixation under drought.

Root System Architectures and Anatomy

Kramer (1969) stated an essential characteristic of drought tolerance: "deep, wide-spreading, much-branched root system". Root systems are usually involved in both drought avoidance and tolerance during water deficits due to the constitutive and plastic characteristics of roots. RSA refers to the shape of the roots and the physical space; and the deeper and wilder root system can avoid tissue dehydration by their ability to acquire more water resource. RSA is also highly plastic to respond rapidly to environmental changes such as water deficit. When plants perceive water deficit stress, roots tend to keep growing and penetrate into deeper soil layers (Hoogenboom et al. 1987; Creelman et al. 1990; Wu et al. 1994). The ability of plants to develop deeper rooting systems under drought stress depends on the tolerance levels of the roots to water deficit stress. Some lines were observed to be able to significantly elongate their rooting depth than some other lines under drought stress in legumes, including soybean (Garay and Wilhelm 1983; Sponchiado et al. 1989). Genetic diversity of RSA has evolved through geographic adaptation of plants. Deep rooting, which is a complex trait affected by growth angle and root length (Araki et al. 2002), plays a crucial role in water uptake from deeper soils to avoid drought under water deficit conditions. Root angle determines the direction of horizontal and vertical distribution of roots in the soil. It is recognized as an adaptive trait for drought avoidance in crops (Mace et al. 2012; Christopher et al. 2013; Uga et al. 2013). In addition to deep rooting, drought stress also induces the plasticity responses of root systems by increasing the number of fibrous roots, decreasing lateral root diameter, and fluctuations in root biomass (Nielsen et al. 1997; Osmont et al. 2007; Meister et al. 2014; Salazar-Henao et al. 2016). Alterations in root anatomy, such as aerenchyma formation in maize (Lynch 2011; Burton et al. 2013), save the energy inputs to allow improved soil penetration and exploration to compensate water deficit (Addington et al. 2006; Maseda and Fernández 2006).

In soybean, the improved RSA was shown to alleviate drought stress by increasing exploration for water and nutrients (Hoogenboom et al. 1987). Natural variation in RSA was reported in soybean, which was suggested to be used for the improvement of drought tolerance (Carter 1989). In the field evaluation, the drought-tolerant Japanese landrace PI 416937 displayed the ability to utilize upper soil horizon with a great network of fibrous roots and was found to have greater lateral root system spread than that of Forrest (Hudak and Patterson 1996). Recently, upon screening of a core set (400 lines) of the USDA Germplasm Collection, several soybean accessions have been identified to have promising RSA

for extensive fibrous rooting, root length or large root angle. Genetic diversity was also observed in root anatomy of soybean and change in root anatomy also affects the water movement through root systems (Rincon et al. 2003). The examination of root anatomy of 41 soybean lines led to the detection of variations in the number of metaxylems in roots. The number of metaxylems was found to be correlated with drought tolerance in soybean, as soybean plants were observed to develop a greater number of metaxylem under drought conditions and the drought-tolerant lines develop obviously more numbers than the drought-sensitive lines (Prince et al. 2017). QTL mapping has been conducted in soybean and many QTLs associated with RSA and drought tolerance have been mapped (Abdel-Haleem et al. 2011; Manavalan et al. 2015; Prince et al. 2015). This information is being used in molecular-assisted breeding to incorporate these RSA traits into elite varieties for drought tolerance improvement.

Water Use Efficiency and Canopy Wilting

Improving water use efficiency is another promising strategy to overcome drought stress. The essential factors to improve water use efficiency are to conserve water in plants and reduce the unnecessary transpiration losses (Turner et al. 2001; Turner 2003). In soybean, researchers made efforts to look for traits associated with water use efficiency, which can allow screening for drought tolerance on a large scale. In 1990, a phenotype of slow canopy wilting under drought and heat stresses was observed in PI 416937, a Japanese drought-tolerant landrace in the maturity group (MG) VI (Sloane et al. 1990). Field evaluation found a contradictory phenomenon that PI 416937 actually used a significantly less amount of water than the drought-sensitive checks (Hudak and Patterson 1996). Further research on water conservation aspects of PI 416937 revealed that this line can limit its transpiration rate under vapor pressure deficit (VPD) above 2.0-KPa compared with other drought-sensitive genotypes (Fletcher et al. 2007; Tanaka et al. 2010; Ries et al. 2012). PI 416937 offered breeding resources for improving drought tolerance in late maturity groups of soybean. Recently, two additional slow canopy wilting landraces (PI 567690 and PI 567731) at MG III were identified after the evaluation of a core set of 250 soybean germplasm lines for canopy wilting and drought tolerance (Pathan et al. 2014). These two lines shared the similar physiological mechanisms of limiting transpiration rate under high VPD as PI 416937 and offered breeding materials for early maturity groups in soybean (Pathan et al. 2014). Moreover, QTL mapping studies performed using drought-tolerant soybean genotypes have identified genomic loci governing physiological traits like slow wilting. For instance, 10 QTLs associated with slow canopy wilting traits have been mapped and subsequently, DNA markers were developed for MAS in breeding (Abdel-Haleem et al. 2012; Hwang et al. 2015; Hwang et al. 2016). The complexity of the canopy wilting trait also indicates that stacking all confirmed QTLs by MAS or genomic selection is necessary to recover the drought tolerance performance shown in the original drought-tolerant exotic PIs.

Sustained N-Fixation Under Drought

N-fixation is highly sensitive to drought stress (Djekoun and Planchon 1991; Adams et al. 2016). Soybean plants transport ureides from nodules to shoots, which make them more sensitive to drought stress than the other legumes transporting amides (Sinclain and Serraj 1995). In soybean, ureides accumulate in leaves and nodules during drought stress and impose a negative feedback to inhibit nitrogenase activity for N-fixation (Serraj et al. 1999; Vadez and Sinclair 2000; Ladrera et al. 2007). Shoot ureide concentration was found to be associated with drought tolerance and used as another indicator of drought tolerance (Desilva et al. 1996; Serraj and Sinclair 1996a, b; Purcell et al. 1998). In the 1990s, Sinclair et al. (2000) screened 3081 soybean PIs for sustained nitrogen fixation, in which eight PIs were identified to have the best performance in sustained N-fixation under drought and have promising drought tolerance related to yield stability. All these soybean genotypes have been used in breeding programs to develop drought-tolerant elite lines (Devi et al. 2014). Recently, a large number of QTLs associated with shoot ureide and nitrogen concentration were mapped in both biparental populations and genome-wide association studies (GWAS) in diverse lines (Hwang et al. 2013; Ray et al. 2013; Dhanapal et al. 2015), which indicated the complexity of N-fixation under drought and suggested that genomic selection should be better suited to improve such complex traits.

1.2.3.2 Waterlogging Tolerance

Recent climate change data with a predicted 30% increase in heavy precipitations by 2030 show that flooding stress will be more severe in the future. Soybean is sensitive to waterlogging, resulting in significant yield reduction, ranging from 46 to 56% (Scott et al. 1989; Oosterhuis et al. 1990; Linkemer et al. 1998). Waterlogging triggers root damage which affects water and nutrient uptake and subsequently causes a reduction in nodulation, impaired photosynthesis, and plant death due to diseases which ultimately results in yield loss (Oosterhuis et al. 1990; Vantoai et al. 1994).

Lack of cellular oxygen in roots was believed as a major component associated with waterlogging stress in soybean. In soybean, aerenchyma formation was also thought to be important for internal aeration of roots to mitigate the root damage during cell anoxia. RSA and plasticity could be another tolerance strategy to compensate root damage during waterlogging and could accelerate root recovery after waterlogging. Previously, a positive correlation was found between total root length and waterlogging tolerance in soybean germplasm lines and one waterlogging tolerant soybean line tends to generate more adventitious/aerial roots than a sensitive line (Kim et al. 2015). Favorable RSA and plasticity in soybean can lead to less waterlogging damage and faster plant recovery after waterlogging stress as revealed through the study of a major waterlogging tolerance QTL in soybean (Ye et al. 2018).

Genetic diversity of flooding tolerance has evolved through geographic adaptation of soybean plants. Shannon et al. (2005) screened a core set of 350 soybean germplasm lines for flooding tolerance at the early reproductive stage. The flooding susceptible lines lost approximately two times more yield compared to the flooding tolerant lines while the exotic PIs have much better tolerance than cultivars. Several cultivated germplasm lines (*Glycine max*) were identified as potential donor sources for the breeding for flooding tolerance, including Archer, Misuzudaiz, PI 408105A, PI 561271, PI 567651, PI 567343, VND2, Nam-Vang, and ATF15-1. In the past few years, lots of QTL mapping has been performed, and several QTLs associated with waterlogging tolerance have been identified (Scott et al. 1989; VanToai et al. 2001; Cornelious et al. 2005; Shannon et al. 2005; Rhine et al. 2010; Vantoai 2010; Nguyen et al. 2012; Ye et al. 2018). Among these QTLs, a major QTL, mapped on chromosome 3, was confirmed at the near-isogenic background and this QTL was reported to increase yield by up to 40% in the field (Ye et al. 2018).

1.2.3.3 Salt Tolerance

Salt stress is one of the major abiotic factors affecting crop growth and production. In general, soybean is sensitive to salt stress (Munns and Tester 2008). Soybean yield could be reduced by 50% when the electrical conductivity of the saturation extract of soil was 9 millimhos/cm (Abel and Mackenzie 1964; Papiernik et al. 2005). Na^+ and Cl^- ions absorption and accumulation in high concentrations causes toxicity in soybean plants, and results in plant death with increasing salt concentration in the soil (Pathan et al. 2007; Phang et al. 2008). Improvement of salt tolerance in soybean is necessary to ensure food security for the world. The success of such improvement depends largely on the discovery and utilization of genetic variation present in the germplasm and characterization of salt tolerance genes and mechanisms.

A large amount of work has been done to investigate salt tolerance in soybean. Based on the responses of soybean plants to salt stress, several phenotyping indices were developed to evaluate salt tolerance levels of soybean plants and the most commonly used indices are based on visual rating, including leaf scorch score, salt tolerance rating, and survival rate. With these phenotyping indices, rich genetic variations of salt tolerance were observed in soybean and salt-tolerant soybean lines were identified over the years (Parker et al. 1983; Yang and Blanchar 1993; Luo et al. 2005; Hamwieh and Xu 2008; Lee et al. 2009b; Chen et al. 2013; Ha et al. 2013; Qi et al. 2014; Do et al. 2016; Xu et al. 2016). The existing genetic diversity in salt tolerance in soybean offers genetic resources to breed salt-tolerant varieties.

QTL mapping for salt tolerance has been focused mainly on soybean seedling stage. The first QTL mapping was performed in an $F_{2:5}$ population derived from the cross of S-100 (salt tolerant) and Tokyo (salt sensitive) and two QTLs were mapped on linkage groups L and N. The QTL on linkage group N (Chr.3) showed the major effect in salt tolerance with a phenotypic contribution of 60% (Lee et al. 2004). Later on, this major QTL was confirmed by multiple studies and a few minor QTLs

were also mapped in different populations (Lee et al. 2004; Chen et al. 2008a; Hamwieh and Xu 2008; Hamwieh et al. 2011; Ha et al. 2013). The major salt tolerance QTL located on Chr.3 (linkage group N), was identified by several research groups using different soybean mapping populations. The underlying gene was cloned to encode an ion transporter and identified to be involved in Na^+ and Cl^- exclusion and homeostasis regulation (Guan et al. 2014; Qi et al. 2014; Do et al. 2016; Liu et al. 2016). This gene is heavily used to improve salt tolerance in current soybean elite germplasm and other new major tolerance resources are needed to enhance the genetic diversity of salt tolerance in soybean.

1.2.3.4 Heat Tolerance

Extreme temperatures cause about 40% reduction in soybean yield (Specht et al. 1999). Heat stress during the vegetative stage affects the growth of soybean. Under heat stress, soybeans in reproductive stages were shown to have increased flower and pod abortion and in later periods of pod-filling stages, prolonged stresses resulted in fewer and smaller seeds with reduced seed vigor (Boyer 1983; Chebrolu et al. 2016). Reproduction of soybean is sensitive to high temperatures (>35 °C), therefore, improving heat tolerance of soybean varieties is crucial to improve the yield (Salem et al. 2007).

Heat stress during reproductive stages such as flowering and seed development significantly decreases soybean yield (Kebede et al. 2012; Redden et al. 2014; Siebers et al. 2015). Increased flower and pod abortion and reduced seed germinability were observed in soybean plants subjected to extreme heat (Boyer 1983; Salem et al. 2007; Chebrolu et al. 2016). Fattened and collapsed pollens were observed in soybean under heat stress, which resulted in lower pollen viability and fertilization rates (Salem et al. 2007). Soybean plants exposed to high temperature (38/28 °C) showed 22.7% reduction in pollen germination and consequently had about 35% reduction in pot setting and the anatomical changes in pollens under heat stress were observed (Djanaguiraman et al. 2013). Evaluation of 44 soybean genotypes from MGs III-IV for heat tolerance helped to categorize into heat-tolerant, -intermediate, and heat-sensitive groups based on pollen viability. Among the 44 genotypes, 13 were identified as most heat tolerant and can be used in breeding programs for heat tolerance at the reproductive stages. Seed development is much more vulnerable than vegetative tissues to heat stress. Heat stress at the pod filling stages of soybean results in seed with less vigor, poor germination and increased incidence of pathogen infection (Hatfield et al. 2011). Genetic diversity and significant differences in germinability under heat stress between the heat tolerant and sensitive lines were reported (Chebrolu et al. 2016). Germination of seeds from the heat-sensitive genotype reduced by 50% under 36/24 °C and completely inhibited under 42/26 °C compared to normal conditions (28/22 °C). In contrast, seed germinability from the heat-tolerant genotypes was unaffected under 36/24 °C, and was reduced by 75% under 42/26 °C treatment compared with

normal conditions (Chebrolu et al. 2016). These identified heat-tolerant lines are good targets for gene discovery for heat tolerance and soybean breeding programs.

1.2.3.5 Cold Tolerance

Cold tolerance is also an important trait to develop climate-smart soybean. The decreased seed yields caused by low temperatures have been attributed to two stages: poor germination and seedling vigor during the early growth stage, abortion of flowers and inadequate grain filling at reproductive stages (Yamamoto and Narikawa 1966). To expand soybean production area, cold tolerance is a key trait, as it is essential for soybean cultivars to adopt low temperature in spring and sudden cold shock at the reproductive stages during summer in the northern parts of the planet, such as Canada and northern Europe. To increase yield in these northern areas with short growing seasons, efforts need to be made to develop varieties showing good emergence and early seedling vigor. Emergence test and early seedling weight have been used to evaluate the soybean germplasm that revealed genetic variation in these two traits among the germplasm lines (Littlejohns and Tanner 1976). Further efforts are needed to characterize the genetic elements controlling these traits and utilize them in the soybean breeding programs, especially for the northern areas.

Low temperatures at the reproductive stages in soybean result in a reduced pod and seed formation (Saito et al. 1970; Lawn and Hume 1985; Gass et al. 1996). The cold tolerance at the reproductive stages is usually evaluated as quantification of pods or seeds (Saito et al. 1970; Hume and Jackson 1981; Lawn and Hume 1985; Gass et al. 1996; Kurosaki and Yumoto 2003) or direct measurement of seed yield (Funatsuki et al. 2003). Genetic loci, *cAPX1*, *T*, *Ln*, *P1*, and *Dt1*, were characterized to control cold tolerance at the reproductive stages in soybean. The soybean maturity loci were also thought to be involved in cold tolerance regulation (Funatsuki and Ohnishi 2009; Toda et al. 2011). These genetic resources provide the potential to improve cold tolerance of soybeans and knowledge on the loci involved and their allelic status in breeding lines would facilitate the use of molecular markers to assist in the development of cold-tolerant varieties at the maturity.

1.2.4 Biotic Stress Tolerance

1.2.4.1 Insect Resistance

Increased temperatures resulted from climate change, could influence soybean insect–pest populations in several complicated and dynamic ways. Insects are cold-blooded organisms and the temperature of their bodies is approximately the same as that of the environment. For this reason, changes in warmth can affect

insect physiology and development directly or indirectly through the physiology or existence of hosts, and impact insect behavior, distribution, development, survival, and reproduction. The precise impacts of increased temperatures on insects are somewhat uncertain, because these changes may favor or inhibit some insect populations. The decrease in pest insect populations would more likely occur when insects are closely associated with a specific set of host crops. Soybean aphid (*Aphis glycines* Matsumura) feeds on soybeans but requires the presence of its overwintering host buckthorn (*Rhamnus cathartica*). Most researchers seem to agree that warmer temperatures will favor insects with a shorter period of reproduction due to their faster ability of adaptation. Entomologists predict additional generations of important insect–pests as a result of increased temperatures. With a 2 °C temperature increase, insects might experience one–five additional life cycles per season, and therefore damage more crops (Yamamura and Kiritani 1998). With these changes of climate, insect–pests may have the ability to spread to new geographical regions, result in the development of insect diversity and an increase in their populations, and an increase in the number of outbreaks. Higher average temperature might result soybean being able to be grown in regions further north and it is likely that some of the insects will follow the expanded crop areas. Based on evidence developed by studying the fossil record, the diversity of insect species and the intensity of their feeding habit have increased historically with increasing temperature (Bale et al. 2002). Insects that spend a long part of their lives in the soil, maybe more gradually affected by temperature changes than those that are above ground because soil provides an insulation that buffers temperature changes more than the air (Bale et al. 2002). These soil-born pests include multiple nematode species (*Heterodera glycines*, *Meloidogyne incognita*, and *Rotylenchulus reniformis*) and insects with larvae form living in the soil like bean leaf beetle (*Cerotoma trifurcata* Forster), multiple wireworms (Melanotus spp., *Agriotes mancus* Say, and *Limonius dubitans* LeConte), and white grubs (*Phyllophaga* spp., *Cyclocephala* spp., and *Popillia japonica* Newman).

More frequent and intensive precipitation events forecasted with climate change may negatively affect many insect populations. The same environmental factors that affect insect pests can affect their insect predators as well as the disease organisms that infect the pests, resulting in an increased attack on insect populations. Fungal pathogens of insects are favored by high humidity and their incidence would be increased by climate changes. Moreover, higher humidity and CO_2 effects on insects can be potentially important considerations in a global climate change setting (Coviella and Trumble 1999; Hunter 2001; Hamilton et al. 2005).

With changes in climate, soybean growers need to meet many challenges related to insect management strategies. Insects will broaden their occurrence in the world, emerge new types, and increase reproduction rates and overwintering survival. Decreased winter mortality of insects due to warmer winters seems to have a positive effect on increasing insect populations. Warmer temperatures could result in extensive insecticide implementations to keep insect populations below economic damage thresholds. Extensive applications of insecticides can result in pest outbreaks to further impose a negative environmental and economic impact.

Additionally, some classes of pesticides, like pyrethroids and spinosad, have been shown to be less effective in controlling insects at higher temperatures (Musser and Shelton 2005). Furthermore, the probability of insects to develop insecticide-resistance will be increased with higher demand in required spraying applications. It also seems that agricultural practices will be also affected by climate change. For example, crop rotation as an insect management strategy could be less effective with earlier insect arrival or increased overwintering of insects. The most optimal and successful action plan for soybean growers is to use integrated pest management practices to track insect population development such as field monitoring, pest forecasting, record keeping, and choosing economically and environmentally sound control measures. Recording insect and crop management over time can evaluate the economic and environmental impact of pest control.

1.2.4.2 Disease Resistance

Many historical and contemporary diseases are emerging as threats to modern agriculture and food security along with climate change. Expression of disease symptoms depends on the interaction between three key components: a susceptible host plant, a widespread and virulent pathogen, and environment that support infection or alter host susceptibility (Scholthof 2007). Alternation of any of these components can dramatically change the consequence and expansion of disease in a given pathosystem. Changes in climate are known to modify disease symptoms in soybean and are involved in new disease emergence (Morgan et al. 2003; Eastburn et al. 2010; Matthiesen et al. 2016; Willbur et al. 2018). Many new soybean pathogens have recently emerged or spread as a direct or indirect consequence of environmental changes around the world (Chang et al. 2015; Murithi et al. 2015; Chang-Sidorchuk et al. 2016; Barbieri et al. 2017; Plasencia-Márquez et al. 2017). In 2004, *Phakopsora pachyrhizi* causing soybean rust was confirmed in Louisiana, making it the first report in the continental United States, and over a decade it spread through most US soybean-growing states (Schneider et al. 2005). The emergence of new diseases such as charcoal root rot, caused by *Macrophomina phaseolina*, is expected to spread to new geographical regions under current climate change scenarios (Sarr et al. 2014).

Plant defense is constantly evolving to respond to disease-causing components: evolving pathogen populations and changes in environmental conditions (Whitham et al. 2016). Climate change alters the susceptibility of the host by inducing signals that modulate gene transcription, cell biology, and physiology. Host specialization is able to limit distribution and saturation of pathogens through genetic resistance. Many resistance genes (*R*-genes) were mapped in soybean genome like *Rpg* genes for bacterial blight (Ashfield et al. 1998), *Rpp* genes for soybean rust (Kelly et al. 2015), and *Rps* genes for Phytophthora root and stem rot (Han et al. 2008). Unfortunately, due to pathogen nature and short life cycle, an adaptation of the pathogen genome to new environment progresses faster than in the plant genome. Some alarming reports emerged recently describing more new virulent pathogen

isolates that defeat *R*-genes in soybean (Khatabi et al. 2012). In this case, partial host resistance and *R*-genes should be combined together into high-yielding cultivars to sustain disease resistance. Effects of climate change on soybean resistance against pathogens have received little attention to date. The major climate change factors affecting soybean disease severity and spread, include warmer temperatures and higher humidity, increase in atmospheric carbon dioxide (CO_2), heavy and unseasonal rains, and drought. More frequent and extreme precipitation events could result in extended periods with conditions favorable for pathogen propagation. Recently, sudden death syndrome (SDS) caused by *Fusarium virguliforme* was reported to be impaired by prolonged flooding and anaerobic conditions (Abdelsamad et al. 2017). Some soybean diseases are favored by cool temperatures and wet soil conditions like *Pythium* spp. and *Fusarium* spp. infection, whereas other pathogens cause more severe symptoms in hot and dry conditions like *Macrophomina phaseolina*, or hot and wet conditions like *Phytophora sojae*. Aggressiveness of many isolates of *Pythium* spp. and *Phytopythium* spp., that cause seed decay, damping-off, and root rot in soybean, were increased as temperature increased from 15 to 25 °C (Radmer et al. 2017). Another research reports that depending on *Pythium* spp., the isolates can be more virulent on soybean at lower or higher temperatures (Matthiesen et al. 2016). The response of soybean to elevated CO_2 and ozone has been studied extensively (Ainsworth et al. 2002; Morgan et al. 2003; Eastburn et al. 2010). Under ambient atmospheric conditions, soybean pathogens can cause annual losses of 424 million metric tons worldwide (Wrather and Koenning 2006; Allen et al. 2017). The effects of elevated CO_2 and ozone were evaluated on three economically important soybean diseases where these atmospheric treatments significantly reduced disease severity of downy mildew caused by *Peronospora manshurica*, mildly increased brown spot severity caused by *Septoria glycines*, and no effect on the incidence of SDS. In addition, higher precipitation and higher daily temperatures in the late spring were associated with increased severity for downy mildew and brown spot (Eastburn et al. 2010). Systemic infection of soybean plants by *Soybean mosaic virus* (SMV) was reduced when plants were exposed to elevated levels of O_3 (Bilgin et al. 2008). Therefore, the specific impacts of climate change on soybean diseases are difficult to predict. It is likely that the increased temperatures may result in a northward expansion of the range of some diseases and cause higher survival of pathogen populations. The significance of soybean disease and climate change cannot be left uncontrolled and unconsidered. More research is underway to protect soybean crop in the future with help of agencies within the United States Department of Agriculture, industry, soybean check-off boards, and universities.

1.2.5 Nutrient Use Efficiency

Climate change is mostly associated with temperature and rainfall regimes. Effects of the extreme temperature and irregular rainfall on nutrient uptake by plant have

not been studied as expected. In a study, Schlesinger and Lichter (2001) have shown that demand for nitrogen (N) increases with the increased CO_2 in the atmosphere. The increased N demand cannot be fulfilled by natural soil processes and that created a deficient condition known as progressive nitrogen limitation (PNL). The PNL occurred with increased CO_2 can be elevated with the supply of N fertilizer (Schlesinger and Lichter 2001). Under the condition of elevated CO_2 and sufficient N supply found to be helpful in increasing productivity of crop plants. In soybean, elevated CO_2 found to be correlated with an increase in symbiotic N but the N uptake from soil or fertilizer was unaffected (Li et al. 2017). The increased symbiotic nitrogen seems to be increased N-fixation efficiency rather than increased nodule formation or nodule biomass. Besides higher N uptake efficiency, mobilization of N from root to shoot and to reproductive tissue is important for the productivity. With increased N supply, yield increases mostly due to the increased number of seed in soybean (Kinugasa et al. 2012). No change in N feeding in soybean has been reported with N supply. Nitrogen fertilizer use under elevated CO_2 not only reduce the natural N-fixation by symbiosis but also raises the concern of effective utilization of the limited fertilizer resources. Manufacturing of N fertilizer is largely depending on natural gas and the resource is estimated to be lasting for another 50 years (Fixen 2009). Although alternative sources have been evolved, those will be costlier. A continuous supply of N will also add-up to the greenhouse effect by increasing release of N_2O and CO_2 from the fertilized soil.

Similarly, Phosphorus (P) deficiency with the changing environmental conditions causes plant stress which leads to changes in strategies to adopt P stress. In soybean, such changes include root morphology and architecture modifications and enhancement of root symbiosis and root exudates induction. There are a number of studies performed on molecular regulation of P stress in soybean and more than 200 genes have been identified in the roots and shoots of soybean seedlings (Hwang et al. 2009; Li et al. 2011; Sha et al. 2012). For example, gene *GmEXPB2 (Glycine max* β-expansins*)* is known to enhance P responsiveness and utilization efficiency by the modification in root system architecture (Valdes-Lopez et al. 2008; Wang et al. 2010; Sha et al. 2016). Additionally, the soybean cultivar BX10 is considered as a P-efficient genotype and a number of early or late P-starvation responsive genes and miRNAs were identified from BX10 based on the transcriptional expression profiles and deep sequencing (Dong et al. 2004). Recently, Sha et al. (2016) identified 37 and 33 unique proteins from soybean root and shoot under high or low P condition, respectively. However, only four of the identified proteins were common in root and shoot which indicates that molecular regulation and response to P stress in different tissue are not similar in soybean.

Despite the availability of genetic and molecular information about P and N uptake, there is still an urge for a comprehensive understanding of molecular mechanisms in response to various nutrient stress in soybean because of the influence of changing climatic conditions.

1.3 Genetic Resources of Climate-Smart Genes

The cultivated soybean (*G. max*) was domesticated 6000–9000 years ago from its wild relative *G. soja*, in East Asia (Carter et al. 2004). Based on morphological, cytogenetic and chloroplast sequence identity several centers of domestication including areas of Japan and China have also been proposed. However, recent whole-genome resequencing and molecular studies indicated Yellow River of China and southern China, as the domestication centers (Guo et al. 2010; Lam et al. 2010; Chung et al. 2014; Zhou et al. 2015). In soybean, the genetic diversity centers are recognized as a primary source of genetic variability. Based on genetic diversity in the accessions China is considered as the primary diversity center, while Korea, Japan followed by countries of South Asia (India, Indonesia, and Vietnam) and Russia is considered as secondary diversity centers. Recently the soybean was introduced to non-Asian countries from primary and secondary diversity centers.

The soybean was first introduced in North America during 1765 (Hymowitz and Harlan 1983). The extensive breeding of soybean was undertaken in China and the USA utilizing the genetic stock from the Chinese origin (Cui et al. 2001). In the USA around 400 soybean cultivars were released for cultivation which is derived from ~80 ancestral lines (Gizlice et al. 1994), most of them introduced from China (Li and Nelson 2001). Even in China, the soybean breeding programs used elite cultivars from North America to broaden the genetic base of modern soybean cultivars (Gai et al. 2015). The Chinese Gene Bank maintains around 28,580 soybean accessions. Soybean accession collection in the USA started in the 1920s but with the initiation of soybean collection in United States Department of Agriculture (USDA) during 1949 led to systematic preservation of soybean (Carter et al. 2004). USDA collected 14,330 germplasm accessions that of which 5216 (36.40%) are from China (Gai et al. 2015).

The primary gene pool (GP1) of soybean consists of *G. max* cultivars, landraces, and its related species *G. soja* genotypes. GP1 is a biological species that can be crossed within the gene pool and produce F_1 progenies having a normal meiotic pairing, normal gene segregation, and complete seed fertility. However, the seed sterility can be attributed to the chromosomal inversions and translocations. In soybean besides having relatively lower diversity, considerable genetic resources have been maintained and characterized. The species from GP2 can produce the F_1 hybrids having some fertility when crossed with the GP1 (Harlan and de Wet 1971). In *Glycine*, no species is assigned to the secondary gene pool (GP2). However, the efforts are underway to explore the GP2 species in the regions of soybean's origin.

The crossing of GP1 with the GP3 results in the sterile or lethal hybrids and gene transfer requires rescue techniques. The tertiary gene pool of *Glycine* consists of 26 wild perennial species indigenous to Australia geographically distant from *G. soja* and G. *max*. Among the GP3 three species, *G. argyrea*, *G. canescens,* and *G. tomentella* were successfully hybridized with *G. max* and F_1 hybrids were rescued and found to be sterile. However, a cross between the *G. max* and *G. tomentella* followed by embryo rescue and further backcrossing efforts led to the

development of BC_2 lines, which showed phenomenal yield increases of nearly 500 kg/ha more than G. max parent (Akpertey et al. 2018).

1.4 Brief on Diversity Analysis

1.4.1 Phenotype-Based Diversity Analysis in Soybean Varieties

The diversity present in crop plants is of great importance to develop elite lines through plant breeding. The genetic diversity can be estimated through phenotypic information, pedigree data, and molecular genotyping using DNA or protein markers. The genetic diversity of soybean analyzed based on pedigree information revealed that the genetic base of North American cultivars is narrower compared to Asian cultivars. In North America, 90% of genes in 258 cultivars were contributed by 26 ancestors (Gizlice et al. 1994), while 90% of genes in 651 Chinese cultivars were contributed by 339 ancestors (Cui et al. 2000). The Chinese national soybean collection of 20,000 accessions was evaluated for 15 phenotypic traits representing a highly valuable resource for breeding (Dong et al. 2004). Evaluation of phenotype of 25 seed, leaf and stem traits of North American and Chinese soybean cultivars have also exhibited narrow genetic base in North American cultivars (Cui et al. 2001).

1.4.2 Genotype-Based Diversity Analysis: Molecular Markers

The assessment of molecular genetic diversity of the soybean cultivars began in the 1980s with the application of restriction fragment length polymorphism (RFLP) technology (Roth and Lark 1984; Apuya et al. 1988). Subsequently, other molecular markers such as random amplified polymorphic DNA (RAPD), microsatellite or SSRs, amplified fragment length polymorphism (AFLP), and SNPs are employed to assess the genetic diversity cultivated and wild soybeans. Li and Nelson (2001) found high genetic diversity in Chinese soybean accessions compared to the Japanese and Korean soybean accessions using RAPD markers. Di- and tri-nucleotide SSR-based genotyping led to the detection of six–eight alleles among a group of 38 *G. max* and five *G. soja* accessions showing highly polymorphic nature of SSRs in soybean (Akkaya et al. 1992). Maughan et al. (1995) used SSRs to genotype 62 *G. max* lines and 32 wild soybeans from Asian origins. The study identified 5–21 alleles at five SSR loci. These studies along with the subsequent experiments suggested SSRs as a reliable marker for analyzing limited diversity found in the soybean accessions (Rongwen et al. 1995; Song et al. 1998). A study

based on AFLP markers showed Japanese cultivars are more distinct from the North American soybeans compared to Chinese accessions, suggesting the use of the Japanese cultivars to broaden the genetic base of North American soybean (Ude et al. 2003). Song et al. (2015) studied a huge number of samples approximately 19,700 soybean accessions from USDA Soybean Germplasm Collection. The collection for this study included more than 1100 wild soybeans from China, Korea, Japan and Russia, and more than 18,000 cultivated soybeans from China, Korea, Japan, and 84 other countries and reported a number of loci in soybean. Additionally, the study identified major candidate regions associated with seed weight on seven chromosomes by utilizing the genotyping data. Furthermore, 106 soybean accessions representing 7 wild, 43 landraces, and 56 elite lines from different countries of origin and domestication were studied using whole-genome resequencing. The study supported the common hypothesis that soybean was domesticated in the China subcontinent and then introduced to the US and other parts of the world on the basis of population structural analysis and phylogenetic analysis (Valliyodan et al. 2016). Recently, Liu et al. (2017) analyzed the diversity among the 277 Chinese accessions and 300 US accessions of soybean using 5361 SNP markers. Population structure and cluster analysis showed that the Chinese soybeans are more diverse than American soybean accessions.

1.4.3 Relationship with Other Cultivated Species and Wild Relatives

The soybean [*Glycine max* (L.) Merr.; $2n = 40$] belongs to family Fabaceae, the tribe Phaseoleae, subtribe Glycininae, and the genus *Glycine* Willd. The subtribe Glycininae consists of about 16 genera, among them. The genus *Glycine* bears distinct morphological and cytogenetic characters and from other genera in the subtribe (Lackey 1977). The genus *Glycine* consists of two subgenera, namely, subgenus *Glycine* Wild and subgenus *Soja*. The subgenus *Soja* consists of three annual species including the cultivated soybean *G. max*, its immediate wild ancestral species, *G. soja* and a weedy form of the soybean *G. gracilis,* indigenous to eastern Asia (Lackey 1981). The subgenus *Glycine* comprises of 15–16 perennial species, found in Australia and Japan. The subgenus *Glycine* is considered as a secondary gene pool for the cultivated soybean and it contains useful agronomic traits.

The subgenus *Soja* (annual type) and subgenus *Glycine* (perennial type) are significantly distinct from each other (Doyle et al. 2003). The attempts to hybridize between the subgenus were unsuccessful (Ahmad et al. 1977; Hood and Allen 1980). Recent molecular phylogenetic relationships confirmed a significant diversification between species from this subgenus (Karasawa 1953; Ahmad et al. 1977; Cao et al. 1996; Doyle et al. 2003; Ratnaparkhe et al. 2011). Within the subgenus *Soja,* the cultivated *G. max* hybridized with *G. soja* and *G. gracilis* and fertile seeds

were produced (Karasawa 1953; Hadley and Hymowitz 1973; Ahmad et al. 1977). The cytological (Wang 1986; Xu 1990) and molecular genetic studies showed close evolutionary relationship between these species (Hui et al. 1996; Powell et al. 1996; Wu et al. 2001).

In a recent study, a total of 106 soybean genomes representing landraces, elite and wild soybean genotypes were resequenced. This approach led to the identification of 10 million high-quality SNPs. In Addition, 159 putative domestication sweeps were found, which comprised of 54.34 Mbp (4.9%) and 4414 genes; 146 regions were involved in artificial selection during domestication. This study provides valuable genomic information for understanding soybean genome structure and its relationship with the wild-type soybean (Valliyodan et al. 2016).

1.4.4 Relationship with Geographical Distribution

Most of the species from subgenus *Glycine* are found in Australia and the South Pacific Islands (Hymowitz and Singh 1987; Shimamoto 2000). However, two species, *G. tabacina* and *G. tomentella*, which are also found in the parts of Philippines, Japan and China, including Fujian and Taiwan, apart from Australia and the associated areas (Hymowitz and Singh 1987; Zhuang 1999). The genus *G. soja* is found in China and in its adjacent areas such as Russia, Korea and Japan (Hymowitz et al. 1998; Zhuang 1999; Shimamoto 2000). *G. gracilis* exhibits several morphological characteristics intermediate to those of *G. max* and *G. soja*. It is recognized as a hybrid between *G. max* and *G. soja* (Hymowitz 1970) and hence it is found in areas where the cultivated *G. max* and its wild ancestor *G. soja* have a sympatric distribution which includes northeast part of China (Hymowitz 1970).

1.5 Population Structures of Soybean in Nature

Linkage disequilibrium (LD) describes the inheritance of an allele of one SNP with an allele of another SNP within a population. The term LD was used to describe changes in the genetic variation within a population over time. The LD concept is the same as chromosome linkage where the markers tend to physically unite on a chromosome throughout all the generations. Linkage disequilibrium, the nonrandom occurrence of alleles at different genomic loci, is affected by several factors and has been of great interest to geneticists. Variation in LD throughout the genome or at a particular-genomic region is affected due to the processes of domestication, mutation, level of inbreeding and selection, confounding effects, population admixture, and population substructure (Rafalski and Morgante 2004). The extent of LD is also reliant on the recombination rate. However, LD in a population decreases due to recombination and can restore equilibrium between the loci in a due course. A strong correlation is anticipated between inter-locus distance and LD

if the recombination rates do not vary across the genome particularly in a constant population size.

The LD decay is influenced by the recombination frequency between the two loci and the number of generations of recombination. In some situations, LD between the SNP alleles on different haplotypes (linked in repulsion phase) is not easily detectable and is tough to define. In such situations, LD descends to a very low level due to independent segregation of haplotypes. Self-fertilizing crop plants like soybean usually show less decay of LD (longer region is in LD) because the recombination is ineffective to cause LD decay in a homozygous genetic background. Whereas, high LD decay (shorter region in LD) is common in an outcrossing crop species like maize. However, vegetatively propagated species, such as potato and sugarcane, show relatively slow LD decay in spite of outcrossing nature of these crops (Raboin et al. 2008).

Cultivated soybean has been reported to have outcrossing rates of <1%, while *G. soja* has an outcrossing rate as high as 13% (Fujita et al. 1997). *G. soja* has high LD decay compared to *G. max* due to the increased recombination rate (Flint-Garcia et al. 2003). The current soybean germplasm is the outcome of several cycles of selection and effective recombination leading to increased LD throughout the entire genome. The landraces resulted from domestication might have increased LD level. Loci governing traits like domestication has extended LD levels mostly because of the selection during the domestication. The fact that the LD decay is found to be associated with domestication-related genes has great importance to understand the domestication process and also the population genetics of the traits. Similar effects may happen on natural selection against several devastating stresses (Vuong et al. 2015). Recent studies performed with next-generation sequencing technology have provided a better understanding of LD decay in soybean. Sonah et al. (2015) have characterized LD decay for the entire set of soybean chromosome using 47,000 SNP data obtained with genotyping-by-sequencing method. The results suggested variation for LD decay within and across the chromosomes. Within the chromosome, the study has found very less LD decay (longer LD) at the centromere and pericentromeric region compared to the gene-rich region (Sonah et al. 2015). Subsequently, a similar finding has been reported in several other reports (Bastien et al. 2014; Iquira et al. 2015).

1.6 Association Mapping Studies

Traditionally, QTLs were mapped in plants using biparental crosses. The population derived from biparental crosses lacks allelic diversity as it deals with genetic variation within the parental lines. This critical limitation of the QTL mapping approach can be overcome by the use of association mapping of unrelated genotypes that have accumulated a large number of crossing-over events since their last common progenitor. Association mapping helps to identify loci controlling phenotypic variations and assists identification of genes underlying observed variation.

With the availability of the whole genome of crop plants, genome-wide association studies (GWAS) gained more importance. Such GWAS are useful to identify candidate loci associated with many traits in animals and plants (Appels et al. 2013; Korte and Farlow 2013). The GWAS analysis followed by candidate gene identification proved to be successful in plant species such as Arabidopsis (Verslues et al. 2014), maize (Li et al. 2013a), and rice (Zhao et al. 2011).

In soybean, high-throughput genotyping techniques provided opportunity to obtain the required number of markers on several hundreds of lines, either through SNP genotyping (Song et al. 2013) or by genotyping-by-sequencing (GBS) approach (Sonah et al. 2013). One of the important efforts on soybean improvement is the development of Illumina Infinium BeadChip (SoySNP50 K iSelect BeadChip), which contained over 50,000 SNPs. This study validated the SoySNP50 K chip with 96 landrace genotypes, 96 elite cultivars, and 96 wild soybean accessions and reported 47,337 polymorphic SNPs and 40,841 of the 47,337 SNPs (86%) had minor allele frequencies $\geq 10\%$ among the landraces, elite cultivars and the wild soybean accessions (Song et al. 2013). The SoySNP50 K iSelect SNP BeadChip is being used in several studies to characterize soybean genetic diversity and linkage disequilibrium, and high-resolution linkage maps construction for the soybean improvement (Hwang et al. 2014; Wen et al. 2014; Zhang et al. 2015). A GBS approach was used to identify >47,000 SNPs on 304 short-season soybean lines. A subset of 139 lines, representing the diversity was phenotypically characterized for eight traits under six environments. Marker coverage was found sufficient to find a significant association between the genes known to control flower, hilum and pubescence color, maturity, plant height, seed weight, seed oil and protein (Sonah et al. 2015). A soybean germplasm containing 189 accessions from 10 countries were used to study association mapping for *P. sojae* resistance. These accessions were evaluated for disease resistance by inoculating with *P. sojae* races 1, 3, 7, 17, and 25. Five accessions were resistant to all the races. The genome-wide analysis identified 32 significantly associated SNPs, which were clustered around genomic loci associated with resistance. Among these SNPs, one SNP was found near the gene *Glyma.14g087500*, a subtilisin protease (Qin et al. 2017). Kaler et al. (2017) evaluated 373 maturity group IV soybean genotypes grown in four different environments for canopy wilting. Over 31,260 SNPs were used for association mapping, among them, significant environment-specific 61 SNP-canopy wilting associations were identified, and 21 canopy wilting SNPs were from more than one environment. Based on significant SNPs, the slowest and fastest wilting genotypes were identified. Several of these SNPs were located within or very close to a candidate gene that had been reported to be involved in transpiration or water transport. Mao et al. (2017) performed association mapping on 91 soybean cultivars from different maturity groups using 172 SSRs and 5107 SNPs. Large-effect loci were found on Gm 11, Gm 16, and Gm 20 as reported in previous studies. Most of the flowering time associated loci were sensitive to photo-thermal conditions. Further within the associated loci, three candidate loci were identified; among them, Gm04_4497001 was found to be a key locus interacting with other loci for regulating flowering time in soybean. A set of

185 soybean accessions was evaluated to identify the QTLs associated with seed protein and oil contents. Using specific length amplified fragment sequencing (SLAF-seq) technology, a total of 12,072 SNPs were detected. Among them, 31 SNPs located on 12 chromosomes were correlated with protein and oil content in seeds. The two SNP markers were related to seed oil content and three SNP were correlated with seed protein content during 2015 and 2016 (Li et al. 2018a).

1.7 Brief Account of Molecular Mapping of CS Genes and QTLs

Due to increasing urgency to develop climate-smart soybean with enhanced yield, breeding strategies have progressed at a massive rate in the past decade. With the advent of molecular genetic techniques, a lot of breeding programs have significantly implemented molecular markers for soybean improvement with regard to seed oil and protein enhancement, drought, flooding, and disease resistance.

Since molecular markers identified genetic variants for different traits quickly and accurately, therefore, markers are important in developing genetic linkage maps, germplasm evaluation, phylogenetic and evolutionary analysis, selection of desired alleles and mapping of genes/QTLs. The first linkage map in crops was constructed in tomato using RFLPs in 1986 by only 57 loci (Bernatzky and Tanksley 1986). RFLP was followed by the development of simpler and inexpensive DNA markers RAPDs (Williams et al. 1990), AFLPs (Vos et al. 1995), and SSRs (Akkaya et al. 1992) which resulted in the selection of desirable lines based on genotype instead of the phenotype. Since SSR markers are less abundant in the genome, SNP markers became popular and enabled the development of highly dense linkage maps and facilitated QTL analysis for nearly every agronomic trait in soybean (https://soybase.org, http://soykb.org). Furthermore, marker-assisted breeding became more applicable to soybean with the availability of sequencing data (Kim et al. 2010; Lam et al. 2010; Schmutz et al. 2010). This revolutionary change in sequencing further facilitated the development of thousands of SSRs and millions of SNP markers. Moreover, a high-density consensus soybean map was developed with 5500 markers including 3792 SNPs (Hyten et al. 2010b). Later on, Song et al. (2013) developed and used Illumina Infinium BeadChip containing 52,041 SNPs to evaluate the entire USDA soybean germplasm collection (Song et al. 2013).

QTL analysis plays a significant role to identify genetic regions which are responsible for phenotypic variation and it requires a large segregating population (biparental mapping population) such as an F_2 population or recombinant inbred lines (RILs). In general, QTL mapping uses a large number of RILs, which are established for at least several generations of inbreeding (typically up to F_6 or F_7) (Takuno et al. 2012). However, RILs are helpful for the detection, but it estimates the effect of single QTL depending on population size. Moreover, the results are

highly population specific for multigenic traits. On the other hand, plants that are homozygous for the unfavorable allele are eliminated in an F_2 population, and plants heterozygouss and homozygous for the favorable allele are advanced for inbred development. This way, frequencies of favorable alleles increase during inbred development the probability of fixation of all or the majority of favorable alleles increase (Bernardo 2010). Furthermore, due to the popularity of QTL mapping, over 2000 QTLs have been mapped in soybean (Table 1.1).

Yield improvement with improved qualities and increased resistance to biotic and abiotic stresses is the major objective of soybean breeding. The maturity group based on latitude (MG 000 to MGX), growth habit (determinate or indeterminate), and seed size (large or small) are most important factors to be considered in soybean breeding program (Pathan and Sleper 2008). A number of studies have been performed to improve yield potential of soybean such as insects and diseases which causes major yield loss. Soybean Cyst Nematode (SCN) is one of the most destructive pests in the USA, many QTLs associated with different races of SCN have been reported and QTLs namely *rhg1* (located on LG G) and *Rhg4* (located on LG A2) are confirmed across the different populations, time and locations and commonly utilized in MAS for SCN screening (Concibido et al. 2004; Vuong et al. 2010). Recently, several efficient and high-throughput SNP markers for SCN resistance have been developed for *rhg1* and *Rhg4* ((Vuong et al. 2010; Kadam et al. 2016). A number of QTLs also have been identified for other pests such as Sclerotinia stem rot (Arahana et al. 2001), sudden death syndrome (SDS) (Iqbal et al. 2001), brown stem rot (Bachman et al. 2001; Patzoldt et al. 2005), and root-knot nematode (Li et al. 2001; Ha et al. 2007). QTL mapping and marker development have progressed not only for insects and pests resistance but also for the resistance against several climatic stress (drought, flooding, and salinity) as well as high-quality seeds (protein and oil content) with improved yield. There are a number of important QTL studies for soybean seed protein and oil content reported QTLs across the different environment and genetic backgrounds, for example, seed oil, protein, and seed size QTL (Hyten et al. 2004a), fine-mapping of soybean protein QTL on chromosome (Chr.) 20 (Nichols et al. 2006). Eskandari et al. (2013) identified QTL for oil content on Chr. 9, which also had a significant positive effect on seed protein composition (Eskandari et al. 2013). For the improvement of soybean meal, Pathan et al. (2013) detected QTL using both SSR and SNP markers for seed protein, oil, and seed weight across genetic backgrounds and environments on Chrs. 5 and 6 (Pathan et al. 2013). In a recent study, QTL analysis was performed for seed protein, oil, and sucrose using 3 K-SNPs. A total of five, nine, and four QTLs were identified for protein, oil, and sucrose content, respectively. The major QTL for protein and oil were mapped on Chr. 20 while novel and major QTL for sucrose content were mapped on Chr. 8 (qSuc_08) (Patil et al. 2018). Additionally, a notable success has been made to map QTLs/genes for abiotic stress such as drought (Mian et al. 1998; Bhatnagar et al. 2005; Molnar et al. 2012), salinity (Lee et al. 2004; Hamwieh and Xu 2008; Do et al. 2018), and flooding tolerance (VanToai et al. 2001; Reyna et al. 2003; Githiri et al. 2006; Nguyen et al. 2012).

Table 1.1 Details of significant QTL mapping studies performed to identify genomic loci for various traits in soybean

Traits	Parents	Population Size	Generation	QTLs	References
Drought	Young × PI416937	120	F_4	5	Mian et al. (1996)
Drought	S-100 × Tokyo	116	F_2	2	Mian et al. (1998)
Aluminum	Young × PI229358		F_4	6	Bianchi-Hall et al. (2000)
Salt	S-100 × Tokyo	106	F2:5	1	Lee et al. (2004a)
Manganese toxicity	Essex × Forrest	100	RILs	3	Kassem et al. (2004)
Phosphorus deficiency	Kefeng 1 × Nanong 1138-2	184	RILs	7	Hamilton et al. (2005)
Drought	Jackson × KS4895	81	RILs	1	Bhatnagar et al. (2005)
Lodging	A5403 × Archer	103	RIL	17, 1	Cornelious et al. (2005)
Lodging	P9641 × Archer	67	RIL	15, 1	Cornelious et al. (2005)
Lodging	Essex × Forrest	100	RIL	1	Kassem et al. (2006)
Lodging	PIs × Beeson/ Kenwood/Lawrence	236	RIL	7	Guzman et al. (2007)
Salt	Jackson × JWS156-1	225	F_2	2	Hamwieh and Xu (2008)
Lodging	G. max 7499 × G. soja PI 245331	120	RIL	1	Li et al. (2008)
Salt	Kefeng1 × Nannong1138-2	184	RIL	8	Chen et al. (2008b)
Drought	Kefeng1 × Nannong1138-2	184	RIL	10	Du et al. (2009a)
Lodging	Zhongdou29 × Zhongdou32	165	RIL	6	Rong et al. (2009)
Drought	Kefeng1 × Nannong1138-2	184	RIL	17	Du et al. (2009b)
Salt	FT-Abyara × C01	96	RIL	1	Hamwieh et al. (2011)
Salt	Jindou No. 6 × 0197	81	RIL	1	Hamwieh et al. (2011)
Aluminum	Kefeng1 xNannong1138-2	184	RIL	9	Korir et al. (2011)
Aluminum	Essex × Forrest	42	RIL	3	Sharma et al. (2011)
Drought	AC Colibri × OT91-3	200	RIL	5	Molnar et al. (2012)
Drought	PI 416937 × Benning	147	RIL	4	Carpentieri-Pipolo et al. (2012)
Seed coat cracking (SCC)	Keunolkong × Sinpaldalkong	117	F_{11}	16	Ha et al. (2012)

(continued)

Table 1.1 (continued)

Traits	Parents	Population Size	Generation	QTLs	References
Drought	Hongfeng 11 × Harosoy	95	BC2F3	18	Zhang et al. (2012)
Seed coat wrinkling	PI 567743 × PI 87623 (Pop1) T-311 × PI 87623 (Pop2) PI 87623 × T-311 (Pop3)	Pop1: 175 Pop2: 066 Pop3:150	F_2	21	Kebede et al. (2013)
Salt	Jackson × JWS156-1	1109	RIL	Validation	Tuyen et al. (2013)
Iron and Phosphorus	Anoka × A7	92	$F_{2:4}$	1	King et al. (2013)
Salt	PI 483463 × Hutcheson		RIL	1	Ha et al. (2013)
Seed isoflavones	Essex × William 82	274	$F_{5:6}$	21	Smallwood et al. (2014)
Total isoflavone	Xiaoheidou × GR8836	184	$F_{2:10}$	21	Zhang et al. (2014)
Seed sucrose content	MFS-553 × PI 243545	220	$F_{2:3}$, $F_{3:4}$, $F_{3:5}$	19	Zeng et al. (2014)
Seed weight	Ohsuzu × Athow (Pop1) PI 593654 × PI 561396 (Pop2)	Pop1:225 Pop2:250	F_6	1	Kato et al. (2014)
Soluble sugar content	V97-3000 × V99-5089	170	$F_{2:3}$	11	Wang et al. (2014)
Seed protein content	R05-1415 × R05-638 (Pop1) V97-1346 × R05-4256 (Pop2)	Pop1:242 Pop:214	F_2	5	Wang et al. (2015)
Stachyose content	Osage × V99-5089	129	$F_{2:3}$	2	Zeng et al. (2015)
Fusarium graminearum Resistance	Magellan × PI 567516C	241	RILs	1	Cheng et al. (2017)
Flowering time and branch number	Toyomusume × Suinong 10	100	F_2	4	Guang et al. (2017)
Seed Protein Content	'Dongnong 46' × 'L-100'	129	RIL	8	Teng et al. (2017)
Sudden Death Syndrome	GD2422 × LD01-5907	129	F_4 derived	4	Tan et al. (2018)

Although QTL mapping has advanced quickly in the past few years, a large number of mapped QTLs cannot be utilized in the breeding because of false-positive QTLs and low accuracy. However, the accuracy can be improved by adapting QTL mapping methods and effective statistical analysis such as single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM), multiple interval mapping (MIM), and Bayesian interval mapping (BIM). Also, a number of QTL mapping software have been developed such as Mapmaker/QTL, QTL Cartographer, PLABQTL, PGRI, MapQTL, QGene, Map Manager, QTLMAPPER, QTLSTA, IciMapping, and QTL network.

The developments in sequencing technologies, statistical approaches, and software resulted in exponential growth in soybean studies to understand plant's response to extreme climatic conditions such as drought, flood, pests as well as disease stress. Consequently, with the advancements of molecular techniques, statistical models, and software development led to the QTL analysis, then to candidate genes identification for biotic, abiotic stress resistance and yield-related traits. However, breeding for stress tolerance traits such as drought is one of the most challenging goals in soybean because of negative correlation between mean performance (stress and nonstress yield average) and stress index (stress and nonstress yield difference), therefore breeding for stress tolerance can lead to loss in yield (Miladinović et al. 2015). Therefore, it is necessary for a soybean breeder to utilize interdisciplinary approaches and tools to develop climate-smart soybean.

1.8 Map-Based Cloning of CS Genes

Map-based cloning approach is the strategy to identify or isolate genes underlying a trait based on their map positions on chromosomes. A robust phenotyping is a prerequisite for successful map-based cloning of genes. A general strategy for map-based cloning involves (a) Development of segregating population for the trait of interest; (b) Phenotypic and genotypic analysis of the segregants; (c) High-resolution mapping; (d) Physical mapping of the loci containing the gene of interest; (e) Identification and isolation of the gene. The F_2 segregating population is used for map-based cloning; however, the use of RILs, and NILs as mapping population is more powerful than the F_2 population.

The availability of physical and genetic maps of soybean, and genome sequence for American cultivar "Williams 82" (Schmutz et al. 2010) greatly accelerated the identification of QTLs and genes controlling agronomically important traits. In soybean, several genes controlling climate-smart traits were identified through map-based cloning approach. Soybean cyst nematode is a major constraint to soybean production. Many reports are available on the identification and mapping of QTL in soybeans showing resistance to SCN from a different germplasm source. QTL on chromosomes 18 (rhg1) and 8 (Rhg4) are the two major QTL that have been consistently mapped and reported from different soybean germplasm. Map-based cloning approach revealed that the major QTL locus, *Rhg4* (for

resistance to *Heterodera glycines* 4) provides resistance to this pathogen (Liu et al. 2012). Further gene silencing, mutation studies, and complementation tests confirm that the gene confers resistance. The gene was found to encode a serine hydroxymethyltransferase enzyme which is structurally conserved and ubiquitous across kingdoms. Both QTL and major (*Rps*) genes showing resistance to *P. sojae* were identified in soybean (Polzin et al. 1994; Bhattacharyya et al. 2005; Sandhu et al. 2005; Gordon et al. 2006). Five *Rps* genes, including the important *Rps1-k*, which confers resistance to most races of *Phytophthora sojae* (Kasuga et al. 1997; Song et al. 2004; Gao et al. 2005; Gao and Bhattacharyya 2008) are mapped to the *Rps1* locus. *Rps1 k* encoding an intracellular coiled coil class of NBS-LRR resistance proteins was cloned by map-based cloning (Gao et al. 2005). Several maturity loci, designated as *E* loci (E1 to E8), controlling flowering time, duration of the reproductive phase (DRP), yield, branching (Kumudini et al. 2007; Sayama et al. 2010; Yamada et al. 2012), chilling resistance (Funatsuki et al. 2005; Takahashi et al. 2005) have been characterized by classical genetics approach. The E4 gene encoding phytochrome A2, protein, was identified through candidate gene approach based on the QTL position on the map (Liu et al. 2008). The *E3* gene, encoding a copy of the phytochrome, *GmPhyA3* was cloned by positional cloning using residual heterozygous line (RHL) (Watanabe et al. 2009). Further, a similar strategy was used to clone soybean maturity locus *E2*, an ortholog of *GI*, *GmGIa* using progeny of an RHL population (Watanabe et al. 2011).

1.9 Marker-Assisted Breeding for CS Traits

Molecular breeding and genetic engineering approaches were successfully employed to develop climate smart soybeans are (Fig 1.1). Marker-assisted selection (MAS) is the indirect selection method where the linked marker is used to transfer important agronomical traits from one genotype to another. Marker-assisted backcrossing is an important strategy in soybean for transferring trait of interest (Concibido et al. 2003; Orf et al. 2004; Lee et al. 2006). The high-throughput genotyping technologies enhanced the process of marker identification and QTL mapping for different traits in soybean. The molecular breeding approaches such as marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) aided in the introgression of the trait of interest in soybean. The Soybean cyst nematode-resistant line, LDX01-1-65(PI636464) was developed using MABC. The *G. soja* accession, PI468916 with poor agronomic traits contained two SCN-resistant QTLs. MABC was used to introgress these QTLs into a recurrent parent, A81-356022. Closely linked markers were employed to select the QTL during each backcrossing. Four rounds of backcrossing were carried out and a BC4F1 was selected which was heterozygous for both the QTLs. A population of BC4F3 lines was derived and the genotypic combination for each QTL was determined using the markers (Diers et al. 2005). Similarly, the SBR resistant soybean lines were developed using MABC by introgressing key *Rpp* genes, Rpp1,

Rpp2, Rpp3, and Rpp4 (King et al. 2016). NILs were developed for individual *Rpp* genes by making backcrosses to soybean cultivar G00-3213. A marker linked to each *Rpp* gene was used for screening progenies during backcrossing process.

The glyphosate-tolerant soybean cultivar Benning (released as 'H7242 RR') developed in less than five years though background selection to recover the recurrent parent genome in a backcross program (Orf et al. 2004). The tolerance to glyphosate by a single transgene facilitated phenotypic selection of plants, while the SSR markers aided in the identification of the tolerant plants with the high proportion of Benning genome during subsequent backcross generations. In another study, the markers linked to three different QTLs from PI 229358 were employed to develop insect-resistant NILs of Benning. These lines provided an opportunity to characterize the individual and combined effects of insect resistance QTLs (Zhu et al. 2008).

Molecular markers linked to a gene controlling particular trait can be used to transfer that trait from one genetic background to another, or pyramid genes (Walker et al. 2002; Walker et al. 2010). Gene pyramiding involves combining favorable alleles controlling the same trait from more than two parental lines. (Melchinger 1990; Huang et al. 1997). Marker-assisted gene pyramiding was successfully carried out to develop durable resistance to several pathogens causing diseases in soybean (Walker et al. 2010). Resistance controlled by a single *R*-gene is likely to be broken by novel biotypes of a pathogen if it is transferred as sole resistance gene into a cultivar. The range of resistance can be increased by gene pyramiding (Nelson 1978; Melchinger 1990; Saghai Maroof et al. 2008). Pyramiding major R-gene with additional resistance alleles using phenotypic assays is difficult while molecular markers linked to the individual genes aid in the selection of plants with multiple resistance genes and to combine them in a single genetic background. SNP associated with southern root-knot nematode resistance allow easy selection of the resistance alleles at major and minor QTLs, facilitating pyramiding multiple genes for an increased level of resistance (Ha et al. 2007). The *Rsv1*, *Rsv3*, and *Rsv4* genes provide resistance to all strains of soybean mosaic virus (SMV), and pyramiding all these genes provide comprehensive SMV resistance (Saghai Maroof et al. 2008; Shi et al. 2009). In another study, Wang et al. (2017) pyramided three SMV resistance genes, R_{SC4}, R_{SC8}, and R_{SC14Q}, from different cultivars. Ten SSRs linked to the resistance genes were used for pyramided breeding. Five F_7 homozygous pyramided plants showed resistance to 21 SMV strains along with desirable agronomic traits. Similarly, markers were used to introgress insect resistance allele from the Japanese soybean accession PI 229358 with a *Bt* protein, cry1Ac toxic to lepidopteran pests of soybean. Yamanaka et al. (2015) developed seven pyramided lines of soybean carrying multiple resistance genes (*Rpp*) to provide a broad-spectrum and higher level of resistance to Asian soybean rust. Higher resistance was found in the pyramided lines, Oy49-4 (*Rpp2* + *Rpp3* + *Rpp4*) No6-12-B (*Rpp4* + *Rpp5*), and No6-12-1 (*Rpp2* + *Rpp4* + *Rpp5*) compared to the genotypes from which the resistance genes were derived. Brzostowski and Diers (2017) stacked resistance alleles from different soybean

accessions, PI88788, PI468916, and PI567516C to develop resistance to the virulent soybean cyst nematode isolates.

1.10 Genomic Resources

Recently, high-resolution genome information has been started to be adopted for germplasm characterization, genetic dissection of agronomic traits, and prediction for breeding value. Along with the development of next-generation sequencing techniques, sequencing and resequencing of plant genomes have expanded dramatically. A complete soybean reference genome was published in 2010 (Schmutz et al. 2010). Then several soybean resequencing projects were initiated to develop soybean genomic resources for more than 1000 soybean accessions (Lam et al. 2010; Zhou et al. 2015; Valliyodan et al. 2016, 2017); unpublished data at the University of Missouri). Genomic resources generated from the recent resequencing of diverse germplasm sets provided powerful tools for characterizing soybean genetic diversity and building a strong foundation for trait/gene discovery to accelerate future breeding for elite cultivars. Comparative genome analysis is also greatly benefited from advances in genome information. Following the release of soybean reference genome "Williams 82" in 2010, high-quality reference genomes for other legume crops, including pigeon pea (Varshney et al. 2012), chickpea (Jain et al. 2013; Varshney et al. 2013; Parween et al. 2015), common bean (Schmutz et al. 2014) and peanut (Bertioli et al. 2015) have been released. The changes in genome structures and genome synteny among the legume species can be acquired through comparative genome analysis. The genes or genomic regions for abiotic stress tolerance cloned in one legume species can be extended into other legume crops by comparing genome structures and synteny.

1.11 Genomics-Assisted Breeding for CS Traits

In recent years, rapid progress in genetics, genomics, and soybean genome sequence information have resulted in the identification of SNPs, copy number variation, and structural variation in soybean germplasm (Kim et al. 2010; Lam et al. 2010; Schmutz et al. 2010) (Table 1.2). The growth of next-generation sequencing and low sequencing cost has revolutionized soybean research and next-generation sequencing approaches (NGS) has been widely utilized in various de novo sequencing, whole-genome resequencing (WGR), genotyping-by-sequencing (GBS), and transcriptomic analysis. These developments have made a significant impact in molecular breeding strategies through marker development such as SSRs (Hwang et al. 2009), SNPs (Kim et al. 2010; Lam et al. 2010; Chung et al. 2014; Zhou et al. 2015; Valliyodan et al. 2016), insertion/deletion (INDEL) markers (Song et al. 2015), specific-locus amplified fragment (SLAF) markers

Table 1.2 Details of whole-genome sequencing efforts in soybean

Sr. No.	Genotypes	No. of lines used	Sequencing depth	Accession Number	Method	No. of SNPs	References
1	*G. max* var. Williams 82	1		GCA000004515.3	De novo sequencing and assembly		Schmutz et al. (2010)
2	*G. soja* var. IT182932	1	~52.07X	SRA009252	Resequencing *De novo* sequencing and assembly	~2.5 Million	Kim et al. (2010)
3	17 *G. soja* and 14 *G. max* (cultivated soybean)	31	×5 depth	SRA020131	Resequencing	6,318,109	Lam et al. (2010)
4	8 *G. soja*, 17 *G. max* (8 landraces, and 9 elite lines/ cultivars)	25		SRP015830	SOAP	5,102,244	Li et al. (2013b)
5	10 *G. max* and 6 *G. soja*	16	>14x	ERP002622	Resequencing	3,871,469	Chung et al. (2014)
6	*G. soja*	7	~111.9X	PRJNA195632	*De novo*sequencing and assembly	3.62–4.72 M SNP per line	Li et al. (2014)
7	10 Semi-wild and 1 *G. soja*	11	9 Semi-wild at ~3X while 1 Semi-wild at ~41X, and 1 Wild at ~55X	PRJNA227063 RX375213	Resequencing *De novo*sequencing and assembly	7,704,637	Qiu et al. (2014)
8	*G. soja* W05	1	~1X	GCA_000722935.2	De novo sequencing and assembly	1,798,504	Qi et al. (2014)

(continued)

Table 1.2 (continued)

Sr. No.	Genotypes	No. of lines used	Sequencing depth	Accession Number	Method	No. of SNPs	References
9	62 *G. soja*, 240 *G. max* (130 landraces, and 110 improved cultivars)	302	>11X	SRP045129	Resequencing	9,790,744	Zhou et al. (2015)
10	*G. max* cv. Enrei	1	22.2X	GCA_001269945.2	Reference-based assembly	1659,041	Shimomura et al. (2015)
11	Wild, Landraces and Elite lines	106	17X	SRP062245	Resequencing	10,417,285	Valliyodan et al. (2016)

(Zhang et al. 2016b). Furthermore, the technical advances and availability of millions of SNPs have facilitated the development of high-density array-based genotyping chips such as Illumina Infinium array (SoySNP50 K iSelect BeadChip) for ~50,000 SNPs (Song et al. 2013), SoySNP6 K Infinium BeadChip (Akond et al. 2013), and the Axiom SoyaSNP array for approximately 180,000 SNPs (Lee et al. 2015), which are being used for the genotyping of soybean lines (Table 1.3).

Furthermore, GBS is one of the popular sequencing-based genotyping approaches which has significantly reduced labor and time and improved precision in the

Table 1.3 List of significant studies to identify SNP markers using various genotyping platforms in soybean

Sr. No	Genotyping platform/ approach	Genotypes	SNPs	References
1	Illumina GoldenGate assay	3 RIL mapping populations	384	Hyten et al. (2008)
2	Illumina GoldenGate assay	3 RIL mapping populations	1536	Hyten et al. 2010b, Vuong et al. (2010)
3	Illumina genome analyzer/ RRLs	444 RILs	25,047	Hyten et al. (2010a)
4	Illumina Genome Analyzer II/ whole-genome resequencing	17 wild and 14 cultivated	205,614	Lam et al. (2010)
5	Illumina genome analyzer/ RRLs	Parental lines of mapping population	39,022	Wu et al. (2010)
6	Illumina genome analyzer/ Reduced Representation Libraries (RRLs)	5 diverse genotypes	14,550	Varala et al. (2011)
7	Illumina Infinium SoySNP6 K BeadChip	92 RILs	5376	Akond et al. (2013)
8	Illumina GAIIx / Genotyping-by-sequencing (GBS)	8 diverse genotypes	10,120	Sonah et al. (2013)
9	Illumina Genome Analyzer II/ whole-genome resequencing	25 diverse genotypes	5,102,244	Li et al. 2013b)
10	Illumina Infinium BeadChip	96 each of landraces, elite cultivars and wild accessions	52,041	Song et al. (2013)
11	Illumina Infinium SoySNP50 K BeadChip	22,000 accessions	52,041	Bandillo et al. (2015)
12	Illumina GoldenGate assay Illumina BeadStation 500	48 $F_{2:3}$ Population	1536	Phansak et al. (2016)
13	Illumina iScan platform/ SoySNP8 K BeadChip	F_2 Population	7039	Ping et al. (2016)
14	Illumina Infinium BeadChip	RIL mapping populations	3343	Patil et al. (2018)
15	llumina BeadArray platform	421 soybean cultivars	1536	Li et al. (2018b)

identification of key genes as compared to the conventional PCR-based genotyping methods and being utilized in several crop species and soybean (Poland and Rife 2012; Sonah et al. 2013). Additionally, GBS also allows the detection of new variants in the population of interest, which can be utilized in future breeding programs. In soybean, a number of studies have explored sequencing-based QTL analyses (Xu et al. 2013; Bastien et al. 2014; Li et al. 2014). Sonah et al. (2015) identified 47,702 SNPs including 2744 InDels using GBS approach in a diverse set of 304 short-season soybean lines. The study further characterized a subset of 139 lines for eight agronomic traits (flower, hilum, and pubescence color, maturity, plant height, seed weight, seed oil, and protein) and identified associated loci (Sonah et al. 2015). In another study, Qi et al. (2014) utilized sequencing-based QTL mapping to map salt tolerance locus in wild soybean accession W05 and the locus was mapped to a 978-kb region on chromosome 3 using 2757 bin markers (Qi et al. 2014). Furthermore, Patil et al. (2016) developed Kompetitive allele-specific polymerase chain reaction (KASP) assays for detecting salt tolerance and seed composition traits based on the subsequent whole-genome sequencing analysis of 106 soybean accessions (Patil et al. 2016; Patil et al. 2017). Moreover, another cost-effective sequencing-based approach, SLAF-sequencing was utilized to study low-phosphate stress QTL in soybean. In this study, the genetic map was generated using 6159 SLAF markers and 85 low-phosphate stress-related QTLs were identified (Zhang et al. 2016b).

Even with the advances in genomics-based technologies, the utilization of these approaches is still restricted because it requires the computational expertise and significant time for data analysis. However, the increasing number of software packages and computational pipeline development will aid the genomics-assisted breeding to develop climate resilient crops.

1.12 Brief on Genetic Engineering for CS Traits

1.12.1 Achievements of Transgenic Approaches

The genetically modified crops significantly increased the yield and production by protecting crops from diseases, pests and abiotic stress factors. The main advantages of climate-smart transgenic plants include reduced crop loss and reduced use of chemicals products in agriculture (Job 2002; James 2011). Since 1996 with the commercialization of biotech crops traits such as herbicide tolerance and insect resistance have gained more interest due to their economic impact. The development of genetically modified soybean with superior yield, tolerance to disease and pest, tolerance to abiotic stress, improved nutritional quality, biofuel production is underway (Lu et al. 2007).

1.12.1.1 Insect Resistance

Insecticidal crystal proteins (δ-endotoxins) produced by entomopathogenic bacterium *Bacillus thuringiensis* (*Bt*) acts as a biopesticide to control lepidopteran, dipteran, and coleopteran larvae (Tabashnik 1994; Peferoen 1997; Hongyu et al. 2000). To date, several plant species including soybean have been transformed with *Bt* gene to impart insect resistance trait. Transgenic soybean expressing *Bt* cry gene showed resistance to many insect pests in laboratory bioassays and field conditions (Parrott et al. 1994; Dufourmantel et al. 2005; Macrae et al. 2005; McPherson and MacRae 2009). Miklos et al. (2007) expressed a synthetic *cry*1A in soybean which showed resistance to lepidopteran pests, *Helicoverpa zea* and *Anticarsia gemmatalis*. A transgenic soybean with high levels of a synthetic *cry1Ac* protein accumulation caused complete *A. gemmatalis* larval mortality and significantly reduced other pests also (Stewart et al. 1996; Walker et al. 2000). Similarly, expression of the synthetic *cry1*Ac gene showed a high level of toxicity to *A. gemmatalis* without affecting the crop yield (Homrich et al. 2008).

The strategy to pyramid the Cry1Ac with the native genes was adopted to increase plant resistance to insects. Several QTLs from soybean lines showing antixenosis and antibiosis resistance (Cregan et al. 1999; Rector et al. 2000). were used to develop transgenic soybean lines by combining QTLs with synthetic Cry1Ac (Walker et al. 2004). The pyramiding Bt lines were found significantly more resistant to lepidopteran pests. The success of Bt crops leads to the employment of different insect-resistant protein-encoding genes such as lectins, plant defense proteins, insect chitinases, α-amylase inhibitors, and defensins (Hudson et al. 2013). Insect-resistant transgenic plants are climate friendly and have the potential to drastically reduce the use of chemical pesticides.

1.12.1.2 Disease Resistance

Viruses and fungi are the most common pathogens affecting soybean and hence they are targeted for the development of disease-resistant soybeans. Resistance to viruses in a different plant species have been achieved using pathogen derived viral coat proteins. When coat proteins are used *in planta*, they interfere with viral assembly controlling their spread. The same approach was used in soybean to develop virus-resistant plants. Soybean resistant to bean pod mottle virus (BPMV) (Di et al. 1996) was developed by introducing a BPMV coat protein. Another study developed soybean resistant to BPMV by transforming a BPMV capsid polyprotein. The transgenic lines showed complete resistance to virus infection with no visible symptoms (Reddy et al. 2001). Similarly, efforts were made to develop soybean mosaic virus (SMV) a devastating virus which causes yield loss up to 90%. Soybean lines conferring pathogen-derived resistance, transgenic plants were produced containing a SMV derived coat protein gene and the 3'UTR (Wang et al. 2001). The coat protein expression was detected in transgenic lines and few lines showed high resistance to infections with the SMV virus. Similarly, soybean dwarf

virus (SbDV) derived sense coat protein gene was used to develop SbDV-resistant soybean plants (Tougou et al. 2006). The resistance was achieved by overexpression of SbDV-CP mRNA, soybean transgenic lines remained symptomless after infection with SbDV.

Sclerotinia stem rot (SSR) caused by the fungus *Sclerotinia sclerotiorum* is one of the important diseases affecting soybeans. This fungus was found to be associated with oxalic acid (OA). Treatment of plants with OA increased symptoms, while OA metabolism resulted in fungal tolerance. Transgenic soybean overexpressing oxalate decarboxylase (OXDC) (Cunha et al. 2010), showed reduced disease progression correlating with the transgene expression levels. The single-chain variable fragment (scFv) antibodies are used as an alternative technology to control fungal infection. The plant can express and assemble antibody fragments. A similar antibody approach was used in soybean to control *Fusarium virguliforme* causing sudden death syndrome (SDS) (Brar and Bhattacharyya 2012). Antibody gene encoding scFv anti-FvTox1was used to create transgenic lines targeting pathogenic toxin Tox1, which reduced disease development.

1.12.1.3 Abiotic Stress Resistance

Drought is a most important abiotic stress factor that affects crop productivity and yield. To understand genetic basis of drought tolerance, research was focused on the study of physiological responses such as water use efficiency, nitrogen fixation, leaf wilting, and root growth. The overexpression of single target genes showed potential for enhancing drought tolerance in Arabidopsis and tobacco model systems, however, the knowledge has not been translated to many crop plants. In soybean overexpression of molecular chaperone binding protein (BiP) showed decreased leaf water potential, leaf wilting, and stomatal closure under drought (Valente et al. 2008). Further, the transgenic plants exhibited decreased rates transpiration and photosynthesis, delayed leaf senescence. The soybean overexpressing the Δ1-pyrroline-5-carboxylate synthase (P5CR) gene from Arabidopsis showed high free proline accumulation resulting in increased tolerance to drought and heat stresses (De Ronde et al. 2004a, b; Kocsy et al. 2005). DREBs belonging to the ethylene-responsive factors (ERF) family of transcription factors play an important role in providing tolerance to abiotic stresses. (Polizel et al. 2011) transformed a drought-sensitive soybean cultivar, BR16 with *AtDREB1A* gene under drought-inducible promoter (rd29A) from Arabidopsis. The transformed plants showed increased chlorophyll, higher stomatal conductance, and enhanced transpiration and photosynthetic rates. The overexpression of GmFDL19, a bZIP transcription factor in soybean caused early flowering and, enhanced tolerance drought and salt stress in transgenic soybean plants. The expression of GmFDL19 was found to be induced by abscisic acid (ABA), polyethylene glycol (PEG 6000) and high salt stresses (Li et al. 2017). Researchers at the University of Litoral developed genetically modified soybeans by transformation of a gene isolated from sunflower (HAHB-4), The transgenic lines were found to tolerate water-stress

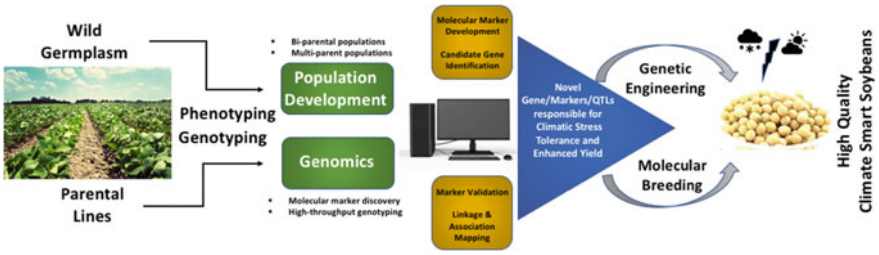

Fig. 1.1 Schematic representation of breeding approach for the development of climate-smart soybeans

(drought) and saline soils. Argentinean government approved HB4 technology for soybean. The HB4 technology improved the yields 13% during severe drought in Argentina and the improvement reached up to 30% in field trials (Mira and Nación 2015; Patiño 2018) (Fig. 1.1).

Aquaporins are the class of transporters involved in the transport of water and other small solutes like ammonia, urea, glycerol, boric acid, silicic acid, H_2O_2, and CO_2 (Tyerman et al. 2002; Maurel et al. 2008; Bienert and Chaumont 2014). These aquaporins belong to major intrinsic protein (MIP) superfamily. Their structure resembles hourglass (Törnroth-Horsefield et al. 2006) with six transmembranes (TM) α helices (helix 1 to helix 6), and five loops that penetrate into the lipid bilayer to make a passage for water movement (Fig. 1.2). Considering their importance, aquaporin-encoding genes are identified in different crop plants including soybean (Deshmukh et al. 2013; Zhang et al. 2013; Deokar and Tar'an 2016; Deshmukh et al. 2016; Song et al. 2016; Shivaraj et al. 2017a, b; Sonah et al. 2017). Several studies have demonstrated the role of aquaporins to improve climate-smart traits in different crop plants (Table 1.4). Recently functionally

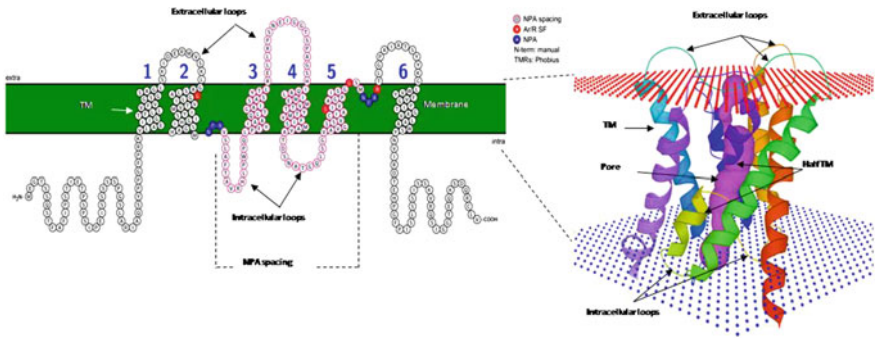

Fig. 1.2 Schematic diagram of the 2D structure and 3D structure of aquaporin GmNIP2-1 identified in Soybean showing six transmembrane alpha-helices and the five inter-helical loops. Modifications in NPA-spacing or Ar/R selectivity filter positions in aquaporins have been shown to change the transport activity and the solute specificity

Table 1.4 Studies demonstrating the role of aquaporins in abiotic stress tolerance in the plants through heterologous expression assays

S. No.	Species	AQP	Transgenic plants	Effect of transgene	References
1	Arabidopsis	*PIP1b*	Tobacco	In normal growing conditions increased vigor but deleterious effect during drought stress and no effect under salt stress	Aharon et al. (2003)
2	Arabidopsis	*PIP1;4*, *PIP2;5*	Arabidopsis and Tobacco	Increased cold tolerance and increased susceptibility to drought	Jang et al. (2007a)
3	Arabidopsis	*TIP1;1*	Arabidopsis	TIP1;1 RNAi plants contained increased apoplastic carbohydrate and high starch, involved in vesicle-based metabolite routing	Ma et al. (2004)
4	Arabidopsis	*PIP2;5* and *PIP1;4*	Arabidopsis	Increase in transpiration rates in response to high irradiance	Lee et al. (2009a)
5	Arabidopsis	*PIP2;2*	Arabidopsis	Enhanced water uptake	Javot et al. (2003)
6	Arabidopsis	*AtTIP1;3*, *AtTIP5;1*	T-DNA insertion mutant Arabidopsis	Abnormal barren silique production, this phenotype is more pronounced under limited nutrient or water supply	Wudick et al. (2014)
7	Arabidopsis	*AtPIP2;1-GFP* and *AtPIP1;4-mCherry*	Arabidopsis	ER-retained AtPIP2;1-GFP interact with other PIPs; decreased root hydraulic conductivity	Sorieul et al. (2011)
8	Arabidopsis	*AtPIP1;2*	T-DNA mutant Arabidopsis	Membrane CO_2 transport regulation	Uehlein et al. (2012)
9	Banana	*MusaPIP1;2*	Banana	Elevated proline and relative water content and higher photosynthetic efficiency, decreased malondialdehyde levels	Sreedharan et al. (2013)
10	Banana	*MaPIP1;1*	Arabidopsis	Increased tolerance to drought	Xu et al. (2014)
11	Banana	*MusaPIP2;6*	Banana	Under salt stress conditions showed higher photosynthetic efficiency and lower membrane damage	Sreedharan et al. (2015)

(continued)

Table 1.4 (continued)

S. No.	Species	AQP	Transgenic plants	Effect of transgene	References
12	Barley	*HvPIP2;1*	Rice	Enhanced internal CO_2 conductance and CO_2 assimilation in the leaves	Hanba et al. (2004)
13	Barley	*HvPIP2;1*	Rice	Increased salt sensitivity in transgenic rice plants	Katsuhara et al. (2003)
14	Barley	*HvPIP2;1*	rice	Enhanced CO_2 assimilation	Katsuhara and Hanba (2008)
15	Barley	*HvPIP2;5*	Arabidopsis	Increased tolerance to salt stress	Alavilli et al. (2016)
16	Cucumber	*CfPIP2;1*	Arabidopsis	Increased tolerance to salt and drought	Jang et al. (2007b)
17	Durum wheat	*TdPIP1;1*, *TdPIP2;1*	Tobacco	Enhanced tolerance to osmotic and salinity stress	Ayadi et al. (2011)
18	*Hydrangea macrophylla*	*HmVALT*, *HmPALT1*	Arabidopsis	Enhanced Aluminum tolerance	Negishi et al. (2012)
19	*Jatropha curcas*	*JcPIP2;7* and *JcTIP1;3*	Arabidopsis	Increased tolerance to salt and drought	Khan et al. (2015)
20	*Mesembryanthemum crystallinum*	*McMIPB*	Tobacco	Affect CO_2 transport, control the regulation of stomata to water deficits	Kawase et al. (2013)
21	*Panax ginseng*	*PgTIP1*	Arabidopsis	Increased ability to tolerate salt stress, drought and cold stress	Peng et al. (2007)
22	Pepper	*CaAQP*	Pepper, overexpression and VIGS	Decreased chilling stress in transgenic plants	Yin et al. (2014)

(continued)

Table 1.4 (continued)

S. No.	Species	AQP	Transgenic plants	Effect of transgene	References
23	Poplar	*PIP1*	Poplar (RNAi)	CO_2 permeability found affected	Secchi and Zwieniecki (2013)
24	Poplar, Tomato	*PtNIP2-1*, *SlNIP2-1* (mutated)	Arabidopsis	Increased silicon uptake and drought tolerance	Deshmukh et al. (2015)
25	Raphanussativus	*RsPIP1;1*, *RsPIP2;1*	Eucalyptus grandis and Eucalyptus urophylla	Improved photosynthesis and growth	Tsuchihira et al. (2010)
26	*Rhododendron catawbiense*	*RcPIP2;1* and *RcPIP2;2*	Arabidopsis	Freezing tolerance and cold acclimation	Bots et al. (2005)
27	Rice	*OsPIP2;1*	Rice	Increased root hydraulic conductivity, salt stress tolerance, seed yield, and seed germination rate	Liu et al. (2013)
28	Rice	*OsPIP1-1, OsPIP2-2*	Arabidopsis	Enhanced tolerance to drought and salt stress	Guo et al. (2006)
29	Rice	*OsPIP2;4*, *OsPIP2;6*, *and OsPIP2;7*	Arabidopsis	Higher biomass accumulation and increased arsenite tolerance and	Mosa et al. (2012)
30	*Sesuvium portulacastrum*	*SpAQP1*	Tobacco	Increased salt tolerance	Chang et al. (2016)
31	*Tamarix hispida*	*ThPIP2;5*	*Tamarix* and Arabidopsis	Increased salt tolerance	Wang et al. (2018)
32	*Thellungiella salsuginea*	*TsTIP1;2*	Arabidopsis	Enhanced tolerance to drought, salt and oxidative stress Increased photosynthesis due to change in leaf mesophyll conductance to CO_2	Wang et al. (2013)
33	Tobacco	*NtAQP1*	Tobacco		Flexas et al. (2006)

(continued)

Table 1.4 (continued)

S. No.	Species	AQP	Transgenic plants	Effect of transgene	References
34	Tobacco	*NtAQP1*	Tobacco	Lowered permeability of the inner chloroplast membrane to CO_2	Uehlein et al. (2008)
35	Tobacco	*NtAQP1*	Tobacco	Increased membrane permeability for CO_2 and water, and increases leaf growth	Uehlein et al. (2003)
36	Tobacco, Arabidopsis	*NtAQP1*, *AtHXK1*	Tobacco	NtAQP1 significantly increased the transpiration rates and growth of AtHXK1-expressing plants	Kelly et al. (2014)
37	Tomato	*SlTIP2;2*	Arabidopsis and tomato	Increased fruit yield, harvest index, and plant mass compared to the control under normal as well as water-stress conditions	Sade et al. (2009)
38	Tomato	*SlPIP2;1*, *SlPIP2;7* and *SlPIP2;5*	Arabidopsis and tomato	significantly higher hydraulic conductivity levels and survival rates under both normal and drought conditions	Li et al. (2016)
39	*Vicia faba*	*VfPIP1*	Arabidopsis	Increased drought tolerance	Cui et al. (2008)
40	Wheat	*TaNIP*	Arabidopsis	Increased salt tolerance	Gao et al. (2010)
41	Wheat	*TaAQP8*	Tobacco	Increased salt tolerance	Hu et al. (2012)
42	Wheat	*TaAQP7*	Tobacco	Increased drought tolerance	Zhou et al. (2012)
43	Wheat	*TaTIP2;2*	Arabidopsis	Enhanced tolerance to drought and salt stress	Xu et al. (2013)
44	Wheat	*TaLsi1*	Arabidopsis	Increased silicon uptake	Montpetit et al. (2012)

Table 1.5 Functional characterization of aquaporin family members in soybean

S. No.	AQP	Function	Methodology	References
1	*GmNIP2-2*	Silicon transport	*Xenopus* oocyte assay	Deshmukh et al. (2013)
2	*GmTIP1;5*	Water transport	*Xenopus* oocyte assay	Song et al. (2016)
3	*GmTIP2;5*	Water and boric acid transport	*Xenopus* oocyte assay	Song et al. (2016)
4	*GmPIP1;6*	Increased salt tolerance and improved yield	Transformation in soybean	Zhou et al. (2014)
5	*GmTIP2;3*	Enhanced osmotic stress tolerance	Heterologous expression in Yeast	Zhang et al. (2016a)
6	*GmSIP1;3*	Increased osmotic stress tolerance	Heterologous expression in yeast and tobacco	Zhang et al. (2017)
7	*GmPIP2;9*	Increased drought tolerance	Transformation in soybean	Lu et al. (2018)

important aquaporin encoding genes were identified and characterized from soybean genome (Table 1.5). The expression analysis of *GmTIP2;3* showed higher levels in the root, stem, and pod. Its accumulation significantly increased in response to osmotic stresses, including polyethylene glycol and abscisic acid treatments. In addition, yeast heterologous expression showed that *GmTIP2;3* could increase tolerance to osmotic stress in yeast cells (Zhang et al. 2016a). Overexpression of *GmPIP1;6* in soybean resulted in enhanced leaf gas exchange in normal conditions compared to wild-type plant. Under salt stress, the transgenic plants showed increased growth and yield relative to wild type in field conditions (Zhou et al. 2014). *GmPIP2;9* overexpression in soybean showed increased tolerance to drought stress in both solution and soil cultures. *GmPIP2;9* overexpression lines under drought stress showed increased net CO_2 assimilation of photosynthesis, transpiration rate, and stomatal conductance compared to wild-type plants. Additionally, field grown overexpression plants exhibited significantly more pod numbers and increased seed size than wild-type plants (Lu et al. 2018).

1.12.1.4 Herbicide Resistance

Weeds are unwanted plants known to reduce yield by competing with crops for water, sunlight, and nutrients, which can be considered as another form of biotic stress. Farmers use different strategies like manual removal, plowing, and application of herbicides to control weeds. Herbicides are chemicals which kill weeds or hinder their growth. Broad-spectrum herbicides kill many types of plants that they come in contact with, while narrow-spectrum herbicides are toxic to a specific group of plant species (Zimdahl 2018). It is important to develop the crop varieties,

which can withstand the toxic effect of herbicides and maximize the benefit of herbicide application. Herbicide-resistant plants provide both economical and ecological benefits. Since the development of herbicide-tolerant soybeans to glyphosate soil tillage has diminished by 23% (Givens et al. 2009). Reduced tilling preserves soil nutrients and organic matter thereby reducing soil erosion. Reduced tillage practices decrease fuel consumption and reduced carbon dioxide emission. The largest reduction in carbon dioxide emissions has come from the adoption of genetically modified herbicide-tolerant soybean (Brookes and Barfoot 2015).

Glyphosate, a broadly used herbicide and is known to inhibit 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), production of several essential aromatic amino acids. Hence research was undertaken to develop glyphosate-resistant soybean called Roundup Ready® by Monsanto. These transgenic soybeans expressed glyphosate-tolerant EPSPS from *Agrobacterium* spp. strain CP4 providing tolerance to the glyphosate (Padgette et al. 1995). Bayer Crop Sci released herbicide-tolerant soybean called Liberty Link® soybean. Liberty Link® soybean was developed to express a gene phosphinothricin-*N*-acetyltransferase (PAT) from bacteria *Strptomyces viridochromogenes*. PAT encodes a glutamine synthetase inhibitor which binds to glutamate, imparting plants resistance to the contact herbicide glufosinate ammonium. Similarly, Pioneer has developed a genetically modified soybean product tolerant to two classes of herbicides, glyphosate and acetolactate synthase (ALS)-inhibiting herbicides. These plants express the glyphosate acetyltransferase and modified soybean acetolactate synthase (GMHRA) protein (Mathesius et al. 2009). The glyphosate acetyltransferase confers tolerance to herbicides containing glyphosate by acetylating glyphosate making it non-phytotoxic, While the GMHRA protein imparts tolerance to the ALS-inhibiting herbicides.

Monsanto has also developed transgenic soybeans that are resistant to herbicide dicamba which controls broadleaf weeds (Behrens et al. 2007). Soybeans transformed with a bacterial dicamba monooxygenase (DMO) gene found to inactivate dicamba, making them tolerant to this herbicide. Syngenta and Bayer Crop Sci are developing an HPPD-inhibitor tolerant soybeans plants. The event consists of a stacking of a gene conferring tolerance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides and a gene for glufosinate tolerance. Inhibition of HPPD arrests the degradation of tyrosine to plastoquinones, which is important for carotenoid biosynthesis, photosynthesis, and tocopherol production (van Almsick 2009). The stacked herbicide-tolerant lines will enable the use of multiple herbicides and will act as an important tool to fight the increasing pressure from resistant weeds.

1.12.1.5 Increased Oil Content

Over the past decade, there has been a growing demand for soybean oil, for edible consumption soy-based biodiesel production. The interest in soybean oil has led to novel metabolic engineering strategies to increase the oil content of soybean seeds. Increasing oil content results in decreased protein content, and vice versa. The attempts made to increase oil content targeted enzymes and substrate pools in the Kennedy pathway involved in the production of triacylglycerols (TAGs).

The overexpression of diacylglycerol acyltransferase (DGAT2) encoding gene from fungi in soybean seeds (Lardizabal et al. 2008) converted diacylglycerols (DAGs) to TAGs. Transgenic soybeans grown at different locations over showed a 1.5% increase in total seed oil without affecting seed protein content. In another study (Rao and Hildebrand 2009), overexpression of the yeast sphingolipid compensation (SLC1) protein in soybean led the conversion of lysophosphatidic acid to phosphatidic acid, which is the precursor of DAG in the Kennedy pathway. Stable overexpression transgenic lines showed 1.5% increased oil content in seeds.

1.13 Recent Concepts and Strategies Development

1.13.1 Gene Editing

Inducing genetic variation in plant genome is the source for increasing genetic diversity and crop improvement. Natural and induced mutations were the only source of introducing new alleles that plant breeders exploited for crop improvement. However, these mutations are distributed randomly in the genome and not always useful. Recent advances in genomics, molecular biology, and genetic engineering have improved our ability to induce precise changes in the plant genome. Gene editing (aka genome editing or genome engineering) describes a suite of techniques that enable precise and targeted modifications (deletions, insertion, gene/base replacement) of host plant genome (Butler et al. 2018). The reagents (endonuclease) required for gene editing includes transcription activator-like effector nuclease (TALENs), zinc-finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR-Cas9).

Particularly, gene editing using CRISPR has emerged as a simple yet most powerful technology due to its high efficacy, simple to design target, cost-effective, and amenable to multiplexing (Jacobs et al. 2015). In CRISPR system, two core components are required to make site-specific change; first, the Cas9 (CRISPR-associated 9 protein) which is a large protein and has endonuclease activity and the second component is guide-RNA (gRNA) which is approx. 100 nucleotide RNA molecule. Both Cas9 and gRNA interact to make Cas9 complex and identify DNA sequence complementary to the gRNA in the genome. When these components introduced to plant cell via *Agrobacterium*-mediated transformation, the Cas9 complex recognizes the target site and it makes double-stranded break (DSB). When DSB created in the eukaryotic cells, the DNA repair mechanism gets activated which facilitate non-homologous end joining (NHEJ) and results in the deletion or insertion at the repair site. Insertion and deletions at the target gene sites cause frame-shift mutation (gene knockout) (Jacobs et al. 2015).

Cermak et al. (2017) developed a comprehensive toolkit that enables targeted, specific modification of monocot and dicot genomes using a variety of genome

engineering approaches. In addition to creating target specific mutations, CRISPR-Cas9 offers a unique advantage to repair a stretch of DNA sequence using homology-directed repair (HDR) and/or base editing. In this process, the CRISPR-Cas9-induced double-strand break can be used to create a knock-in, rather than a target gene knockout when a donor template provided (Gaj et al. 2016; Curtin et al. 2018). The precise insertion of a donor template after double-strand break can be altered to fix a mutation. However, one significant remaining challenge in plant genome engineering is achieving high-frequency gene editing by HDR. Recently, several groups have engineered Cas9 (dCas9, nCas9) for programmable editing of DNA base (Reviewed by (Eid et al. 2018). This advanced technology has provided an opportunity to edit single-base and accelerating functional characterization of novel genes and trait discovery to cope with abiotic stresses.

In soybean, these CRISPR and TALEN technologies have been successfully used to knockout genes involved in important agronomic and seed composition traits (Haun et al. 2014; Du et al. 2016; Curtin et al. 2018). Haun et al. (2014) used TALEN to improve soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Recently, Curtin et al. (2018) used both CRISPR/Cas9 and TALEN reagents to generate heritable mutations in small RNA processing involved in drought tolerance in soybean. They created a bi-allelic double mutant for soybean paralogs GmDrb2a and GmDrb2b, and Dicer-like2 genes. Notably, the study showed that Gmdrb2ab mutant plants were significantly more sensitive to drought stress than wild-type soybean plants suggesting a functional role of these genes in water stress. Moreover, utilization of a hairy root transformation system (*Agrobacterium rhizogenes)* is now possible to evaluate the efficacy and efficiency of multiple targets in a high-throughput setting (Cermak et al. 2017).

1.13.1.1 Concerns and Compliances About Gene Editing and Genetically Modified Crops

Modern biotechnology has provided a wide range of options to improve nutrition, climate resilience, and productivity. However, the technology and popularity of genetically modified (GM) crops have created social and ethical contradictions between consumers, farmers, researchers and policymakers. GM crops are wildly accepted for cultivation in the US and other parts of the world for either food crops (e.g., soybean, corn, canola) or nonfood crops (e.g., cotton). On the other hand, Europe has concerns about GM crop cultivation (Maghari and Ardekani 2011). However, it is needed to understand that there is a big distinction between gene editing in crops and classically defined GM crops. GM refers to insertion of a gene (foreign gene) from an external source such as viruses, bacteria, animals, or plants (usually unrelated species). While, in gene editing technology, a new gene is not transferred into a target crop plant, rather, genome editing technology tool is used to alter the function of a preexisting gene inside the plant genome. More importantly, gene editing technology is similar to the widely accepted ‘mutation breeding’ and moreover it is much faster and precise. Despite this fact, many other agencies such

as Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) in the US also plays a major role in the regulation and these agencies are considering the issues. It is increasingly clear that the USDA will not regulate the genome edited plants for cultivation (Waltz 2018). But gene-edited plants now are subjected to tough GM regulation in the European Union (https://www.nature.com/articles/d41586-018-05814-6).

1.13.2 Nanotechnology

Recent advancements in nanotechnology have opened up a novel application in agriculture and the scientific data indicates its potential to positively impact on the development of climate-resilient crops (Srilatha 2011; Fraceto et al. 2016). Nanoparticles can be synthesized from metal or metal oxide through physical or chemical processes and these nanoparticles are being studied to assess their potential in plant growth and development, protection from biotic and abiotic stresses. Nanotechnology has been successfully implemented in fertilizer applications, wastewater treatment, nanosensor, etc. (Srilatha 2011). Similarly, this technology opens large scope for diverse applications in fields of crop biotechnology and the potential benefits could be exploited mitigating abiotic stress and boosting agriculture productivity (Saxena et al. 2016).

Researcher has used silicon nanoparticles (SiNPs) to enhance abiotic stress tolerance via increased nutrient uptake, enhancement of antioxidant enzyme activity and by the formation of a thin layer in the apoplast, which helps the plant to resist various stresses (Liang et al. 2007; Saxena et al. 2016). A study showed than SiNPs (Na2Sio2) absorbs fasters without toxic effect in maize plant and exhibited a promoting effect on plant growth. In another study, Sedghi et al. (2013), demonstrated that nano zinc oxide have the potential to increase seed germination rate in soybean under water stress. Further, they concluded that application of these nanoparticles under drought condition decrease seed residual fresh and dry weight, suggesting its potential effect as seed reservoirs to seedling growth and enhance drought tolerance (Sedghi et al. 2013).

1.14 Brief Account on the Role of Bioinformatics as a Tool

Bioinformatics plays important role in curation deposition and organization of data to understand biological phenomena. The importance of bioinformatics tools rose sharply with the expansion of high-throughput molecular biology and genomic techniques. Currently, several repositories specific to Soybean genome and expression data are available (Livingstone et al. 2016.

1.14.1 Gene and Genome Databases

1.14.1.1 Phytozome (http://www.phytozome.net/soybean)

The JGI (Joint Genome Institute) released the soybean genome sequence. This genome was sequenced by whole-genome shotgun sequencing, and subsequently, the genome sequence was assembled (Jaffe et al. 2003). The Phytozome houses the whole-genome sequence of soybean and it also provides tools explore the genome through browsing interface. The data can be downloaded using the BioMart tool. Users can perform BLAST analysis against many plant genomes including soybean. Each gene has been annotated with, KOG, PFAM, PANTHER, KEGG RefSeq, UniProt, TAIR, and JGI assignments.

1.14.1.2 SoyBase (http://soybase.org/index.php)

The USDA-ARS initiated a central repository, SoyBase for genetics data and related resources. It is a map-based database containing different classes of data such as markers, maps, QTLs, locus, etc. SoyBase and the Soybean Breeder's Toolbox database include tools to browse the genetic map, physical map, and sequence map of the soybean. There are also many search pages and BLAST analysis to collect and analyze the data.

1.14.1.3 SoyGD (http://soybeangenome.siu.edu/)

The Soybean Genome Database (SoyGD) provides genomic information in with respect to the genetic map representing linkage groups based on loci and markers (Shultz et al. 2006). This browser allows users to visualize the physical and genetic maps of soybean. The search interface also enables to locate a specific region of interest based on region or landmark.

1.14.2 Soybean Omics Databases

1.14.2.1 Gene Networks in Seed Development (http://estdb.biology.ucla.edu/seed/)

This database is a collaborative effort between the Goldberg laboratory at UCLA and the Harada laboratory at UCD stores information about all the genes involved in soybean seed development. The soybean and Arabidopsis Affymetrix GeneChips, Laser Capture Microdissection (LCM), and next-generation high-throughput sequencing technologies were employed to profile the mRNA

sets from different seed regions from distinct developmental stages. Tools are available to browse and analyze gene expression data based on different seed developmental stages.

1.14.2.2 The Soybean Genomics and Microarray Database (SGMD) (http://psi081.ba.ars.usda.gov/SGMD/Default.htm)

The SGMD contains EST and microarray data to analyze the interaction of soybean with the major pest, soybean cyst nematode. The database contains more than 50 million rows of DNA microarray data and around 20,000 ESTs (Alkharouf and Matthews 2004). The analytical tools are in place to show the result with statistical measurement.

1.14.2.3 SoyXpress (http://soyxpress.agrenv.mcgill.ca/)

SoyXpress provides a link between the gene expression data from Affymetrix chips with related information like transcriptome data, Gene Ontology terms, and KEGG (Kyoto Encyclopedia of Genes and Genomes) metabolic pathways. It also contains many search interfaces, which enables users to browse by GenBank accession number, EST ID, Affymetrix probe ID, SwissProt protein ID, GO term or EC enzyme number (Cheng and Strömvik 2008).

1.14.2.4 The Soybean Proteome Database (SPD, http://proteome.dc.affrc.go.jp/soybean/)

Contains proteome data and their annotations from several organelles under flooding, salt, and drought stress. It comprised of the data generated by analyzed by two-dimensional polyacrylamide gels and gel-free proteomics technique.

1.14.2.5 Soybean Knowledge Base (SoyKB) (http://soykb.org/)

It is a comprehensive soybean genomics resource. SoyKB contains integrated data of genomics, transcriptomics, proteomics, and metabolomics along with gene function and biological pathway annotations. It contains information on SNPs, genes, microRNAs, and metabolites.

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Chapter 2
Genetic Solutions to Improve Resilience of Canola to Climate Change

Harsh Raman, Rajneet Kaur Uppal and Rosy Raman

Abstract Climate change and the accompanying impacts of global warming such as with rising temperatures and water shortages at crucial plant development stages poses a great challenge to the productivity and profitability of agricultural crops. In order to improve the resilience of canola to climate change, an integrated approach for breeding climate-smart varieties is required. Although the majority of the current breeding targets for canola improvement programs remain largely unchanged, emerging climate uncertainties reinforced the development of high yielding resilient varieties for tolerance to excessive drought, frost, heat, and waterlogging. Ecological, evolutionary adaptation, and selective breeding processes have provided a range of natural variation in 'climate smart traits' in canola and its closely related species. In this review, we focus on the extent of natural variation in various adaptation and productivity traits, and recent genetic and genomic innovations to confront climate uncertainties. Further understanding of the genetic determinants underlying resilience traits, increasing genetic diversity by creating desired mutations, and the deployment of prediction based breeding methods will accelerate the development of climate smart varieties for improving productivity and profitability of canola.

Keywords Drought resistance · Climate change · Stress tolerance · Genetic variation · QTL mapping · Genetic technologies

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2.1 Introduction

Canola (also known as rapeseed, oilseed rape, *Brassica napus* L., $A_nA_nC_nC_n$ genome, $2n = 4\times = 38$) is the third important oilseed crop in the world, followed by soybean and palm, contributing approximately 13% of global oil supply (https://www.fas.usda.gov/commodities/oilseeds). Canola belongs to the family *Brassicaceae* and originated as a result of spontaneous hybridization between *Brassica rapa* ($2n = 2\times = 20$) and *Brassica oleracea* ($2n = 2\times = 18$) (Nagaharu 1935). Three types of canola are grown in the temperate and subtropical regions; the winter type is planted in Europe and parts of China, the spring type is mainly grown in the United States and Canada, the semi-winter/spring-type canola is grown in Australia, China, and India.

Canola has a high plasticity, with a long-flowering window and adaptation to diverse production systems ranging from low-input to highly intensive farming. For example, in the US, canola production is concentrated on Northern Plains where a drier and shorter growing season prevails, while high-input intensive cultivation is often practiced in Europe and other parts of the world that have ample water availability; either via natural rainfall or via irrigation. In Australia, it is mainly grown as a broad-leaf rotation crop in cereal producing regions that have a rainfall variation from ~300 mm to >450 mm. However, its cultivation mainly relies on the residual soil moisture. Insufficient soil moisture in the sowing window directly impact sustainability of this valuable crop used for vegetable oil, stockfeed, and biodiesel production markets. Occasionally, growers practice dry sowing of canola in anticipation of follow-up rain events. Canola yields vary from year to year; ranging from less than 1 ton/ha under water-limited conditions to ~6 to 8 ton/ha under irrigated conditions, respectively, and are highly affected by various abiotic and biotic stresses. Of them, drought and heat are considered as major limitations to crop productivity worldwide (Ceccarelli et al. 2010; Smith and De Smet 2012).

2.1.1 Effects of Climate Change

Climate change poses an unprecedented challenge for global food security and livelihoods of rural communities. Impacts of global warming include changes in rainfall patterns; more frequent drought and flood, and other extreme weather events (Rosenzweig and Hillel 1995; BOM 2007; Gornall et al. 2010; Luo et al. 2010). However, these changes are not new and have been affecting plants and human civilizations from several thousand years (Ceccarelli et al. 2010). Climate change occurring at present is likely to adversely impact on the global food production, food quality, biodiversity; incursion and severity of insect, pest and diseases; and availability of soil nutrients (Garrett et al. 2006; Ceccarelli et al. 2010). The negative effects of climate changes on crop productions are likely to be more severe in future as global temperatures are predicted to rise. On a global scale,

crop yield reductions ranging from 21 to 40% due to drought stress have been reported (Daryanto et al. 2016). Global surface temperatures are expected to rise between 0.5 and 4.5 °C within next six decades (IPCC 1995). It is estimated that for each 1 °C increase in night temperature, crop yields may decline by 10% (Peng et al. 2004; Liu et al. 2016a). Global warming due to elevated CO_2 levels could also impact oil quality. Water stress and heatwaves especially at flowering and pod-filling stages often reduce seed yield and oil content in canola (Mailer and Cornish 1987; Champolivier and Merrien 1996; Walton et al. 1999a; Sinaki et al. 2007), undervaluing the profit-margins to canola growers. In a recent study, Namazkar et al. (2016) showed that elevated levels of CO_2, O_3 and temperature; individually or in combination reduce the fatty acid composition of canola; up to a 45% reduction was observed in α-Linolenic acid (ω3, a fatty acid essential for human nutrition). This study also showed that an elevation in CO_2 and temperature could lead to 58% reduction in the oil yield per hectare, and ω3 by 77%. With the onslaught of climate impact on productivity, demand of canola for vegetable oil, stockfeed and biodiesel industries is going to accelerate to support growing population of over 9 billion people by 2050 (Godfray et al. 2010); warranting a significant improvement in crop production on already limited arable land.

Only a few attempts have been made to investigate the climate-resilient traits in canola as compared to major cereals like maize and wheat. Concerted efforts are thus required to develop new cultivars resilient to climate variability for stable yield production under stress environments. In the past, majority of the breeding programs have been producing improved varieties having high oil quality and resistance to diseases and herbicides. This selective breeding practice may have narrowed down the allelic diversity (Cowling 2007) for climate resilience traits within the breeding germplasm. Understanding the distribution and extent of natural variation in adaptive and productivity traits and their interaction with environment is essential for genetic improvement (Alonso-Blanco et al. 2009). In this context, we review the (i) extent of natural variation in various traits which underpins the canola productivity, especially that are directly subjected to climate uncertainties such as drought resistance, tolerance to elevated temperatures (heat) and waterlogging and (ii) the genetic basis of existing natural variation in canola germplasm, so that genetic solutions for preparedness to extreme weather could be prioritized. We also provide a summary on various phenotyping, genotyping and biotechnological tools available to understand complex traits involved in canola productivity.

2.2 Natural Variation in Grain Yield

Developing stable high yielding varieties across the target and changing environments is the primary objective of breeding programs. However, selection for high yield is confounded by plethora of environmental factors and evolutionary adaptive traits such as pod length, number of seeds/pod, seed weight/pod phenological

attributes I flowering time, maturity, plant height and architecture); nutrient use efficiency; abiotic stresses (drought, heat, waterlogging, and frost); toxicities to minerals (Mn^{2+}/Al^{3+}) and response to insect-pest and diseases). Therefore, genetic dissection of grain yield per se is quite challenging. Identification of natural variation and underlying genetic basis (loci/gene) controlling various component traits that contribute to high grain yield will enable the indirect selection.

In recent years, breeding high yielding varieties especially under water-limited/waterlogged and heat stress environments has become one of the important targets in canola improvement programs. For medium to high rainfall areas, breeders often target varieties that have high plant biomass, increased leaf area, delayed maturity for high yield potential, while smaller plant canopy, reduced leaf area, early vigor, early-mid flowering, and early maturing varieties are bred for Mediterranean environments to escape terminal drought and heat such as in South-Eastern/Western Australia, southern Europe, Middle Asia, South Africa and the USA (e.g., southern California) and South America. Rapid grain filling can also be used as a target trait to enhance grain yield in these environments. Currently, a limited number of breeding programs focus on developing varieties, especially hybrids for hostile environments. It is possible that stress-resistant varieties may not achieve the high yield potential under non-stress environments, due to trade-off in stress-related traits. However, higher yield could be achieved under stress environments via deployment of climate smart crops using traditional (natural and artificially-induced variation) and biotechnological methods (Fig. 2.1).

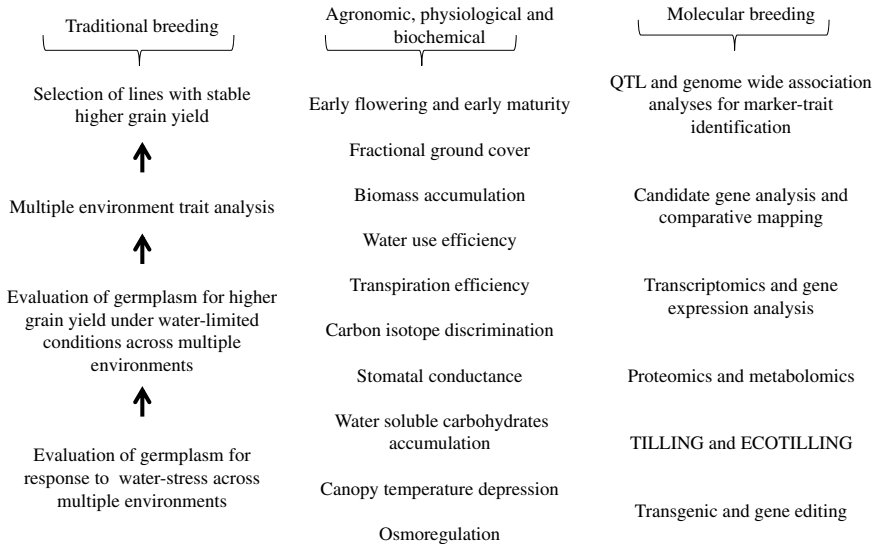

Fig. 2.1 Approaches for developing drought-tolerant varieties in canola

2.3 Natural Variation in Drought Resistance Traits

Drought refers to a period of water deficit that is related to changes in soil conditions, leading to plant water deficit. Various factors such as water availability, soil water holding capacity and rate of evapotranspiration, determine the timing and duration of drought episode to occur (Jones 1992; Larcher 1995). Drought stress impairs stomatal conductance, transpiration rate, nutrient and water relations, photosynthesis, and partitioning of assimilates, which result in significant reduction in crop yield (Farooq et al. 2009; Osakabe et al. 2014). Impaired mitosis, cell elongation, and expansion result in reduced plant height, leaf area, and crop growth (Nonami 1998; Kaya et al. 2006). Drought stress affects canola at all stages of plant development (Fig. 2.2); right from seedling to pod filling (Chaves et al. 2002; Norouzi et al. 2008). However, moisture shortage and elevated temperature, especially during flowering and pod filling stages are more critical and result in significant yield loss and reduced oil content (Mailer and Cornish 1987; Morrison 1993; Champolivier and Merrien 1996; Gan et al. 2004; Sinaki et al. 2007). Plant water deficits also impair radiation interception and radiation use efficiency which reduce harvest index (Earl and Davis 2003). Drought-induced yield reduction has been reported in many crop species, including canola (Morrison 1993; Champolivier and Merrien 1996; Gan et al. 2004; Sinaki et al. 2007; Cattivelli et al. 2008).

To 'survive' and 'thrive' under limited water availability and produce seeds for the subsequent generations, plants have evolved three strategies for drought resistance: drought escape, dehydration avoidance and dehydration tolerance (Levitt 1972; Turner 1986). 'Drought escape' is the ability of a plant to complete the life cycle before the onset of severe drought stress and relies on developmental plasticity traits; early flowering and maturity. This type of response is well documented in several plants including cereals (Bruce et al. 2002), legumes (Chaves et al. 2002) and canola (Franks 2011; Raman et al. 2016c). 'Dehydration avoidance' is the ability of plants to postpone or avoid tissue water deficit for a short-term survival (Ludlow 1980) and relies on increased water uptake by root modifications, reduced water use or increased water use efficiency (WUE) attributed to leaf waxiness, plant size, small or closed stomata and reduced photosynthesis (Ludlow 1989; Blum 2005). Plants with deep and extensively branched root system (Gowda et al. 2011), roots with low hydraulic conductivity (Passioura 1983), limited transpiration due to reduced leaf area (Vadez et al. 2013), stay green (Christopher et al. 2008; Vadez et al. 2014), and low stomatal conductance (Fischer et al. 1998; Richards et al. 2007; Kholová et al. 2010a, b; Kumagai and Porporato 2012; Vadez et al. 2013) show drought avoidance for short-term survival. However, under prolonged drought conditions, avoidance mechanisms eventually fail to prevent the dehydration of the plant tissue (Ludlow 1980). 'Drought tolerance' refers to adaptation allowing the plant to withstand drought stress, with or without reduction in performance. Drought tolerance, i.e., increased WUE and maintenance of metabolic activities at low tissue water potential through osmotic adjustment is imparted due to accumulation of both intercellular organic and inorganic solutes such as soluble

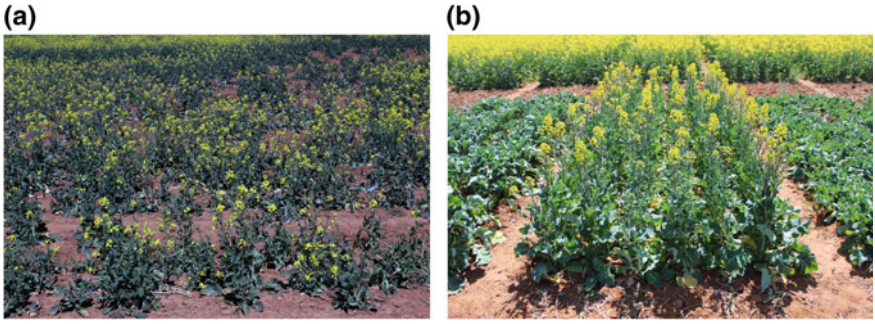

Fig. 2.2 **a** Canola crop showing low emergence and poor growth under water stress conditions at the research farm of Wagga Wagga Agricultural Research Institute. **b** Spring type canola variety, Yudal display early flowering, a drought escape trait (plants were >70 cm tall and showed early flowering) compared to winter type canola variety, Darmor-*Bzh* grown at Wagga Wagga, Australia. Some of the DH lines from Darmor-*Bzh*/Yudal (plants <10 cm) showed precocious flowering). Photos were taken on 19th September 2019 by Harsh Raman, after 120 days of sowing. Seeds of Darmor-*Bzh* and Yudal accessions were provided by Dr. Regine Delourme, INRA, France

sugars, proline, glycine betaine; change in phytohormone levels (e.g., ABA) and enzymes to maintain turgor in plants (Morgan 1977, 1984; Levitt 1980; Davies et al. 1994; Chimenti et al. 2006; Ma and Turner 2006).

Drought resistance mechanism often operates at different times of drought stress; drought escape occurs before the onset of drought, whereas drought tolerance and avoidance mechanisms operate during the drought stress period. However, under severe water stress conditions, all described mechanisms fail to sustain plant growth achieve 'economic' grain yield. For practical breeding, achieving high grain yield and high oil content in varieties remain the key target trait for canola breeding under water-limited conditions.

2.3.1 Flowering Time

Drought escape occurs when phenological development such as flowering time and maturity is matched with periods of soil moisture availability (Araus et al. 2002) in the Mediterranean environments where growing season is restricted by terminal drought and high temperatures in such as in South-Eastern/Western Australia, Southern Europe, Middle Asia, South Africa, and parts of North America (USA) and South America. This mechanism of plant adaptation may have evolved in nature; demonstrated experimentally in *B. rapa* populations (Franks et al. 2007, 2016; Franks 2011).

Genetic variation for flowering time exists among winter, spring, and semi-winter types in canola and has been exploited globally in breeding programs

to develop short-duration varieties. In Australia, several early flowering spring varieties have been developed for commercial cultivation such as 43C80(CL), ATR-Stingray, CB-Telfer CB-Trilogy, Diamond, Hyola 404RR, and Sturt TT. Deployment of these varieties has proven to be an effective strategy for minimizing yield loss from terminal drought conditions. However, these varieties need to be sown at optimum time for the development of sufficient vegetative growth to maximize grain yield through remobilization of resources (Kirkegaard et al. 2016). Generally, short season, early flowering varieties have lower yield potential compared to mid-season/late-flowering varieties under non-water stress situations. However, some early flowering and early maturing varieties also yield higher under both water-limited and optimal growing seasons (wet), suggesting that these varieties may have WUE genes. Field evaluation of 145 doubled haploid (DH) lines derived from two Australian varieties, Skipton and Ag-Spectrum, showed that early flowering lines had high negative correlation with grain yield ($r = -0.6$), suggesting that early flowering lines yield higher in rainfed environments across 2013 and 2104 (Raman et al. 2016c). It is observed that even early flowering varieties fail to make economic yield, if they experience severe drought conditions during early stages of plant development during early stages of plant development.

2.3.2 *Water Use Efficiency (WUE)*

WUE is often considered as a drought avoidance trait (Condon et al. 2004; McKay et al. 2008) and refers to the ability of the plant to produce biomass per unit of water applied (rainfall/irrigation). It has been widely used for measuring crop performance in water-limited conditions (Passioura 1977; Richards et al. 2002) and is expressed as:

$$\text{WUE} = \text{water transpired} \times \text{transpiration efficiency (TE)} \times \text{harvest index}.$$

This framework is not based on the notion of 'drought resistance' (Levitt 1972), but rather on traits which directly or indirectly improve WUE either by increasing water extraction or by limiting water use, thus improving grain yield. Each component of this framework can be considered as a target for genetic improvement (Condon and Richards 1993). Several studies have identified significant genetic variation in WUE within crop species, including canola (Richards and Thurling 1978; Farquhar and Richards 1984; Teulat et al. 2001; Condon et al. 2004; Hall et al. 2005). WUE can be measured at leaf, whole plant, and crop levels. Different methods such as infrared gas exchange analysis, neutron moisture meter, gravimetric, weighing lysimeter, chlorophyll fluorescence, thermal imaging, and automated weighing, and watering platforms such as phenoscope are used to evaluate germplasm for WUE (Ludlow and Muchow 1990; Bechtold et al. 2010, 2013; Tisne et al. 2013; Easlon et al. 2014; Vadez et al. 2014; McAusland et al. 2016; Ryan et al. 2016; Ferguson et al. 2018).

Gas exchange analysis has been widely used to measure variation in TE, as both leaf transpiration and stomatal conductance could be simultaneously recorded; however, measuring internal leaf CO_2 levels of individual leaf is quite time-consuming, variable and laborious. Jackson et al. (2016) have shown that multiple measurements for leaf CO_2 are necessary to reduce error-rate and increase broad-sense heritability. Therefore, this method is unsuitable for measuring WUE in large field plots. Additionally, gas exchange measurements at individual leaf, do not correlate well at plant and crop levels. Therefore, evaluation of germplasm for improved WUE should be based at the crop/plot level under field conditions. Rainout shelters can be utilized to mimic the drought stress environment as prevails under field conditions and provide an option to control water regime precisely. However, rainout shelters may create a 'micro-environment' that could influence the performance of specific genotype(s), and contribute to genotypic × environment interactions. Nevertheless, genotypes of interest can be identified for different performance traits such as high biomass, early vigor, and high grain yield in response to water stress under rainout shelters. Those genotypes are then analyzed in much greater detail to discover physiological traits responsible for the observed differences, eventually leading to gene discovery of drought tolerance traits.

The ratio of natural isotopes of $^{13}C/^{12}C$ or $\delta^{13}C$ (CID, carbon isotope discrimination) could be used as a proxy for WUE; the least value for $\delta^{13}C$ represent plant with the greatest WUE (Farquhar and Richards 1984). This simple and rapid screening method provides a long-term measure of WUE as compared to other methods such as gaseous exchange measurements based on single leaves. In water-stressed conditions, stomatal closure results in decrease in transpiration and CO_2 in leaves which consequently decrease in assimilation. This forces ribulose-1, 5-bisphosphate carboxylase oxygenase (RUBISCO) enzyme to discriminate against ^{12}C, which results into increase in ^{13}C (Farquhar and Richards 1984; El-Soda et al. 2014).

The $\delta^{13}C$ has been extensively used to assess natural variation in WUE in Arabidopsis (Easlon et al. 2014) and agricultural crops including canola (Matus et al. 1995; Condon et al. 2004; Hall et al. 2005). Luckett et al. (2011) showed that a limited genetic variation exists for $\delta^{13}C$ among 63 accessions of canola; Triazine tolerant (TT) genotypes exhibited higher $\delta^{13}C$ compared to non-TT accessions; as TT varieties have inherent low photosynthetic capacity and low biomass (Beversdorf et al. 1988). However, no obvious relationship between $\delta^{13}C$ and grain yield was found. Matus et al. (1995) also did not find any relationship between $\delta^{13}C$ and grain yield in canola. In wheat, $\delta^{13}C$ is negatively correlated with TE (Farquhar and Richards 1984; Rebetzke et al. 2002), therefore, genotypic selection for low $\delta^{13}C$ is recommended for higher biomass and grain yield under drought stress. However, the relationship between improved TE and grain yield is variable depending on the growing environment (Condon et al. 1987; Fischer et al. 1998). A studies has shown that sampling stage of tissue collection may impact on the relationship between $\delta^{13}C$ and grain yield variation in canola; $\delta^{13}C$ signature may vary with the phenological development (Raman et al. unpublished).

In some studies, positive correlations between TE and specific leaf weight (Nageswara et al. 1995; Brown and Byrd 1997; Byrd and May 2000), and leaf

chlorophyll content (Lambrides et al. 2004; Fotovat et al. 2007; Arunyanark et al. 2008) were reported, suggesting that these simple-to-measure traits could be used for selecting superior TE lines. Positive genetic correlations between WUE and flowering time were reported in Arabidopsis, where late-flowering plants have improved WUE (Juenger et al. 2005; McKay et al. 2008; Lovell et al. 2013; Kenney et al. 2014). In Australia, at least three wheat varieties, Drysdale, Rees and Scout, having high WUE have been released for commercial cultivation. These varieties were selected on the basis of $\delta^{13}C$. Incremental genetic gains in grain yield can be made in the canola breeding programs, once the relationship between TE and $\delta^{13}C$ is validated.

2.3.3 Canopy Temperature

Leaf surface temperature measured with thermal imaging and infrared thermometer can be used as a surrogate for stomatal conductance for screening large populations in the field. In water and heat stressed conditions, plants with cooler canopies or high canopy temperature depression (CTD) use more soil available water, and thus avoid negative effects of stress on grain yield (Blum et al. 1989). CTD is deployed for screening germplasm for drought and heat tolerance (Blum et al. 1989; Reynolds et al. 2009; Guo et al. 2015; Pandey et al. 2017).

2.3.4 Early Vigor

Accumulation of plant biomass, normally measured as aboveground biomass, at early growth stages, is an important adaptation trait for crop species subjected to terminal drought/heat stress in Mediterranean environments. Genotypes with early vigour accrue more growth when evaporative demand is low, leading to high WUE. Increased early vigor is reported to reduce soil evaporation; ensuring conservation of water for transpiration and plant growth, and increase nutrient uptake and weed competition (Turner and Nicolas 1987; López-Castañeda et al. 1996; Coleman et al. 2001; Botwright et al. 2002; Rebetzke et al. 2004, 2007; Ward et al. 2007; Pang et al. 2014; Ryan et al. 2015; Wilson et al. 2015). It also improves radiation interception to maximize photosynthesis and increase crop growth, biomass, and grain yield especially in the environments, where crop duration is short (Regan et al. 1997). In canola, Zhang et al. (2016) reported that vigorous genotypes with thicker leaf and more leaf area intercept more radiation and higher biomass in vegetative and reproductive stages. Although larger leaves may be beneficial during early development, the same trait has trade-off at the end of growing season (Richards 1991). Therefore, a combination of greater early vigor and TE traits has been suggested to maximize the amount of biomass accumulated for a given quantity of available water (Wilson et al. 2015).

Genetic variation for early vigor has been determined in canola germplasm using traditional biomass cuts and the normalized difference vegetative index (NDVI) before the flowering stage (Holzapfel et al. 2009; Moroni et al. 2009; Cowley et al. 2014; Raman et al. 2014c). Positive correlations, ranging from 0.31 to 0.80, between early vigor and grain yield were reported (Cowley et al. 2014). This study also showed good correlations between NDVI and yield for dry (0.48) and wet environments (0.52), suggesting that NDVI can be used as a 'proxy' for improving grain yield in canola across environments.

2.3.5 *Root Traits*

Root architectural traits, such as root weight (root density, root number, root length) and diameter of xylem vessels, play a critical role for increasing crop yield under water stress (Richards and Thurling 1978; Richards and Passioura 1989; Tuberosa et al. 2002; Craine et al. 2013). The importance of deep and vigorous root system for higher yield has been described in several crops (Gowda et al. 2011; Henry et al. 2011; Tuberosa et al. 2011; Varshney et al. 2011). Measuring root traits is expensive, laborious and difficult under field conditions. Several techniques such as 'shovelomics' (Trachsel et al. 2011), minirhizotrons (Johnson et al. 2001), hydroponics (Hatzig et al. 2014), root architecture analysis (Landi et al. 2002), and root pulling force; as a proxy for root mass in canola (Fletcher et al. 2015) have been investigated. In a recent study, high-throughput phenotyping was performed to investigate root traits such as root length, and root number; which showed a positive correlation (r = 0.3–0.5) with seed yield under field condition (Thomas et al. 2016). However, this method is not widely adopted in canola breeding programs yet. Selection of varieties for fast-growing deep roots could improve water uptake and grain yield under conditions when moisture is stored in deeper soil layers, particularly in crops with limited capacity to adjust osmotically. However, root-related traits have low heritability and difficult to phenotype accurately. For example, Fletcher et al. (2015) reported broad-sense heritability of 0.16–0.25 in a DH population of canola (IMC106RR/Wichita) under dry and wet treatments, respectively.

2.3.6 *Water Soluble Carbohydrates*

Accumulation and remobilization of water-soluble carbohydrates (WSC, e.g., sucrose, glucose, fructose, and fructan) from stem and leaves has been shown to improve grain-filling (a proxy for improving WUE), particularly, under terminal drought and heat stress conditions in cereals (Foulkes et al. 2007; Rebetzke et al. 2008b; Gupta et al. 2011). In canola, genotypic variation for WSC and its contribution to grain yield in contrasting seasons, suggests greater remobilization toward developing grain in dry season (Zhang and Flottman 2016a, b). However, there was

no significant relationship between WSC and grain yield because of confounding effects of late-flowering genotypes. Berry and Spink (2006) suggested that contribution of WSC to canola yield could be increased up to 10% to achieve a yield of 6 ton/ha in European environments. Green canola pods also contribute to photosynthesis along with accumulation and remobilization of pre-flowering carbohydrate stem reserves (Hua et al. 2012). Therefore, selection of longer pods, in addition to stem WSC has been suggested to improve sink size (Lewis et al. 2001; Hawkins et al. 2005; Samizadeh et al. 2010; Qi et al. 2014). Utility of this trait under drought and heat stress environments remains to be investigated. To date, no significant progress in genetic analysis for WSC content in canola has been made. This could have partly attributed due to expensive and laborious estimation of WSC in large segregating mapping populations using wet chemistry and large G × E interactions. To enhance the throughput and reduce the cost of phenotyping, Raman et al (2014c) developed a method based on Near-infrared spectroscopy which showed a very high correlation with WSC estimation based on wet chemistry method.

2.3.7 Osmotic Adjustment

Osmotic adjustment has been associated with adaptation under drought stress conditions in several crops including sorghum (Jones 1978), wheat (Morgan 1977), sunflower (Chimenti et al. 2006), and *Brassica* species (Kumar et al. 1984; Wright et al. 1997; Kumar and Singh 1998; Wright and Morgan 1998; Niknam and Turner 1999; Ma et al. 2004; Norouzi et al. 2008; Gunasekera et al. 2009; Guo et al. 2015; Pandey et al. 2017). The screening methods for osmotic adjustment are not straightforward, and generally require pressure chambers to measure leaf water potential which limits their application in screening large populations grown under field conditions. Polyethylele glycol (PEG)-induced hydroponic screening system has been extensively used to induce osmotic stress at early seedling stage in various crop species, including canola (Hatzig et al. 2014). This system allows cost-effective screening and genetic analyses for large populations (Blum et al. 1980; Kato et al. 2008). Root and shoot parameters are measured for evaluation of seedling vigor under control and stress conditions (Zhang et al. 2015a). Hatzig et al. (2014) showed increase in ABA and proline under osmotic stress in canola but did not find significant differences in hormonal changes between drought-resistant and drought-sensitive genotypes. Niknam et al. (2003) reported that Brassica genotypes with low osmotic adjustment impose 40% yield penalty under drought stress whereas genotypes with high osmotic adjustment had only 10% yield loss due to water stress. Better yield could be due to the buffering action of osmotic adjustment against sterility induced under drought stress (Ma and Turner 2006). Research suggests that osmotic adjustment improve grain yield under water stress situations, however, further research is required to demonstrate the value of osmotic adjustment as a target trait in canola breeding programs.

2.4 Heat Tolerance

Heat stress, particularly, at flowering and pod filling stages affects canola yield. High temperatures (>27 °C) narrow down the flowering window, reduce fertility (Bjorkman and Pearson 1998; Morrison and Stewart 2002; Garlinge 2005), and increase canopy respiration and water stress, while post-flowering stress seriously affect the extent and rate of grain-filling which reduce grain yield and oil content by 3–5% (Angadi et al. 2000; Aksouh et al. 2001; Aksouth-Harradj et al. 2001; Garlinge 2005; Annisa et al. 2013). Hocking and Stapper (1993) reported 1.5% reduction in oil content for each 1 °C rise in temperature. Heat stress impairs various metabolic processes such as membrane thermo-stability, respiration, transpiration, and photosynthesis by disrupting PSII-mediated electron transfer, changing fluidity of thylakoid membranes leading to leakage of PSII light-harvesting complexes and loss of chlorophyll (Gan et al. 2004; Ristic et al. 2007; Prasad et al. 2008).

Two mechanisms for heat tolerance: acquired tolerance, ability of plant to survive under lethal temperatures after exposure to mild temperature known as 'acclimation/avoidance' and inherited tolerance, evolutionary selection for high temperature known as 'adaptation' have been described (Kramer 1980; Wahid et al. 2007; Hanumappa and Nguyen 2009). Short-term acclimation mechanism includes traits related to change in leaf orientation, leaf cooling via transpirational loss of water and alteration of membrane lipid compositions. In many crop plants, adaptation trait such as early maturity is closely correlated with smaller yield losses under high temperatures, which may be attributed to an escape mechanism (Adams et al. 2001).

Genetic variation for heat tolerance exists among *Brassica* species, in the order of *B. rapa* > *B. napus* > *Brassica juncea* (Angadi et al. 2000; Aksouh-Harradj et al. 2006; Annisa et al. 2013). Several phenological, physiological, and biochemical traits such as bud temperature depression, chlorophyll fluorescence (Cowley and Luckett 2011), increased chlorophyll a:b ratio, and decreased chlorophyll: carotenoids ratio, increase in electrolyte leakage and membrane stability, stay green, and accumulation of heat shock proteins (HSP) have been used as indicators for heat tolerance (Blum and Ebercon 1981; Queitsch et al. 2000; Young et al. 2004; Camejo et al. 2005; Wahid and Ghazanfar 2006; Wahid et al. 2007; Lopes and Reynolds 2012; Annisa et al. 2013; Guan et al. 2014; Talukder et al. 2014). To address the impact of rising global temperatures on crop productivity, the rate of food production needs to be increased significantly in the recent future.

2.5 Other Productivity and Adaptive Traits

Canola is very sensitive to waterlogging, causing deprivation of oxygen which impacts root respiration, water uptake and nutrient uptake (Jackson and Drew 1984). Waterlogging not only occurs due to excessive water supply (rainfall/irrigation) but also attributed to ancient soils where water does not penetrate into the

deeper layers. It can cause yield losses of up to 50% compared to well-drained soils (Walton et al. 1999b). Genetic variation for tolerance to waterlogging, resistance to lodging and various diseases, nutrient use efficiency and toxicities to soil acidity mainly caused by Al^{3+} and Mn^{2+} ions (Kochian 1995; Raman and Gustafson 2010; Delourme et al. 2011; Delhaize et al. 2012; Li et al. 2014c; Han et al. 2015; Raman et al. 2017a) exists in canola germplasm. This resource has been exploited in the breeding programs by selecting traits of interest directly or indirectly by while selecting high yielding breeding lines in stress-prone environments.

2.6 Epigenetic Variation in Productivity and Adaptation Traits

Epigenetic features are also reported to enhance grain yield in canola. Hauben et al. (2009) showed that energy use efficiency can be selected for improvement of seed yield. Results showed that low respiration lines with a high energy use could increase up to 8% seed yield compared to control lines, while the low-energy lines with high respiration reduce seed yield by up to 10%. Recently, Verkest et al. (2015) showed that canola epilines enhance energy use efficiency, drought tolerance, and nitrogen use efficiency. Research findings suggested that heritable epigenetic variation, caused by DNA methylation/histone modification could be incorporated into improvement programs.

2.7 Genetic Dissection of Natural Variation

2.7.1 Molecular Marker and Mapping Technologies

Understanding the genetic basis of natural variation in traits of agronomic importance has played a crucial role in the development of improved varieties using traditional and biotechnological methods. Different marker systems such as random amplified polymorphic DNA, RAPD (Somers et al. 2001; Hawkins et al. 2005); restriction frament length polymorphism, RFLP (Parkin et al. 1995; Butruille et al. 1999); amplified fragment length polymorphism, AFLP (Vos et al. 1995; Honsdorf et al. 2010); simple sequence repeat, SSR (Lowe et al. 2004; Piquemal et al. 2005); diversity arrays technology, DArT) (Raman et al. 2012); sequence-related amplified polymorphism, SRAP (Li et al. 2007; Sun et al. 2007); restriction site associated DNA, RAD (Miller et al. 2007; Baird et al. 2008; Chen et al. 2017); intron-polymorphisms (Panjabi et al. 2008); single nucleotide polymorphism, SNP (Trick et al. 2009; Dalton-Morgan et al. 2014; Clarke et al. 2016); genotyping by sequencing, GBS (Elshire et al. 2011; Rahman et al. 2016); DArTseq (Raman et al. 2016b), and specific-locus amplified fragment sequencing, SLAF-Seq (Geng et al. 2016)

have been employed for revealing genetic diversity, construction of genetic linkage maps and identifying trait marker associations in canola.

Genetic loci associated with traits of agronomic importance have been dissected primarily through two forward genetics approaches; QTL (quantitative trait loci) and GWAS (genome-wide association studies). In addition, bulked segregant analysis has also been used to map qualitative as well as quantitative traits such as seed weight in canola (Geng et al. 2016; Hua et al. 2016; Wang et al. 2016). GWAS based on linkage disequilibrium (Flint-Garcia et al. 2003; Jannink 2007) is increasingly being used to identify alleles for flowering time, resistance to pod shatter and blackleg, seed germination and vigor, seed weight and seed quality, and tocopherol content (Jestin et al. 2011; Fritsche et al. 2012; Li et al. 2014a; Raman et al. 2014b, 2016a, b; Hatzig et al. 2015b). Traditional QTL mapping requires a segregating population derived from limited (two or more) parents, while GWAS relies on historic recombination events occurred over time in a 'natural' population. For QTL analysis, majority of the studies utilized either DH or intercross (F_2 or $F_{2:3}$) populations. In recent years, some groups are using multi-parental populations such as multi-parent advanced generation intercross (MAGIC) and nested association mapping (NAM) populations to unravel genetic control of trait of interest in genetically diverse backgrounds (Kover et al. 2009; Liu et al. 2016b). Various statistical and quantitative genetic tools based on single marker regression, interval and composite interval mapping, and whole genome average interval algorithms mapping are routinely being employed to investigate the genetic basis of natural variation in traits of interest in canola (Snowdon and Friedt 2004; Zhou et al. 2014). A range of QTL effects were detected: QTL which are stable across environmental, with a small G × E effect (environmentally stable QTL), environment-specific QTL, with the QTL having an effect in one environment but no effect in another environment (Bagheri et al. 2012).

In recent years, several genomes of *B. napus* as well as its ancestral and related species such as *B. rapa*, *B. oleracea*, *Brassica nigra* and *B. juncea* have been sequenced (Wang et al. 2011b; Chalhoub et al. 2014; Liu et al. 2014; Parkin et al. 2014; Yang et al. 2016). Additionally, genome of *Brassica carinata* has also been sequenced at the AAFC, Canada (Isobel Parkin, pers com.). Developments in next-generation sequencing technologies and bioinformatics have made possible to perform whole genome resequencing and develop assays to target region(s) of interest such as sequence captures, which are being applied to uncover natural variation for a range of traits in canola (Edwards et al. 2013; Schiessl et al. 2014; Schmutzer et al. 2015). These genomics innovations empowered research community to map markers of interest on the physical maps of *B. species* and identify genes/alleles controlling phenotypic trait variation. In addition, canola research has benefitted immensely from the landmark innovations made in *Arabidopsis thaliana*, which relates to *Brassica*, and diverged approximately 14.5–20.4 million years ago (Lagercrantz et al. 1996b; Rana et al. 2004; Town et al. 2006; Chalhoub et al. 2014; Liu et al. 2014; Parkin et al. 2014). Colinearity and congruence of several loci between the reference genomes have made possible to isolate paralogs of various

genes and to relate with phenotypic variation for a range of traits in canola (Wang et al. 2009a, 2011a; Zou et al. 2012; Raman et al. 2013a; 2016b; Shah et al. 2018).

2.7.2 *Mapping Genes for Productivity and Adaptation Traits*

2.7.2.1 Grain Yield

Thousands of QTLs associated with seed yield and yield-related traits such as seed weight, pod (silique) number/plant, seed number/pod, pod length, pod density, harvest index, lodging, and seed quality attributes were mapped in canola populations using QTL mapping (Udall et al. 2005; Quijada et al. 2006; Chen et al. 2007; Li et al. 2007; Radoev et al. 2008; Shi et al. 2009; Basunanda et al. 2010; Fan et al. 2010; Zhang et al. 2011; Yang et al. 2012; Shi et al. 2015; Lu et al. 2016; Raman et al. 2016c; Chao et al. 2017) and GWAS approaches (Li et al. 2014a, b; Zhou et al. 2014; Hatzig et al. 2015a). Herein, we describe a few examples in more detail (see Table 2.1); Shi et al. (2009) identified 85 QTLs for seed yield and 785 QTLs for yield-related traits (flowering time, maturity, plant height, branch number, pod number, seed number per pod, seed weight, biomass yield per plant) in DH and reconstructed F_2 canola populations derived from Tapidor (winter)/Ningyou7 (semi-winter), evaluated under 10 environments. On an average, 71.9–92.5 of 'consensus' QTLs mapped within the confidence intervals were associated with all the eight traits investigated. This study concluded that either QTLs for seed yield/related traits map in a cluster or have pleiotropic effects. Other studies also showed colocalization of QTLs for seed/grain yield and other traits such as flowering time (Long et al. 2007; Shi et al. 2009; Raman et al. 2016c), plant height, maturity, and nutrient uptake efficiency (Ding et al. 2012; Hua et al. 2016) illustrating the pleiotropic effects. It is also possible that relatively smaller size of populations may not have been adequate to determine whether consensus QTLs for different traits are linked or as a result of pleiotropic effects. In addition, epistatic interaction among loci for grain yield and its related traits have been documented (Radoev et al. 2008). In silico analysis of yield-related QTLs showed an uneven distribution across the canola genome, most of them were mapped on chromosome A03 and the least were on chromosome C06 (Zhou et al. 2014). Genetic analysis studies revealed that detection of QTLs for grain yield vary in the magnitude and allelic effect across environments. These findings reiterate that yield is a complex trait and governed by quantitative genes with G × E interactions.

2.7.2.2 Flowering Time

Several research groups have mapped genetic loci associated with variation in flowering time in *B. napus* (Ferreira et al. 1995; Osborn and Lukens 2003; Hou et al. 2012; Zou et al. 2012; Raman et al. 2013a, 2014a) and its related species;

Table 2.1 QTL for yield and related traits identified in various *Brassica napus* populations

Mapping population	Population type	Population size[a]	Phenotyping environment	Phenotype scored	QTL identified	Reference
Westar/Ceres	128	IBL	2	FT,PH,TSW	4–9	Butruille et al. (1999)
Sallux/Gaoyou	282	DH	4	FT,MT,PH	5–12	Zhao et al. (2005)
RV128/P1804	144	DH	2	FT,PH, SY, TW, TSW	5–6	Quijada et al. (2006)
RV289/P1804	148	DH	3	FT,PH, SY, TW, TSW	7	Udall et al. (2006)
TO1141/P1804	160	DH		FT,PH, SY, SL, TW, TSW	7	Udall et al. (2006)
Quantum/No2127-17	258	DH IF2	3	HPB, PH, LMI, SD, SL, FB	30 22	Chen et al. (2007)
SI-1300/Eagle	184	$F_{2:3}$	2	PH, HPB, LMI, ELM!, SMI, SDMI, FB, SFB, SS, SP, TSW, SY	3–15	Li et al. (2007)
Express617/R53	250	DH	4	SY, SPS, TSW		Radoev et al. (2008)
Tapidor/Ningyou7	202 101 crosses	DH F_2	10	BY, FT, MT, NPB, PH, SY, SPS, SPP, TSW	23 (BY)-201 (FT)	Shi et al. (2009)
Express617/V8	250	DH	4–5	PH, SY, TSW	3–16	Basunanda et al. (2010)
Hickory/JA177	190	DH	2	TSW	9	Fan et al. (2010)
J7046/J7005	190	F_2	2	TSW	3–17	Fan et al. (2010)

(continued)

Table 2.1 (continued)

Mapping population	Population type	Population size[a]	Phenotyping environment	Phenotype scored	QTL identified	Reference
Tapidor/Ningyou7	202 101	DH F_2	3	BY, FT, MT, NPB, PH, PY, SY, SPS, SPP, TSW	1022	Shi et al. (2011)
04-1139/05-1054	221	F_2	–	SPS, SPP, TSW	3–5	Wang and Guan (2010)
HZ396/Y106	140	DH	3	SPS, SL, TSW	26	Zhang et al. (2011)
Quantum/No.2127	186	RIL	3	SL, TSW	2–8	Yang et al. (2012)
HZ396/Y106	807	DH/ near-isogenic lines	2	SPS, SL, TSW	1	Zhang et al. (2012)
F_1 derived from 9 crosses (10 parental lines)	390 (DH population 9 to 93/cross)	DH	4	FT, PH, PC, Oil content, glucosinolates, dry matter content and grain yield	1–5	Wurschum et al. (2012)
Zhongshuang11/No. 73290	184 F2	F_2, $F_{2:3}$, $F_{2:4}$	2	SW, SL	18–51	Li et al. (2014b)
Diverse lines	576	GWAS	1	SW, SL	6–8	Li et al. (2014b)
Diverse lines	472	Inbreds	2	SW	2	Li et al. (2014a)
Express/SWU07	261 233	DH F_2	3	SL, SW	20–21	Fu et al. (2015)

(continued)

Table 2.1 (continued)

Mapping population	Population type	Population size[a]	Phenotyping environment	Phenotype scored	QTL identified	Reference
Skipton/Ag-Spectrum//Skipton		DH	2	Grain yield	11	Raman et al. (2016c)
KenC-8/N53-2	348	DH	8	BY, SW, PH, BH, FBN, LMI, PMI, SY	226	Zhao et al. (2016b)
Tapidor/Ningyou7	182	DH	Up to 19	BY, BN, DT, FBN, RBH, FT, MT, PH, PY, SY, SN, SW, PN on main inflorescence, TSW	366	Luo et al. (2017b)

[a]*IBL* Inbred backcross lines. *PH* Plant height, *HPB* Height of primary branch, *LMI* Length of main inflorescence, *ELMI* Effective length of main inflorescence, *SMI* No of siliques on main inflorescence, *SDMI* Silique density on main inflorescence, *FB* No of first branches, *SFB* Number of siliques on first branch, *SP* No of siliques per plant, *SS* No of seeds per siliques, *TSW* Thousand seed weight, *YP* Yield per plant, *DH* Doubled haploid

B. rapa (Kole et al. 2001, 2002; Schranz et al. 2002; Lou et al. 2007; Zhao et al. 2010), *B. oleracea* (Kennard et al. 1994), *B. juncea* (Zou et al. 2016) and *B. carinata* (Zhang et al. 2017) mapping populations. Research has shown that flowering time is controlled by multiple loci with varying allelic effects. For example, Long et al. (2007) identified a large number of 'statistically significant' and 'micro-real' QTLs (up to 42) associated with flowering time in the DH and RC-F_2 populations derived from a cross between Tapidor and Ningyou7 that were evaluated in 14 environments. This study revealed a major QTL; *qFT10-4* on chromosome A10, explaining 50% of the phenotypic variation for flowering time in a spring-environment. This QTL corresponds to the *FLOWERING LOCUS C* (*FLC*) on chromosome A10*, BnFLC.A10* gene (Hou et al. 2012). Genetic analysis of two diversity panels; BnASSYT lines (374) and BnAHDS (*Brassica napus* Australian Homozygous Diversity set: 300) and 10 DH populations from ATR-Cobbler/BLN3363, BLN2762/Surpass400, Charlton/Monty, Skipton/Ag-Spectrum//Skipton, 06-5101, 11-5107, 11-5329, RP04/Ag-Outback, Tarcoola-22/Tarcoola-69 and R1/R2, evaluated across multiple field experiments (2015, 2016, and 2017) suggested that genetic architecture of flowering time is complex and likely to be regulated by different pathways (Raman et al. 2018a, b).

Four major pathways involved in flowering time control: the photoperiodic, the vernalization, the autonomous, and the gibberellic acid pathways have been revealed in Arabidopsis and several genes responsible for phenotypic diversity have been identified (Koornneef et al. 1991, 2004; Balasubramanian et al. 2006; Alonso-Blanco et al. 2009; Amasino 2010). Canola genome has undergone 72× multiplication since the origin of angiosperm and 6× polyploidization since diversification from Arabidopsis (Chalhoub et al. 2014). Several paralogs of Arabidopsis flowering time genes have been identified in *Brassica* species that are related with natural variation in flowering time (Lagercrantz and Lydiate 1996; Lagercrantz et al. 1996a; Tadege et al. 2001; Lin et al. 2005; Wang et al. 2009a, 2011a; Yuan et al. 2009; Zhao et al. 2010; Hou et al. 2012; Wu et al. 2012; Nelson et al. 2014, 2016). For examples, at least nine copies of *FLC* on chromosomes A02, C02, A03, C03, A10, and C09 (Fig. 2.3) and six copies of *FLOWERING LOCUS T* (*FT*); *BnA2.FT2* on chromosome A2; *BnA7.FT.a,* and *BnA7.FT.b* on chromosome A07; *BnC2.FT* on chromosome C2, *BnC6.FT.a* and *Bnc6.FT.b* on chromosome C06 (Fig. 2.4) result in functional divergence affecting flowering time between winter and spring cultivars (Wang et al. 2009a; Hou et al. 2012; Zou et al. 2012; Raman et al. 2013a, 2014a, 2016b, c). Mutation in *BnC6FTa* and *BnC6FTb* paralogs altered the flowering in *B. napus* accessions (Guo et al. 2014). Different paralogs seem to control natural variation for flowering time in different genetic backgrounds.

Despite several studies on flowering time in canola, loci associated with early flowering under contrasting water regimes have not been precisely identified yet in order to understand gene network involved in drought resistance mechanisms. A negative relationship between flowering time and grain yield has been found in canola (Raman et al. 2016a, b, c), suggesting that higher yield can be achieved in early flowering lines as compared to late ones. It remains to be tested whether this

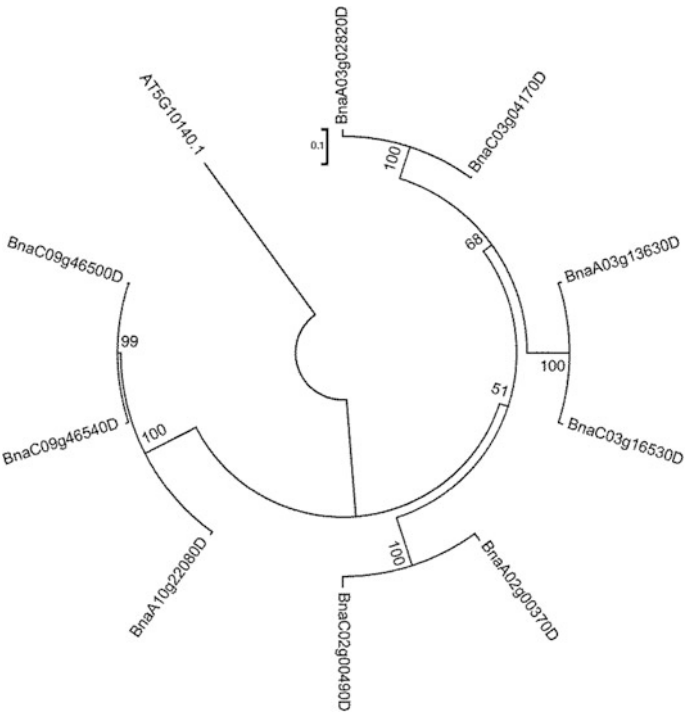

Fig. 2.3 Molecular diversity among nine paralogs of the *FLOWERING LOCUS C* (*FLC, A. thaliana, ID: AT5g10140*), in canola. *FLC* sequences were retrieved (http://www.genoscope.cns.fr/brassicanapus/) representing nine copies 'with suffix 'Bna' reported previously (Zou et al. 2012). Phylogenetic tree was constructed using the neighbor-joining method implemented in the Mega X package

correlation remains valid across different agricultural environments. In addition, flowering time has implications for a variety of other traits such as plasticity in water use efficiency; carbohydrate availability, plant vigor, resistance to diseases and yield (Ni et al. 2009; Graf et al. 2010; Lovell et al. 2013; Kenney et al. 2014; Wei et al. 2014). Pleiotropic effects of *FLC* on WUE and nitrogen content (Loudet et al. 2003), and *ELF3* on bolting date, rosette diameter and leaf number in response to shade-avoidance (Brachi et al. 2010), have also been reported in Arabidopsis.

2.7.2.3 WUE

QTLs for WUE have been mapped using $\delta^{13}C$ in Arabidopsis (McKay et al. 2008) and some crop such as barley (Teulat et al. 2002), wheat (Rebetzke et al. 2008a), rice (Takai et al. 2006), soybean (Deshmukh et al. 2014). QTL for internal CO_2 measurements in leaf (Kapanigowda et al. 2014), and stomatal conductance in maize (Lebreton et al. 1995) have also been identified. In *B. oleracea,* Hall et al. (2005)

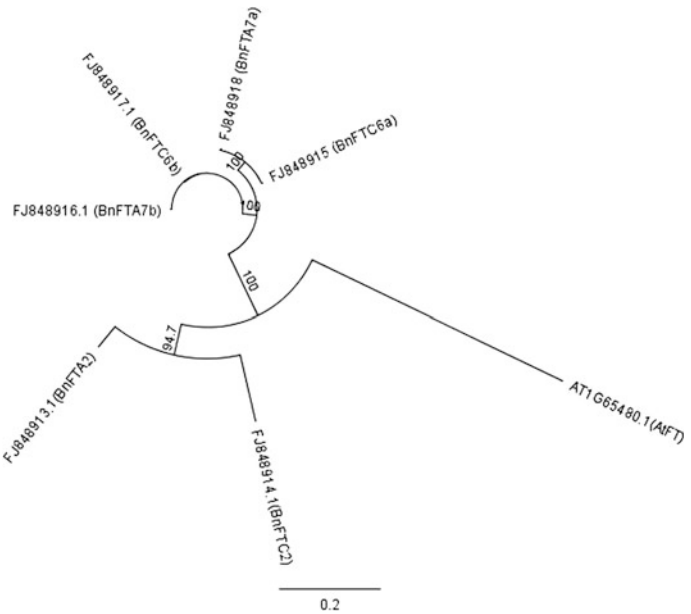

Fig. 2.4 Molecular diversity among six paralogs of the *FLOWERING LOCUS T* (*FT, A. thaliana, ID: AT1G65480*), in canola. *FT* sequences (Wang et al. 2009a, 2012) were retrieved from NCBI. Phylogenetic tree was constructed using the neighbor-joining method implemented in the Mega X package

reported a number of QTLs associated with $\delta^{13}C$, WUE, leaf conductance, photosynthetic assimilate rate, leaf thickness and leaf nitrogen. In canola, QTLs associated with $\delta^{13}C$ variation have also been identified in three Australian DH populations derived from Skipton/Ag-Spectrum, Charlton/Monty and Tarcoola-22/Tarcoola-69 (Raman et al. 2018b). The identified QTLs are currently being validated for their genetic effects across agricultural environments (Raman et al. unpublished). Fletcher et al. (2015) mapped 20 QTLs associated with drought escape (flowering time) and drought avoidance (root architectural traits such as dry mass of tap root, dry biomass of lateral roots, dry mass, tap root diameter, tap root length) and grain yield on chromosomes A03, A10, and C02 in a DH population from IMC106RR/Wichita. All the QTLs for grain yield, flowering time, and root pulling force were colocated, except on chromosome C07, suggesting that both drought escape and drought avoidance mechanism (root system size) are associated with WUE. Fletcher et al. (2016) further saturated the QTL region on A10 with molecular markers generated with the Illumina Hiseq approach and found that the *Bn.FLC.A10* gene for flowering time in Tapidor/Ningyou7 population (Hou et al. 2012), is also implicated in drought tolerance in the DH population derived from IMC106RR/Wichita, suggesting pleiotropic role of *Bn.FLC.A10*.

2.7.2.4 Drought Avoidance Traits

QTLs for drought avoidance have been identified in several crops including wheat (Quarrie et al (1994), barley (Honsdorf et al. 2014), rice (Quarrie et al. (1997), and maize (Hao et al. 2010, 2011). In canola, Zhang et al. (2015b) identified 16 loci for tolerance to water stress, imposed by PEG 600 among 140 accessions. This study further identified 79 differentially expressed genes under control and stress treatments in two canola varieties. However, the relationship between PEG-mediated stress and drought tolerance under field conditions was not reported.

2.7.2.5 Early Vigor and WSC Accumulation

Nguyen et al. (2018) mapped QTLs associated with components of early vigor; germination rate, shoot length and seedling fresh weight in a DH population of canola from Express (high vigor genotype)/1012-98 (low vigor genotype). However, no common QTL associated with these traits was identified, suggesting that early vigor is a complex trait. Raman et al. (2018a) mapped QTLs for early vigor using NDVI in a DH population derived from Skipton/Ag-Spectrum and identified nine QTLs associated with NDVI on chromosomes A06, A07, C02, C03, C04, C06, and C09. Positive correlation between NDVI and biomass accumulation, and grain yield was found, suggesting that NDVI can be used as an indicator for selection of higher grain yield in canola. In an independent study, Raman et al. (2014c) also evidenced that several QTLs are associated with variation in WSC accumulation in a DH population from Skipton/Ag-Spectrum.

2.7.2.6 Waterlogging Tolerance

Some progress on genetic mapping of QTLs for waterlogging has been made in canola. For example, Li et al. (2014c) mapped DH population from a cross between, No2127-17 (waterlogging-tolerant/275B (drought-resistant) and line Huyou15 (waterlogging-sensitive)/5900 (drought-sensitive) and identified 26 and 31 QTLs for waterlogging tolerance and drought tolerance, respectively. Some of the QTLs for waterlogging tolerance-related traits overlapped with QTLs for drought resistance-related traits, indicating that the genetic bases of waterlogging tolerance and drought resistance were related to some degree in this DH population, hinting toward converging molecular cross-talk of abiotic stress signals.

2.7.2.7 Other Traits

Genomic regions for shoot ionome, lodging resistance, plant architectural traits such as branch angle, main inflorescence length proportion, branch height, and branch segment proportion, and resistance to pod shattering, blackleg, and

Sclerotinia stem rot have been mapped in canola (Snowdon and Friedt 2004; Delourme et al. 2011; Raman et al. 2013b, 2016a; Bus et al. 2014; Larkan et al. 2016; Wang et al. 2016). Natural variation for Mn^{2+} tolerance also exists in canola germplasm (Wratten and Scott 1979; Moroni et al. 2003). Genetic analyses of segregation populations showed that a single major gene controls Mn^{2+} tolerance in canola (Moroni et al. 2003; McVittie et al. 2011). Recently, Raman et al. (2017a) mapped the locus for Mn^{2+} tolerance in a DH population from Darmor-*bzh*/Yudal on chromosomes A09/C08 and identified a suite of molecular markers based on DArTseq and SNPs. Physical mapping of linked markers revealed a candidate gene, *BnaA09g37250D* (in 'Darmor-*bzh*'assembly) which showed significant sequence similarity with a gene encoding for cation efflux protein.

2.8 Application of Markers in Canola Breeding

Breeding programs need to stay ahead to address imminent challenges posed by climate variability and global warming and develop elite varieties with quick turnover so growers have access to well-adapted crop varieties to keep up with the demand (Atlin et al. 2017). In recent years, marker-assisted selection has been successfully deployed to enrich alleles for qualitative and quantitative traits, explaining a small proportion of phenotypic variance into elite varieties and increase genetic gains in breeding programs. However, genomic selection (GS) methodologies will be more useful for selection of especially quantitative traits, having low heritability in breeding programs (Meuwissen and Goddard 2010). Recent advances in plant genomics, high-throughput genotyping platforms and next-generation sequencing technologies described above, have made possible to use predictive breeding methods in large breeding populations, comprising several thousands of individuals (Norman et al. 2018). It has also been demonstrated that even low-density (few hundred to few thousand) markers are also good enough to achieve high genomic prediction accuracies in breeding populations with strong linkage disequilibrium, and thus offer more cost-effective method for the routine application of GS (Raman et al. 2016b; Werner et al. 2018). The next-generation breeding methods offer to increase the rate of genetic gain, response to selection, reduced cost of selection and time per breeding cycle and fast-track new crop varieties with traits adapted to climate variability. Molecular markers linked with traits of interest can be used to increase selection accuracy. Speed breeding method can be deployed to increase selection intensity as well as to reduce time per breeding cycle.

GS entails the selection of breeding progenies on the basis of predicted performance (genomic estimated breeding values, GEBVs) derived from a statistical model associating genotypic and phenotypic information. These predictions are then used to envisage the performance of the candidates for selection based solely on genotype rather than on phenotype. However, larger datasets of genomic and,

phenotypic (multi-environment, multi-year) information are preferred to maximize the effectiveness of genomic prediction of the candidate populations. Although the cost of genotyping is getting cheaper/data point as technologies advance, yet cost of accurate phenotyping is high mainly due to expensive labor. In recent years, high-throughput phenotyping platforms for field trials such as phenomobile, and aerial vehicles are becoming available to phenotype a large number of plots, e.g., for canopy temperature, and NDVI/biomass (Liebisch et al. 2015; Haghighattalab et al. 2016; Rutkoski et al. 2016), however, their current use in practical breeding program is limited.

GS has been successfully applied in plant breeding programs for developing drought-resistant varieties in maize, wheat, sugarcane, chickpea, pigeon pea and groundnut (Cerrudo et al. 2018; Dias et al. 2018). GS has been used to expand the limited genetic diversity in the gene pools of the commercial breeding programs by mining the novel alleles from the landraces or crop relatives in the world gene banks (Crossa et al. 2016; Cowling et al. 2017). This approach may allow the maintenance of genetic diversity while making genetic progress in breeding programs.

Several predictive models such as ridge-regression best linear unbiased prediction (RR-BLUP), best linear unbiased prediction (G-BLUP), BayesA, BayesB, BayesC, BayesCr, Bayesian LASSO. Machine Learning methods and Random Forest (RF), have been utilized for GS in different crops. Generally, GS models are single trait models, however, multivariate models have also been implemented (Jia and Jannink 2012). GS models in crop breeding need to accommodate spatial variation in field trials, genotype by environment interactions, and nonadditive effects for better prediction (Oakey et al. 2016; Luo et al. 2017a). Selection on optimal haploid value (based on haplotype) has been proposed to develop ultimate DH line with best allele combination (Daetwyler et al. 2015).

In canola, testcross performance for a number of important agronomic traits including seedling emergence, days to flowering, lodging, oil and seed yield, seed oil content and seed glucosinolate content was predicted using RR-BLUP model in combination with 500 cross-validations for each trait (Jan et al. 2016). High prediction accuracy for seed oil content (0.81) and oil yield (0.75) was observed. Studies have shown that the knowledge of QTLs associated with traits of interest (seed yield and flowering) in genomic prediction models further improved prediction accuracy (Raman et al. 2016b; Luo et al. 2017b). Further research includes developing better prediction models, handling a large volume of genotypic data, comprising millions of SNPs being generated with resequencing approach and multiple data point captured with high-through phenotyping platforms, identification of target populations of specific environments, understanding effects due to genotype × environment × management, epistasis and pleiotropic interactions, to develop varieties with superior combination of alleles, surpassing existing yield limits, while incorporating alleles for tolerance to biotic and abiotic stresses.

2.9 Progress Toward Tailoring Climate Smart Canola

2.9.1 Map-Based Cloning and Candidate Genes

It is believed that conventional breeding has limited potential to develop high yielding crops which can produce stable grain yield under changing climate and meet the global demand. Therefore, identification of candidate genes for productivity and adaptive traits and their deployment in new varieties is necessary.

2.9.1.1 Grain Yield

Genetic analysis studies revealed thousands of QTLs associated with grain yield and yield components (see Table 2.1). Recently, two seminal studies; cloning the AUXIN-RESPONSE FACTOR 18 (*ARF18*) and SUPPRESSOR WITH MORPHOGENETIC EFFECTS ON GENITALIA7 (*BnaC9.SMG7b*) revealed the molecular bases of seeds weight and silique length, and number of seeds per silique in canola. Both studies demonstrated that these traits are governed by single major genes (Li et al. 2015a; Liu et al. 2015). The first study by Liu et al (2015) showed that *ARF18* (*BnaA.ARF18a*, BnaA09g55580D) gene controls natural variation for seed weight and silique length in canola population derived from ZY72360/R1 on chromosome A09. Transgenic lines with high expression levels of *ARF18* had low seed weight, suggesting that seed weight was regulated via auxin-response pathway. Previously, QTLs for both seed weight and silique length were mapped 1.7 Mb apart on chromosome A09 in the GWAS panel of 576 canola inbred lines (Li et al. 2014b). The second study by Li et al. (2015a) cloned a major QTL, *qSS.C9* that controls number of seeds per silique using a map-based cloning strategy and identified a gene, *BnaC9.SMG7b* on chromosome C09 that shows homology with *SMG7* of *A. thaliana*). Evidence for gene function was obtained by knocking-down of the *BnaC9.SMG7b*, following RNAi approach which significantly reduced the number of functional ovules per silique in canola. This study further performed haplotyping of 84 accessions which were sequenced covering 3.48 Kb region of *BnaC9.SMG7,* including promoter region. Results showed that the favorable *BnaC9.SMG7b* 'alleles' are prevalent in modern germplasms, suggesting that this locus has been a major selection target for canola improvement.

2.9.1.2 WUE

Several genetic loci implicated in stomatal signaling (Xie et al. 2006; Vahisalu et al. 2008; Yamamoto et al. 2016), stress response transduction pathways (Iuchi et al. 2001; Satoshi et al. 2001; Shinozaki and Yamaguchi-Shinozaki 2007; Aubert et al. 2010; Behnam et al. 2013; Yuan et al. 2014), cell wall composition (Liang et al. 2010) and WUE (Masle et al. 2005; Rossel et al. 2006, 2007) have been

identified and confirmed for their gene functions. Torii et al. (1996) identified a leucine-rich repeat receptor-like kinase (LRR-RLK) gene, *ERECTA*, which is shown to regulate TE in Arabidopsis (Masle et al. 2005). This study mapped a major QTL and further showed that *ERECTA* controls TE through coordination between transpiration and photosynthesis via regulating stomatal density, epidermal and mesophyll development and porosity of leaves. Paralogs of *ERECTA* (AT2G26330.1) have been identified in various crop plants including canola; which map on homeologous chromosomes A09 (coordinates: 28,500,117-28,511,190 bp) and C08 (coordinates: 31,680,918-31,686,937 bp) representing *B. napus* cv. Darmor-*bzh* genome assembly (http://www.genoscope.cns.fr/blat-server/cgi-bin/colza/webBlat). However, in canola, the relationship between WUE and *ERECTA* paralogs has yet to be established.

In wheat, Zheng et al. (2015) identified two homologs of *ERECTA: TaER2* and *TaER1*, and further analyzed their expression patterns in flag leaves at heading and grain-filling stages. Correlation analysis revealed that the expression of *TaER1* and *TaER2* at both growth stages was negatively associated with stomatal density, transpiration rate, and $\delta^{13}C$, while significant positive correlation was found with flag leaf area, photosynthetic rate, instant WUE, biomass production and grain yield. There were stronger correlations for *TaER1* at grain-filling stage than *TaER2* at heading stage.

Studies have shown that *ERECTA* play diverse roles in plant growth/development processes such as inflorescence development, inflorescence architecture, organ shape, epidermal stomatal patterning, ovule development, and resistance to the necrotrophic fungus, *Plectosphaerella cucumerina*, and bacterial wilt, caused by *Ralstonia solanacearum* (Godiard et al. 2003; Shpak et al. 2003; Llorente et al. 2005; van Zanten et al. 2009; Meng et al. 2012; Pillitteri and Torii 2012; Bemis et al. 2013). Recently, it was shown that overexpression of *ERECTA* gene confers heat tolerance in rice and tomato, possibly by protecting cells from heat-induced cellular damage and cell death (Shen et al. 2015). This study initially performed QTL analysis of a recombinant inbred line (RIL) population of Arabidopsis for heat tolerance and identified two QTLs: *qHat2-1* (R^2 = 30%) and *qHAt2-2* (R^2 = 23–28%). Using chromosomal segment substitution lines, a major QTL (*qHat2-1*) was fine-mapped and identified *ERECTA* was identified a candidate gene for heat tolerance in Arabidopsis.

Another success story from QTL identification to gene function relates to ABA signaling genes. ABA accumulation has been associated with various biological processes including seed dormancy, stomatal regulation, transpiration, and drought tolerance. ABC transporter genes are shown to be responsible for ABA transport and functioning of guard cells (Kang et al. 2010; Kuromori et al. 2010, 2011). The *ERA1* gene encoding β subunit of farnesyltransferase is implicated in ABA signaling that controls seed dormancy, stomatal closure, and growth inhibition (Cutler et al. 1996). Wang et al. (2005) manipulated the level of β-subunit of Arabidopsis farnesyltransferease in canola cv. DH12075 via antisense technology under a drought-inducible promoter rd29A. Transgenic plants showed enhanced ABA sensitivity as well as reduction of stomatal conductance and water transpiration under drought stress. There was no trade-off penalty to plants, as transgenic plants

had the same yield compared to control. However, transgenic plants yielded more under moderate stress than the control. Data obtained from multiple field trials in different locations suggested that conditional downregulation of the *ERA1* in canola significantly increased the yield compared to wild-type control under drought stress at the time of flowering.

In a subsequent study, Wang et al. (2009b) reported the specific downregulation of *FTA* (encoding the α-subunit shared between protein farnesyltransferase and protein geranyltransferase-I in canola using the Arabidopsis hydroxypyruvate reductase (AtHPR1), which expresses specifically in the shoot, promoter driving an RNAi construct also resulted in yield protection against drought stress in the field. Under water-limited conditions, transgenic lines had 11–20% higher yield compared to control lines, suggesting that there was no yield penalty under well-watered conditions. Other ABA signaling genes such as abscisic acid–responsive kinase gene, *ATMPK12* was also shown to affect WUE and had pleiotropic effects on guard cell size: larger stomata lead to higher stomatal conductance and lower WUE in natural populations (Des Marais et al. 2014). Overexpression of a bZIP transcription factor *GhABF2* from cotton and *BnaABF2* from canola, significantly improved drought and salt stress tolerance (Liang et al. 2016; Zhao et al. 2016a), while silencing made cotton plants more sensitive to PEG-mediated osmotic and salt stress (Liang et al. 2016).

Overexpression of *LOS5/ABA3* encoding a molybdenum cofactor, essential for activating aldehyde oxidase, which is involved in ABA biosynthesis, improved drought tolerance in cotton and maize (Yue et al. 2012; Zhang et al. 2016). Overexpression of ABC transporter, *AtABCG25* gene reduced transpiration rate, enhanced drought tolerance without affecting growth, probably resulting from maintenance of water contents over the common threshold for survival after drought stress treatment (Kuromori et al. 2016). Besides, *HARDY*, *BnMAPK1*, *OsNAC10*, and *LEA2* enhanced the drought tolerance/WUE in different crops including rice, canola, and cotton (Karaba et al. 2007; Jeong et al. 2010; Weng et al. 2014; Magwanga et al. 2018).

Yang et al. (2011) manipulated the level of GRAS protein; constitutive overexpression of *BnLAS* in Arabidopsis resulted in inhibition of growth and delayed leaf senescence and flowering time. Interestingly, transgenic plants exhibited enhanced drought tolerance and increased recovery after exposure to dehydration treatment. The stomatal density on leaves of the transgenic plants increased significantly due to the smaller cell size. However, the stomatal aperture on the leaves of the transgenic plants reduced significantly compared with wild-type plants. These results clearly show that significant improvement in drought tolerance could be made via transgenic approach.

2.9.1.3 Heat Tolerance

Rapid upregulation of heat shock genes is considered as a hallmark of high-temperature stress (Finka et al. 2011). Overexpression of several heat shock

genes including *HSP10* has been shown to improve heat tolerance in transgenic plants of Arabidopsis, tobacco, tomato, rice, soybean, and cotton (Queitsch et al. 2000; Grover et al. 2013; Burke and Chen 2015). Besides, upregulation of genes involved in osmolytes accumulation such as proline, glycine betaine, soluble sugars, and sugar alcohols, signaling and perception of abiotic stresses have also been employed to increase tolerance to high temperature. Recently, Li et al. (2015b) identified a major QTL for heat tolerance in *Oryza glaberrima*, *Thermo-tolerance 1* (*OgTT1*), which encodes an α2 subunit of the 26S proteasome involved in the degradation of ubiquitinated proteins. Overexpression of *OgTT1* was associated with enhanced thermotolerance in rice, Arabidopsis and *Festuca elata*. Qi et al. (2018) showed that the overexpression and knockdown of *CmCPL1* encoding RNAPII CTD phosphatase-like 1, increased and diminished the tolerance of chrysanthemum to heat stress, respectively.

2.9.1.4 Other Traits

In addition to cloned genes described above, other genes associated with plant architectural traits such as dwarf *BREIZH* (*Bzh*), *BnGID1*, *BnDWF1, Bna.A02.CLV2*, *Bna.A09.SLY2*, and *Bna.C07.AHK4* have either been fine-mapped or cloned in canola populations (Foisset et al. 1995; Liu et al. 2010; Li et al. 2011; Zeng et al. 2011; Cai et al. 2016). These genes are being exploited in the breeding programs to develop short statured varieties to improve harvest index. Comparative mapping has also facilitated the development of gene-specific markers for many loci such as *BnAP2* and *SUT* underlying QTLs for seed weight and harvest index (Li et al. 2007; Cai et al. 2012).

Several candidate genes for Al^{3+} tolerance in wheat, barley and sorghum have been cloned and associated with Al^{3+} tolerance (Sasaki et al. 2004; Raman et al. 2005; Sasaki et al. 2006; Wang et al. 2006; Furukawa et al. 2007; Magalhaes et al. 2007; Wang et al. 2007; Raman et al. 2008, 2010; Ryan et al. 2009, 2010). In canola, homologs of wheat aluminum malate transporter (*TaALMT1)* gene for Al^{3+} resistance have been identified but their role in conferring tolerance to aluminum is unknown (Ligaba et al. 2006). Recently, Zhang et al (2017) showed that overexpression of *BoALMT1,* an Al-induced malate transporter of cabbage (*B. oleracea*) in transgenic Arabidopsis results in enhanced Al^{3+} tolerance and increase malate secretion. Organic acids such as malic and citric acids are implicated in chelation of Al^{3+} and formation of nontoxic OA-Al complex (Kochian 1995; Ryan et al. 2009).

Transgenic approach based on RNAi has been successfully used to reduce sinapine content in canola by downregulating *BnaXSGT* (UDP-glucose:sinapic acid glucosyltransferase) and *BnaX.REF1* (sinapaldehyde dehydrogenase/coniferaldehyde dehydrogenase) genes (Hüsken et al. 2005; Mittasch et al. 2013). Overexpression of microRNA, 156/SPL gene has shown to enhance grain quality, biomass yield, starch content, forage digestibility (could be used for dual purpose canola), and improved radiation use efficiency in corn (Jiao et al. 2010; Chuck et al. 2011; Fu et al. 2012).

The developments in QTL mapping, map-based cloning, and genetic transformation technologies suggested that a single QTL/gene has the potential to improve grain yield by manipulating the gene expression involved in adaptive and productivity traits. However, some genes may have trade-off costs to other useful traits as shown with MPK4 that improve photosynthesis and grain yield but affect other useful traits such as ABA-induced stomata closure and disease resistance (Hettenhausen et al. 2012).

Climate change and canola pests/pathogens

Climatic change could alter lifecycle stages and the rates of development and distribution of pests and pathogens (Chakraborty and Newton 2011; Dixon 2012). Increase rainfall events will impact the application of chemicals and their efficacy to control insect-pests (e.g., diamondback moth and aphids) and diseases. Temperature variations could alter the severity and spread of diseases (Madden et al. 2007) such as clubroot caused by *Plasmodiphora brassicae* (Dixon 2009). Climate change could also impact the efficacy/durability of both qualitative (*R*) and quantitative resistance (QR) genes of major diseases. In canola, at least 16 *R* genes and several QR genes for resistance to resistance to *Leptosphaeria maculans*, causing blackleg resistance have been reported (Raman et al. 2013b, 2016a; Larkan et al. 2016; Kumar et al. 2018). More recently, two *R* genes; *Rlm2*, and *LepR3* for blackleg resistance have also been cloned in canola (Larkan et al. 2013, 2015). Huang et al. (2006) have shown that temperature and leaf wetness duration affect the expression of *Rlm6* gene for resistance to blackleg; this gene is shown to be ineffective in conferring resistance at elevated temperature. Badawy et al (1992) also reported that *Rlm1* mediated resistance to *L. maculans* is ineffective at 27 °C. Deployment of temperature dependent *R* genes will have consequences, especially in temperate canola growing regions. In addition to *R* genes, quantitative resistance has also been shown to be affected by high temperature in controlled environment conditions (Huang et al. 2009). Authors showed no difference between Darmor (effective quantitative resistance) and Eurol in stem canker severity at 25 °C, whereas QR was effective in Darmor at 15 °C. Therefore, efforts need to be made for developing new climate-ready varieties having durable resistance to current and novel pests.

2.9.2 Proteomics Approaches

Proteomics approaches have also been utilized to investigate the response of leaf proteome to long-term drought (28 days) in four canola cultivars (Urban et al. 2017). In the water-savers cultivars, Californium and Cadeli, proteins related to nitrogen assimilation, ATP and redox homeostasis were increased under stress, while in the water-spenders cvs. Navajo and Viking, carbohydrate/energy, photosynthesis, and stress-related and rRNA processing proteins were increased upon stress.

Mohammadi et al. (2012) used a comparative proteomics approach to reveal protein expression profiles of drought tolerant and sensitive parental lines and their F_1 hybrid. Findings indicated that H^+ATPase, plasma membrane-associated cation-binding protein, HSP90, and elongation EF-2 factor have a role in the drought tolerance of canola

2.9.3 Targeting Induced Local Lesion in Genomes (Tilling)

This approach has been used to expand the genetic base of canola by creating a new source of genetic variation. Potential of this approach has been exploited commercially in canola, where *IND* (*INDEHISCENT*) gene involved in pod shatter resistance was manipulated to reduce seed loss before and during harvesting (Laga et al. 2008). Several pod shatter resistant (PodGuard) varieties such as IH51RR and InvigorR5520P are commercially grown in Australia which are less prone to seed shattering and better suited for mechanical harvesting. Besides, useful mutants having low sinapine content—an anti-nutritional compound), early flowering and grain yield were identified in canola (Harloff et al. 2012; Guo et al. 2014; Braatz et al. 2018).

2.9.4 Genome Editing

Knowledge on the genes and their network involved in adaptive and productivity traits and the response to abiotic and stress factors provides new opportunities to exploit the gene editing technologies such as Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENS) and clustered regularly interspaced, short palindromic repeats (CRISPRs) for genetic improvement in canola. CRISPR/Cas9 relies on complementarity of the guide RNA to a specific sequence and the Cas9 endonuclease activity to generate targeted mutation, and thus desired gene function. This system has been used to edit genes involved in drought stress in wheat, rice, maize and tomato (Jinrui et al. 2017; Shi et al. 2017; Wang et al. 2017; Kim et al. 2018; Miao et al. 2018) and multiple gene copies at the same time in *B. napus and A. thaliana* (Mao et al. 2013; Yang et al. 2017). In a recent study, Yang et al. (2018) knocked out the canola homologs of *CLAVATA3* (*BnCLV3*) and their related receptor genes; *CLV1* using the CRISPR/Cas9 system. The double mutation of *BnCLV3* produced more leaves and multilocular siliques with a significantly higher number of seeds/silique and a higher seed weight than the wildtype and single mutant plants. These gene edits can be used to increase canola seed grain.

2.10 Opportunities

Conventional breeding has been extremely successful to develop superior varieties of canola, ensuring adequate supply of vegetable oil, and meal for stockfeed, for meeting demand of a growing population.

Traditional tandem truncation selection procedures are not efficient, therefore new selection procedures such as index and genomic selection need to be implemented in the breeding programs (Pesek and Baker 1969; Clark et al. 2013; Cowling et al. 2017; Gorjanc et al. 2018).

In recent years, canola hybrid varieties having wide range of herbicide resistance against Triazine, Imidazole and Roundup are becoming much more popular in recent years, therefore, robust alleles which caused heterosis, in addition to other traits of agronomic importance need to be incorporated in the breeding lines for variety development. Future efforts need to focus on traits that reduce yield gap between potential yield under non-stress, and ecological stress environments (Long et al. 2015). There is a limited genetic variation for some agronomic traits in canola germplasm such as WUE, resistance to Sclerotinia stem rot and clubroot, tolerance to waterlogging and resistance to pod shatter, which could be exploited from related species such as *B. carinata* (Raman et al. 2017b). New genomic tools such as physical maps of Brassica genomes, genome sequencing technologies, bioinformatic pipelines, and discovery of trait-marker associations especially for quantitative traits are already available and these resources will further enhance the rate of progress of breeding programs, including via breeding strategies such as 'breeding by design'. High-throughput phenotyping platforms along with new breeding methods such as, CRISPR and speed breeding (Watson et al. 2018) could be incorporated in the breeding program to accelerate product delivery to canola growers.

2.11 Conclusions

Natural variation in crops as a result of ecological, evolutionary and domestication processes provides a great wealth of germplasm for crop improvement and understanding the functional basis of allelic variation for a range of traits and genetic bottlenecks posed by human and ecological niches. Breeding programs have been selecting elite canola varieties under optimal condition (medium to high rainfall areas) for canola cultivation and not for water-limited low rainfall environments. As a result, the current breeding germplasm is likely to be biased for high-value elite alleles, the, desirable alleles suitable for hostile environments must be exploited from either diverse canola germplasm or related species. Diverse set of canola germplasm is being exploited using classical genetics, plant breeding and genomics tools to develop high yielding varieties for meeting food and energy demands. In addition, chemically induced genetic and epigenetic mutants, and natural occurring epialleles provide excellent resources to understand the gene

function as well as a new source of germplasm for canola improvement (Harloff et al. 2012; Guo et al. 2014).

Dissection of genetic loci and their functional role has provided insights on the architecture of a range of traits involved in adaptation and canola productivity. The major challenge is how to utilize this vast knowledge of QTLs and their networks, particularly QTLs with small effects, specific to a certain environment and show epistatic interaction. Enrichment of QTL alleles for quantitative traits may enlarge the gene pool for breeding. Genomic selection methodologies in conjunction with high-throughput phenotyping platforms for screening large breeding populations and speed breeding methodologies will enhance the genetic gains as demonstrated recently in canola and other crops. Irrespective of the new breeding methodologies, breeding programs have to rely on highly accurate phenotyping for marker–trait associations (QTL, GWAS) for improving prediction accuracies for genomic selection, as well as candidate genes for transgenic and gene editing technologies. No doubt, field screening of diverse germplasm for quantitative traits such as tolerance to drought and heat stress is extremely difficult and challenging due to unpredictable weather pattern across sites, different phenology as well as various nutrient stresses. However, various proxy traits such as CID for WUE, NDVI for biomass accumulation/grain yield, and thermal imaging for heat tolerance could complement the selection of elite lines. Tremendous research effort has been done in Arabidopsis and other model crops; the knowledge generated can be translated into canola for product development to meet current and future demands.

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Chapter 3
Climate-Smart Groundnuts for Achieving High Productivity and Improved Quality: Current Status, Challenges, and Opportunities

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Abstract About 90% of total groundnut is cultivated in the semi-arid tropic (SAT) regions of the world as a major oilseed and food crop and provides essential nutrients required by human diet. Climate change is the main threat to yield and quality of the produce in the SAT regions, and effects are already being seen in some temperate areas also. Rising CO_2 levels, erratic rainfall, humidity, short episodes of high temperature and salinity hamper the physiology, disease resistance, fertility and yield as well as seed nutrient levels of groundnut. To meet growing demands of the increasing population against the threats of climate change, it is necessary to develop climate-smart varieties with enhanced and stable genetic improvements. Identifying key traits affected by climate change in groundnut will

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be important for developing an appropriate strategy for developing new varieties. Fast-changing scenarios of product ecologies as a consequence of climate change need faster development and replacement of improved varieties in the farmers' fields to sustain yield and quality. Use of modern genomics technology is likely to help in improved understanding and efficient breeding for climate-smart traits such as tolerance to drought and heat, and biotic stresses such as foliar diseases, stem rot, peanut bud necrosis disease, and preharvest aflatoxin contamination. The novel promising technologies such as genomic selection and genome editing need to be tested for their potential utility in developing climate-smart groundnut varieties. System modeling may further improve the understanding and characterization of the problems of target ecologies for devising strategies to overcome the problem. The combination of conventional breeding techniques with genomics and system modeling approaches will lead to a new era of system biology assisted breeding for sustainable agricultural production to feed the ever-growing population.

Keywords Climate-smart crop · Groundnut · Biotic and abiotic stress · Genomics-assisted breeding · Genetic and association mapping · Wild relatives

3.1 Introduction

Agriculture is primarily dependent on the variables of climate and associated undesirable consequences, which impact food production across the globe. In other words, the agricultural industry is one of the most sensitive sectors to climate change (CC), because it can influence the nature and characteristics of vegetation,

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limiting agricultural productivity (Enete and Amusa 2010). Global climate change has become an important issue for the world agronomy and food security. Climate change is a consequence of "Global Warming" showing its influence worldwide, and agriculture is the most vulnerable sector to this variability and change. The effect of climate change on agronomy is more devastating than it is presumed. The surging levels of CO_2 and its effects have been addressed in many crops. Increasing variability in rainfall, rising and fluctuating environmental temperature, changes in soil texture and composition are the more intensive components of climate change that cause severe agricultural loss. Recent reports suggest climate change may benefit some plant species especially of grassy origin by augmentation of growing seasons and increased CO_2 (Zhu et al. 2018). However, the question remained unanswered: how the plants will adapt to the warmer world with various environmental calamities like drought and flood, and more intense disease severity and their sporadic spread. Modern research involves modeling which uses development, dissemination, and application of "CO_2 × Temperature × Water" datasets generated for essential crop plants.

Groundnut (*Arachis hypogaea* L.), or peanut, is one of the most important oilseed and food crop. The domesticated groundnut originated in the region of southern Bolivia and northwestern Argentina on the eastern slopes of the Andes (Simpson et al. 2001). About 95% of the total area is in the semi-arid tropics (SAT) (FAO 2017), where elevated high atmospheric temperatures in daytime often go beyond 35–38 °C. Over the past few decades, groundnut production and area under cultivation have increased significantly. Globally, groundnut is grown in more than 100 countries in an area of 27.6 Mha with a production of 43.9 million tons (FAO 2017). Groundnut has a unique and rare feature, geotropism, i.e. flowering, pollination, and hybridization happen on the aerial part followed by formation of a peg (gynophore) from the flower's pedicel. The peg, containing the growing embryo, quickly grows downward by penetrating into the soil, leading to the development of the seed, surrounded by a hard shell (subterranean pod). This feature protects seed from many unfavorable conditions specific to aerial seeds, such as wind and bird damage. Even though geotropism is beneficial, pods inside the soil are targets for several soil-borne pathogens such as *Sclerotium rolfsii* (stem rot), *Botrytis cinerea* (gray mold), *Pythium spp*. (pod rot), *Rhizoctonia solani* (damping off, seed decay, root rot), *Sclerotinia minor* and to a lesser extent *S. sclerotiorum* (sclerotinia blight) *Verticillium dahlia* (verticillium wilt), and *Aspergillus flavus* (causes preharvest aflatoxin contamination) (Thiessen and Woodward 2012).

Climate change is considered a problem for the future, nevertheless, it has already reduced groundnut productivity and quality significantly in some areas due to increased incidence of high temperature and drought stress at critical growth stages such as flowering and seed development (Akbar et al. 2017). Additionally, climate change can also cause rapid degeneration of texture and other physical properties of soil that have a direct influence on pod development. For instance, intense rainfall in Zambia has leached the soil and led to acidification, making the

soil less suitable for cultivation of groundnut (http://fsg.afre.msu.edu/climate_change/). This has severe effects on the agronomy and for resource-poor farmers who mainly grow groundnut under rainfed conditions. The groundnut area in India during 1988–89 was 8.3 million ha with a production of 9.6 million tons. The area gradually decreased to current 5.36 million ha (2017–18) while the productivity has gone up with the total production of 8.94 million tons (FAO 2017). It is expected that by 2025, climate change will reduce the groundnut yield globally by 11–25% (van Duivenbooden et al. 2002). The consequence of climate change has been observed globally, highlighting the pressing need for designing climate-smart (CS) crops which can withstand these unfavorable conditions and enhance sustainable agriculture to achieve food security.

The improved crop genetics has increased crop production and quality to meet food and nutritional security of the growing population and enabled cultivation of crops under various biotic and abiotic constraints. As in the current scenario, the development of new CS groundnut varieties in the face of climate change is one of the most suitable options for sustainable production and quality. The first requirement is to conduct studies to identify biotic and abiotic constraints associated with production and quality under climate change scenarios. Climate change is affecting the frequency and scale of abiotic stress to deteriorate ecosystem, reinforcing the increased incidence of pests infestation/diseases, thus abating global food security. Therefore, identification of CS traits for combating climate change requires a combination of both genetic and physiological studies including crop modeling. For simultaneous improvement of multiple traits, it is necessary to identify and deploy molecular markers for faster generation of CS varieties. Plant researchers have developed markers for several biotic and abiotic responses in different crop species followed by their deployment in molecular breeding including groundnut. However, the current efforts are considered to be not good enough to understand and evaluate agronomic traits under a rapidly changing environments followed by accelerated development of CS varieties. Therefore, an integrated breeding approach is required which can allow selection of multiple desirable alleles aiding gene pyramiding, as well as deployment of genomic selection (GS) breeding approaches for achieving higher genetic gains through developing climate-ready groundnut varieties. This chapter reviews the status and perspective for achieving high productivity and improved quality of groundnut under global climate change.

3.1.1 Food, Nutrition, Energy, and Environment (FNEE) Security

The food, nutrition, energy and environment (FNEE) security should be simultaneously addressed sooner than later in the changing scenario of climate change (Kole 2017). Agriculture is highly dependent upon the climate to produce the food,

fiber, feed, and oil necessary to sustain human life. Climate change may reduce the nutritional quality of crops which can lead to multiple forms of malnutrition, with countries including high rates of child undernutrition, anemia, etc. Although, in some cases, rising CO_2 levels may increase crop yield, higher levels of CO_2 can reduce nutritional quality (Kumar et al. 2017; Zhu et al. 2018). Specific studies on the impact of climate change on oil and nutritional quality needs to be conducted to know the measurable impact in groundnut.

3.1.2 *Effects of Global Warming and Climate Change on Groundnut Production*

There are numerous traits which hamper individually or together the genetic potential of crops such as abiotic stresses like heat, drought, erratic rainfall, water logging, cold, salinity, iron, and phosphorous deficiency, and biotic stresses like *Aspergillus* contamination, foliar diseases (early and late leaf spot), tomato spotted wilt virus (TSWV), bacterial wilt and stem rot (Fig. 3.1).

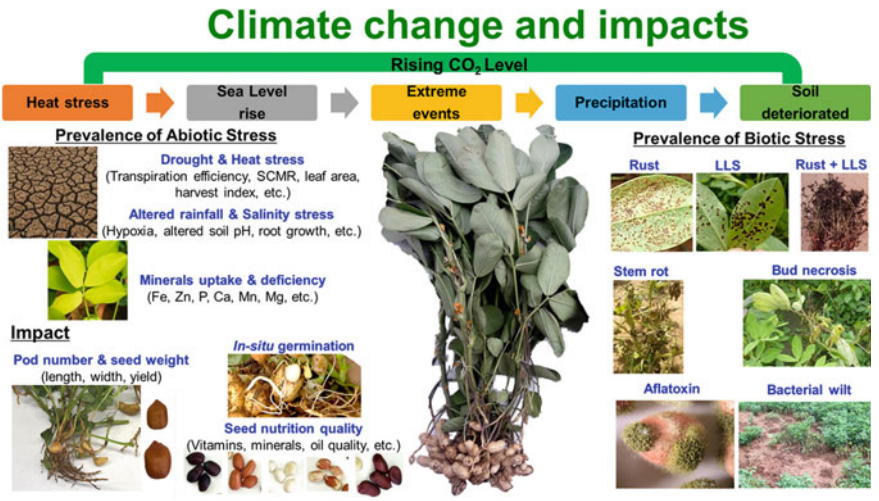

Fig. 3.1 Major effect of climate change on groundnut crop. Anthropogenic activities driven climate change limit groundnut productivity and quality due to prevalence of drought, heat and salinity stress, and altered precipitation, which has also increased the occurrence of devastating diseases

3.1.3 Impacts of Abiotic Stresses on Groundnut Growth Causing Yield Penalty

Groundnuts are often exposed to high temperature (>40 °C) for short periods especially during the reproductive stage. Additionally, with present trends of global warming, it is predicted that increase in mean air temperatures of 2–3 °C can reduce the groundnut yields by 23–36% (Hundal and Kaur 1996). The optimum temperature for groundnut is between 25 and 30 °C for growth processes and 28–33 °C for flowering and maturity (Ketring et al. 1982; Ong 1986; Williams and Boote 1995). Mean daily diurnal temperatures warmer than 30 °C are very common in the SAT, particularly in the Sahelian zone of West Africa (Sivakumar et al. 1993), and occasionally are experienced in the southern USA (Ketring 1984). Continuous exposure to elevated temperature has been reported to reduce apparent photosynthesis (Ketring et al. 1982), vegetative growth and leaf area (Ketring 1984), partitioning of dry matter between roots and shoots (Wood 1968) and between vegetative and reproductive structures (Ong 1986; Nigam et al. 1994), and water use efficiency (Craufurd et al. 1999). The reproductive stage is very sensitive to temperature; high temperature can increase flower production (Wood 1968) but reduces pollen viability and number (de Beer 1963), and fruit-set (Ketring 1984). The critical day temperature for these processes is about 36 °C (Prasad et al. 2000). Similarly, cold tolerance in groundnut has been examined (Benedict and Ketring 1972; Fu et al. 1988; Bell et al. 1991; Bhagat et al. 1992) and the temperatures below 18–20 °C reduce or delay germination leading to poor plant stand, and delayed maturity (Bhagat et al. 1992).

Several studies have been performed that examined the joint effect of elevated CO_2 coupled with heat stress; results were conflicting, but might be due to use of different methodologies (Bannayan et al. 2009; Booker et al. 2007; Burkey et al. 2007; Tu et al. 2009; Vu 2005). An increase in pCO_2 (400–700 ppm) influenced the vegetative stage through the increase of leaf area index and total biomass at harvest in growth chambers; however, seed yield declined from 24 to 53% at current temperature levels (Bannayan et al. 2009). At the highest temperature (ambient +5 °C), the effect on seed yield was genotype-specific, increasing in one case at the 700 ppm CO_2 level but was nonetheless 81% lower than current temperature levels. Another study using outdoor open top chambers determined that doubling of ambient pCO_2 could increase the photosynthetic rate by 23% but decreased stomatal conductance by 42%, and increased total biomass by 15% (Booker et al. 2007). It was demonstrated that current ozone (O_3) levels had detrimental effects on the groundnut yield, but an increased pCO_2 can reduce the effects of higher O_3 concentrations expected from air pollution (Booker et al. 2007). Another combined study demonstrated 27% yield enhancement at 710 ppm CO_2 and 46 ppb pO_3, an increase of 15% yield when pCO_2 was increased by 100% and pO_3 levels were halved, and a 60% yield increase when pCO_2 was doubled in the presence of doubled ozone; in the later case, pod yield was

still 9% lower than observed under ambient O_3 levels (Burkey et al. 2007). Further, it was demonstrated that, although elevated pCO_2 increases the net carbon assimilation rate, correlation with yield increase was proportionally less, and elevated pCO_2 did not change whole plant N content, suggesting that factors (such as N) could limit productivity (Tu et al. 2009). Examining groundnut leaf photosynthesis behavior to elevated CO_2 and temperature conditions revealed that the photosynthesis rate, and sugar and starch accumulation were significantly increased due to the increased Rubisco photosynthetic efficiency (Vu 2005). Another study found a similar response across different groundnut genotypes grown in open top chambers which showed that the rising atmospheric pCO_2 (from 350 to 600 ppm) significantly increased photosynthesis, stomatal conductance, transpiration and biomass production, counteracting the inhibitory effect of salinity (Ratnakumar et al. 2013).

Haider et al. (2015) used experimental data generated in the 2013 season as input into the simulation model CROPGROW. The model was run independently for temperature warming and rising CO_2 over the years. Results showed yield reduction (from 9 to 52%) with a temperature increase (ambient +5.44 °C). In contrast, simulation for CO_2 showed an increase in yield (+36%) with rising atmospheric pCO_2 (from 390 to 650 ppm). Based on these outputs, groundnut seed yield reduction was most likely to be accentuated by higher temperatures, while this negative effect might be counteracted by rising pCO_2. However, the model could be improved by including experimental data conducted in different locations, combining stresses and contrasting genotypes. The "Coordinated Climate-Crop Modelling Project (C3MP)" was introduced aiming to improve predictions of groundnut performance by considering the effect of pCO_2, high temperatures, and water deficit simultaneously (Ruane et al. 2014). Unlike groundnut seed productivity, the question regarding forage quality under climate change has been ignored. One of the studies addressed it and found stem digestibility was reduced under high-temperature environments, but elevated pCO_2 had no effect on the quality-related traits analysed (Newman 2003). These results indicate that as pCO_2 increases coupled with temperature, groundnut forage value might be reduced.

Among the C3 plants, leguminous species seem to benefit the most with an increase in CO_2 levels through compensatory N_2 fixation (Rogers et al. 2009). Thus, enhanced physiological performance, growth, and yield are expected at enriched CO_2 environments. However, nitrogen fixation by leguminous plants is reduced by moisture stress due to a reduction in the content of leghaemoglobin and root nodule activity. In addition, dry weight of nodules was significantly reduced and nodule formation was delayed in water deficit-stressed leguminous plants (Reddi and Reddy 1995).

In groundnut, leakage of solutes as a consequence of membrane damage is a common phenomenon observed during drought and heat stress (Guo et al. 2006). Biochemical compounds such as glycine betaine and proline accumulate whenever there is water-deficit stress. Accumulation of proline is greater in the later stages of drought stress and therefore its concentration is considered a good indicator of moisture stress (Reddi and Reddy 1995). Drought stress has a strong effect on

protective compounds like phenols, phytoalexins, and antifungal proteins. Aflatoxin contamination risk increases with increasing seed maturity, decrease in moisture content, and increasing soil temperature (Guo et al. 2008). As the seed moisture content decreases during drought, the capacity of seed to produce phytoalexins decreases resulting in *Aspergillus* invasion and aflatoxin production (Guo et al. 2008). The concentration of some of the enzymes that are induced in response to fungal attacks, such as chitinases, osmotins, peroxidases, and proteases, are also reduced during drought stress through cell membrane-mediated mechanisms (Guo et al. 2008). Drought stress mediated-fungal infection lacks host-mediated defense, exacerbates aflatoxin formation in the seeds (Guo et al. 2005). Thus, breeding for drought tolerance has been accepted as one of the strategies for developing aflatoxin-tolerant groundnut cultivars, which would minimize not only water usage but also expand groundnut production in marginal and sub-marginal soils (Holbrook et al. 2000). However, not all authors agree with the linkage between drought tolerance, and aflatoxin resistance; some claim that it is either weak or does not exist (Hamidou et al. 2014; Nigam et al. 2009). Success in this effort has been slow due to several factors such as lack of genetic resources and information on the relationship or interaction between the pathways affected due to drought and/or pathogen invasion, high environmental and genotype × environment interaction, and differences in pathways among components of aflatoxin contamination (Nigam et al. 2009). To date, few groundnut cultivars with natural pre-harvest resistance to aflatoxin production have been identified through field screening. Under drought stress, nutrient availability hinders potential yields in groundnut, for example, in phosphorus-deficient conditions (Nagamadhuri et al. 2017a, b and 2018). However, few reports on the enhanced performance of some groundnut genotypes are mentioned in Table 3.1. The N, P, and K uptake of groundnut is reduced by drought stress (Kulkarni et al. 1988).

Although climate change is sometimes considered synonymous with water-deficit stress, climate change also results in more extreme weather fluctuations, including flooding. Waterlogging is a major yield-limiting factor for groundnut in humid regions (Jackson and Drew 1984). Waterlogging significantly reduces the leaf area, leaf water potential, and chlorophyll content, which decreases photosynthesis rate and yield (Bishnoi and Krishnamoorthy 1992). Saline soil causes reductions in yield (by 10–20% depending upon the salt concentration) and hence, it is one of the important abiotic constraints to groundnut production. Large areas of saline soils are under-utilized worldwide for crop production (Oputa 1981). In the Niger Delta area of Southern Nigeria, about 30% of the soils are saline with electrical conductivities greater than 0.5 ms/cm. Screening of the groundnut germplasm for salinity tolerance has identified several tolerant groundnut genotypes (Singh et al. 2008) (Table 3.1).

Table 3.1 Climate-smart groundnut varieties/genotypes can be used as resistance sources for the improvement of various abiotic and biotic stresses

Trait(s)	Variety/Line/Genotype	References
Resistance to biotic stress responses		
Root-Knot nematode (*Meloidogyne* spp.)	COAN	Simpson and Starr (2001)
Kalahasty Malady	Tirupati-3	Mehan et al. (1993)
Peanut bud necrosis disease (PBND), stem rot	Kadiri 3, ICGS 11, ICGS 44, ICGS (FDRS) 10, ICGV 86325, DRG 17, CSMG 884	Ghewande et al. (2002)
Tomato spotted wilt virus (TSWV), early leaf spot, late leaf spot, rust	Florida 07, C-99R, Florida Runner, UF 91108, GPBD 4	Branch (1994), Culbreath et al. (1997), Gorbet and Tillman (2009), Gowda et al. (2002)
Groundnut rosette disease (GRD)	Samnut 24 (ICIAR19BT), Samnut 25 (ICGX-SM 00020/5/10), and Samnut (26ICGX-SM 00018/5/P15/P2)	Ajeigbe et al. 2015
Bacterial wilt disease	Zhonghua 42, Zhonghua 6, Tianfu 11, Zhonghua 21, Yueyou 92	Yu et al. (2011)
Low aflatoxin contamination and in vitro seed colonization (IVSC)	J-11, 55-437, ICG 7633, ICG 4749, ICG 1326, ICG 3263, ICG 9407, ICG 10094, ICG 1859, ICG 9610, Zhonghua 93–76	Nigam et al. (2009), Yu et al. (2011)
Abiotic stress tolerance		
Drought and heat tolerance	ICGV 91114, ICGV 87846, ICR 48, ICGV 00350, 55-437, GC SRV 1-3, ICGV 87281, ICGV 92121, GG-2, ICG 12991 and 12CS-116	Mayeux et al. (2003); Vindhiyavarman et al. (2014); Craufurd et al. (2003); Yadav et al. (2017); Hamidou et al. (2017)
Iron deficiency chlorosis (IDC) resistance	ICGV 86031	Pattanashetti et al. (2018)
Salinity tolerance	NRCG 6450, NRCG 7206, NRCG 2588, TMV 2 NLM, JNDS-2004-15	Singh et al. (2008)
Cold tolerance	Gangapuri, M13, ICGS 44, ICGS 76	Upadhyaya et al. (2009)
Phosphorus use efficiency	TCGS 1624, TCGS 1616, Greeshma, TCGS 1517, TCGS 1157, TCGS 1398, ICGV 00350, ICGV 05155 and ICGV 06146	Nagamadhuri et al. (2017a); (2017b); (2018)

3.1.3.1 Impacts of Biotic Stresses Leading to Yield Penalty

The groundnut crop is affected by several biotic factors including fungi, bacteria, viruses, mycoplasma, and nematodes. Major foliar fungal diseases in groundnut

include early leaf spot (ELS) (*Cercospora arachidicola*), late leaf spot (LLS) (*Cercospora personatum*), rust (*Puccinia arachidis*), web blotch (*Phoma arachidicola*), Alternaria leaf spot (*Alternaria alternate*), and leaf blight (*Cercospora canescens*). Major diseases that target seed and seedlings include *Aspergillus* crown rot or collar rot (*Aspergillus niger*), yellow mold (*A. flavus*), dipodia collar rot (*Lasiodiplodia theobromae*), and *Rhizoctonia* damping off (*R. solani*). The major diseases affecting stem, root, and pods include stem rot (*S. rolfsii*), *Sclerotinia* blight (*S. minor*), *Botrytis* blight (*B. cinerea*), *Fusarium* wilt (*Fusarium oxyporum*), charcoal rot (*Macrophomina phaseolina*), and pod rot (caused together by *Fusarium solani*, *S. rolfsii*, *B. cinerea*, *Sclerotinia* spp, and *Verticillum* spp). The major disease caused by bacteria is bacterial wilt (*Ralstonia sonancearum*) which is prominent in China, Indonesia, and Vietnam. Among diseases caused by viruses and mycoplasma are peanut mottle, peanut stripe, peanut clump, peanut bud necrosis, tomato spotted wilt, groundnut rosette (caused by two viruses namely *Groundnut rosette assistor virus* and *groundnut rosette virus,* together with a satellite RNA) and peanut stunt. Root-knot is caused by the root-knot nematode *M. arenaria, M. hapla, M. javanica,* and *M. incognita*); other nematodes, such as groundnut pod nematode (*Ditylenchus africanus*) are prevalent in limited regions of the world, such as Southern Africa (De Waele et al. 1989).

Higher levels of moisture in the air (humidity) promote the severity of rust, LLS, and many other diseases of fungal origin. Around 80% of groundnut cultivation in India is during the rainy season, during which these fungal diseases spread more rapidly. Foliar diseases affect net photosynthesis and are distributed across the humid climates and cause major yield losses, especially when the crop is infected by both the diseases. Therefore, foliar diseases including both rust and LLS reduce groundnut yield upto 40–60% (Subrahmanyam et al. 1984).

Climate change has a huge impact on many of the above-listed traits which adversely impact the performance of groundnut productivity, and oil and nutritional quality. The improved varieties need to address the prevailing issues in a given climatic condition in order to mitigate the adverse impact of climate change on groundnut.

3.1.4 Genetic and Genomic Resources for Mining Climate-Smart Genes

3.1.4.1 Genetic Diversity in *Arachis* Species

The genus *Arachis* includes 80 species which are divided into nine sections namely *Arachis, Erectoides, Heteranthae, Rhizomatosae, Extranervosae, Caulorrhizae, Procumbentes, Triseminatae, and Trierectoides* (Krapovickas and Gregory 1994;

Valls and Simpson 2005). These sections have been divided on the basis of morphology, geographic distribution, and cross-compatibility relationships. Among these nine sections, the polyploidy events occurred and evolved independently in the sections *Arachis* and *Rhizomatosae* (Krapovickas and Gregory 1994). Multiple polyploidy events are suggested from diploid species of sections *Erectoides* and *Arachis* giving rise to tetraploid species in section *Rhizomatosae* (Krapovickas and Gregory 1994; Valls and Simpson 2005). Of these sections, section *Arachis* is the biggest one consisting of 32 species including the cultivated groundnut *A. hypogaea*. In section *Arachis*, only two species (*A. hypogaea* and *A. monticola)* are tetraploid ($2n = 4x = 40$), three species (*A. decora, A. palustris, and A. praecox*) are aneuploid ($2n = 2x = 18$), while the remaining species are diploid ($2n = 2x = 20$) (Krapovickas and Gregory 1994; Valls and Simpson 2005). Further, on the basis of branching habit, the cultivated groundnut varieties have been categorized into two subspecies (*hypogaea* and *fastigiata),* six varieties (*hypogaea, fastigiata, hirsuta, vulgaris, peruviana,* and *aequatoriana)* and four common market types (Spanish, Virginia, Valencia, and runner) (Krapovickas and Gregory 1994). The primary gene pool of groundnut is very narrow and includes all the genotypes of cultivated tetraploid species, *A. hypogaea*, in addition to the closely related tetraploid species, *A. monticola*. The remaining genotypes in section *Arachis* belong to the secondary gene pool and they have very low cross compatibility. This has resulted in a huge genetic bottleneck for natural hybridization, limiting the exchange of genetic variation or diversification of the cultivated gene pool (Sharma et al. 2017). Recently, efforts have been made for developing genetically diverse tetraploid groundnut lines termed as "synthetic groundnuts" (Mallikarjuna et al. 2011). These synthetics have great potential for diversifying the primary and cultivated gene pool.

3.1.5 Genetic Resources in Arachis Species

Thousands of groundnut accessions are conserved in different countries including India (15,445 accessions), USA (9310 accessions), and China (7837 accessions). Further, Brazil (1220 accessions), USA (1200 accessions), India (477 accessions), and Argentina (472 accessions) conserve and maintain the largest collections for wild groundnut species (see Pandey et al. 2012). In order to develop manageable germplasm sets in breeding programs, core collections were developed by ICRISAT (1704 accessions), USDA (831 accessions), and China (576 accessions). This set was further reduced by ICRISAT (188 accessions), USDA-ARS (112 accessions), and China (298 accessions) and was named mini-core collections. In addition, ICRISAT has also developed a composite collection (based on phenotypic data, geographic origin, and taxonomy) and a Reference Set (ICRISAT mini-core + other diverse cultivated and wild accessions) (see Pandey et al. 2012). In addition to these germplasm resources, different breeding programs across the world have developed several biparental (recombinant inbred lines, interspecific

lines, etc.) and multiparent populations (such as multiparent advanced generation intercross and nested association mapping panels) for use in trait mapping and gene discovery (see Holbrook et al. 2013, Pandey et al. 2016). To achieve faster generation of improved varieties from the above material, precise phenotyping together with high-throughput genotyping are deployed to identify diagnostic markers for molecular breeding (Agarwal et al. 2018).

3.1.5.1 Genomic Resources in *Arachis* Species

The groundnut crop has just crossed the transition from insufficient to high abundance of genomic resources. Multiple publications are available (see Pandey et al. 2012; Varshney et al. 2013; Burow et al. 2013; Pandey et al. 2014a, b; Holbrook et al. 2016; Vishwakarma et al. 2017a) where details of these genomic resources are provided in detail. There are six major genomic resources that are decisive for groundnut breeding—(i) sequencing and development of reference genome for diploid progenitor species, i.e., *Arachis duranensis* (Bertioli et al. 2016; Chen et al. 2016b) and *A. ipaënsis* (Bertioli et al. 2016) of cultivated tetraploid groundnut; (ii) development and validation of high density genotyping array "Axiom_*Arachis*" with >58 K highly informative single nucleotide polymorphisms (SNPs) by ICRISAT and University of Georgia (Pandey et al. 2017a); (iii) development of gene expression atlas by University of Georgia (Clevenger et al. 2017) and ICRISAT (see Pandey and Varshney 2018); (iv) genome-wide simple sequence repeat (SSR) and insertions/deletion markers (Wang et al. 2018; Vishwakarma et al. 2017b); (v) trait linked diagnostic markers for use in molecular breeding (see Vishwakarma et al. 2017a) and (vi) development of training set for initiating GS breeding in groundnut (see Pandey and Varshney 2018). These resources are very crucial for conducting high-resolution trait mapping, marker development, candidate gene discovery, and molecular breeding including GS breeding in groundnut.

3.2 Classical and Traditional Breeding for Climate-Smart Crops

3.2.1 Selection of Precise Traits and Breeding Climate-Smart Groundnut Varieties

As the reproductive stage is more vulnerable for high-temperature stress, the number of fertile flowers, pegs, and pod-forming pegs are important climate-smart traits to be selected for groundnut improvement. Pod yield, 100 seed weight, pod growth rate (PGR) under heat stress traits are highly affected and can be used as selection criteria to identify heat-tolerant groundnut genotypes, as can more direct

measures such as pollen viability or acquired thermotolerance (ATT) (Burke 1994, 2001; Gomez et al. 2011). Accession ICGS 76 was identified as heat-tolerant on the basis of ATT. Partitioning of the photosynthate to the pods is one of the key processes affected by high-temperature stress in groundnut. Groundnut genotypes accumulate higher photosynthetic products under high temperatures, but only heat-tolerant genotypes can partition them to pods (Akbar et al. 2017). Groundnut genotypes ICGV 07246, ICGV 03042, ICGV 06039, ICGV 07012, ICGV 06040, ICGV 06424 and ICGV 07038 were identified as heat-tolerant lines based on stress tolerance index and pod yield performance (Akbar et al. 2017).

Transpiration (T), transpiration efficiency (TE), specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), dry weight, harvest index, canopy conductance, carbon discrimination ratio, seed weight are important measures of agronomic response to yield under water deficit (Bhagsari and Brown 1976; Hubick et al. 1986, 1988; Wright et al. 1988; Nageswara Rao and Wright 1994; Nageswara Rao et al. 1995; Songsri et al. 2009; Puangbut et al. 2009; Nautiyal et al. 2002; Ravi et al 2011). Physiological traits such as relative water content (Barrs and Weatherly 1962) and cell membrane stability (Blum and Ebercon 1981) are linked with drought and temperature tolerance. Some biochemical traits associated with yield include cell viability (Calkins and Swanson 1990), chlorophyll content (Hiscox and Israelstam 1979), superoxide dismutase (SOD) activity (Beyer and Fridovich 1987) and proline content (Bates et al. 2005). The level of proline and glycine betaine is metabolic indicators to recognize drought tolerance in crop plants (Guo et al. 2008).

3.2.2 Advantage of Genomics Assisted Breeding (GAB) Over Traditional Breeding

Despite achieving good success by conventional breeding, this process is very time-consuming and laborious, and typically takes 10–15 years or so to deliver an improved variety. An important drawback is that continuous exploitation of traditional breeding methods in similar species narrow down the generic variation among modern cultivars, making them vulnerable to biotic and abiotic stress.

Though traditional breeding has addressed improvement for both biotic and abiotic stresses, strategies of adaptation to climate change need very precise phenology and ideal resistance sources. Instead of using biparental populations which involve less allelic diversity and fewer recombination events, in the last few years, GAB has begun to utilize next-generation mapping populations such as NAM (nested association mapping) (McMullen et al. 2009) and MAGIC (multiparent advanced generation intercross) (Mackay and Powell 2007, Cavanagh et al. 2008). Now multiparent populations are being developed in groundnut at ICRISAT and elsewhere, which will be of great use in breeding and trait mapping due to the presence of higher frequency of recombination events. In this context, the

development of trait-associated markers using genetic mapping and association studies is key for deployment of GAB. High-density genotyping well supported by accurate phenotyping of genetic populations facilitate discovery of genomic regions and linked markers. GAB deploys these linked genetic markers for performing selection in the early generation of breeding material leading to fixation of multiple desirable alleles faster as compared to conventional breeding.

Most of the climate-smart traits with less heritability are difficult to quantify; here GS could be a useful approach. GS takes benefit of large population size and dense genotyping with advanced GS models. Bigger population size ($\sim$2000) increases the efficiency of GS, Kernel Hilbert space regression and Bayes B models are the most robust models for GS identified in soybean (Xavier et al. 2016). In India, necessary initiatives have been taken at ICRISAT together with National Agricultural Research System (NARS) partners for deploying GS breeding in groundnut its potential in achieving higher genetic gains.

3.2.3 *High-Throughput- and Cost-Effective Genotyping and Phenotyping Key for Faster Development of Groundnut Varieties*

Genotyping for association and linkage analysis is a very crucial step in mapping studies. The first linkage map of groundnut was developed using RFLP markers in a diploid $\times$ tetraploid cross (Kochert et al. 1996) comprising the A-genome of groundnut. The first linkage map covering all linkage groups of tetraploid groundnut was developed from a cultivated $\times$ amphidiploid cross, with 370 RFLP markers (Burow et al. 2001). The very first genetic map involving solely cultivated groundnut had 191 SSR loci (Varshney et al. 2009). Over the last decade, application of genotyping-by-sequencing approach using whole genome re-sequencing (WGR) or skim sequencing has revolutionized plant genotyping and development of high-density genetic map. Recently, Pandey et al. (2017a) developed a microarray named as "Axiom_*Arachis*" SNP array with 58,233 highly polymorphic SNPs which is now available in the public domain for high-throughput genotyping.

Phenotyping of traits for breeding objectives is important for improving the accuracy of genetic analyses. High-throughput phenotyping will be needed for measuring genetic influences contributing to quantitative phenotypic variation across developmental stages, seasons, and locations (Fahlgren et al. 2015). Rapid phenotyping platforms developed so far include infrared sensors for imaging plants, spectroradiometry, Light Detection and Ranging (LiDAR), NDVI (Normalized Difference Vegetation Index)—the ratio of red to near-infrared, hyperspectral, thermal, fluorescence, and 3D laser imaging (Lobet et al. 2017; Tardieu et al. 2017).

3.3 Groundnut Genetic Diversity for Improving CS Traits

Groundnut has rich germplasm resources, but most of the accessions are tetraploid with a narrow genetic base because of a single polyploidization event during domestication and selective breeding (Kochert et al. 1996). The low genomic diversity has been confirmed by various genotyping assays including RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeats) and re-sequencing (Kochert et al. 1996; de Carvalho Moretzsohn et al. 2004; Burow et al. 2009; Bertoli et al. 2016; Chen et al. 2016a, b; Nayak et al. 2017a).

Prolonged use of a similar resistance source (such as the use of GPBD 4 for rust and LLS resistance, or COAN for root-knot nematode resistance) for variety improvement pose risks to food security as pathogens are evolving efficiently. Therefore, designing reliable and sustainable CS varieties solely from the primary (cultivated) gene pool is risky. Now breeders have used secondary gene pool for more sustainable groundnut production. However, the major limitations during introgression of useful alleles from the secondary pool are ploidy level, cross-incompatibility, and linkage drag (resulting into the co-inheritance of undesired traits), which obstruct and slow down the improvisation of elite cultivars possessing CS traits. In order to achieve this, breeders have developed groundnut synthetics lines such as amphidiploids synthetic derived from "*A. duranesis* × *A. batizocoi*" and "*A. magna* × *A. batizoco*'" (Mallikarjuna et al. 2011) which were used to introgress foliar disease resistance into popular varieties ICGV 91114, ICGS 76, ICGV 91278, JL 24, and DH 86 (Kumari et al. 2014; Sharma et al. 2017). This approach has huge potential for the exploitation of diploid wild relatives for developing CS varieties.

3.3.1 Genetic Diversity in Wild Species

Arachis wild species are known to own alleles for numerous agronomic important traits such as resistance to fungal and virus diseases, insect pests, and abiotic stress. Considerable effort has been made to identify donor lines with biotic stress tolerance. Resistance to 25 pests and diseases conditions has been demonstrated in wild species (see Stalker and Simpson 1995). Wild *Arachis* species *A. batizocoi* and *A. duranensis* are known to be involved in conferring resistance to rust, *A. cardenasii* for LLS and *A. batizocoi, A. cardenasii,* and *A. diogoi* for root-knot nematodes (Nelson et al. 1989, 1990). *A. stenosperma* has strong resistances against rust, leafspots, and root-knot nematodes (Proite et al. 2008; Leal-Bertioli et al. 2015).

Phenotypic diversity for cold and heat tolerance among 38 wild accessions of *Arachis* was evaluated (Nautiyal et al. 2008), and *A. paraguariensis* and *A. glabrata*

were demonstrated to be cold- and heat-tolerant genotypes, respectively; whereas *A. appressipila* was identified as both heat and cold susceptible. Both earliest flowering and high SPAD chlorophyll meter reading (SCMR) traits were reported in wild accessions of *A. pusilla* and *A. villosa* (Upadhyaya et al. 2011).

Various efforts have been made to introduce alleles from wild species. The earliest consequential efforts were those of Smartt and Gregory (1967) and Stalker et al. (1979), crossing *A. cardenasii* with *A. hypogaea*, generating a triploid, which was treated with colchicine to form a hexaploid. Backcrossing this to the tetraploid and selfing had the effect of eliminating extra chromosomes, producing a series of tetraploid introgression lines (Stalker and Moss 1987). This population was the basis of the first marker map of groundnut (Halward et al. 1993), and of the CS lines which have donated resistance alleles to GPBD 4 which has been the source of interspecific resistance alleles to LLS and rust (Gowda et al. 2002). Derived breeding lines ICGV 99001 and ICGV 99004 had resistance to LLS, while ICGV 99003 and 99005 had resistance against leaf rust disease caused by *P. arachidis* (Singh et al. 2003).

A second successful introgression program was the development of the synthetic amphidiploid TxAG-6, from a three-way cross to form a sterile diploid, then doubling with colchicine to create a fertile tetraploid; this amphidiploid [*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)]^{4x} (Simpson 1991), was used in a backcrossing program and was a donor for root-knot nematode resistance to six modern cultivars so far (see Table 3.1). Resistance to ELS is also present in this population (Burow et al. 2011), as well as high oil content (Wilson et al. 2017).

Other amphidiploids have been derived from crosses *A. duranensis* × *A. batizocoi* and *A. magna* × *A. batizocoi* (Mallikarjuna et al. 2011) which were used to introgress foliar disease resistance into popular varieties ICGV 91114, ICGS 76, ICGV 91278, JL 24, and DH 86 (Kumari et al. 2014; Sharma et al. 2017). High levels of LLS and rust resistance were identified from two interspecific lines (ILs) populations raised from ICGV 91114 × ISATGR 121250 and ICGV 87846 × ISATGR 265-5 which are synthetics derived from *A. duranensis* Krapov. & W.C. Greg. × *A. ipaensis* Krapov. & W.C. Greg., and *A. kempf-mercadoi* W.C. Greg. & C.E. Simpson × *A. hoehnei* Krapov. & W.C. (Sharma et al. 2017). Phenotyping-based diversity analysis has identified several resistance lines by screening some interspecific hybrids derived from cross between *A. hypogaea* × *A. cardenasii*. Resistance to bacterial wilt has also been transferred from diploid species into the cultivated species. Therefore, exploitation of these diploid species is important to design CS varieties.

3.3.2 Genetic Diversity in Cultivated Species

A significant source of genetic variation for screening groundnut is the core, mini-core, and reference collections that have been developed by several germplasm resource centers. By screening these, sources of tolerance or resistance to traits needed for CS varieties have been identified. ICRISAT also investigated 184

accessions of the mini-core collection, and identified 18 superior accessions with high SCMR and better water use efficiency according to SLA (specific leaf area) measurements (Upadhyaya et al. 2011), which can be used in cultivar development in breeding program for drought tolerance (Upadhyaya 2005), early maturity (Upadhyaya et al. 2006), and cold tolerance (Upadhyaya et al. 2009). Likewise, resistance to several abiotic stresses have been identified, such as tomato spotted wilt (Anderson et al. 1996), ELS (Isleib et al. 1995), Sclerotinia blight (Damicone et al. 2010), reduced preharvest aflatoxin contamination (Holbrook et al. 1998), and foliar disease resistance (Dwivedi et al. 2002). Very recently, phenotype-based clustering was made for 11 quantitative characters present in groundnut germplasm including early flowering, days to maturity, etc. (Bhakal and Lal 2015).

Till now, several groundnut tetraploid accessions were screened to identify resistance and yield source (Upadhyaya et al. 2011; Janila et al. 2013). Phenotype-based diversity analysis has identified several lines which can be used as a donor for particular traits. ICRISAT also identified accessions ICG 1703, ICG 4995, ICG 10920, and ICG 13917 as resistance sources for multiple foliar diseases including ELS. ICGV 99001 and ICGV 99004 are the potential donors for LLS resistance, while ICGV 99003 and 99005 were identified to have resistance to rust (Singh et al. 2003). For aflatoxin contamination, nearly 2000 groundnut accessions were screened which identified several accessions viz. ICG 1326, ICG 3263, ICG 3336, ICG 3700, etc., with considerable resistance to pre-harvest aflatoxin production. The seven best groundnut accessions, ICG 13603, ICG 1415, ICG 14630, ICG 3584, ICG 5195, ICG 6703, and ICG 6888, over 6 years (2008–2013) consistently accumulated very low levels of aflatoxin (<4 μg kg − 1). These seven accessions could be potential sources for understanding the resistant mechanisms and can be further used in developing resistant cultivars or introgressing resistance in popular released varieties (Waliyar et al. 2016). Above finding has encouraged ICRISAT to develop a MAGIC population for achieving stable resistance against aflatoxin contamination by bringing three mechanisms together, i.e., resistance to pre-harvest aflatoxin contamination, aflatoxin production and in vitro seed colonization. Similarly, a MAGIC population and two NAM populations have also been developed which can be used for mapping and breeding for yield and oil content under drought and heat conditions. Till date, several accessions have been identified which are potential donors for multiple biotic and abiotic stresses (Table 3.1) and can be used in groundnut breeding programs in developing climate-ready crop varieties. An ethyl methanesulfonate (EMS) derived mutant TMV 2-NLM showed higher SCMR (43.56) when compared with its parent TMV 2 (24.59), which is a very popular and well-adapted variety (Hake et al. 2017).

3.4 Discovery of Marker-Trait Associations Through Genome-Wide Association Studies (GWAS) in Groundnut

During the past few years, GWAS (Genome-Wide Association Study), a novel genetic approach based on linkage disequilibrium mapping in populations of distantly related accessions has hastened association of traits with genomic regions and provided a method to screen large germplasm pools without the need for making large numbers of combinations of biparental matings. The GWAS approach has been used to identify genetic variants associated with various traits in a wide range of crops including cotton, maize, soybean, tomato (Abdurakhmonov et al. 2009; Poland et al. 2011; Lin et al. 2014; Han et al. 2016). Estimates of the extent of linkage disequilibrium were to approx 10 cM (Belamkar et al. 2011) or 15–20 cM (Pandey et al. 2014a, b). The latter performed a GWAS study using 300 accessions consisting of diverse germplasm and elite cultivars for 50 major agronomic traits of groundnut, and reported more than 500 highly significant MTAs linked with 36 traits with very high phenotypic variance (~90%). Deployment of the newly available reference genome for cultivated tetraploid groundnut will enhance the precision of MTA detection and candidate gene discovery for complex traits including domesticated traits. A GWAS using 17,338 SNPs with 11 agronomic traits related to the domestication of groundnut provided insight into the complicated genetic architecture for traits such as height of main stem, number of branches, seed size and weight, etc. (Zhang et al. 2017). More studies are required to understand the genetic control for yield and seed-related traits and identify candidate genes related to disease resistance for future breeding programs. Use of GWAS is expected to provide additional alleles for traits with more robust genotyping platforms, including the groundnut *Arachis* Axiom array chip. GWAS has created a substantial need for downstream studies including genetics, biochemistry, and other relevant studies to confirm the genotype–phenotype associations to elucidate the underlying mechanisms for complex traits including yield and stress responses.

3.5 Molecular Mapping for CS Traits Through Linkage Mapping Approaches

Diverse genetic populations have served to make linkage maps and to map agronomic and yield-related traits such as fresh seed dormancy, seed weight, leaf canopy, yield, haulm yield, transpiration rate, and water use efficiency. The genetic maps are being used for mapping resistance to diseases such as TSWV, ELS, LLS, rust, and GRD. The first genetic map in cultivated groundnut for drought tolerance was constructed by using 135 polymorphic SSRs on a RIL population derived from (TAG 24 × ICGV 86031) (Varshney et al. 2009). Evaluation of 10 important traits in the RILs led to the identification of 65 main effect QTLs with 3–35% phenotypic

Table 3.2 List of major QTLs identified for important traits in groundnut

S. N.	Traits studied	Major QTLs identified	Phenotypic variation explained (%)	References
Biotic stress resistance				
1.	Late leaf spot (LLS)	31	10.27–67.98	Sujay et al. (2012); Wang et al. (2013); Agarwal et al. (2018)
2.	Early leaf spot (ELS)	7	10.48–47.38	Agarwal et al. (2018)
3.	Leaf rust	8	10.68–82.96	Khedikar et al. (2010); Sujay et al. (2012)
4.	Resistance to *Aspergillus flavus* invasion	3	10.5–22.7	Liang et al. (2009)
5.	Aphid vector of groundnut rosette disease	4	10.05–76.1	Herselman et al. (2004)
6.	Resistance to tomato spotted wilt virus (TSWV)	17	10.64–35.8	Qin et al. (2012); Wang et al. (2013); Agarwal et al. (2018)
7.	Root-knot nematode	7	11.9–22.1	Burow et al. (2014a, b)
8.	Bacterial wilt (BW)	4	12–22	Peng et al. (2010); Zhao et al. (2016)
Abiotic stress tolerance related traits				
9.	Transpiration (T)	5	10.3–18.17	Varshney et al. (2009); Ravi et al. (2011); Gautami et al. (2012)
10.	Transpiration efficiency (TE)	4	12.3	Ravi et al. (2011); Gautami et al. (2012)
11.	Specific leaf area (SLA)	6	11.02–13.29	Varshney et al. (2009); Ravi et al. (2011)
12.	Leaf area (LA)	1	11.51	Ravi et al. (2011); Chopra et al. (2018)
13.	SPAD chlorophyll meter reading (SCMR)	12	10.6–19.53	Varshney et al. (2009); Ravi et al. (2011)
14.	Biomass	3	15.58–20.32	Ravi et al. (2011)
15.	Canopy conductance (ISC)	3	11.92–22.24	Ravi et al. (2011)
16.	Total dry matter (TDM)	1	22.39	Gautami et al. (2012)
17.	Harvest index	1	18.1	Fonceka et al. (2012)
18.	Hundred pod weight	2	15–17	Fonceka et al. (2012)
19.	Hundred seed weight	2	12.4–14.9	Fonceka et al. (2012)
20.	Haulm weight	2	13.5–17.5	Fonceka et al. (2012)

(continued)

Table 3.2 (continued)

S. N.	Traits studied	Major QTLs identified	Phenotypic variation explained (%)	References
21.	Pod number	2	9.6–12.6	Fonceka et al. (2012)
22.	Total biomass	2	11–16.6	Fonceka et al. (2012)
23.	Stress Tolerance Indices (STI)—Hundred pod weight	2	13.9–16.8	Fonceka et al. (2012)
24.	STI—Hundred seed weight	2	15.5–16.2	Fonceka et al. (2012)
25.	STI—Haulm weight	2	16.4–17.1	Fonceka et al. (2012)
26.	STI—Pod number	2	10.4–19.4	Fonceka et al. (2012)
27.	STI—Pod weight	1	12.3	Fonceka et al. (2012)
28.	STI—Seed number	1	11	Fonceka et al. (2012)
29.	STI—Seed weight	1	11.5	Fonceka et al. (2012)
30.	STI—Total biomass	2	10.8–20.1	Fonceka et al. (2012)
Agronomic and yield component traits				
31.	Shoot dry weight (ShDW)	2	14.4–22.09	Gautami et al. (2012)
32.	Haulm weight	2	10.4–33.3608	Ravi et al. (2011); Fonceka et al. (2012)
33.	Harvest index	2	11–40.1	Gautami et al. (2012); Fonceka et al. (2012)
34.	Pod mass/plant	3	13.1–18.3	Liang et al. (2009)
35.	Mature pods/plant	2	11.9–12.3	Liang et al. (2009)
36.	Hundred pod weight	2	15.1–20.6	Fonceka et al. (2012)
37.	Hundred seed weight	2	15.7–16.3	Fonceka et al. (2012); Chopra et al. 2018
38.	Pod weight	1	11.7	Fonceka et al. (2012)
39.	Shell weight	1	12.6	Fonceka et al. (2012)
40.	Seed number	1	14.5	Fonceka et al. (2012)
41.	Seed weight	1	11	Fonceka et al. (2012)
42.	Total biomass	1	13.2	Fonceka et al. (2012)
Other morphological traits				
43.	Flowering date	1	19.5	Shirasawa et al. (2012)
44.	Angle of branch	2	11.9–23.2	Shirasawa et al. (2012)
45.	Length of pod	2	20.5–28.2	Shirasawa et al. (2012); Chopra et al. 2018
46.	Width of pod	2	15.2–25.5	Shirasawa et al. (2012)
47.	Pod width	5	12.2–20.1	Fonceka et al. (2012)
48.	Seed length	1	12.5	Fonceka et al. (2012)
49.	Seed width	2	14.2–23.7	Fonceka et al. (2012)

(continued)

Table 3.2 (continued)

S. N.	Traits studied	Major QTLs identified	Phenotypic variation explained (%)	References
50.	Pod length	3	24.24–26.11	Chen et al. 2016b
51.	Pod width	1	16.14	Chen et al. 2016b
52.	Seed length	7	11.15–20.80	Chen et al. 2016b
53.	Seed width	2	12.60–14.43	Chen et al. 2016b
Seed and oil quality				
54.	Oil content	14	10.2–14.18	Selvaraj et al. (2009); Sarvamangala et al. (2011); Pandey et al. (2014a, b); Shasidhar et al. (2017); Wilson et al. (2017)
55.	Protein content	3	10.2–13.4	Liang et al. (2009); Sarvamangala et al. (2011)
56.	Carbon discrimination ratio	1	12.15	Ravi et al. (2011)
57.	Oleic acid	12	10.71–38.41	Pandey et al. (2014a, b); Shasidhar et al. (2017); Wilson et al. (2017)
58.	Linoleic acid	11	11.98–39.5	Pandey et al. (2014a, b); Shasidhar et al. (2017); Wilson et al. (2017)
59.	Oleic/linoleic (O/L) acid ratio	3	10.82–45.63	Pandey et al. (2014a, b); Shasidhar et al. (2017); Wilson et al. (2017)
60.	Palmitic acid	11	10.56–37.37	Wang et al. (2015); Shasidhar et al. (2017);; Wilson et al. (2017)
61.	Stearic acid	7	17.8–40.57	Wang et al. (2015); Shasidhar et al. (2017);; Wilson et al. (2017)
62.	Arachidic acid	6	28.32–36.93	Wang et al. (2015); Shasidhar et al. (2017);; Wilson et al. (2017)
63.	Gadoleic acid	9	11.17–26.14	Wang et al. (2015); Wilson et al. (2017)
64.	Behenic acid	4	12.37–13.56	Wang et al. (2015); Shasidhar et al. (2017);; Wilson et al. (2017)
65.	Lignoceric acid	8	10.03–12.61	Wang et al. (2015); Shasidhar et al. (2017);; Wilson et al. (2017)

variance (Ravi et al. 2011). In a separate study, several QTLs for yield-related traits were reported under water-deficit conditions (Faye et al. 2015) (Table 3.2).

Lack of fresh seed dormancy (FSD) can result in 10–50% yield loss during harvest, especially in Spanish varieties (Varman and Raveendran 1991). A mapping effort using DArT and DArT-seq markers approach identified two major QTLs associated with FSD along with a genetic map of 2423.12 cM with 1152 loci and a map density of 2.96 cM/loci using a RIL population (ICGV 00350 × ICGV 97045)

(Vishwakarma et al. 2016). Similarly, a RIL population from TMV 2 × TMV 2-NLM showed considerable variability in SCMR, indicating that the population may be used for mapping drought tolerance (Hake et al. 2017). QTL mapping in this population detected a minor QTL on AhVI flanked by two transposable element markers (AhTE0251-AhTE0249). Currently, the genetic map is being saturated by mapping newly developed AhTE markers (Gayathri et al. 2018).

For nematode resistance, using an advanced backcross-QTL approach, Burow et al. (2014a, b) identified seven QTLs in the BC_3F_1 generation of the TxAG-6 [*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)] cross. Markers to one QTL dating back to 1996 have been used for selection for resistance; identification of additional QTLs will give breeders the potential to pyramid multiple QTLs for a more durable resistance or to use other genes with different mechanisms of resistance. Earlier, sequencing information was used to identify numerous candidate genes associated with QTLs conferring resistance to root-knot nematode and rust (Bertioli et al. 2016). Detailed study of QTL hotspots for root-knot nematode resistance loci of 6.1 Mb (A02) and 7.6 Mb (A09) length suggested nearly 38 and 54 NBS-LRR genes introgressed from *A. stenosperma* and *A. cardenasii,* respectively. Fifty-one NBS-LRR genes were identified in the QTL regions conferring rust resistance to groundnut transferred through introgression of genomic segments derived from *A. cardenasii, A. magna,* and *A. stenosperma* (Leal-Bertioli et al. 2015). Similarly, three candidate genes were identified for the *Ma-1* root-knot nematode resistance gene (Clevenger et al. 2017).

For resistance to foliar diseases, a RIL population namely TAG 24 × GPBD 4 was used to identify major QTLs for both the foliar fungal diseases (Khedikar et al. 2010; Sujay et al. 2012) followed by marker development and validation. These markers were later on deployed in molecular breeding for development of improved groundnut varieties with enhanced resistance to foliar fungal diseases (Varshney et al. 2014; Kolekar et al. 2017). Next-generation sequencing has provided another method for QTL analysis—the QTL-seq approach, which combines bulked segregant analysis (BSA) with genome sequencing; this has the capability to produce detailed sequence information of the genomic loci linked to the trait, including nearby candidate genes. Pandey et al. (2017b), reported a genomic locus on chromosome A03 linked with rust and LLS resistance in groundnut by analyzing RILs developed from the TAG 24 × GPBD 4 cross. Further, detailed analysis of these loci resulted in the identification of 30 non-synonymous SNPs in affecting 25 candidate genes for rust, and 14 SNPs affecting nine candidate genes for LLS resistance (Pandey et al. 2017b). Also, another effort using a RIL population (Tifrunner × GT-C20) identified 11 QTLs for TSWV, 22 QTLs for LLS and 9 QTLs for ELS, with phenotypic variation explained (PVE) ranging from 15.6 to 47.2% (Pandey et al. 2017c). Interestingly, out of 42 QTLs, 34 QTLs identified were on the A-genome, suggesting its major role in conferring resistance towards TSWV, LLS, and ELS.

3.6 Molecular Breeding for Climate-Smart (CS) Trait

Marker-assisted backcrossing (MABC) was used successfully in developing the second root-knot nematode-resistant groundnut variety NemaTAM, released for cultivation in the USA (Simpson et al. 2003). This variety was superior to the first nematode-resistant cultivar COAN, processing higher yield due to the breaking of linkage drag between low yield and nematode-resistant (Simpson and Starr 2001). Markers have been used for developing several resistant varieties then. A QTL and differential gene expression study suggested introgression of a large chromosomal segment derived from *A. cardenasii* remains in the nematode-resistant cultivars, possessing two linked nematode resistance genes (Clevenger et al. 2017; Burow et al. 2014a, b). Previously, *FAD2* alleles have been identified which govern high oleic acid trait (Pandey et al. 2014a, b; Chu et al. 2007, 2009; López et al. 2000; Jung et al. 2000). For instance, the nematode-resistant cultivar Tifguard was used as a recurrent female parent while Georgia-02C and Florida-07 served as donor parents for the high-oleic trait to develop a variety, Tifguard High 'O/L' that has resistance to nematode and possesses high-oleic trait (Chu et al. 2011), which was released in 2017 as TifNV-High O/L (Holbrook et al. 2017). Likewise, a cultivar "Webb" has been released which has combined root-knot nematode resistance and the high-oleic trait, and also moderate resistance to Sclerotinia blight (caused by *S. minor* Jagger) (Simpson et al. 2013).

Similarly, through MABC approach, a QTL region from linkage group *Ah*XV (82.62% of phenotypic variation) for rust resistance was introgressed from cultivar GPBD 4 into three rust susceptible varieties, i.e., ICGV 91114, JL 24, and TAG 24 (Varshney et al. 2014). Several introgression lines (ILs) were developed after two–three backcrosses from all the three crosses with improved rust resistance and having significantly increased (56–96%) pod yields compared to the susceptible parents in infected environments (Varshney et al. 2014). Additionally, foliar disease-resistant breeding lines were developed using TMV 2 (foliar disease-susceptible parent) and GPBD 4 (foliar disease-resistant parent) (Kolekar et al. 2017). Among them, two homozygous backcross lines TMG-29 and TMG-46 showed enhanced resistance to LLS and rust diseases, with a score of 3.0 for both diseases, and 71.0 and 62.7% increases in pod yield per plot, respectively, over the check TMV 2. These foliar disease-resistant and high yielding lines can be released as commercial varieties or can be used as genetic resources in the groundnut improvement.

Through MABC approach, two *FAD2* mutant alleles from SunOleic 95R were transferred into the genetic backgrounds of ICGV 06110, ICGV 06142, and ICGV 06420. Eighty-two MABC and 387 MAS derived introgression lines (ILs) were developed with elevated oleic acid varying from 62 to 83%. Oleic acid increased by 0.5–1.1-folds, with concomitant reduction of linoleic acid by 0.4–1.0-fold and palmitic acid by 0.1–0.6-fold among ILs compared to recurrent parents (Janila et al. 2016; Zhang et al. 2018).

Marker-assisted selection based on GS is another approach for crop improvement. The major advantage of this approach is that the minor alleles can be captured and used in the trait selection (Meuwessin 2001). For groundnut improvement, ICRISAT has initiated GS-assisted breeding by developing a training population (TP) with 340 advanced breeding lines followed by multiseason and multilocation phenotyping for important agronomic traits. These resources will further facilitate GS-assisted breeding in groundnut.

3.7 Map-Based Cloning of Climate-Smart (CS) Genes

Understanding the mechanisms by which plants perceive and transduce the stress signals to initiate adaptive responses is essential for engineering crop plants for stress tolerance. Genetic engineering strategies rely on the transfer of one or multiple genes that are either involved in signaling and/or regulatory pathways. Bioengineering stress-signaling pathways to produce stress-tolerant crop is one of the major goals of modern agricultural research. For instance, efforts are ongoing to develop a high-resolution linkage map for fine mapping and map based cloning of *RGA121* and *Ahsw* (Liu et al. 2013). Reference genome sequences for both diploid progenitors and tetraploid cultivar are in the public domain (Bertioli et al. 2016; Chen et al. 2016a, b). As the tetraploid sequence has depended upon physical mapping, its accuracy can be expected to be greater than the diploid sequence maps, and can potentially be used in place of BAC-based physical mapping and chromosome walking. Indeed, Clevenger et al. (2017) identified three candidate genes controlling root-knot nematode resistance in groundnut (Burow et al. 1996; Garcia et al. 1996), based on substitution mapping and use of genomic sequences. These genome sequences are very crucial for such research activities; thus in coming years, groundnut research community may witness the map-based gene cloning to identify key regulatory genes.

3.8 Structural and Functional Genomic Resources for Breeding CS Traits

Functional genomics and biotechnological techniques serve as important tools for the discovery of key genes controlling CS traits through QTL-seq and transcriptome analysis for the cultivar improvement (Clevenger et al. 2017; Brasileiro et al. 2014). The sequencing of groundnut progenitor's V14167 (A-genome, A. *duranensis*) and K30076 (B genome, *A. ipaensis*) was completed by the International Peanut Genome Initiative (IPGI) (Bertioli et al. 2016). Another progenitor species of the A-genome (*A. duranensis;* PI475845) was sequenced by the Diploid Progenitor Peanut A-Genome Sequencing Consortium (Chen et al. 2016b). The genotype

V14167 was originated in Argentina while the other two genotypes PI 475845 and K30076 were originated in Bolivia.

A gene atlas was developed from 22 different types of tissue representing the developmental pattern of groundnut. Transcriptome analysis resulted into identification of 8816 putative homeologous gene pairs, 9000 alternative splicing events, and over 6000 non-coding RNAs, which is a valuable resource for groundnut research (Clevenger et al. 2016). Transcriptomes of 22 accessions (12 cultivated, 8 diploid and one tetraploid wild species, and one synthetic amphidiploid) were also obtained, providing a transcriptome atlas, giving evidence for 67,098 to 79,214 genes among tetraploid accessions, as many as 30,673 to 44,760 unique transcripts among diploid wild species (Chopra et al. 2016). From 3318 to 10,832 SNPs were identified from cultivated species transcriptome data, whereas 168,289 SNPs were found among diploid species. Further, about 50,324 protein-coding gene models were predicted using transcriptome sequences in *A. duranensis* (Chen et al. 2016b) in contrast to 39,088 identified by Chopra et al. (2016). Of the 50,324 gene models, ~90% matched entries in publically available databases. In contrary, the diploid genome sequences (Bertioli et al. 2016) suggest only 36,734 genes for *A. duranensis* and 41,840 genes for *A. ipaënsis*.

Microarrays were used to identify 62 genes up-regulated in aflatoxin resistant cultivars, and 22 putative *Aspergillus*-resistance genes constitutively expressed in resistant cultivars (Guo et al. 2011). RNA-Seq was also performed to understand the interaction between hosts and pathogens to identify differentially expressed genes (DEGs) controlling resistance to IVSC. This study provides information on a group of genes involved during host–pathogen cross-talks (Nayak et al. 2017a, b). When compared with other oilseeds and plant species, *A duranensis* showed the highest similarity to legumes with gene numbers comparable with *Medicago truncatula* (50,894), which is lower than soybean (tetraploid *Glycine max*, 56,044), but higher than other legumes. A total of 5251 putative transcription factors belonging to 57 families were identified, comprising 10.4% of the predicted *A. duranensis* genes, slightly higher than soybean, and much higher than most plant species analyzed. Certain Tfs, such as B3, E2F/DP, FAR1, GeBP, HSF, NAC, S1Fa-like, and STAT were present in high proportions in *A. duranensis.* Families such as ARR-B, CAMTA, DBB, MIKC, and NF-YA, were less frequent in *A. duranensis* when compared to other plant species (Chen et al. 2016a, b). These genomic and transcriptomic resources form the basis of functional genomics studies for developing a better understanding of target traits including climate-smart traits such as aflatoxin contamination, drought and heat tolerance, and foliar disease resistance.

3.9 Genetic Engineering for CS Traits in Groundnut

Genetic engineering could play a major role to develop crops more resilient to climate change, although consumer resistance, higher cost, and regulatory approval limit the commercialization of genetically modified (GM) crops that are primarily part of human diet. Currently, most of the GM crops commercially available have added traits that protect plants from pests or make them resistant to herbicides. Establishment of a high-efficiency in vitro regeneration system is a prerequisite for genetic engineering for groundnut improvement. Several studies reported the establishment of groundnut regeneration systems by using different explants and growth medium compositions (Brar et al. 1994; Cheng et al. 1997; Yang et al. 1998; Radhakrishnan et al. 2000a, b; 2001; 2002, Magbanua et al. 2000; Sharma and Anjaiah 2000; Matand and Prakash 2007; Shan et al. 2009).

Recently, coat protein-mediated resistance to PBND has been demonstrated in transgenic groundnut (Mehta et al. 2013). Genetic transformation of the *mtlD* gene into groundnut variety GG−20 provided tolerance against drought (Bhauso et al. 2014) and salinity stress (Patel et al. 2016). This transgenic event imparted drought stress tolerance even at maturity stages and improved groundnut yield through a complex molecular mechanism involving a cascade of stress signaling and growth-regulating genes. Additionally, tolerance was also achieved in drought susceptible groundnut variety JL24 by overexpressing the gene *DREB1A* (Bhatnagar-Mathur et al. 2007). Similarly, overexpression of a gene *hydrogen pyrophosphorylase* confers higher root and shoot weights, altered transpiration and photosynthesis rates, and yield under water deficit (Qin et al. 2013). Plants transformed with gene *isopentenyltransferase*, enzyme of the cytokinin biosynthetic pathway, had higher photosynthetic and transpiration rates in the growth chamber, and higher yields under water deficit (Qin et al. 2011).

Aflatoxin contamination in groundnuts poses major challenges for vulnerable populations globally. Groundnut achieved a high level of aflatoxin resistance by overexpressing antifungal plant defensins genes—*MsDef1* and *MtDef4.2*, and through host-induced gene silencing of aflatoxin biosynthetic genes *aflM* and *aflP* (Sharma et al. 2018). Major groundnut seed storage proteins are allergens, for example, Ara h 1 and Ara h 2, which can cause serious allergic reactions in a small percentage of the human population including 2–3% of children in the US (Sicherer and Sampson et al. 2014). Although so far no report is available regarding the adverse impact of climate change on allergen proteins, such variation in protein profile cannot be ignored, thus require further investigation.

In groundnut, an increased insect tolerance in transgenic plants was observed by transferring cowpea trypsin inhibitor gene into Luhua-11 and Fenghua-2 (Xu et al. 2003; Zhuang et al. 2003), two widely used groundnut cultivars (Liu et al. 2005). Through the RNA interference (RNAi) approach, a *FAD2* gene was silenced to reduce the content of linoleic acid and increase the oleic acid content and stability of groundnut oil (Zhang et al. 2007; Huang et al. 2008). Seeds from the transgenic plants showed an increased O/L ratio (Huang et al. 2008).

3.10 Genome Editing: A Promising Approach for Developing CS Varieties Much Faster

The rate of crop improvement for CS traits must increase to ensure global food security for the growing population and to address rapidly changing climatic conditions. Genome editing can generate a targeted allelic series of trait associated genes and regulatory elements, creating a set of variable phenotypes for breeding within a single generation. Disrupting genic and regulatory regions is particularly effective for engineering quantitative traits. While qualitative traits can be more difficult to engineer using disruption, precise base editing may allow an efficient path to improving qualitative traits if protein function can be accurately modeled. Genome editing provides novel opportunities to improve crop productivity by modifying existing alleles that control important traits such as stress responses and nutrient assimilation and nutrient-use efficiency (Gaj et al. 2013; Kamthan et al. 2016). In the past years, the CRISPR/Cas9 system has been shown to be effective in a wide range of crop species, including maize, orange, potato, rice, sorghum, tobacco, tomato, and wheat (Bortesi and Fischer 2015). In rice and tomato, CRISPR/Cas9 can introduce homozygous mutations in the first generation of transformants (Shen et al. 2014; Zhang et al. 2014), potentially accelerating crop improvement.

In groundnut, there are no reports of implementing genome editing to improve groundnut against climate change. Genome editing has the potential to succeed where GM approaches have failed. Genome editing has been used successfully by numerous small companies, and costs lower than that of GM approach (Nickel 2018). Additionally, regulatory agencies such as the USDA in the US have considered that most genome-edited traits do not need special regulatory approval, giving the potential for far lower regulatory costs. However, its acceptance in Europe is restricted as they were classified under similar regulatory directive 2001, a law imposing hurdles to develop and accept GM crops (Callaway 2018). It remains to be seen what obstacles may exist in the developing countries.

3.11 Summary

Climate change has been occurring noticeably for decades in certain regions, such as the Sahel, and will certainly pose serious challenges for groundnut productivity, quality, and nutritional value. In this context, it is necessary to equip high-yielding groundnut varieties with improved climate-smart traits using an integrated breeding approach, i.e., using all the updated knowledge and modern technologies including physiological evaluation and genomics. Further, faster development of CS varieties in groundnut and their replacement of older varieties in farmers' field will be key to maintaining area under groundnut cultivation across the globe, and maintaining or expanding yields per area. The crop is going to face an immense challenge in

meeting production needs in the farmers' field under climate change conditions in coming years, and this problem will be perplexed by population growth greater than expected previously especially in the developing countries. However, advance technologies are going to help to a great extent in making a selection of breeding lines and breeding improved varieties in less time. Also developing, testing and deploying the technologies which are in pipeline will further strengthen our effort towards adapting to climate change in the coming years.

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Competing financial interests

The author(s) declare no competing financial interests.

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Chapter 4
Sunflower and Climate Change: Possibilities of Adaptation Through Breeding and Genomic Selection

Dragana Miladinović, Nada Hladni, Aleksandra Radanović, Siniša Jocić and Sandra Cvejić

Abstract Due to its ability to grow in different agroecological conditions and its moderate drought tolerance, sunflower may become the oil crop of preference in the future, especially in the light of global environmental changes. In the field conditions, sunflower crop is often simultaneously challenged by different biotic and abiotic stresses, and understanding the shared mechanisms contributing to two or more stresses occurring individually or simultaneously is important to improve crop productivity under foreseeable complex stress situations. Exploitation of the available plant genetic resources in combination with the use of modern molecular tools for genome-wide association studies (GWAS) and application of genomic selection (GS) could lead to considerable improvements in sunflower, especially with regard to different stresses and better adaptation to the climate change. In this chapter we present a review of climate-smart (CS) traits and respective genetic resources and tools for their introduction into the cultivated sunflower, thus making it the oil crop resilient to the extreme climatic conditions and well-known and emerging pests and diseases.

Keywords Helianthus annuus · Climate change · Stress resistance · Breeding · MAS · Genomic selection · New techniques

4.1 Introduction

Due to its ability to grow in different agroecological conditions and its moderate drought tolerance, sunflower may become the oil crop of preference in the future, especially in the light of global environmental changes. Even though simulations showed an increase of sunflower yield for northern parts of Europe in view of the predicted climate changes, the negative effects on sunflower yield may occur in southern latitudes (Debaeke et al. 2017). The future of sunflower is probably related

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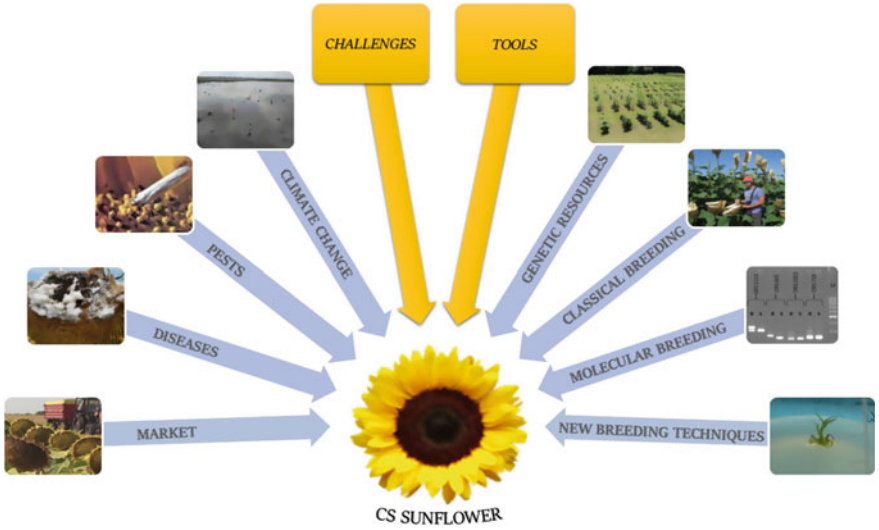

Fig. 4.1 Breeding for climate-smart (CS) sunflower—challenges and tools

to its potential adaptation to climate change (García-Vila et al. 2012). Consequently, more attention should be paid to breeding for better adaptation with regard to climate changes. These traits should include not only improvement in drought tolerance, but also the introduction of pest resistance, salt tolerance and changes in plant architecture for better adaptation (Dimitrijević and Horn 2018).

During the past 15 years, the sunflower yield increase through genetic advance has been slower than before, suggesting that the current resources and breeding methods might not bring suitable solutions in a context of climate change (Vear 2016). However, the sunflower crop stands a very good chance of surviving a changing environment with its broad genetic base and the emerging technologies that will allow the mining of traits and genes from the vast and relatively unexploited wild crop relatives' gene pool. That is why sunflower crop has been proposed as a potential model crop for adaptation to a changing environment (Seiler 2018). In the future, it is expected that integrative approaches combining omic technologies (genomics, transcriptomics, proteomics, metabolomics, and phenomics) using bioinformatic tools will facilitate the identification of target genes and markers for complex traits and facilitate sunflower adaptation to the changing environment (Fig. 4.1).

In this chapter, we present a review of CS traits and respective genetic resources and tools for their introduction into the cultivated sunflower, thus making it the oil crop resilient to the extreme climatic condition and old and emerging pests and disease.

4.2 Prioritizing Climate-Smart (CS) Traits

4.2.1 Flowering Time and Length of Vegetation

Crop adaptation to regional climates is often highly dependent on flowering time and its sensitivity to environmental signals (Henry and Nevo 2014). Flowering time is a quantitative trait, which is also a critical agronomic trait. Its evolution was crucial for the domestication and spread of many crop species into new climatic regions (Colledge and Conolly 2007; Fuller 2007; Izawa 2007). The seasonal timing of flowering is a critical determinant of plant fitness and crop yield, and genetic variation of photoperiod response in the wild relatives of crop plants, sunflower included, may provide an important resource for tailoring crops to thrive in local or future climates (Henry and Nevo 2014).

In Europe, sunflower germinates in spring and flowers in the mid-summer to mid-autumn depending on the genotype. Both wild and cultivated forms, exhibit a surfeit of diversity in flowering time and its regulation by environmental factors. How this variation evolved over complex history of sunflower early domestication and very recent improvement efforts is a complicated story in terms of both selection and mechanism (Blackman 2013). Photoperiod and temperature are the primary environmental influences regulating the transition to flowering (Goyne et al. 1989). Broader surveys of wild populations and cultivated accessions now clearly demonstrate that all three major classes of photoperiod response—day neutrality, short-day response, and long-day response are observed in *Helianthus annuus* (Wien 2008; Blackman et al. 2011).

Genetic and environmental controls of flowering in sunflower are certainly complex and mostly undefined. The flowering phenotype in sunflower has been assessed as a period of flowering in days and is determined by days from sowing to flowering (DSF or STF) or days from emergence to flowering (DTF) in most genetic studies. DSF or DTF is an important trait because cultivars with certain ranges of growth cycle length provide optimum yield in specific environments. Photoperiod and temperature have major effects on STF/DTF and could be important sources of genotype × environment interaction (Leon et al. 2001). Polygenic inheritance patterns have been reported for DSF in most studies (Stoenescu 1974; Machacek 1979; Leon et al. 2000), although there is evidence of genetic factors with major qualitative effects (Jan 1986). Additive gene action has the greatest influence on flowering (Miller et al. 1980; Alvarez et al. 1992), but dominant effects have also been noted (Jan 1986). Understanding genetic factors influencing DSF could improve the breeding method and ability to investigate and manipulate other traits in selection programs.

In sunflower breeding programs, plant height at flowering, flowering date, and humidity percent of seed at harvest are characters regularly measured, mostly to avoid very tall or very late material and to have a good knowledge of an inbred line or a hybrid (Vear 2016). The date of flowering is important in determining the period when the plant is most susceptible to drought and to *Sclerotinia* capitulum

attack. Earliness is also an important trait and determines the date at which sunflowers can be harvested (Kaya 2016). Early-maturing hybrids have the shortest possible period from butonization to flowering. They are convenient for cultivation in northern regions, but also suitable for drought-affected regions. However, earlier sunflower hybrids generally have a lower leaf area index, total evapotranspiration, as well as yield potential than later hybrids (Fick and Miller 1997).

Developing earlier or very early hybrids or arranging sowing depending on the starting of dry period in summer season could also be used as a strategy for avoiding drought stress. Within a hybrid, differences between sowing dates affect mostly the duration of the vegetative stage. Late sowing dates shorten the vegetative stage, whereas the inverse response is found in the early sowings. Changing sunflower cultivars to take advantage of the opportunities offered by climate change or to minimize the vulnerability to extreme conditions could be an additional strategy that should be combined consistently with the change of planting date (Debaeke et al. 2017).

Sunflower response to photoperiod is less known, largely because it behaves as a short-day plant in one stage and as a long-day in another (Hall 2001). Furthermore, there are also some photoperiod-insensitive genotypes (Sadras et al. 2000). By use of association mapping and linkage mapping, Cadic et al. (2013) identified quantitative trait loci (QTLs) and/or causative mutations involved in the control of flowering time in the cultivated sunflower and identified eight regions associated with flowering time that were in common between both approaches.

4.2.2 Root Characters

For drought tolerance selection in breeding programs, genotypes need to be developed with such attributes as reduced transpiration or increased water uptake from the soil using powerful roots (Kaya 2016). Although sunflower is a deep-rooted crop, water stress is one the most reducing factors of sunflower seed yield because sunflower is generally cultivated in drylands and rainfed regions (Pekcan et al. 2016).

Development of more productive and drought-tolerant lines in breeding programs requires the knowledge of root traits and functions and their effect on sunflower productivity, as depth-efficient roots for more water uptake are one of the indicators of physiological drought tolerance (Comas et al. 2013; Kaya 2016). Little is known about morphological and physiological root parameters of sunflower, since it is rather complicated to study root traits, for it is extremely difficult or sometimes impossible to extract the complete roots of field-grown plants (Nagarathna et al. 2012). Root traits such as root length and diameter, root length density, root volume, fresh and dry root weight, and also total dry matter are significant indicator of sunflower root drought tolerance (Rauf 2008; Nagarathna et al. 2012; Comas et al. 2013). Since plant root is the most important organ to obtain moisture and nutrients from deep layers of soil, sunflower genotypes with

longer roots exhibit higher level of tolerance to drought stress condition (Angadi and Entz 2002). The number of roots plays a critical role for efficient water uptake for higher crop yield under drought stress in particular soils. Limited water availability results in decreased leaf growth and leads to decreased relative dry matter portioning into the root and shoot/root ratio (Rauf and Sadaqat 2008).

In sunflower, most of the root traits are controlled by multiple genes, often with a degree of epistasis or interaction effects that can change with environmental conditions (de Dorlodot et al. 2007; Cooper et al. 2009). Comas et al. (2013) found that root length and root to shoot ratio have higher contribution of additive gene action under drought stress when compared with no-stress regimen while traits like shoot length and root weight showed decreased contribution of additive genes under drought. Root-shoot communication in drying soil is mediated by the stress hormones, abscisic acid, and cytokinins (Robertson et al. 1985; Abida et al. 1994; Shashidhar et al. 1996).

The interspecific hybridization method gives the breeders the opportunities to introduce desirable genes from wild sunflower species into cultivated sunflower. Several data are available on morphological and physiological root parameters of sunflower wild relatives under abiotic stress. *Helianthus petiolaris* had an increased percentage of deep roots in response to water deficits (Sobrado and Turner 1986). A large range of genetic variation for root morphology has been described by Seiler (1994) among annual *Helianthus* species, including the cultivated sunflower.

4.2.3 Heat Tolerance

Selection for heat resistance is an integral part of many breeding programs and cultivation of sunflowers in conditions of high temperature caused by climate change can be achieved by avoiding adverse conditions or by breeding varieties and species with increased resistance to heat shocks.

The mean global temperature increased by 0.6 °C in the twentieth century (Folland et al. 2001) and is expected to increase between 1.7 and 4.9 °C by 2100 (Cubasch et al. 2001). Changes in the global temperature would cause significant changes in the crop production and the yield of annual crops (Alexandrov and Hoogenboom 2000). In sunflower breeding, it is very important to know the effect of high temperatures on sunflower plant, in order to be able to determine the right breeding methods, targets, and selection criteria and to choose suitable breeding material for selection for heat resistance (Škorić 2009). Heat stress often negatively impacts fertilization, seed number per head, rate and duration of seed and embryo growth, seed weight and seed oil characteristics (Chiementi et al. 2002; Prasad and Staggenborg 2008). Higher air temperature can negatively affect sunflower growth by inducing shorter developmental stages. The increased temperatures to be expected from the ongoing climate change will also probably lead to early senescence and diminish oxidative protection in sunflower primary leaves (De la Haba et al. 2014). Temperatures above 27 °C reduce nectar production, while those

above 33 °C completely stop nectar production (Terzić et al. 2017). Sunflower demonstrates high adaptability to higher temperatures by increasing transpiration, which keeps the leaves relatively cool. However, the transpiration rate can only be increased if enough water is supplied by a deep and well-developed root system. Therefore, such root type, tolerance to intensive transpiration, increased capacity of a plant to produce more pollen, high seed filling rate, and rapid synthesis of oil under hot conditions are important criteria for selecting sunflower tolerant to high temperatures (Seiler 2012; Škorić 2012).

Sunflower breeders should also choose a strategy, parameters, and germplasm suitable for selecting genotypes resistant to high temperatures, as well as air and soil drought in their breeding programs, to develop tolerant lines and hybrids (Fick and Miller 1997; Rauf 2008; Škorić 2009; Škorić 2012). Shorter crop duration with increasing temperature could be compensated for by using long-cycle cultivars combined with early sowing dates (Debaeke et al. 2017). Regarding germplasm, *Helianthus argophyllus* is a useful source of heat tolerance traits. It has silver leaves that reflect sunbeams and reduce transpiration (Tavoljansky et al. 2004; Warburton et al. 2017). Seiler (2012) indicated that *H. anomalus* Blake, *H. deserticola*, *H. nuttallii,* and *H. petiolaris* could be used as germplasm for heat stress studies, as well.

4.2.4 Cold Tolerance

Škorić (2009) determined that each plant species, more particularly each genotype, has an optimum range of temperatures for its normal growth and development. These specific temperatures depend not only on the genotype but also on the stage of growth and development of a given genotype. When temperature moves beyond this optimal range, it generates temperature stress, i.e., temperature interferes with plant performance. Temperature stresses may be grouped into the following two categories: chilling stress and freezing stress (Singh 2000).

Sunflowers mainly encounter cold temperatures at early stages of development such as germination, emergence, and the 2–3 leaf stage, especially in early plantings and during the maturation period of sunflower production at higher altitudes. That is why it is important to increase its resistance to cold in the early stages of growth and development, i.e., at germination, emergence and the stage of 2–3 leaf pairs, so as to enable successful early sowing (Škorić 2009). Chlorophyll content and specific leaf area have been genetically associated with cold tolerance, so they could be used as the selection criteria in sunflower breeding programs (Fernández-Martínez et al. 2009; Sala et al. 2012; Škorić 2012).

Conventional breeding methods have met with limited success in improving the cold tolerance of important crop plants involving interspecific or intergeneric hybridization (Sanghera et al. 2011). Early sowing date in early spring, which is characterized by a low and fluctuating temperature regimen, has increased the importance of earliness for low-temperature tolerance in sunflower. The ability of

sunflower plants to gain frost tolerance after exposure to a period of low temperature is still poorly understood (Hewezi et al. 2006). The lowest soil temperature for germination and plant growth of sunflower is about 6 °C, but seedlings at the cotyledon stage can resist slight frosts, whereas older plants may lose leaves or become branched because of partial destruction of the terminal bud (Vear 2010). In Brazil, for instance, sunflower can be planted as the first crop at the beginning of the rainy season (winter–spring), due to its tolerance to low temperatures in the early stages of growth (Castro and Leite 2018).

Sources of cold resistance should be sought exclusively in the wild *Helianthus* species that are found growing wild in the mountains where winters are harsh and springs are cold. Tetreault et al. (2016) examined variation in cold acclimation capacity and freezing tolerance among three natural populations (Texas, Kansas, and Manitoba) of the perennial sunflower species. Freezing tolerance was the highest in plants from the northernmost latitude under both non-cold-acclimated and cold-acclimated experimental conditions. The plants from all populations retained the ability to increase freezing tolerance through the process of cold acclimation.

4.2.5 Drought Tolerance

One of the major limitations for agricultural productivity around the world is water stress, particularly in warm, arid, and semiarid regions. Drought is the most restraining factor in sunflower production worldwide, severely reducing yield, oil volume, oil quality, and other important yield traits (Hladni et al. 2018). Therefore, it is important to identify the physiological, molecular and genetic components of sunflower hybrids resilience to environmental variation, with a special focus on water stress in the context of climate change (Debaeke et al. 2017).

Breeding for resistance to drought and high temperatures is an important objective in many sunflower breeding programs (Hladni 2016). Cultivated sunflower has narrow genetic base and is deficient in drought-survival mechanisms which were lost during the process of selecting plants for high yields. However, being a crop with medium water requirements (Ky < 1), sunflower has the ability to tolerate a short period of drought. Measuring photosynthetic performance index and leaf temperature in early phases of development can be used in breeding programs to develop sunflower hybrids better adapted to water stress (Kulundžić et al. 2016). Stay-green sunflower hybrids have such attributes as higher rate and quantum yield of photosynthesis in leaves under drought conditions and increased the content of total soluble proteins. In order to develop drought-tolerant lines and hybrids in sunflower breeding, breeders need to be aware of the relationship between drought resistance traits and yield and apply effective screening methods for these traits. Genotypes should have such advantages as enhanced leaf area, earliness, and earlier stomatal closure. Drought tolerance has been considered as a valid breeding target to partially compensate for the loss in yield. However, this has yet to be exploited

(Vear 2010). Onemli and Gucer (2010) showed that the number of leaves and root weight were the best selection criteria to determine drought resistance at early vegetative stage. There are numerous strategies utilized in breeding for drought stress in sunflower, such as the induction of earliness for drought escape, or by moving the sowing date (early or late sowing), modification of certain plant traits that leads toward drought resistance, and introduction of drought-tolerant traits associated with high yield (Onemli and Gucer 2010; Škorić 2012).

Wild relatives of cultivated sunflower such as *H. anomalus* and *H. deserticola*, native to drought-prone environments, are potential sources of drought resistant traits for improving sunflower crop productivity under water-limiting conditions (Seiler and Marek 2016). Up to now, *H. argophyllus* was the most used species for drought resistance breeding of cultivated sunflower, it is quite easy to be crossed with cultivated sunflower. When choosing wild species, it is important to use the ones that inhabit desert areas, like *H. deserticola* whose name demonstrates the kind of conditions it can withstand (Hladni and Miklič 2012; Vear 2016; Seiler and Marek 2016). Traits connected with drought resistance include fast growth and flowering rate, lower plant height in the maturity, increased photosynthesis activity, and stoma conductivity. *H. deserticola* shows both increased photosynthesis activity and stoma conductivity so it is an excellent candidate for the adaptation to desert environments. *Helianthus anomalus* has frequently been recognized as drought tolerant (Seiler 2007) and was identified as a target species, particularly for abiotic stress tolerance and adaptation to extreme soil properties (Kantar et al. 2015). It also appears to be more tolerant to nutrient stress than its ancestral parents, based on its slower relative growth rate and higher nutrient-use efficiency (Brouillette and Donovan 2011). Sunflower wild relatives also allow for detailed study of the physiological processes involved in the survival mechanisms of desert-inhabiting species, such as Bowsher et al. (2016) for desert-adapted *Helianthus niveus* (Benth.) Brandegee ssp. *Tephrodes*.

4.2.6 Flooding and Submergence Tolerance

Plants require water for growth and development, but excessive water can negatively affect their productivity and viability (Tamang and Fukao 2015). Global warming is seen as one of the causes of an increase in floods as well as their unexpected occurrences, regimes, and localizations (El-Khoury et al. 2014). Waterlogging affects 10% of the global land area and it is one of the most important constraints imposed on agricultural crop production (Patel et al. 2015). Duration of flooding is a major factor in determining plant survival following oxygen deprivation (Lenssen et al. 2004; Colmer and Voesenek 2009). The intensity of the flood stress depends on the flooding duration, crop variety, growth stage, soil type, fertility levels, pathogens, and flooding conditions (Sullivan et al. 2001). Future experiments assessing waterlogging and submergence responses of plants should include the combination of different flooding regimes. This would contribute to a

better understanding of the costs and benefits related to particular combinations of traits conferring tolerance in variable flooding scenarios. Thus, a better comprehension of plant functioning underwater excess, in a context that indicates a higher flooding occurrence in the years to come, would be helpful for breeding programs (Bailey-Serres and Voesenek 2008).

Sunflower is a flood sensitive species; therefore, floods have a major impact on the reduction of sunflower production in the world (Else et al. 2001; Islam and McDonald 2004). When the original root system is flooded, adventitious roots develop to prevent injury to the shoot and contribute to plant survival during flooding (Kramer 1951). Aerenchyma formation facilitates internal gas-phase transport. This mechanism is essential for sunflower survival in waterlogged or flooded environments (Allen 1997). Hypoxic flooding of sunflower roots leads to rapid ethanol synthesis in the roots (i.e., anaerobic respiration), while it is metabolized by alcohol dehydrogenase (ADH) in both the shoots and the roots to avoid its accumulation (Jayasekera et al. 1989). In Japan, sunflower cultivation in rotation with rice in the paddy field is very important to study the effects of excess water on sunflower plants growth, seed yield, and oil quality. Higher soil moisture conditions tended to decrease the growth, yield, oil content, and the oleic acid content. Waterlogging during the period between flowering and maturity decreases the oleic acid content in sunflower (Yasumoto et al. 2011).

Although it has been observed that flooding affects the growth of sunflower, the response of the plant to high water is still unknown, and further research is needed to identify the physiological mechanisms responsible for the responses of sunflower to excess soil moisture (Yasumoto et al. 2011). Population genetics, bioinformatics, and reverse genetics will help to identify genes or quantitative trait loci (QTL) both from cultivated and wild sunflowers that can enhance sunflower adaptation in abiotic stress-prone environments (Ortiz 2015).

4.2.7 Salinity Tolerance

Soil salinity is a limiting factor for sunflower production. However, in many countries, sunflower is often grown on soils of mild salinity (Hladni 2010). The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutrition imbalance, specific effects salt stress and a combination of these factors. All these factors cause adverse pleiotropic effects on plant growth and development at physiological, biochemical, molecular and whole plant levels (Rasool et al. 2013).

A large number of researchers studied the influence of increasing levels of soil salt concentrations on sunflower plant. Increased soil salinity was found to cause reduction in sunflower leaf area, dry matter and ultimately yield of salt-sensitive lines (Katerji et al. 1994). It has also affected the sunflower seed oil content (Ashraf and Tufail 1995; El-Kheir et al. 2004; Flagella et al. 2004; Di Caterina et al. 2007). Increasing levels of salinity have led to the reduction of seed number per head and

weight of seeds per plant and significantly reduced seed yield (Flagella et al. 2004; Di Caterina et al. 2007). Cell survival, seed germination, dry matter accumulation, leaf death or senescence, leaf ion content, leaf necrosis, root growth, and osmotic regulations could be used as the selection criteria for salinity studies (Singh 2000; Lexer et al. 2004; Fernández-Martínez et al. 2009; Seiler 2012; Škorić 2012). The agronomical parameters used for estimation of salt tolerance in sunflower are yield, survival, plant height, leaf area, leaf injury, relative growth rate, and relative growth reduction (Ashraf and Harris 2004).

Different methods have been proposed to mitigate negative effect of salinity on sunflower. NaCl priming was found to be a useful method for improving salt tolerance of sunflower seeds (Bajehbaj 2010). Sodium, boron, manganese, and magnesium exclusion coupled with greater calcium uptake can also contribute to salt tolerance (Lexer et al. 2004). Foliar application of K^+ increased growth and yield of sunflower under salinity (Akram et al. 2009), while foliar application of ascorbic could mitigate the adverse effects of salinity on sunflower (Khan et al. 2013). Application of proline spray on sunflower leaves alleviated the adverse effects of salt stress on sunflower growth by maintaining ion and water availability and protecting sunflower photosynthesis against salt-induced oxidative stress (Khan et al. 2014). These results suggested that foliar application of nutrients could be used to improve sunflower tolerance to salinity by alleviating the adverse effects of salinity on growth and reproductive yield (Nusrat and Rafiq 2012). Calcium is believed to play an important role in stress tolerance and may be responsible for the observation of salt-tolerance QTLs (Lexer et al. 2003).

Salt tolerance in sunflower can be attributed to one major gene along with possible recessive modifier genes (Miller and Gulya 1995). Lai et al. (2005) identified six genes that may be responsible for the uptake regulation of mineral ions in sunflower. The identified genes are designated *HT089*, *HT175*, *HT185*, *HT215*, *HT 216,* and *HT227*. *H. paradoxus* is believed to be a hybrid that originated between 75,000 and 280,000 years ago (Welch and Rieseberg 2002) from a cross of two salt-sensitive species, *H. annuus* and *H. petiolaris* (Lexer et al. 2003). This species own genes which enable transport of potassium and calcium, suggesting that these genes contribute to the adaptation of this species to salinity (Edelist et al. 2009).

Many wild *Helianthus* species naturally grow in saline soils and could be used by breeders as sources of genes for resistance to salinity by applying effective screening methods. Heirloom cultivars and local landraces with unique morphological and physiological traits that can be useful in sunflower production through breeding can also be sources of resistance to salt tolerance in sunflower (Kaya 2016). At the same time, they also provide breeders with the genetic variability necessary for the successful realization of the breeding programs (Tan et al. 2016). *H. paradoxus* which inhabits sporadic salt marshes in the USA has three times more stable salt (up to 1300 mM) than cultivated sunflower and also exhibits high salt tolerance with having higher leaf succulence and leaf sodium sequestration (Karrenberg et al. 2006; Edelist et al. 2009). The United States Department of Agriculture (USDA) recognized the need for the creation of salt resistant

germplasm with a cultivated background and released two lines from an interspecific cross of *H. paradoxus* and *H. annuus*. These lines were registered as HA 429 and HA 430 (Miller and Seiler 2003).

4.2.8 Disease Resistance

Sunflower is attacked by many diseases and pests (insects and birds) that threaten its production. The main problem in modern sunflower production is the existence of a large number of diseases that cause a significant reduction in yield. According to the nature of the causative agent, all sunflower diseases can be classified into mycoses, bacterioses, viroses, and parasitic weeds (Aćimović 1998). Mycoses are diseases that are caused by various parasitic fungi and they cause the greatest damage in sunflower production. It is believed that over 40 fungi attack the sunflower, but not all of them cause economically significant damage, and not all are present in all areas of sunflower cultivation. Diseases causing the most losses worldwide are *Sclerotinia* head rot and stalk rot, *Phomopsis* stem canker, rust, and downy mildew. Some diseases are serious in only a few countries such as *Verticillium* wilt in Argentina or white rust (*Albugo*) in South Africa. Most sunflower diseases are caused by pathogens specific to sunflower, such as *Phomopsis helianthi*, *Alternaria helianthi*, and *Plasmopara halstedii*. Some of the most serious diseases, however, are caused by pathogens with wide host ranges, such as *Sclerotinia sclerotiorum* that occurs on 137 different genera of plants (Seiler and Gulya 2016).

Current results in sunflower breeding for disease resistance could be classified into four groups: genetic resistance to diseases such *as P. halstedii*, *Puccinia helianthi*, *Verticilium*, and *Erysiphe cichoracearum*; high level of tolerance to diseases such as *Phomopsis*, *Macrophomina*, *Albugo*, and *Alternaria* spp.; satisfactory tolerance of diseases such as *Phoma macdonaldii* and *S. sclerotiorum*; and partial disease tolerance against *Rhizopus* spp., *Botrytis cinerea*, and other pathogenic fungi (Kaya 2016). Increasing resistance to the dominant diseases is one of the basic tasks of sunflower breeders, which must be solved in order to make sunflower ready for the upcoming changes in climate. That is why it is necessary to achieve long-term tolerance or resistance to a particular pathogen (Jocić et al. 2012). *Phomopsis*—stem canker (tolerance) and downy mildew (resistance) can serve as examples of successful resolution of disease susceptibility problems, as well as breeding work on the creation of sunflower genotypes with durable disease resistance.

4.2.8.1 Stem Canker

Stem canker, caused by *Diaporthe/P. helianthi* Muntanola-Cvetković Mihaljčević et Petrov, is one of the most widely distributed and most damaging diseases of

sunflower. When the meteorological conditions during sunflower-growing period become favorable for disease development, the damages caused by pathogen cause considerable reductions of seed yield and oil content. Its large-scale occurrence was first registered in the Vojvodina Province (Serbia) and Romania in 1980 when it caused large economic damage to sunflower production. Soon afterward, it was observed in most sunflower-growing countries in Europe (France, Hungary, Slovakia, Bulgaria, Ukraine, Russia, and Italy). In the early 1980s, its presence was reported in the USA, Canada, Argentina, Uruguay, Australia, Iran, and some other countries (Škorić 2016). First identified in Serbia in 1980 by Mihaljčević et al. (1980), new species was described as *Diaporthe helianthi* Muntanola-Cvetković et al. for the teleomorphic state and *P. helianthi* Muntanola-Cvetković et al. for the anamorphic one (Muntanola-Cvetković et al. 1981). The pathogen softens up the plant tissue, destroys the stem and causes wilting, premature drying, and stem breakage (Aćimović and Štraser, 1981).

The best way of controlling the fungus is to grow resistant cultivars (Mihaljčević et al. 1982). Commercial hybrids with a high level of tolerance to *Diaporthe/P. helianthi* are available since Škorić (1985) obtained highly tolerant inbred lines. In the year of *Phomopsis* epidemic 1981, only four lines out of the entire breeding material (around 5000 inbred lines) of the Institute of Field and Vegetable Crops (IFVCNS) demonstrated high tolerance to stem canker. The lines have different genetic origins. Two of them were made by crossing the cultivated sunflower with *H. tuberosus*, one originated from a local population from Morocco, and the fourth one was derived from the cross *H. argophyllus* × Armavirski 9345. Three of them were converted to cms form and labeled cms Ha 74, cms Ha-BCPL, and cms Ha 22. The remaining one was converted to a restorer line and labeled SNRF-69. On the basis of these inbred lines, the first *Phomopsis* tolerant hybrids, NS-H-43, NS-H-44 and NS-H-45, were developed at IFVCNS. A parallel program dealt with the accumulation of genes carrying tolerance or resistance to stem canker. Within that program, (Ha 74 × Ha 22) × Ha-BCPL were used to develop female lines and ((Ha 74 × Ha 22) × Ha-BCPL) × SNRF-69 were used to develop restorer lines. In subsequent studies, the existence of the resistance *Phomopsis* stem cancer was also found in the perennial wild relatives of sunflower *H. maximiliani*, *H. pauciflorus*, *H. hirsutus*, *H. resinosus* and *H. tuberosus* (Škorić 1985; Dozet 1990) but these sources of resistance did not have practical application in sunflower breeding. The presence of resistance to *Phomopsis* in wild species of the genus *Helianthus* has also been confirmed by Griveau et al. (1992) who determined that several families descended from *H. argophyllus*, *H. debilis*, *H. petiolaris* and *H. exilis* were tolerant to *Phomopsis*.

Genetic studies have not obtained a definitive answer on the mode of inheritance and number of genes that control *Phomopsis* tolerance and sunflower breeders still need more information about the type of inheritance and number of genes involved in *Phomopsis* resistance. The first report on the genetics of sunflower resistance to *Phomopsis* came from Vranceanu et al. (1983) who noted that the resistance was controlled by several genes with partial dominance and that it was positively correlated with stay-green trait. Škoric (1985) reported that the resistance to *Diaporthe/*

P. helianthi is most probably controlled by at least two or more complementary genes, and the mode of inheritance is intermediate or partial dominance. Tourvieille et al. (1988) found that resistance might be recessive or dependent on interactions between genes and polygenic nature of resistance. Furthermore, he found that different types of resistance seem to be active in the plant tissues of leaf and stem. Vranceanu et al. (1992) stated that resistance was primarily controlled by partial dominance and that three genes or gene groups are involved in the resistance, and in fact, there are a few genes (3–7) that control it. Langar et al. (1997) observed two kinds of *Phomopsis* tolerant genotypes: permissive genotypes, which had a large delay in reaction against a fungus and nonpermissive genotypes, which possessed the ability to stop fungus development at the beginning of the attack. Degener et al. (1999) reported that different types of resistance are active in the plant tissues of leaf and stem, and this resistance in both tissues is mainly controlled by additive gene action. Langar et al. (2000) suggested that resistance on leaves is influenced by one major gene and that at least two complex factors influence resistance in petiole and stem tissues. It could be concluded that combinations of inbred lines with the best levels of resistance give the best hybrids and that it should be possible to obtain increased resistance by selecting combinations from different sources. Škorić (2016) reported that *Phomopsis* resistance is positively correlated with *Macrophomina* and *Phoma* resistance as well as with drought tolerance.

It is interesting to note that the situation in the population of this parasite is stable and that, based on the examination of various isolates of *P. helianthi*, it has been established that there are no significant differences between isolates of different geographical origin and that occasional strong attacks of *Phomopsis* in some regions of sunflower production are the result of favorable weather conditions for its development, and not changes in the pathogen population (Jocić et al. 2004). *Phomopsis* literary data have lately been mainly related to its occurrence in countries where there has been no strong attack in the previous period, such as Argentine and Uruguay (Huguet 2006), Australia (Thompson et al. 2011), and the United States (Methew et al. 2012).

4.2.8.2 Downy Mildew

Downy mildew is a sunflower disease caused by *P. halstedii* (Farl.) Berlese et de Toni, an oomycete with high virulence, aggressiveness, and a great potential in developing new races. Downey mildew infection can be primary (soilborne and seedborne) and secondary (airborne) (Aćimović 1998). Primary infection causes more severe symptoms and the decay of a plant, while secondary infection usually results in small angular spots on leaves without limiting the sunflower yield (Jocić et al. 2009). Under humid conditions, oospores germinate into zoosporangia, which liberate flagellate zoospores. Spores move toward the roots and penetrate into the tissue, thereby initiating primary infection (Virányi and Spring 2011). Infected

plants tend to be insufficiently developed, dwarfed, with chlorotic leaves covered with white mycelium (Dussle et al. 2004). The extent of the damage that the pathogen causes is influenced by several factors:

(1) environmental conditions, primarily humidity and temperature (Aegerter et al. 2003; Tourvieille et al. 2008b);
(2) potential of pathogen for host inoculation, i.e., zoosporangia viability (Meliala et al. 2000; Tourvieille et al. 2000); and
(3) genetic resistance or susceptibility of the crop.

Efficient control of downy mildew requires understanding fundamental biology, molecular mechanism of infection, development and reproduction of the pathogen (Sakr et al. 2009; Sakr 2010), as well as identification and determination of position and function of the host resistance genes.

Until 1980, only two races of downy mildew were known–race 100 in Europe and race 300 in the USA. After the races from the USA came in contact with the races from Europe, a number of races emerged in different parts of the world (Tourvieille et al. 2000; Sakr 2010). Until the present day, 24 races of downy mildew have been identified in Europe and 36 worldwide (Virányi et al. 2015). The frequency of resistant pathogen individuals often starts increasing after the intensive use of an effective method of control. While controlling the sensitive pathogen subpopulations, disease-control measures enable the progress of the resistant subpopulations, which soon become dominant. In order to prolong resistance duration, several control measures need to be applied simultaneously.

Genetic resistance of sunflower to downy mildew can be classified into two categories:

(1) partial resistance which is controlled by minor genes (QTLs), and tends to influence the extent of disease development and
(2) qualitative or monogenic resistance, conferred by the major *Pl* genes.

As far as genetic resistance is concerned, durable resistance could be achieved by combining quantitative with monogenic resistance (Vear et al. 2008), or by introducing genes from different clusters and with different origin in a single genotype (Jocić et al. 2010). Partial resistance, also called field resistance, general, quantitative or horizontal resistance, is usually not race-specific and is assumed to be polygenic. The use of partial resistances does not appear to have a strong effect on pathogen populations as the monogenic resistance. By combining the partial resistance provided by minor genes with specific resistance genes, durable resistance could be achieved (Tourvieille et al. 2008a). It has been shown that QTLs provide tolerance to a number of pathogens such as *S. sclerotiorum* (Vasić et al. 2004), *D. helianthi* (Mihaljčević et al. 1982; Škorić 1985), *Phoma macdonadlii* (Vear et al. 2008), and *Orobanche cumana* (Pérez-Vich et al. 2004). Al-Chaarani et al. (2002) found four putative QTLs. The three major QTLs were located on LG 1, 9, and 17, and explained 54.9% of the total phenotypic variance. Vear et al. (2008) reported two QTLs located on LG10 and LG8, and suggested microsatellite markers ORS613 and ORS389 for their detection.

Resistance genes denoted *Pl* genes provide complete resistance to the pathogen. As the *Pl* genes are race-specific (Miller and Gulya 1988; Vear et al. 2008), there is a great probability that new races of downy mildew will overcome them in a relatively short period of time. Even though monogenic resistance is not durable, introgression of *Pl* genes is still among the most efficient methods of controlling downy mildew. Genes that confer resistance to downy mildew are dominant and often form clusters. Until the present day, a number of *Pl* genes have been reported ($Pl_{1\text{-}15}$, Pl_v, Pl_w, $Pl_{x\text{-}z}$, M_w, M_x, Pl_{arg}, and $Pl_{HA\text{-}R4}$) and position of 11 genes has been determined on the SSR genetic map. It is usually the case that new resistance genes provide resistance to earlier races of the pathogen. Molecular markers have become a valuable tool in downy mildew resistance breeding programs (Kaya et al. 2012). Molecular techniques such as restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), insertion–deletion (INDEL), tartrate-resistant acid phosphatase (TRAP), and single-nucleotide polymorphism (SNP) markers, enabled construction of genetic maps (Gentzbittel et al. 1995; Tang et al. 2002; Yu et al. 2002; Yu et al. 2003; Lai et al. 2005) that greatly facilitated mapping of *Pl* genes. Molecular markers facilitate detection of *Pl* genes, make breeding faster and more reliable, and are becoming an essential part of the selection process (Imerovski et al. 2011; Miladinović et al. 2011).

Downy mildew resistance genes are often found in wild sunflower species. From these wild species, inbred lines have been developed, which are then used as donors of resistance genes. Thus, Pl_6 was introduced from wild ecotype of *H. annuus* (Miller and Gulya 1991), Pl_5 from *H. tuberosus* (Vranceanu et al. 1981), Pl_7 from *H. praecox* (Miller and Gulya 1991), and Pl_8 and Pl_{arg} from *H. argophyllus* (Miller and Gulya 1991; Seiler et al. 1991). According to Seiler (2012), complete resistance to the downy mildew pathogen was found in annual wild sunflower species *H. annuus*, *H. argophyllus*, *H. debilis*, and *H. petiolaris,* and perennial *H. decapetalus*, *H. divaricatus*, *H. eggertii*, *H. giganteus*, *H xlaetiflorus*, *H. mollis*, *H. nuttallii*, *H. scaberrimus*, *H. pauciflorus*, *H. salicifolius* and *H. tuberosus*. Along with resistance genes, lines developed from wild sunflower species also inherit a number of undesirable characteristics. When the donor lines are used in commercial breeding programs they have to be crossed with inbred lines which have desirable agronomical traits (Jocić et al. 2010).

Even though a large step has been made in understanding the complex interaction of the *Plasmopara* and sunflower, as well as the mechanisms of sunflower resistance, a number of questions remain unanswered. One of the problems that need to be addressed is the classification of pathogen races. It would be of a great significance if a unique molecular method for pathotype identification would be proposed. With the use of molecular markers, race composition in different sunflower cultivating regions could be determined with a higher accuracy. Additionally, tracking the pathogen population dynamics, i.e., new race occurrence would become possible.

4.2.9 Nutrient-Use Efficiency

The most important mineral elements for sunflower development are macro elements (N, P, K, Ca, Mg, and S) and microelements (Fe, Mn, Zn, Cu, B, Cl, and Mo) (Blamey et al. 1987). However, N, P, K, and S are considered to be limiting factors for sunflower development and growth. In order to take advantage of the great genetic potential of sunflower hybrids, it is necessary to provide optimum amounts of these elements during the entire vegetation season. It is also necessary to know the requirements for certain elements in all the stages of sunflower growth and development. The most efficient way of ensuring sufficient amounts of nutrients is timely and rational use of mineral fertilizers. Estimates of overall efficiency of applied fertilizers in sunflower have been reported to be about or below 50% for N, below 10% for P, and about 40% for K (Baligar et al. 2001).

Climatic changes affect the absorption and metabolism of nutrients in sunflower, which is reflected in seed yield and oil content and quality. The impact of atmospheric CO_2, heat, and stress due to drought affect the development of sunflower plants and their productivity. However, the interaction between these abiotic factors and their effect on photosynthesis, the development of plants, and transpiration are still insufficiently unexplored in sunflower (Debaeke et al. 2017). Mangin et al. (2017b) studied abiotic stress due to the lack of nitrogen and found a strong correlation between tolerance to drought and nitrogen. The lack of nitrogen in the early stages of sunflower development can reduce the number and surface area of the leaves (Gimenez et al. 1994). The loss of nitrogen from the leaf results in reduced photosynthetic activity, slow growth and slow seed filling (Hall et al. 1995). Zeng et al. (2014) found that using moderate amounts of nitrogen fertilizers, in the phase from seedling to butonization, resulted in the maximum photosynthetic rate and maximum dry matter yield.

Potassium plays the most important role in cell osmotic balance and maintaining the turgor. Therefore, it affects the development of all organs, especially roots, stems, and leaves. Photosynthesis and transcription affect the potassium availability indirectly because cations are involved in cellular turgor, as well as the opening and closing of the stoma (Connor and Hall 1997). The largest part of the potassium is returned to the soil by the harvesting of harvest residues, and a small portion remains in the seed. Contrary to potassium, the adoption of phosphorus is most intense at the time of seed filling. Phosphorus plays a key role in the transfer of energy through ATP. It is also a component of phospholipids in cell membranes and nucleotides.

Due to climate change, the management of certain nutrients has changed in some regions and growing systems (Brouder and Volenec 2008). Akram et al. (2009) have observed the improvement of sunflower growth due to exogenously applied K_2SO_4, which led to increased photosynthesis capacity, water-use efficiency, leaf turgor, and relative water content. There is a growing evidence that plants suffering from stressful environmental conditions, such as drought, have a growing demand for potassium as it is needed to maintain the fixation of photosynthetic CO_2 (Cakmak 2005).

The nutrient-use efficiency (NUE) of varieties (crops) could be increased by the existence of appropriate genotypic variability, techniques and selection criteria (Graham 1984). Identification of varieties with higher tolerance to suboptimal levels of nutrients from the soil offers the possibility of increasing the potential for crop production on marginal lands of low nutritional value around the world (Baligar and Fageria 1997). In sunflower breeding programs, the following plant properties should be taken into account: the ability to produce near-maximum yields on low-level soil nutrients and the root system that efficiently uses large amounts of soil nutrients in order to create varieties with high NUE that can contribute to sustainability and environment protection (Clark and Duncan 1991). The potential for the creation of varieties with superior NUE depends largely on: (i) the genetic variability in the species present for that particular NUE-regulated property and (ii) the development of a methodology for the precise quantification of physiological parameters reflecting an effective NUE (Baligar et al. 2001).

4.2.10 Water-Use Efficiency

Water-use efficiency (WUE) is the ratio of total carbon in the plant (total of photosynthesis, biomass, or yield) and the total loss of water due to transpiration (Blum 2005). The increase in WUE is most often referred to as a mechanism of plant response to moderate and severe water deficiency in the soil and is in the focus of many programs that aim to increase the tolerance of crops to drought. WUE is also an indicator of various water-use strategies in plants and adaptability to different climatic conditions (Keenan et al. 2013). Sunflower is a plant species with moderate water requirements and has the ability to tolerate short-term drought, partly recovering from stress, and with reduced water absorption has moderate yield reduction (García-Vila et al. 2012). There are two reasons for this, the first one is that the sunflower forms a deep root system that allows it to draw water from the deeper layers of the soil, and the other is that it has the ability to regulate the water transpiration from leaves in the case of water deficiency. However, the long-term water deficit, which is increasingly common due to climate change, can negatively affect the growth of sunflower plants, and consequently seed and oil yields (Ahmad et al. 2014).

Sunflower water requirements can be divided into two phases. The first phase is before the flowering (pre-anthesis) and water deficit is mainly reflected by the small leaf area index and withered leaves. The second phase, which, after flowering (post-anthesis), combines groups of factors such as accelerated aging of the leaves, withering and the reduction of the stoma (Connor and Hall 1997). Although the lack of water affects all development phases of the sunflower, the maximum yield reduction occurs when in drought during the reproductive phase (Reddy et al. 2003; Vijay Kumar 2004). Vijay Kumar (2004) studied the impact of irrigation on sunflower seed yield in four phases: 15–20 days after sowing, at the start of butonization, at flowering, and at seed maturity and found that the maximal seed yield can be obtained by irrigation at flowering stage.

The best agronomical approach to solving water deficit in plants is irrigation. Other methods include mulching to reduce soil evaporation, better weed control, crop rotation, improved cultivation technology to increase the infiltration rate, but these measures increase production costs. Water deficit could be overcome by changes in plant morphology or some other properties, such as seed properties, harvest index, root system, and leaf hydraulics that will increase WUE. Hence, the creation of high-WUE genotypes is the most efficient and cheapest strategy to deal with the problem of water deficit (Rauf 2008).

The existence of genetic variability is a precondition for breeding to increase WUE. Markulj Kulundžić et al. (2016) studied 12 sunflower genotypes and found 2 genotypes with greater tolerance to the water content in soil than others based on the maximum quantum yield of photosynthesis II (Fv/Fm), the photosynthetic efficacy index (PIABS) and the temperature of the leaf. Canavar et al. (2014) examined the reaction of four sunflower hybrids to the lack of water in a nonirrigated and irrigated environment and found a statistically significant difference in terms of WUE in all hybrids in both environments. The same authors suggested that the selection for a higher WUE can be done *via* an indirect isotope $\Delta^{13}C$ which is of a great importance in sunflower breeding programs aimed at generating drought-tolerant genotypes. Lambrides et al. (2004) also studied sunflower hybrids based on $\Delta^{13}C$ content. Crossing of genotypes with low and high $\Delta^{13}C$ content produced the progeny classified into two gene pools; with low $\Delta^{13}C$ and high $\Delta^{13}C$. Genotypes from low $\Delta^{13}C$ gene pools had significantly higher yields compared to genotypes of a high $\Delta^{13}C$ gene pool in water stress conditions (Condon et al. 2004).

4.2.11 Greenhouse Gas Emission and Carbon Sequestration

Combustion of fossil fuels results in the emission of carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) that act as obstacles for thermal radiation and prevent it from leaving the Earth's atmosphere, thus creating the so-called greenhouse effect (Pachauri and Reisinger 2007). Agricultural production itself is a significant producer of the gases responsible for the greenhouse effect (Johnson et al. 2007). The greatest influence is CO_2 (57%), while CH_4 and N_2O are influenced by 27 and 16%, respectively. As a result of the emission of gases, there is an increase in the global surface temperature of the Earth and global warming and uncertainty regarding their future impact on the climate (Pimentel and Pimentel 1996). Reducing energy from fossil fuels in agricultural systems is important because of the reduction of atmospheric emissions of greenhouse gases, which helps prevent global warming. It is necessary to identify the crop production method that would maximize energy efficiency and reduce greenhouse gas emissions (Tzilivakis et al. 2005).

CO_2 is the primary carbon source for plant growth and development. A higher concentration of atmospheric CO_2 can stimulate the photosynthetic process, promote plant growth and productivity without increasing the demand for water for

plant transplantation. It has been proven that C3 plants produce more biomass and harvest products in a high CO_2 environment compared to C4 plants (Long et al. 2006). In C3 plants, where the sunflower belongs, increased atmospheric CO_2 affects the growth and yield of plants mainly through the increase in photosynthesis rate and carbon assimilation (Griffin and Seemann 1996). In the future, if CO_2 is increased, the sunflower should become more efficient in absorbing sunlight, using its energy to convert CO_2 into carbohydrates and save water (Debaeke et al. 2017). This has been reported in several studies describing that elevated CO_2 significantly affects the increase in biomass (24–68%), as well as the final seed yield of sunflower. Recently, Rinaldi et al. (2015) showed that the increase in CO_2 from 370 to 760 ppm led to an increase in the net photosynthesis rate of more than 60, 7% reduction of stomatal conduction, water savings of 0.074 L/m^2 (leaves)/h (owing to the loss of transpiration) and consequently to the improvement of the sunflower crop water-use efficiency (WUE).

4.2.12 Genome Plasticity

Living organisms are defined by the genome they possess. The control of gene expression in response to the environmental impact determines whether the organism can survive the changing conditions and compete for the resources it needs to reproduce (Bennett 2004). The need for increased agricultural production in the coming decades in the climate change scenario requires new approaches to create new varieties that are more resilient and more efficient in resource use (Prohens et al. 2017). Over the past 15 years, sunflower yields have increased through genetic progress but slower than before, indicating that existing resources and methods of breeding cannot bring adequate solutions in the context of climate change (Vear 2016). The sunflower genome has recently been sequenced and forms the basis for future research programs to exploit genetic diversity to improve biotic and abiotic resistance to stress conditions, while addressing agricultural constraints and human nutritional needs (Badouin et al. 2017). This sunflower has the potential to become a plant model for adaptation to climate change.

Wild relatives are the source of variability for many properties of interest in breeding, especially tolerance to abiotic and biotic stresses. It is believed that the potential of wild species in plant breeding has largely remained unused (Prohens et al. 2017). However, wild sunflower species have played a significant role in the development of sunflowers and will continue to do so in the future as one of the primary sources of genetic diversity. They are adapted to a wide range of different habitats and have significant variability for most biotic and abiotic properties. The habitats of wild sunflower species are diverse, with species growing in deserts, grasslands, swamps, forests, mountains, roads and fields (Seiler et al. 2017).

Interspecific hybridization is used to transfer properties from wild species into the cultivated sunflower. The use of interspecific hybridization in sunflower breeding dates back to 1916 when a Russian scientist Saziperov produced a hybrid

between *H. annuus* and *H. argophyllus* in an attempt to develop sunflower with rust resistance (Cockerell 1929). Interspecific hybridization in Russia was continued by Galina Pustovoit on perennial *H. tuberosus* (Kaya et al. 2012). Discovery of cytoplasmic male sterility (CMS) and fertility restoration gene (*Rf*) in *H. petiolaris* paved the way for the development of single-cross hybrids, but it also resulted in a genetic bottleneck in sunflower germplasm, as all contemporary hybrids have the same source of male sterility and genes for the restoration of fertility. The diversity of the sequences present in the wild sunflower species has made cultivated sunflower genome and provided new alleles for desirable properties. However, with desirable traits, negative alleles for oil content, seed yield, and plant architecture are also introduced from the wild into the cultivated sunflower, thus slowing down breeding process, as it would take several generations to create new and useful recombinants (Warburton et al. 2017).

4.3 Resources of CS Genes

Prediction of climate change effects on genetic resources has become an extremely active research area and has an important role in warning scientists and decision makers about possible future risks (Bellard et al. 2014). One of the key issues in discussion on the ecological effects of climate change is whether the species will be able to adapt quickly enough to keep up with the rapid pace of climate change (Lavergne et al. 2010; Salamin et al. 2010). Regardless of the plant species, the basic mechanisms for adaptation to climate change in genetic terms are an adaptation to new conditions by mutations or wide crossings in order to improve species/genotype plasticity.

4.3.1 Mutations

Mutations are the primary source of variability in all organisms. Variability caused by induced mutations does not differ from the variability caused by spontaneous mutations during evolution (Sigurbjornsson and Micke 1974), although the frequency of induced mutations is 100 times higher than the frequency of spontaneous mutations. The use of induced mutations in breeding is called mutation breeding, which implies the creation of mutant varieties and the direct or indirect use of mutations in crop improvement. Mutations are also one of the primary sources of new genes that play an important role in the development and improvement of plant species. Induced mutations can be used in two ways: by direct use of mutants and by using mutants in crosses.

Due to the global change of climatic conditions, the breeding for increased resistance to abiotic stress conditions and improved product quality is gaining importance. The basic strategy in plant breeding using induced mutations is to

improve already adapted variety by changing one or two main traits, such as plant height, earliness, disease resistance, which will contribute to the yield improvement and quality of the existing variety. Improvement of the yield and quality of new varieties also leads to an increase in the economic value of these varieties.

The genetic variability of sunflower can be increased by mutations (Cvejić et al. 2014). Mutations can occur spontaneously in nature without human influence. One of the most famous spontaneous mutations in sunflower is the mutation of a cultural sunflower with a chrysanthemum-type blossom (Fick 1976). Although this mutant had its commercial application in horticulture, the biggest commercial significance in sunflower production has been the spontaneous mutations that provide resistance to herbicides. Wild *H. annuus* populations with spontaneous mutations for tolerance to imidazolinone herbicides (Al-Khatib et al. 1998) and the sulfonylurea herbicides (Al-Khatib et al. 1999) were found. Owing to breeders, these mutated genes were introduced into cultivated sunflower (Jocić et al. 2011).

The frequency of spontaneous mutations is very low, that is why induced mutations have a greater significance in increasing genetic variability of sunflower. Induced mutations are successfully used to increase the genetic variability of cultivated sunflower, and a large number of researchers use induced mutations in breeding programs and created numerous mutants with altered agronomic properties (earliness, dwarf growth, thinner hull, altered content and oil quality) (Savin and Stepanenko 1968; Saadat et al. 1974; Sarafi 1976; Soldatov 1976; Lofgren and Ramaraje 1982; Ivanov et al. 1988; Christov 1995; Osorio et al. 1995; Miller and Vick 1999; Kalaydzhyan et al. 2007). Mutations cause a wide range of inherited changes in sunflower. Most of these changes are manifested in morphological characteristics, oil quality and resistance to diseases, herbicides, and other stresses.

4.3.1.1 Morphological Traits

Adaptation of sunflower to existing climate changes is manifested through breeding for the changed morphological traits and developmental phenophases (earliness, deeper root system, shorter genotypes with lower leaf mass, etc.). Induced mutations can serve as a source of traits needed for adaptation to environmental stresses and create genetic variability for certain properties. Early genotypes have advantage in sunflower production as they endure unstable weather conditions in spring, summer and autumn. Early mutants were reported by many authors (Gundaev 1971; Plotnikov 1971; Voskoboinik and Soldatov 1974; Girigaj et al. 2004; Cvejić et al. 2015). Girigaj et al. (2004) isolated promising mutant lines using the pedigree method and used them in the hybrid development program by crossing lines with different maturity groups. Gundayev (1971) and Voskoboinik and Soldatov (1974) created induced mutants of shorter vegetation, with thinner hull and shorter stem. Plant height is one of the most studied morphological characteristics, and dwarf mutants are the most common product of induced mutations (Cvetkova 1970; Christov 1995; Jambhulkar 2002; Cvejić et al. 2015). Shorter plants are agronomically more desirable, for their easier redistribution of nutrients and easier

combining. Mutants with shorter stem and larger head diameter were also produced by Leclercq (1985) using gamma rays. Other useful mutations were also produced, such as increased 1000-seed weight (Savin and Stepanenko 1968), as well as higher leaf area and plant height (Cvetkova 1970).

4.3.1.2 Disease Resistance

The use of induced mutations in sunflower, unfortunately, did not give significant results in increasing resistance to diseases. Positive results were achieved only in resistance to rust (*P. helianthi* Schvein.), *A. helianthi* (Hansf.) Tub. et Nish., broomrape (*O. cumana* Vallr.) and downy mildew (*P. halstedii* (Farl.) Berl. and de Tony). Treating sunflower seeds at different stages and with different doses of ethyl methanesulfonate (EMS) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG), Lofgren and Ramaraje Urs (1982) obtained early maturity and rust-resistant sunflowers in the M_2 and M_3 generations. De Oliveira et al. (2004) used gamma rays (150 and 165 Gy) and EMS (0.05%) for the treatment of sunflower seeds and produced mutants with a satisfactory level of tolerance to *A. helianthi*. Mutant resistant to race E of broomrape were produced by treatment of immature embryos by ultrasound (Encheva et al. 2008). Encheva and Shindrova (2011) obtained sunflower genotypes resistant to downy mildew (race 330) by combining induced mutations and embryo culture.

4.3.2 Reverse Genetics

The targeting-induced local lesions in genomes (TILLING) methodology allows focus on specific genes or genomic regions, bypassing the problems of other functional genomic tools (such as T-DNA knockouts or iRNA) that are required for the generation of transgenic plants. The development of TILLING technology can also lead to the identification of new alleles that can be a direct value for crop improvement.

TILLING populations for a high-throughput identification of EMS-induced point mutations in the sunflower genome have successfully been obtained (Sabetta et al. 2011; Kumar et al. 2013). This allows the detection of induced and natural polymorphisms (SNP) in sunflower plants and even rare recessive mutations. Thus, an exciting tool for advanced and reverse sunflower genetics has been developed that will contribute to a global understanding of organization and regulation of sunflower plants. This new approach to the creation and presentation of a sunflower library in order to determine the properties that can rapidly generate genetic diversity within a population enables effective detection of recessive mutations that have largely been missed with the classical methods of selection (Kumar et al. 2013).

4.3.3 Wild Species

The basic method in modern sunflower breeding is the recurrent selection between elite genotypes (inbred lines, varieties, hybrids), which resulted in the creation of hybrids with higher yields, but also the reduction of genetic variability (Jocić et al. 2015). It has been found that the elite breeding material represents only 50–60% of the genetic variability found in the wild relatives of sunflower (Mandel et al. 2011). In order to achieve further progress in breeding, it is necessary to increase genetic variability, especially when considering climate change, human population growth and changes in the food production system (organic production). One of the potential methods for increasing genetic variability in sunflower is the use of wild relatives in the process of breeding.

Sunflower belongs to the family Asteraceae, the genus *Helianthus*. In the genus *Helianthus* there are 51 species, 14 of which are annual, 37 perennial, and 19 subspecies (Vear 2011). The use of wild relatives in sunflower breeding has a very long tradition and the first recorded case dates from 1916 when a Russian scientist Sazyperow crossed *H. annuus* with *H. argophyllus* in order to find resistance to rust (Seiler et al. 2017). The introduction of interspecies hybrids as source material in sunflower breeding was accomplished by Galina Pustovoit (Pustovoit and Gubin 1974), who crossed *H. tuberosus* with cultivated sunflower. In this way, the genetic variability of sunflower was significantly increased and the varieties created by this method have served as a source of resistance to downy mildew, broomrape, rust, *Verticillium*, and other sunflower diseases, and also as initial populations for creating inbred lines in the process of hybrid formation. The most significant contribution of wild relatives to sunflower breeding is certainly the discovery of cytoplasmic male sterility in *H. petiolaris* Nutt. (Leclercq 1966), and the genes for the restoration of fertility in the wild *H. annuus* (Kinman 1970) and *H. petiolaris* (Leclercq 1971), which enabled the introduction of hybrids into sunflower production.

The process of increasing the existing genetic variability of sunflower by using wild relatives is a long-term process that requires a lot of resources and work—from collecting of wild relatives, their maintenance, their testing and finding of the desired genes to the valorisation in breeding programs. Therefore, the most commonly used method for the introduction of traits from wild relatives into cultivated sunflower is targeted crossing, which involves finding the genes of interest in a particular wild relative (resistance to disease, herbicide, etc.) and transferring it to a cultivated sunflower. One of the basic problems of using wild relatives in sunflower breeding is that, besides the desired genes, a number of unwanted properties are introduced into the cultivated genotypes, due to their interconnectedness. These unwanted properties often lead to deterioration of agronomically important traits. In addition to this problem, the work on the practical application of the wild relatives in breeding is connected with a series of difficulties, a review of which is given in Table 4.1.

Table 4.1 An overview of challenges, advancements, bottlenecks, and strategies of crop wild relatives (CWR) use (Dempewolf et al. 2017)

Challenges to using CWR	Advancements facilitating use
Interspecific crossability	Tissue culture and embryo rescue
Missing data on species' basic biology (ploidy levels, life history traits, taxonomy)	Ongoing research
Lack of understanding of gene-trait relationships	Ongoing genomics research
Predicting how allelic combinations will be expressed in different cultivated crop backgrounds	Development of introgressed materials
	Basic research into epistasis
Transferring gene(s) of interest from wild to cultivated backgrounds with precision	Advanced embryo rescue techniques
	Transgenic technologies
	Marker-assisted selection (MAS)
Funding constraints	Efforts to communicate the value of CWR, particularly in the context of climate change, will raise the profile of pre-breeding programs and help secure resources and build capacity
Human capacity limitations	
Bottlenecks in the pre-breeding process	Strategies for advancement
Underrepresentation of wild species diversity in genebank collections	Increase collection, informed with gap analyses
Unidentified and misidentified accessions in gene banks	Improve documentation of incoming materials (collection of passport data, consistent taxonomy)
	Compare accessions using genomics data
Lack of characterization and evaluation data for genebank accessions	Mechanisms to ensure feedback to gene banks from screening
	Systematic and coordinated characterization and evaluation efforts; compilation of multilocation evaluation data
	Bioinformatics initiatives to manage data and make it accessible
Restricted access to materials under development	National-level legislation facilitating access
Disconnect among research communities	Increased coordination among actors along the pre-breeding continuum
Lack of clarity regarding roles and responsibilities	

In the context of the emerging climate changes in the future, it is certain that the most significant contribution of the wild relatives to the adaptation of cultivated sunflower will be in the area of introduction of pathogen, pest and parasite resistance. Overviewing the published studies, Seiler and Marek (2011) found that resistance to pathogens and parasites was described in 44 papers for annual and 119 papers for perennial wild sunflower species. Resistance to various pathogens was described in 12 out of 14 annual species with *H. argophyllus*, *H. petiolaris*, wild *H. annuus*, *H. debilis* and *H. praecox* being considered the most promising ones.

H. tuberosus, *H. pauciflorus*, *H. maximiliani*, *H. resinosus*, *H.* divarcatus, and *H. nuttallii* were the most interesting among perennial wild sunflower relatives. In other words, it is possible to say that potential sources of resistance to the most important sunflower pathogenic fungi were found, such as *Alternaria*, *Septoria*, powdery mildew, charcoal rot, *Verticillium*, *Phomopsis*, rust, downy mildew, and *Sclerotinia*; as well as parasitic weed broomrape; sunflower mosaic virus and insects, such as sunflower moth, steam weevil, sunflower beetle, red seed weevil, and banded sunflower moth (Seiler et al. 2017). Table 4.2 gives an overview of a part of potential new sources of different genes from wild relatives for improving cultivated sunflower production.

Table 4.2 Some examples of traits for which potentially useful genetic variation has been reported in the wild *Helianthus* species, along with their natural habitat (Warburton et al. 2017)

Trait	Species	Habitat	References
Oil concentration and fatty acid profile	*H. anomalus* and *H. deserticola*	Utah, Arizona, and Nevada	Seiler (2007)
Increased α-linolenic acid	*H. porter*, *H. atrorubens*, and *H. schweinitzii*		Seiler (2012)
Broomrape resistance	*H. anomalus*, *H. agrestis*, *H. debilis* subsp. *Cucumerifolius*, and *H. exilis*	Desert southwest USA (*H. agrestis* only grows in Florida)	Fernández-Martínez et al. (2000)
Sclerotinia sclerotiorum resistance	*H. maximiliani*	Central USA	Rönicke et al. (2004)
Sclerotinia stalk rot, *Phomopsis* stem canker, *Phoma* black stem, and charcoal rot	*H. tuberosus*	Eastern Canada and North Dakota	Seiler (2010)
Drought and heat resistance genes (low cell membrane injury, high epicuticular waxes and high leaf hair density)	*H. argophyllus*	Coastal region of Texas	Hussain et al. (2017)
Single dominant gene resistant to race G of broomrape (*Orobanche cumana* Wallr.)	*H. debilis* subsp. *tardiflorus*	Gulf coast and some inland areas of Florida, Georgia, and Alabama	Jan et al. (2014)
Head and stalk rot resistance	*H. californicus*, *H. schweinitzii*, *H. maximiliani*,	California, North and South Carolina, Great Plains in central North	Liu et al. (2010)

(continued)

Table 4.2 (continued)

Trait	Species	Habitat	References
	H. giganteus, and *H. grosseserratus*	America, eastern USA and eastern and central Canada, eastern and central parts of Canada, and the USA	
Salt resistance	*H. paradoxus*	West Texas and New Mexico salt marshes	Seiler (2007)
Drought resistance and high-water-use efficiency	*H. anomalus*	Southwestern USA	Seiler (2012)
Abiotic stress tolerance	*H. deblis* Nutt., *H. anomalus* Blake, and *H. divaricatus* L	Atlantic and Gulf Coasts of USA, Southwestern USA, North America, Ontario, Quebec, Illinois, Florida, and Louisiana	Fernández-Martínez et al. (2000)
Sunflower moth resistance	Wild sunflower (*H. annuus* L).	Albany, California	Rönicke et al. (2004)
Herbicide tolerance (sulfonyl urea; imazethapyr)	*H. praecox* accession 1823 and *H. nuttallii* NUT05	USA	Seiler (2010)

4.4 Molecular Tools for Introduction of CS Traits

4.4.1 Linkage Maps

The first molecular maps of cultivated sunflower were created by use of RFLP markers. Berry et al. (1995) used an F_2 population obtained from a cross between HA89 × ZENB8 and covered 1380 cM (centiMorgans) of sunflower linkage map by use of 213 probes and MapMaker version 3.0 (Lander et al. 1987). The same year, Gentzbittel et al. (1995) used three F_2 and two BC_1 populations derived by crossing three lines HA89, CX, RHA266, and PAC2 in different combinations and mapped 180 enzyme probes covering 1150 cM by use of GMendel 3.0 (Holloway and Knapp 1993) and MapMaker 3.0 (Lander et al. 1987). While Berry et al. (1995) mapped the markers on 17 LGs which is the haploid number of cultivated sunflower genome, Gentzbittel et al. (1995) mapped markers on 23 LGs on the "merged" RFLP map widely known as CARTISOL map. Few years later, Jan et al. (1998) developed more RFLP probes and mapped in the F_2 developed from a cross RHA 271 × HA 234 covering 1129 cM. Soon, Gentzbittel et al. (1999) published a composite sunflower RFLP map based on seven mapping F_2 populations, three of which have been used in creating RFLP map (Gentzbittel et al. 1995). The new

crosses included SD × PAC1, SD × CP73 175, CP73 × PAC1, GH × PAC2. Maps differed in length and LG number which ranged from 14 to 21. Map length of the newly analyzed F_2s was shorter compared to the first three crosses used in the previous study, mostly because Kosambi function was used instead of Haldene function. Composite map constructed by MapMaker/EXP 3.0 was based on the information obtained from all seven crosses in which 1115 individuals were tested. The map contained 17 LGs and four small fragments and was 1573 cM long, which was the longest map obtained so far.

Later on, Peerbolte and Peleman (1996) added 523 amplified fragment length polymorphism (AFLP) markers to RFLP maps previously developed by Gentzbittel et al. (1995), while Gedil et al. (2001b) integrated RFLP and AFLP maps by use of F_2 derived from the cross HA370 × HA372. Until the end of the 1990s, only 81 of all developed probes were publicly available (Berry et al. 1997). Therefore, Gedil et al. (2001b) broadened the number of available RGC probes and DNA sequences. The authors compared locus order from previously reported maps and generated a map by use of GMendel (Holloway and Knapp 1993) and MapMaker (Lander et al. 1987). At the end, the integrated map was constructed with 104 RFLP and 296 AFLP markers covering 1325 cM, (Stam 1993; Stam and Van Ooijen 1996).

The first SSR maps were published at the beginning of the twenty-first century. Tang et al. (2002) used F_7 recombinant inbred lines (RILs) developed from RHA280 × RHA801 cross and covered 1368.3 cM of sunflower map. The map was constructed with 459 SSR marker loci and contained 17 LGs which the authors presumed correspond to 17 chromosomes of the sunflower haploid genome. In addition, the total number of developed SSRs was 879, 579 of which were polymorphic among 4 tested sunflower inbred lines: RHA280, RHA801, PHA, and PHB. A year later, Yu et al. (2003) published a comprehensive study in which the authors cross-referenced public reference RFLP maps with the maps that they constructed on three mapping populations: F_2 from HA 370 × HA 372 (Gedil et al. 2001b), RILs from PHA × PHB, and RILSs from RHA 280 × RHA 801 (Tang et al. 2002). For mapping of these populations, different types of markers were used: publicly available SRR "ORS" markers (designed by Gedil (1999); Yu et al. (2002), and Tang et al. (2002) and "CRT" markers that are CARTISOL SSRs, RFLP "UB" markers (developed by Jan et al. (1998) and Gedil et al. (2001b), and INDEL "ZVG" markers (developed by Berry et al. (1997) and Gedil et al. (2001b). The length of the sunflower map was estimated in RHA280 × RHA801 map by calculating length obtained from SSR and INDEL markers, while RFLP markers were used to fulfill the telomeric region in several LGs where there were no SSR mapped. The total length that was calculated was 1522.7 cM (1423 cM the length from SSR and INDEL map and 99.7 cM from RFLP). This size is similar to the one reported by Gentzbittel et al. (1999). The number of publicly available unique SSR markers reached 600 at that time, which was a significant improvement for the sunflower molecular community. The same year, Tang et al. (2003) constructed the first 657-locus composite SSR map. The authors added to the published genetic linkage map 80 SSR marker loci from the HA 370 × HA 372 F_2, PHA × PHB RIL and HA 89 × ANN 1238 F_2 maps reported by Burke et al. (2002)

and Yu et al. (2003). Additionally, the authors created 13 6-locus multiplex PCRs for simultaneous detection of 78 SSR marker loci that covered all the 17 LGs (spanning to 1067 cM or 75% of the composite map length) thus facilitating and cheapening such analyses as genotyping, diversity or association studies. SSR markers have been developed by different authors and mainly used for polymorphism screening (Paniego et al. 2002; Chapman et al. 2008; Heesacker et al. 2008). Talia et al. (2010) used previously described AFLP markers, three INDEL markers and some of the previously described SSRs (ORS and HA) and newly developed ones for the construction of genetic linkage map. The map was constructed by analyzing F_2 obtained from a cross RHA 266 × PAC2 (Fusari et al. (2008)) by use of Carthagene 0.999 (Schiex and Gaspin 1997) and Mapmaker 3.0 (Lander et al. 1987). Sequences and information of publicly available SSR markers can be found in the NCBI and they represent a valuable tool for sunflower molecular biologists (Dimitrijević and Horn 2018).

In time, different types of markers have been added to the existing linkage maps. TRAP markers were added to the previously developed RIL mapping population derived from RHA 280 × RHA 801 cross by Hu (2006), Hu et al. (2007) who added in total 403 markers to the previously developed SSR map by Tang et al. (2002) and Yu et al. (2003). SNP markers enabled further improvement of the existing linkage maps. SNPs were created from expressed sequence tag (EST) or transcriptome information (Lai et al. 2005; Bachlava et al. 2012, respectively). Bowers et al. (2012) used the Illumina Infinium SNP array (Bachlava et al. 2012) for genotyping of four mapping populations (HA412-HO × RHA415, HA412-HO × ANN1238, RHA280 × RHA801, NMS373 × Hopi (PI369359)) and created a consensus map that consisted of 10,080 loci. SNP number for use in sunflower studies increased after the development of SNPs by restriction site-associated DNA sequencing (RAD sequencing) and genotyping by sequencing (GBS) (Talukder et al. 2014; Celik et al. 2016, respectively).

4.4.2 Association Mapping

After successful application of association mapping in medicine (Puffenberger et al. 1994; Carlson et al. 2004), association analysis started to be applied in plant research. In sunflower, initial association studies started to be published in the 2010s with the wide use of SSR markers, development of high-throughput SNP methods and association panels. Two approaches used in the association studies include:

(1) genome-wide association mapping that enables analysis of the entire plant genome and identification of markers linked to a certain phenotype or
(2) candidate–gene association mapping that includes tracking and analysis of particular genes.

Differences between using classical linkage mapping tools and association mapping are that the first requires developing F_2 population in order to analyze linkage, while the second approach is based on the exploitation of historical recombination and thus it is not necessary to develop mapping populations, which is a time-consuming process. Another important difference is that linkage mapping provides information of nonrandom association between alleles, and it does not imply that the marker correlates to a particular phenotype, while in association mapping researchers search for a statistical significance between a marker and a phenotype of interest (Gupta et al. 2005). The mapping resolution is higher by use of association mapping and a higher number of alleles are analyzed, however, the analyzed population structure can cause the appearance of false positive results. In order to remove suspicious results, validation of results can be obtained by combining association and linkage studies (Li et al. 2014; Shi et al. 2017).

The largest sunflower gene banks are located in Serbia, USA, and Russia (Atlagić and Terzić 2014; Gavrilova et al. 2014; Marek 2016; Seiler et al. 2017). Gene banks have set a good basis for sunflower breeding as a source of desirable traits such as stress resistance genes, *Rf* and *cms* genes. Recently, significant efforts have been made in creating association panels that capture as much genetic variation as possible and those panels have been used in molecular association studies. These populations have mostly been used in the detection of association between molecular markers and plant architecture traits, flowering time and stress resistance genes.

Initial association study in sunflower included 94 inbred lines consisting of public and Instituto Nacional de Tecnología Agropecuaria (INTA) sunflower breeding program genotypes (Fusari et al. 2012). For the candidate–gene approach, 43 candidate genes were chosen for *Sclerotinia* head rot resistance. These genes have previously been identified by transcript profiling in both sunflower and rapeseed (Zhao et al 2007). Candidate gene, *HaRIC_B*, showed significant association with head rot resistance. Miladinović et al. (2014a, b) associated a band amplified by primer C12 with resistance to mid-stalk rot in wild sunflower.

Davar et al. (2012) used a diverse panel consisting of 32 sunflower lines of different geographical origins, including RILs and mutant lines, to assess the number of regions in sunflower genome that could be linked to the resistance to black stem (*P. macdonaldii*). Seven isolates from France were used in the study and 1–4 SSR markers were linked to resistance to a particulate isolate in an association study. However, the identified markers did not pass the more stringent bar of statistical significance.

Mandel et al. (2013) performed genotyping and phenotyping of chosen traits (the number of days to flower and the total number of branches per plant) in an association mapping population that comprised 271 sunflower lines of different origin and that captured 90% of the allelic diversity of cultivated sunflower (Mandel et al. 2011). The authors found variable linkage disequilibrium (LD) across sunflower genome and reported elevated LD in regions that have been under selection by the breeders or were important in demystification. Such regions were found on LGs: 1, 5, 8, 10, 13, 16, and 17. Some of the named LGs harbors dominantly inherited resistance genes or resistance QTLs.

Concerning branching, Mandel et al. (2013) found significant associations of 17 regions on 12 LGs: 2, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, and 17. Some of the identified regions were located on LGs where there were previous reports of existence of QTLs, while some were novel. A larger region that spans ca. 25 cM carried a major branching locus on LG10, on which Bachlava et al. (2010) mapped a recessive branching allele B-locus. Concerning flowering time, significant associations were found in 10 regions on eight LGs: 1, 3, 4, 9, 10, 12, 13, and 17. Again, some were novel, while others have previously been reported by linkage mapping. Some of the association found (such as on the LG8 for branching) were established by use of kinship-only model, and were not verified, most likely due to the population structure. Therefore, as mentioned several models can give different results and validation is sometimes needed in detection of true association. An interesting study was published by Cadic et al. (2013) who compared linkage analysis and association mapping by using RIL population derived from XRQ × PSC8 and collection of 384 inbred lines from INRA and breeding companies. As previously shown, these authors also reported different level of LD across sunflower linkage map, with high LD found on LG8 and LG10, which happened due to selection efforts for disease resistance breeding on LG8 and branching on LG10. Based on LD reported, it was estimated that 12,857 SNPs are needed to properly cover the entire sunflower genome and obtain good resolution. Association and QTL detection discovered by association and linkage analysis, respectively varied between different environments. Additionally, not all QTLs linked to flowering time were confirmed by association. In total eight regions were in common by use of both approaches and five potential candidates were identified in these regions by association peaks.

Nambeesan et al. (2015) used association study to discover genomic regions associated with different types of branching. The authors have so far identified the unidentified regions and reported that different regions were associated with apical and mid-apical branching compared to basal and mid-basal branching. Recently, Darvishzadeh (2016) analyzed population structure and LD in a population comprised of 106 pure sunflower lines originating from research centers from Europe, the USA and Iran. Great phenotypic diversity was observed in the assessment of 14 agro-morphological traits, while genetic variation between groups formed by molecular analysis was low. The authors found association of several SSR and retrotransposon markers with examined traits such as kernel ratio, petiole length, flowering date 100-seeds weight. SSRs, such as P9491 and P822, were found to be associated with several traits and are thus desirable in marker-assisted selection (MAS).

4.4.3 Mapping of Quantitative Trait Loci (QTLs)

Quantitative traits have always been in a spotlight of research since the majority of traits of interest are inherited quantitatively. Development of linkage maps and different types of molecular markers set the basis for linkage analysis and mapping

of QTLs associated to climate-smart traits, such as disease resistance and drought tolerance (Miladinović et al. 2014a, b).

4.4.3.1 *Sclerotinia Sclerotiorum* Resistance

S. sclerotiorum (Lib.) de Bary is one of the most devastating sunflower diseases that can cause significant seed yield reduction of up to 100% (Sackston 1992). *S. sclerotiorum* is a fungus from class Ascomycete that is widespread in sunflower cultivation areas and can infect sunflower roots, buds, and head. Thus, it can cause distinct diseases: wilt and stem rot and head rot. There is almost no limitation for its advancement, except for dry and hot weather (Aćimović 1998).

Controlling *Sclerotinia* by chemical means has proven to be inefficient, while breeding for resistance shows better results. However, there is a large impediment for achieving total resistance. The reason for this is that resistance toward *S. sclerotiorum* is quantitatively inherited. Thus, molecular biologists need to identify and develop markers for QTLs that (1) explain as much of phenotypic variation as possible; (2) are stable across different environments; and (3) can be transferred to other sunflower crosses. In addition, markers associated to QTL should be less than 1 cM distant from the QTL in order to assure the efficiency of the marker and avoid possible crossovers (Zhang et al. 1999). In order to facilitate QTL detection, breeders need to develop large mapping populations (Mićić et al. 2005a).

Initial identification of *Sclerotinia* resistance QTLs involved the use of 73 RFLP markers. Mestries et al. (1998) identified several QTLs associated with sunflower resistance to white stem and head rot in a cross between GH and PAC2 by examining F_2, F_3, and F_4 generations. Mapmaker/EXP version 3.0 (Lander et al. 1987), with a minimum LOD score of 3.0 and a threshold recombination value of 0.45 was used for linkage analysis and two methods of QTL detection were applied: analysis of variance and interval mapping. QTLs detected for the reaction to the leaf test were located on different LGs depending on generation analyzed, while for the capitulum the same QTLs were identified in all three generations examined. Those were located on LG A and LG M. Some of the QTLs were common for both types of reaction, while some were unique. The obtained percentage of phenotypic variability explained by the QTLs identified for both traits was higher by use of interval mapping than ANOVA (10% by ANOVA but up to 40% by interval mapping). In the majority of cases, overdominance acted for QTLs for leaf reaction. Gentzbittel et al. (1998) used candidate–gene approach and identified a protein kinase gene as a cosegregating marker to a *Sclerotinia* resistance QTL that explained up to 50% of phenotypic variation across three crosses. Later on, Bert et al. (2002) used a segregating population obtained from a cross XRQ × PSC8 for the construction of a sunflower linkage map consisting of 19 LGs by use of RFLP, AFLP and some candidate genes of the CARTISOL MAP (Gentzbittel et al. 1995, 1998, 1999). The authors detected 15 resistance QTLs to white rot by use of the interval mapping method at a LOD threshold of 3. Phenotypic variance explained

by individual QTLs ranged between 9 and 41.2% for *Sclerotinia* resistance. QTLs were correlated to four parameters: mycelium on leaves and capitulum, percentage attack and latency index. Another study conducted by Bert et al. (2004) combined detection of markers linked to the resistance QTLs to white and black rot (P*homa macdonaldii*) by use of RFLP and AFLP markers. Concerning white rot, the authors identified seven QTLs associated with terminal bud attack and four QTLs for capitulum attack. Individually, QTLs for terminal bud attack explained less than 10% of the phenotypic variation, while those for capitulum attack explained up to 20%.

With the development and availability of SSR markers, new linkage maps that incorporate QTLs for *Sclerotinia* resistance were developed. Mićić et al. (2004) used, at that time, novel markers to identify the ones linked to midstalk resistance QTLs by analyzing F_3 population obtained from a cross NDBLOS × CM625. The authors obtained a 16-LG map and mapped QTLs on 14 LGs of sunflower. Only two LGs (LG8 and LG15) showed the presence of common QTLs for all three traits underlying midstalk rot resistance (leaf and stem lesion and speed of fungal growth). Phenotypic variance explained by individual QTLs for these three traits ranged between 2.51 and 34.67%. A year later, Mićić et al. (2005b) used RILs derived from the same cross as in Mićić et al. (2004) for validation and analysis of the predictive ability of the previously identified QTLs. The authors reported highly significant genotypic correlation between the two analyzed populations for morphological traits tested (leaf length and stem lesion) 0.5 and 0.64, respectively. The two most significant and stable QTLs that were singled out as the most promising for MAS were QTLs for stem lesion on LG8 and LG16 explaining 26.5% of the genotypic variance, respectively. In addition to validating QTLs in the two different populations obtained from the same cross, Mićić et al. (2005a) employed selective genotyping (SG) for identification of mid-stalk resistance QTLs in TUB-5-3234 by use of F_3 population obtained from a cross TUB-5-3234 × CM625. Some QTLs were validated; however, some were unique for a specific population. The authors singled out large QTLs on LG8 and LG4 as a starting point for MAS in combination with phenotypic selection. By combining results obtained from multiple studies (Mestries et al. 1998; Bert et al. 2002; Mićić et al. 2005a, Rönicke et al. 2005) LG10 carries QTLs for resistance to both, stalk and head, rot making this LG perspective for MAS. Further on, Yue et al. (2008) identified nine and seven QTLs for disease incidence and disease severity, respectively by use of TRAP, INDEL and SSR markers in a cross HA 441 × RHA 439. QTLs were located on 10 LGs in 13 chromosomal regions.

The development of SNPs enabled further improvement of the existing linkage which led to the work of Amoozadeh et al. (2015) who used both SSR and SNP markers to create a sunflower linkage map and identified 14 QTLs conferring partial resistance to two *S. sclerotiorum* isolates. The authors found a QTL linked to the *glutathione S-transferase* gene on LG1 that could be a good candidate for MAS of partial resistance to basal stem rot. Recently, Qi et al. (2016a) employed genotyping by sequencing (GBS) in order to identify regions of introgression from wild to cultivated sunflower. Wild sunflowers *H. argophyllus* and *H. petiolaris* were

chosen due to their resistance to basal stalk rot. The authors created six introgression lines from *H. argophyllus* and two from *H. petiolaris* and identified introgression segments of the wild genome in cultivated sunflower on LGs: 3, 8, 9, 10 and 11 and LG 8, respectively. Livaja et al. (2016) developed and used a genotyping array based on Illumina® Infinium assay, 25 K SNP genotyping array, for analysis of complex traits, such as midstalk rot resistance. The authors combined phenotypic results of *S. sclerotiorum* resistance from Mićić et al. (2005b) with the newly developed chip abundant in SNP markers and identified six QTLs in total for resistance traits (stem lesion length, speed of fungal growth, and leaf length with petiole) that individually explained between 8.1 and 35.2% of phenotypic variability. LG8 harbored QTLs that explained the largest portion of the variability.

4.4.3.2 *Phoma Macdonaldi* Resistance

P. macdonaldii (Boerema) is a fungal disease that attacks all sunflower parts and causes black rot. It is distributed throughout sunflower cultivation regions and has different levels of negative impact on sunflower production, mainly by reducing sunflower seed and oil yield, and premature ripening (Seassau et al. 2010). Humid and moist climate further influences higher level of infection severity. *Phoma* is thus a large problem in regions where sunflower is grown in oceanic or semi-oceanic climates, as is in France (Velasco et al. 2015).

Resistance to black rot is quantitative and with a strong additive effect (Roustaee et al. 2000). Bert et al. (2004) detected four minor QTLs by composite interval mapping (CIM) and two by simple interval mapping (SIM), which explained 20% of variability in total, by use of RFLP and AFLP in an F_2-F_3 obtained from a cross FU × PAZ2. Contrary to Bert et al. (2004) who used F_2 population, Al-Chaarani et al. (2002) employed RILs obtained from a cross PAC 2 × RHA 266 to create an AFLP linkage map of sunflower and detect markers linked to resistance QTLs. Seven QTLs were found on seven LGs: 3, 6, 8, 9, 11, 15, and 17 which explained 92% of the phenotypic variation (individual QTLs explained between 6 and 17% of the phenotypic variation). A few years later, Alfadil et al. (2007) added SSRs to previously created AFLP map and identified specific and nonspecific QTLs for resistance to four *P. macdonaldii* basal stem and root necrosis isolates: TA2, TA4, TA6 and TA9 collected in France. In total 27 QTLs were detected and positioned by CIM in QTL Cartographer 1.16 (Basten et al. 2002). Individual QTLs explained 7 to 29% of the phenotypic variation. Thirteen QTLs were isolate-specific (on LGs: 5, 6, 8, 12, 13, and 15), while 14 were nonspecific. Out of all detected markers linked to the resistance QTLs to different basal and stem necrosis isolates, the authors singled out HA3555 on LG12 and E33M48_26 on LG6 and E33M48_20 on LG13 as potentially useful in MAS for the introduction of tolerance to different isolates. The same year Darvishzadeh et al. (2007) detected isolate-specific and nonspecific resistance QTLs in the same mapping population. The authors detected 10 QTLs, four of which were nonspecific (on LGs 5 and 15) and three were specific for isolate MP8 and another three QTLs specific for MP10 isolate. Detected QTLs

showed moderate effect and explained from 6 to 20% of the phenotypic variance, individually. Four SSRs were singled out by the authors for the potential use in MAS: HA3700, SSU25, ORS1097, and ORS523_1 which encompassed QTLs for partial resistance to black stem isolates. In addition, the results of both studies showed that different QTLs can be detected by use of different pathogen isolates.

Recently, Bordat et al. (2017) exploited SSRs, INDELs and SNPs for detection of QTLs for resistance to *P. macdonaldii* at different stages in RILs developed from two crosses XRQ × PSC8 and FU × PAZ2. For early symptoms, three QTLs on LG5 and LG10 were detected, while for later symptoms, QTLs were located on LGs: 5, 10, and 15. Individually, the detected QTLs explained 15.6 to 19.9% of the phenotypic variability.

4.4.3.3 *Phomopsis* Resistance

Phomopsis stem canker (*D. helianthi* Munt.-Cvet. et al.) is a fungal disease widely distributed in non-arid sunflower cultivation areas (see Sect. 4.2.8.1 for more details).

One of the initial molecular analyses of *Phomopsis* resistance in sunflower included the use of RFLP and AFLP markers linked to resistance QTLs (Bert et al. 2002). The authors detected 15 resistance QTLs on LGs: 3, 4, 8, 10, 14, 11, and 17 (CARTISOL map) by use of the interval mapping method with a LOD threshold of 3. Individual QTLs explained from 7.2 to 34.7% of the phenotypic variation in the cross XRQ × PSC8. LG8 of the CARTISOL map carried QTLs for resistance to *Sclerotinia* and *Phomopsis* suggesting a common component in the resistance mechanism for both pathogens. Langar et al. (2004) used RILs obtained from a cross HA 89 × LR 4 cross for detection of the resistance and susceptibility QTLs by use of AFLP and direct amplification of length polymorphism (DALP) markers with CIM (PLABQTL software, Utz and Melchinger 1995). Resistance QTLs were distributed on at least eight LGs. One major and two significant QTLs associated with final expansion rate of lesions on leaf blades were found on LG6. Individual QTL explained 8 and 28% of the phenotypic variation. Resistance QTLs detected differed depending on the type of inoculation (artificial infection of leaves or seminatural infection) and were not located in the same regions, which confirmed the previous hypothesis that different mechanisms govern resistance on leaf blades, petioles and stems (Langar et al. 2002). As in many studies that include QTL detection, in this study, resistance QTLs was found in resistant line LR4 as well as in susceptible parental line HA 89.

4.4.3.4 Water Stress Tolerance

Abiotic stress has always been a subject of numerous studies in crops, however with the current climate change that we are witnessing, this topic is becoming even more popular in crop improvement. The Intergovernmental Panel on Climate Change

(IPCC) predicted an increase in CO_2 concentration, which should lead to increase of global temperature and possibly lead to changes in precipitation patterns (Pachauri and Reisinger 2007; Pachauri and Meyer 2014). Even though sunflower is a moderately drought tolerant crop that can be grown without systematic irrigation (Debaeke et al. 2017), there is a lot to be improved, especially bearing in mind that water shortage in the flowering time can cause significant seed yield damage (Rauf 2008).

Drought tolerance is controlled by several genes and mechanisms, and therefore it is a quantitative trait. Thus, sunflower molecular breeders need to put a lot of effort in the identification of tolerant QTLs that could be used in MAS, but also in order to understand how many genes are involved in drought tolerance. Drought tolerance is independent from yield properties and therefore identification of proper molecular markers to be used in MAS for introduction of desirable genes in high yield genotypes would be most beneficial for sunflower breeders and crop improvement (Chiementi et al. 2002). Additionally, identification of QTLs associated to agro-ecologically important QTLs in drought tolerant genotypes may be combined with drought resistance QTLs in the creation of high-yield drought-tolerant crops. The first study that involved the use of molecular markers in the identification of drought-tolerant QTLs was published by Jamaux et al. (1997). The authors used two families of sunflower lines that had contrasted in osmotic adjustment and differed in relative water loss of excised leaves to map QTLs by RAPD (random amplified polymorphic DNA), RFLP, and sequence-tagged site (STS) bulked analysis. Another study included detection of QTLs associated with water status (stomatal conductance, predawn leaf water potential, transpiration, and relative water content) and photosynthesis (leaf chlorophyll concentration, net photosynthesis, and internal CO_2 concentration) (Herve et al. 2001). In this study, 19 QTLs were mapped by use of AFLP markers. Detected QTLs described between 8.8 and 62.9% of the phenotypic variance of each examined trait. QTLs that co-localized with water status and stomatal movement were found on LGs: 5, 8, and 14.

One of the most significant studies of drought tolerance in sunflower was Kiani et al. (2007a, b; 2008; 2009). The authors exploited RILs developed from a cross PAC-2 × RHA 266 in a comprehensive study of sunflower reaction to water stress. AFLP and SSR markers were used in QTL mapping which was done by CIM in QTL Cartographer, version 1.16 (Basten et al. 2002). Kiani et al. (2007b) analyzed water status traits (relative water content, leaf water potential, turgor potential, osmotic potential, and osmotic potential at full turgor) and osmotic adjustment in two treatments: well-watered and water-stressed. Twenty-four, 32, and 8 QTLs were associated with well-watered, water-stressed conditions, and osmotic adjustment, respectively. Detected QTLs explained 6–29% of the phenotypic variation individually. Five QTLs were in common for two treatments. Some of the identified QTLs were common for several traits. In addition to QTL mapping, Kiani et al. (2007a) performed expression analysis in determining drought tolerance in sunflower for which they have chosen four RILs and their parents. QTLs were

associated with water status and gas exchange, while graphical genotyping was performed in GGT software (Van Berloo 1999) in order to present QTL location inherited from each parent. Several QTLs were associated with expression of water status traits and net photosynthesis rate. In the expression studies, four genes were selected based on the previous knowledge of their function in water stress conditions in other plant species: aquaporin, dehydrin, leafy cotyledon1-like protein, and fructose-1,6 bisphosphatase. Water stress caused decrease in aquaporin and fructose-1,6 bisphosphatase gene expression, and an increase in dehydrin and leafy cotyledon1-like gene expression. However, this increase was not associated with water status directly. Several detected QTLs were associated with expression of the examined genes, and this was the first study to show possible role of leafy cotyledon1-like protein in water stress response in sunflower. A year later, Kiani et al. (2008) established that prolonged water stress causes reduction of potential photochemical efficiency of photosystem II electron transport and mapped markers associated with four chlorophyll fluorescence parameters in well-watered and water- addition, as a result of all the research conducted, the authors identified overlapping QTLs for some chlorophyll fluorescence parameters and plant water status mainly on LG7 and LG16. All the experiments were conducted in the greenhouse and validation of the identified markers in field conditions and in different genetic backgrounds were recommended. Finally, Kiani et al. (2009) analyzed agronomical and yield parameters in two water stress conditions in both, greenhouse and the field. The most interesting region that was stable under three water treatments and controlled yield were located on LG14. Use of marker from this region, ORS391 in combination with a marker that is the closest to the common QTL for plant status and osmotic adjustment, ORS523_1 was recommended for pyramiding of QTLs that are associated with yield and drought tolerance.

Abdi et al. (2012) used SNP-based cleaved amplified polymorphic sequence (CAPS) markers and high-resolution melting (HRM) for improving QTL map developed by Kiani et al. (2007a). The authors mapped between one and 11 QTLs per examined agro-morphological trait (plant height, stem diameter, leaf area duration, leaf number per plant, petiole length, head diameter, grain yield per plant, number of achenes, 100-grain weight and total dry matter per plant) in water-stressed and well-watered conditions. Each QTL explained between 0.23 and 48.89% of the phenotypic variation and regions with desirable QTLs were compared to the previously published works (Kiani et al. 2007a; 2008; 2009). Regions that carry co-localized QTLs for several traits were found on LGs: 5, 10, 14, and 17 and thus markers associated to these detected QTLs could be a valuable tool in marker-assisted breeding. Owart et al. (2014) used a RIL population obtained from a cross cmsHA89 × Ann1238 and mapped 20 QTLs for 10 characters in the control treatment and 24 QTLs for 9 characters in the low water treatment. QTLs explained 10.5–55.3% and 10.5–31.7% of the phenotypic variation for each trait. Adiredjo et al. (2014) identified QTLs associated with water-use efficiency and carbon isotope discrimination in leaves in addition to QTLs associated with biomass and cumulative water transpired in greenhouse experiments on 148 RILs obtained from a cross XRQ × PSC8. The authors identified regions as the most

promising for MAS on LG6 and LG13 that carried QTLs associated to examined traits, of which carbon isotope discrimination was found to be as the most important one and in high negative correlation with water-use efficiency.

4.4.4 Major Genes

4.4.4.1 Downy Mildew

Downy mildew is caused by oomycete *P. halstedii* (Farl.) Berl. et Toni. The severity of infection depends on climatic conditions and conditions of cultivation. The more humid the vegetation period is, the higher the predicted severity of the infection (see Sect. 4.2.8.2 for details).

Introduction of downy mildew resistance genes in sunflower is relatively easy since the majority of downy mildew genes are monogenetic dominant genes, meaning that Mendelian rules apply when it comes to conversion of susceptible to resistant form. Downy mildew resistance genes or *Pl* genes are distributed throughout sunflower genome on different LGs. Two linkage groups most abundant in *Pl* genes are LG8 and LG1 with five (Pl_1, Pl_2, Pl_6, Pl_7, and Pl_{15}) and four (Pl_{13}, Pl_{14}, Pl_{16}, and Pl_{arg}) resistance genes, respectively (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997; Slabaugh et al. 2003; Mulpuri et al. 2009; Wieckhorst et al. 2010; Bachlava et al. 2011). This is followed by LG13 containing three resistance genes Pl_5, Pl_8, and Pl_{21}, LG4 with two *Pl* genes: Pl_{17} and Pl_{19}, and Pl_{18} is localized on LG2 (Bert et al. 2001; Bachlava et al. 2011; Vincourt et al. 2012; Qi et al. 2015a; 2016b; Zhang et al. 2017). Except for Pl_{arg} and Pl_{18}, the other *Pl* genes reported so far are clustered.

Discovery of resistance gene and identification of molecular markers closely linked or cosegregating to the *Pl* genes has been a major task for sunflower pre-breeders, breeders, and biotechnologist. The first *Pl* gene that was discovered and described was designated as Pl_1 gene and was positioned on LG8. Back then, SSR-based linkage map of sunflower did not exist and the first markers that were used for mapping of Pl_1 gene were RAPD and RFLP markers. Mouzeyar et al. (1995) used these markers to map Pl_1 gene that confers resistance to *P. halstedii* race 100 on LG1 of CARTISOL sunflower map (consensus RFLP map) by bulked segregant analysis (BSA) of F_2 obtained from GH × RHA 266, of which RHA 266 is the donor of the resistance gene Pl_1. The closest linked RFLP markers were located at 5.6 and 7.1 cM on each side of Pl_1 gene. This gene was later mapped on LG8 of the sunflower SSR map and further efforts of mapping the gene included the development of CAPS marker which was linked to the gene but did not completely cosegregate (Gedil et al. 2001a).

Most significant efforts were put into mapping of Pl_6, Pl_8 and Pl_{arg} genes. Pl_6 was for a long time sufficient in controlling *P. halstedii*, however in the late twentieth century new, more virulent races appeared. At present, Pl_{arg} and Pl_{15} are resistant to all currently identified races. Pl_6 gene is a part of a large cluster

(*Pl*$_1$, *Pl*$_2$, *Pl*$_6$, and *Pl*$_7$) on LG8. Markers that were developed for its detection include STSs, SSRs and CAPS. Out of 13 developed STSs markers for detection of *Pl*$_6$, Bouzidi et al. (2002) marked Hap1 as a very useful marker for MAS. Later on, Panković et al. (2007) developed CAPS markers based on previously developed STS marker, Hap3. In addition, two dominant SSR markers ORS 166 and ORS 1043 cosegregated with *Pl*$_6$ (Slabaugh et al. 2003; Panković et al. 2007). The majority of the mentioned markers have been successfully used in MAS for downy milder resistance breeding (Jocić et al. 2010; Dimitrijević et al. 2010). *Pl*$_5$/*Pl*$_8$ cluster was initially analyzed by use of STSs (Radwan et al. (2004), some of which have later been tested in different genotypes (Dimitrijević et al. 2011). Later on, Bachlava et al. (2011) identified SSCP (single-strand conformational polymorphism) markers RGC251 and RGC15/16 in close vicinity of the *Pl*$_8$ gene (0.3 cM downstream and 0.4 cM upstream, respectively). Concerning *Pl*$_{arg}$ gene, the majority of identified and developed markers belong to SSR, SNP and NBS–LRR (nucleotide-binding site–leucine-rich repeat) RGCs type. Dussle et al. (2004) and Wieckhorst et al. (2010) identified flanking and cosegregating markers to the gene, respectively. Cosegregating markers ORS 716, HT722, and HT211 in addition to NBS–LRR RGCs (RGC151, RGC52a and RGC52b) markers were identified by Wieckhorst et al. (2010), while Imerovski et al. (2014) confirmed cosegregation of previously reported SSR markers and reported another cosegregating SSR ORS 675 and validated the markers across different sunflower genetic backgrounds. ORS 716 proved to be the most useful marker in MAS.

Some of the remaining *Pl* genes have been mapped by use of SSRs, RGCs, and SNPs. SSRs ORS 1008 and HT636 were linked to both, *Pl*$_{13}$ and *Pl*$_{16}$ genes (Mulpuri et al. 2009; Liu et al. 2012). Bachlava et al. (2011) who mapped RGCs linked to *Pl*$_8$, also mapped RGCs closely positioned to *Pl*$_{14}$ gene. The authors exploited BAC (bacterial artificial chromosome) library which was constructed by digestion of high molecular weight genomic DNA of the sunflower line HA 383 with *Hind*III (Miller and Gulya 1995). For specific RGCs Bachlava et al. (2011) developed overgo probes and identified positive BAC clones which were verified by colony PCR. High information content fingerprinting was performed in order to build contigs by use of FPC software (Soderlund et al. 1997), while BAC-end sequences were obtained by cloning and used for SSRs mining and annotation studies. The study showed that RGC206 and RGC188 are 1.6 and 2.6 cM distant from *Pl*$_{14}$, respectively. Overgo probe RGC203 was used for detection of positive BAC clones, one of which was cloned, shotgun sequenced and structurally annotated. Another gene that was mapped by use of both SSRs and SNPs was *Pl*$_{17}$ gene on LG4 (Qi et al. 2015a). The closest markers were SSR marker ORS963 and SNP marker SFW04052 that were mapped 0.8 and 2.1 cM from *Pl*$_{17}$, respectively. Similarly, flanking SNPs and SSRs were mapped to *Pl*$_{18}$, *Pl*$_{19}$, and *Pl*$_{20}$ genes: 10 SNPs and two SSRs to *Pl*$_{18}$, two SSRs and two SNPs to *Pl*$_{19}$, and four SNPs to *Pl*$_{20}$ (Qi et al. 2016b; Zang et al. 2017; Ma et al. 2017).

With the development of SNP markers in sunflower, sunflower biotechnologists not only used the recently available tools for detection of the newly discovered resistance genes, but also exploited them for examining some of the "older" ones,

such as Pl_8 and Pl_{arg} due to their effectiveness in controlling downy mildew infections. Thus Qi et al. (2017) employed previously developed SNPs (Bowers et al. 2012; Pegadaraju et al. 2013; Talukder et al. 2014) and SSRs for saturating the region surrounding Pl_8 and Pl_{arg} by use of F_2 populations derived from crosses HA 434 × RHA 340 and HA 89 × RHA 464, respectively. In addition, some of the identified cosegregating markers were validated on a large sunflower panel that comprised of 548 sunflower lines. For Pl_8 gene two SNP markers: NSA_000423 and NSA_002220 could be used in diagnostic of 87% of sunflower lines where RHA 340 line was used as a donor of Pl_8 gene, while for introgression of Pl_{arg} into the majority of genetic backgrounds, nine SNPs could be used as potential diagnostic tools.

4.4.4.2 Rust Resistance

P. helianthi Schwein is a fungal disease causing rust in sunflower and can cause significant yield loss in sunflower production in infected fields. It is mostly spread in North America, Argentina, South Africa, and Australia. Climate conditions have a significant impact on the initial infection process and on the speed of the progression of the rust infection advancement (Gulya et al. 1990). Increased night temperature and moisture of the leaf significantly influence the germination of the spores. Ninety percent of the spores will germinate if leaf is wet for 3 hours at 25 °C or if the leaf is wet for 8 hours at 20 °C (Gulya et al. 1990). After the penetration, the temperature becomes the key factor in further advancement of the disease.

Significant efforts have been put in discovering resistance genes and mapping them in order to facilitate the introduction of the resistance genes into susceptible sunflower germplasm. So far, genetic control of the disease showed to be effective against rust since most of the rust resistance genes (*R* genes) are monogenic dominant. Ten rust resistance genes have been mapped on five sunflower LGs: R5 on LG2, R_1 on LG8, *R12,* and R_{13} on LG11, R_4, R_{11}, R_{adv}, R_{13a} (R_{HAR6}), and R_{13b} on LG13 and R_2 on LG14 (Bachlava et al. 2011; Qi et al. 2011; 2012; Gong et al., 2013a; Bulos et al. 2014, Zhang et al. 2016). Since the initial molecular studies were conducted at the end of the last century, RAPD and sequence characterized amplified region (SCAR) markers were used for mapping of the R_1 and R_{adv} genes (Lawson et al. 1996; 1998). SCAR marker $SCT06_{950}$ developed by Lawson et al. (1998) was linked to R_1 gene and useful in diagnostics of R_1 gene in different genetic backgrounds, except for sunflower line MC29 that contains R_2 and R_{10} genes. Recently, Qi et al. (2015b) mapped R_2 gene from MC29 (USDA) which was maintained in the Sunflower Research Unit of USDA-ARS, Fargo, North Dakota. Two SNPs, SFW01272 and NSA_002316 were mapped 1.8 and 2.8 cM from R_2 gene, respectively, on LG14. Due to an evident distance between the closest marker, SFW01272, and the rust resistance gene, the authors recommended using two closest markers in MAS. In this way, molecular breeders should be able to minimize selection of false positives.

Different types of molecular markers were developed and identified for detection of *R* genes on the LG13 which is most abundant in *R* genes compared to other LGs. Two markers, INDEL ZVG61 and SSR ORS 581, were mapped 0.8 and 2.1 cM from R_4 gene on the lower end of LG13, respectively (Qi et al. 2011). Due to the dominant nature of the identified SSR and the codominant nature of INDEL, use of both markers in MAS was recommended. Since the lower end of LG13 harbors the second largest region rich in NBS-LRR resistance genes, several more resistance genes have been mapped in this region. That is the case with another *R* gene, R_{HAR6}, which was discovered in confectionary sunflower population HA-R6. According to Bulos et al. (2013) ZVG61 is distal to the gene at a distance of 0.7 cM, while ORS581 was proximal at a distance of 1.5 cM. Gong et al. (2013a) examined the relationship between rust resistance genes in HA-R6 and RHA397, R_{13a} (R_{HAR6}) and R_{13b}, respectively. The authors mapped both genes in the lower end of LG13 and reported a set of common cosegregating markers for both genes (ORS191, ORS316, ORS581, ZVG61, and ORS464). Another gene was associated to R_4 gene, and that is Pu_6. The two genes are linked at a genetic distance of 6.25 cM. Bulos et al. (2014) mapped ORS316 distal to Pu_6 at a distance 2.5 cM and ORS224 proximal to the gene at a distance of 4.8 cM. Another gene mapped in this region is R_{adv}. Lawson et al. (1998) developed a SCAR marker, $SCX20_{600}$, cosegregating with R_{adv}, which was later on mapped distal to fertility restoration gene, Rf_1 (Yu et al., 2003). Subsequently, Bachlava et al. (2011) identified RGC markers tightly linked to R_{adv} that was found to be at a genetic distance of 8.2 cM apart from downy mildew resistance gene Pl_8. Besides, Pl_8 and Rf_1, Qi et al. (2012) examined R_{11} gene in the lower end of LG13 and mapped it closely to fertility restoration gene Rf_5. The SSR ORS728 was proved to be a common marker for R_{11} and Rf_5 genes, while ORS45, was mapped 1 cM proximal to R_{11}, thus the best marker for MAS. Based on all previous studies there are several *R* and *Pl* genes clustered in the lower end of LG13. Thus Gong et al. (2013a) proposed the existence of two subclusters in this region: R_{adv} and R_{11} form subcluster I, while R_4, $R_{13a/b}$, Pl_5, Pl_8 form subcluster II. Since LG13 is rich in *R* genes, the introduction of several *R* genes in this region in one sunflower genotype may lead to increase of the resistance of the genotype. For *R* gene pyramiding, Qi et al. (2015c) employed publicly available and proprietary SNPs for saturation of regions surrounding R_4, R_5, R_{13a}, and R_{13b} genes. The authors mapped SFW03654 at a distance of 0.6 cM from R_5 gene, while they also mapped three cosegregating proximal and four distal markers to R_4 gene at a distance of 0.7 cM and 0.6 cM, respectively. SNP marker SFW05743 was mapped 0.2 cM proximal to R_{13a}, while SFW00757 cosegregated with R_{13b}. In addition, the authors identified "double-resistant" genotypes in F_2 population carrying R_5 and R_{13a}. Similarly, Qi et al. (2015c) used SSRs and SNPs for detection of "double-resistant" genotypes with R_2 and R_{13a} genes in confection sunflower. Improved genotypes showed higher resistance level in comparison to genotypes that harbor only one *R* gene.

Analyses of the other rust resistance genes were performed recently, and therefore all studies involved using newly available SNPs that enabled saturating the region surrounding investigated *R* genes. Thus, Talukder et al. (2014) fine

mapped the region surrounding R_{12} by use of SNPs and identified five SNP markers (NSA_000064, NSA_008884, NSA_004155, NSA_003320, and NSA_003426) at a distance of 0.83 cM from the gene. By performing fine mapping, the authors enabled more precise MAS since, in the previous study ORS1227 and ZVG53 were mapped 3.3 cM and 9.6 cM from the gene, respectively with the CRT275 marker being the closest to R_{12} gene at a distance of 1 cM (Gong et al. 2013b). Another *R* gene, R_{14} was mapped in the vicinity to R_{12}. R_{14} is positioned between ORS1227, which is 1.6 cM proximal to the gene and ZVG53 that is 6.9 cM distal from R_{14} (Zhang et al. 2016). Even though the origins of the two resistance genes differ, the authors reported that the closest SNP marker to the R_{14} gene, NSA_000064, could not enable differentiation between R_{12} and R_{14} gene. Other markers were too distant to be used in MAS of R_{14} gene.

4.4.5 Genomic Selection

Past efforts to improve plant tolerance to drought, high salinity, and low-temperature through breeding and genetic engineering have had limited success owing to the genetic complexity of stress responses. Genome-wide prediction, also known as genomic selection (GS), has been proposed as a method to improve the breeding efficiency of quantitative and polygenic traits in plants. GS utilizes genotypic and phenotypic data collected on a breeding population to calculate the quantitative measure of each individual's value as a parent for future cycles of breeding, referred to as a genome-estimated breeding value (Spindel and McCouch 2016). The advantage of genomic selection over traditional phenotype-based selection is one of efficiency in both time and money. The accuracy of the genome-estimated breeding value (GEBV) is directly proportional to gain from selection, because accurate GEBVs allow a breeder to make selection decisions that increase the proportion of top-performing offspring crossed and recombined each generation (Hayes et al. 2009; Heffner et al. 2010).

In all crops, sunflower included, breeding for complex polygenic traits is still challenging as the complex nature of polygenic traits such as yield and resistance to the most abiotic and biotic stresses requires a multipronged approach to breed new varieties with stable and enhanced yield in the environment affected by climate change. High-throughput genomics and phenomics combined with GS offer rapid and targeted improvement of populations and identification of parents for rapid genetic gains and improved stress-resistant varieties. Using these approaches together with appropriate genetic diversity, databases, analytical tools, and well-characterized climate change scenarios, weather, and soil data, new varieties with improved stress resistance corresponding to farmer preferences can be introduced into target regions rapidly (Khan et al. 2016).

Exploitation of available plant genetic resources in combination with the use of modern molecular tools for genome-wide association studies (GWAS) and the application of genomic selection could lead to considerable improvements in

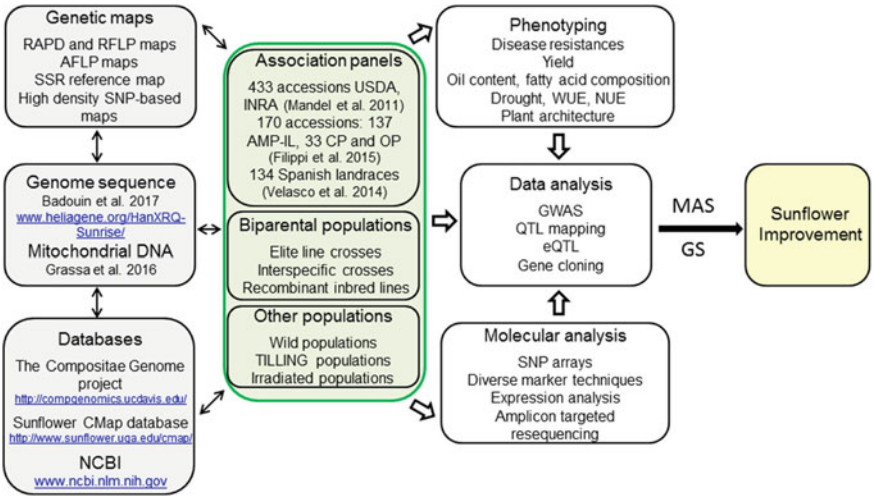

Fig. 4.2 Schematic overview of the resources available in sunflower for marker-assisted selection (MAS) and future genomic selection (GS) (Dimitrijević and Horn 2018)

sunflower. However, only in the recent years, plant and genomic resources have become available in sunflower comparable to other crops (Dimitrijević and Horn 2018) (Fig. 4.2). In sunflower, GS has so far been used for prediction of hybrid performance (Reif et al. 2013), hybrid oil content (Mangin et al. 2017a), and *Sclerotinia* mid-stalk rot (Livaja et al. 2016). The results obtained in these studies showed that GS could lead to major improvement of breeding efficiency compared to the classical general combining ability (GCA) modeling when either one or both parents are not well-characterized. Although GS is still expensive and unavailable for many researchers and breeders, the first step in genomic selection with regard to hybrid performance and hybrid oil content have shown that genomic selection can successfully address complex quantitative traits in sunflower and will help to speed up sunflower breeding programs in the future (Dimitrijević and Horn 2018).

4.5 New Breeding Techniques for CS Traits

4.5.1 Genetic Engineering

The application of genetic engineering for improving the existing and introducing new traits into cultivated sunflower is limited, mainly due to the difficulty of regenerating plants in a reproducible and efficient way. The existing techniques need substantial improvement because the regeneration response is strongly affected by genotype and culture conditions (Vasić 2001). Besides that, concerns raised about the environmental risk after the release of transformed plants prevented

the appearance of genetically modified (GM) sunflower on the market (Cantamutto and Poverene 2010).

Everett et al. (1987) were the first to obtain transformed sunflower plants using *Agrobacterium*-mediated transformation, followed by Malone-Schoneberg et al. (1994) and Bidney et al. (1992) who combined microprojectile bombardment with uncoated particles and *Agrobacterium tumefaciens* coculture. Moyne et al. (1988) used polyethylene glycol (PEG)-induced uptake of vectors for transformation of sunflower protoplasts, but no plants were regenerated from the calluses obtained in the process. Some attempts were made to transform sunflower via microprojectile bombardment with DNA-coated particles (Hunold et al. 1995; Laparra et al. 1995) or electroporation (Kirches et al. 1991), but again no transgenic plants were obtained. Different approaches to sunflower genetic transformation varied in their success (see Moschen et al. 2014 for review). Consequently, all reports on transgenic sunflowers published to date are based on *Agrobacterium*–mediated gene transfer to embryonic axes of mature embryos with subsequent plant regeneration from the meristematic region (Everett et al. 1987; Schrammeijer et al. 1990; Escandón and Hahne 1991; Grayburn and Vick 1995; Burrus et al. 1996; Müller et al. 2001; Molinier et al. 2001; Mohamed et al. 2006; Ikeda et al. 2005; Lewi et al. 2006; Radonic et al. 2006; Dağüstü et al. 2008; Radonic et al. 2008; Neskorodov et al. 2010; Pradeep et al. 2012; Sujatha et al. 2012). Recently, Zhang (2016) reported using low inoculum with long coculture with *Agrobacterium* (LI/LC) as efficient tool for optimization of sunflower transformation and production of large numbers of GFP-expressing shoots.

In spite of various methods used, the number of genes introduced into sunflower using transgenic approaches remains low. Among the different methods, only the use of explants derived from shoot apex was able to produce transgenic sunflower carrying important agronomic traits.

4.5.1.1 Biotic Stress Tolerance

The so-called OXO-transgenic sunflower plants, with introduced wheat germin *gf2.8 OXOgene* (Lane et al. 1991), were the first transgenic sunflower plants with successfully introduced resistance to diseases (Lu et al. 2000, Scelonge et al. 2000). This gene was found to confer increased level of resistance to *S. sclerotiorum* both in laboratory and field conditions (Bazzalo et al. 2000; Hu et al. 2003). This was also the first example of a successful transgene-mediated fungal resistance mechanism in plants.

Overexpression of pathogenesis-related (PR) proteins has been found to lead to increased resistance to pathogens in several crops. Using this approach, transgenic sunflower has been created with overexpressed tobacco β-1,3-glucanase gene and resistant to *A. helianthi* (Kumar et al. 2011). Using the same system, Radonic et al. (2008) tried to obtain sunflower resistant to both *Verticilium dahila* and *S. sclerotiorum* by introducing antifungal genes, including *gln2* (a glucanase) from *Nicotiana tabacum*, a chitinase (*ch5B*) from *Phaseolus vulgaris*, an osmotin gene

(*ap24*) from *N. tabacum*, and a gene coding for a ribosome inhibitor protein (*rip*). However, transformation efficiency was rather low and depended on DNA sequence used. Furthermore, there were some examples of transgenic instability as some T_1 plants which expressed the transgene lost it in the following generation.

Lectin or proteinase inhibitor genes have been used to engineer sunflower with insect resistance (Schuler et al. 1998). Transgenic sunflowers resistant to *Spilosoma virginicia* and *Rachiplusia* were obtained by introduction of Cry1Fgene ("Bt" gene) isolated from *Bacillus thuringiensis*. *Cry1AC* gene was used to develop a transgenic line of Bt sunflowers by Pioneer Hi-Bred and Dow AgroSciences which produce CrylAc protein that is lethal to Lepidopteran (moth) larvae (Snow et al. 2003). However, it was found that "gene flow" from the transgenics could have detrimental effects on the native lepidopteran herbivores and other populations of coleopteran and dipteran herbivores (Snow et al. 2003).

4.5.1.2 Abiotic Stress Tolerance

Although sunflower is considered tolerant to abiotic stress, its improved stress tolerance would allow cultivation of this crop across an even larger area than currently utilized. There have been few attempts to introduce genes for drought and salt resistance into sunflower. Cheng et al. (2009) transformed the drought and salt resistance gene *P5CS* into sunflower and obtained six transformed buds which were resistant to kanamycin, thus showing that successful gene transfer and expression had occurred. However, no fertile transgenic plants were obtained. Tishchenko et al. (2014) introduced dsRNA-suppressor of proline dehydrogenase gene, based on the *ProDH1* gene of *Arabidopsis*, into sunflower plants in order to increase tolerance level to water deficiency and salinity. Watanabe et al (2005) incorporated yeast metallothionein gene (*CUP1*) into sunflower and managed to select heavy metal-tolerant lines of the transgenic sunflower calluses thus confirming that transgenics could be used to obtain abiotic stress tolerance in sunflower.

To date, no application for the market introduction of a genetically modified sunflower has been made (ISAAA 2018).

4.5.2 Genome Editing and Nanotechnology

Development of new breeding techniques, such as genome editing, could provide new perspectives for more efficient sunflower breeding. Generally, climate-smart traits, such as tolerance to various abiotic stresses, are complex phenotypic traits controlled by polygenes, and it is usually necessary to study more than a single gene or single class of genes to understand molecular mechanisms underlying respective tolerance. Genome editing could be very useful to evaluate and validate the strength of the predictive value of a given candidate gene by easily transferring its best alleles into a set of different genetic backgrounds representative of the

diversity of the genetic material used in the selection schemes (Nogué et al. 2016). As multiple genes can be individually engineered at the same time, genome editing also provides a way to modify linked genes or QTLs that are usually difficult to segregate due to the limits of meiotic recombination (Flavell 2010).

As in genetic transformation, regeneration efficiency could be bottleneck for the effective use of genome editing techniques in sunflower breeding. Different approaches have been used to overcome this problem. Ikeda et al. (2005) studied the applicability of small and branching varieties of sunflower for plant regeneration and gene introduction and found that these small and easily transformed sunflower varieties are useful subjects for molecular genetic experiments. More recently, Zhang (2016) proposed low inoculum with long coculture (LI/LC) transformation and regeneration optimization approach for the production of large numbers of GFP-expressing sunflower shoots. Recent breakthrough in sunflower genome sequencing (Badouin et al. 2017) is expected to facilitate the use of genomics and other new breeding techniques, including genome editing, and work on understanding the molecular mechanisms underlying key traits related to abiotic stress resistance.

Nanotechnology provides new agrochemical agents and new delivery mechanisms to improve crop productivity and it can boost agricultural production. Its applications include: (1) nanoformulations of agrochemicals for applying pesticides and fertilizers for crop improvement; (2) the application of nanosensors/nanobiosensors in crop protection for the identification of diseases and residues of agrochemicals; (3) nanodevices for the genetic manipulation of plants; (4) plant disease diagnostics; (5) animal health, animal breeding, poultry production; and (6) postharvest management (Sekhon 2014). Up to date, there are no reports on use of nanodevices for genetic manipulation and improvement of climate-smart traits of sunflower. However, this technology has found its use in mitigation of negative effects of drought, low soil fertility and nutrients deficiencies on sunflower crop through use of different nanoformulations, some of which are already commercially available (Seghatoleslami and Forutani 2015; Janmohammadi et al. 2016; Ma and Tyro 2016; Tassi et al 2017; Torabian et al. 2017).

4.6 Future Prospects: Meta-Analysis and Bioinformatics

Novel approaches in genotyping and phenotyping enabled more efficient data collection for identification of quantitative characters and elucidation of genetic basis of agronomically important traits in sunflower. The flip side of these new approaches is the risk of drowning in the massive amounts of data. That is why it is essential to use proper approaches for data management and integrated analysis of differently collected data, as in the study of Moschen et al. (2016) who used integrated transcriptomic and metabolomic approach to elucidate mechanisms related to the senescence process. (Moschen et al. 2016).

In the field conditions, sunflower crop is often simultaneously challenged by different biotic and abiotic stresses. Hence, understanding the shared mechanisms contributing to two or more individually or simultaneously occurring stresses is important to improve crop productivity under foreseeable complex stress situations (Ramu et al. 2016). Multiple stress approach has recently been used to model the impact of multiple abiotic stresses on sunflower oil yield (Mangin et al. 2017b). The authors developed stress indicators to characterize 14 environments for three abiotic stresses (cold, drought, and nitrogen) using the SUNFLO crop model and phenotypic variations of three commercial varieties. The computed plant stress indicators at variety level better explained yield variation than descriptors at the climatic or crop levels. Ramu et al. (2016) used a meta-analysis to dissect molecular mechanism behind multiple individual stress and combined stress tolerance in sunflower, using publicly available transcriptome datasets from individual stress studies. Meta-analysis revealed 526 up- and 4440 downregulated genes in all combined stresses. Amongst these genes, 17 were induced under all combined stresses (pathogen, NaCl, drought, cold and methyl viologen stress) in tolerant genotype showing that under combined or multiple stresses, the meta-analysis can identify stress-responsive candidate genes.

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Chapter 5
Progress Toward Development of Climate-Smart Flax: A Perspective on Omics-Assisted Breeding

S. M. Shivaraj, Priyanka Dhakate, Humira Sonah, Tri Vuong, Henry T. Nguyen and Rupesh Deshmukh

Abstract Flax or linseed is one of the oldest crops that is economically valued for both fiber and oil. The bast fiber derived from flax is having high tensile strength, which is used for the production of linen clothes. Its seed oil contains a high amount of polyunsaturated fatty acids, which is beneficial for human health. Flax is the third largest fiber-yielding crop and one of the five major oil producing crops in the world. Like other crop plants, flax faces many biotic and abiotic stresses that can affect its productivity. Conventional breeding methods in flax focused on stabilizing yield and other important traits. Consequently, application of molecular markers to analyze genetic diversity in flax and identify quantitative trait loci (QTLs) for different agronomic traits augmented flax breeding. After the revolutionary improvements in sequencing technology, several plant genomes have been sequenced including flax. However, less effort has been made to utilize the available genetic resources for flax improvement. The available resources provide an opportunity to utilize advanced tools like genome-wide association studies (GWAS) and genomic selection to increase the precision of plant selection for flax breeding. In addition to breeding, genetic engineering techniques allow the introduction of novel traits by manipulating candidate genes such as transcription factors, protein-encoding genes, and transporters. A holistic approach involving diverse bioinformatics, breeding, and genetic engineering technologies will greatly facilitate the introduction of climate-smart traits into flax varieties to sustain their productivity in the scenario of global climate change.

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5.1 Introduction

Flax (*Linum usitatissimum* L.), commonly known as linseed, is one of the oldest crops that is economically valued both as a fiber crop and an oilseed crop. Flax belongs to the genus *Linum*, of the family Linaceae. The genus comprises more than 100 annual and perennial species. Cultivated flax ($2n = 2x = 30$), is an annual, self-pollinated crop plant. Flax is the third largest fiber-yielding crop and one of the five major oil producing crops in the world. The species is assumed to be originated in either Indian or Middle East region (Vavilov 1951) and later spread throughout the Asia and Europe (Green et al. 2008).

Flax species experienced divergent selection resulting in fiber (fiber flax) and oil (oilseed flax) types, which differ significantly in morphology, physiology, and agronomic performance (Diederichsen and Ulrich 2009). Fiber flax cultivars are taller, less branched, while linseed-yielding flax cultivars are shorter, more branched (Green et al. 2008). Obtaining both fiber and oil from the same crop is not possible because the fiber becomes more lignified and less flexible after flowering. The high quality of the fiber is obtained before seed set (Deyholos 2006). Flax provides raw materials for food, medicine, textiles, and other industries. The cellulose-rich bast fiber derived from the part of the flax phloem is having high tensile strength, which is used for the production of linen clothes. Its seed (linseed) oil contains a high amount of polyunsaturated fatty acids (PUFA), more specifically omega-3 fatty acids (~55%), which is beneficial for human health (Simopoulos 2000). Flaxseed is an undervalued source of protein. However, it contains about 22% protein that can release physiologically beneficial bioactive peptides upon digestion. The unique drying properties and fatty acid composition make linseed oil suitable for the preparation of paints and varnishes (Przybylski 2005). The meal left after the oil extraction from linseed contains ~35–40% protein and ~3–4% oil, which is used as a feed for livestock. Flaxseeds are rich in lignans those are having anticancer properties.

Despite the high economic prospects and valuable health-related properties of flax, its improvement is undertaken through breeding, and other genetic techniques are far behind other fiber and oilseed crops. The low genetic diversity (Fu et al. 2002a, 2003; Cloutier et al. 2009) and difficulties with production of intraspecific hybrids with other species (Green et al. 2008) limited molecular breeding and genomic tools (Cloutier et al. 2011, 2012) affect yield and quality improvement in flax. Conventional breeding of flax focused on improving yield under different environments, increasing oil quality and quantity, enhancing resistance to major wilt and rust diseases (Kenaschuk and Rowland 1993; Mpofu and Rashid 2001), and breeding for lodging resistance. Flaxseed is a promising candidate to cater for

sustainable oilseed, fiber, and protein sources. This chapter discusses the origin, production, agronomy, genetics and breeding, and biotechnology of flax, with a focus on the development of climate-smart cultivars.

5.2 Prioritizing Climate-Smart (CS) Traits

5.2.1 Flowering Time

Flowering time is a complex trait that is governed by approximately 300 genes that are associated with at least five endogenous and exogenous pathways. These include photoperiod, aging, vernalization, and autonomous flowering pathways (Cheng et al. 2017). Therefore, there is a need to decipher the underlying genetic and molecular basis of flowering to aid the development of early flowering and consequently early maturing lines in crop plants. Early crop maturity is an important agronomic trait that equips a plant to evade various abiotic and biotic stresses such as drought, disease, frost, and heat (Shivaraj et al. 2018; Tyagi et al. 2018a, b). The typical life cycle of a flax plant is 90–150 days long wherein 45–60 days comprises vegetative phase followed by a 15–25 days flowering period after which a maturation period takes about 30–40 days (Diederichsen and Richards 2003). Shortening the time that flax stands in the field can be achieved by development of early flowering and early maturing flax varieties that are desirable to augment efficient flaxseed production. Traditional breeding methods take more than ten years to commercialize a new crop variety. Therefore, the forward genetics approaches such as marker-assisted breeding and reverse genetic approaches like transgenics are amenable methods to develop viable new flax varieties. Maturity is a quantitatively inherited trait; however, genetic basis that determines maturity in flax is still not well understood. However, it was found that early flowering flax genotypes were obtained when germinating seedlings had been treated with 5-azacytidine (Fieldes 1994) indicating that major genes governing flowering time in flax are under epigenetic control (Gehring and Henikoff 2007; Suzuki and Bird 2008). Further, it was found that stable early flowering flax plants displayed heritable changes in the genotypes as they flowered approximately 7 days earlier and were relatively shorter than the wild-type control (Fieldes and Harvey 2004). Further, the vegetative phase in early flowering lines was reduced, and their DNA was found hypomethylated as compared to the control plants (Fieldes et al. 2005; Brown et al. 2008).

5.2.2 Fiber and Oil Quality

The flax plant consists of approximately 25% seed and 75% leaves and stem (Lay and Dybing 1989). Flaxseeds are a nutritionally rich source of short chain PUFA, omega-3 fatty acid: α-linolenic acid (ALA), insoluble and soluble fibers, proteins,

and an array of antioxidants, phytoestrogenic lignans (Oomah 2001; Gebauer et al. 2006; Touré and Xueming 2010; Ivanov et al. 2011). Flaxseed oil is composed of less saturated fatty acids (9%), moderate monosaturated fatty acids (18%), and more of polyunsaturated fatty acid (73%) (Cunnane et al. 1993). ALA is the major fatty acid (39.00–60.42%) in flaxseed oil followed by oleic, linoleic, palmitic, and stearic acids (Pellizzon et al. 2007). The flaxseed contains 20–30% of protein comprising approximately 80% globulins and 20% glutelin (Hall et al. 2006).

The cellulose-rich bast fiber derived from flax is having high tensile strength, which is used for the production of linen clothes. Flax fiber is extracted from the upper layer of the stem, which is extracted by chemical or mechanical methods. A flax fiber is having less density with good mechanical properties (Singh et al. 2011). Flax fiber is soft, flexible, and lustrous and is stronger than cotton fiber; however, it is less elastic compared to cotton fiber (Singh et al. 2011). Flax fiber is also used as raw material for the production of high-quality paper (Carter 1993).

5.2.3 Biotic and Abiotic Stress Tolerance

To achieve sustainability in agriculture, it is imperative to develop and raise crops that are tolerant to increasing abiotic stresses in light of climate change. Plants face a constant threat from various abiotic and biotic stresses which affect their productivity. The plant responds to these stresses via complex endogenous signaling pathways and numerous adaptations. The cross talks between these pathways determine the productivity and reproductive success of the plant under such environmental conditions. Flax, like any other crop, faces many abiotic stresses that can affect its productivity. These abiotic stresses are discussed below.

5.2.3.1 Drought Tolerance

To withstand drought and/or water deficit conditions, plants have many survival adaptations. Understanding these mechanisms is important to develop crop plants tolerant to such conditions. In an attempt, Quéro et al. (2015) studied the mechanisms involved in β-aminobutyric acid (BABA) induced drought tolerance in plants. Metabolomic and ionomic profiling in flax leaves revealed that BABA induces a reorganization of solute content that leads to increased accumulation of nonstructural carbohydrates and proline, and also decrease in inorganic solutes. Therefore, BABA treatment was found to induce changes that led to an improvement of flax plants to drought stress (Quéro et al. 2015). Ansari et al. (2016) studied the interaction of seed treatment and mycorrhizal fungi under different irrigating conditions. It was found that unlike other plant species, in flax mycorrhizal fungi may aid growth in both stress and nonstress conditions. However, the use of salicylic acid may reduce growth, indicating that an expected synergistic effect of these treatments on flaxseed growth under drought tolerance does not exist. Aghdam

et al. (2016) elucidated the impacts of different concentrations of nanosized (10–25 nm) titanium dioxide (TiO_2) on flax growth under stress and nonstress conditions. It was found that TiO_2 at low concentration improved the flax growth under water deficient conditions. In addition, the increased seed oil and protein content were obtained in flax plant that was treated with nano-TiO_2 at 100 mg/l. Therefore, application of nano-TiO_2 particles at low concentrations was found to ameliorate drought stress damage in flax plants as well as aid the drought tolerance by improving physiological processes. Both the reverse and forward genetics approaches are used to develop stress resilient crops. In the same context, an attempt was made to develop a drought-tolerant cell line of flax cv. Blanka by incorporating drought responsive element binding protein 2A (*DREB2A*) gene in the plant genome (Tawfik et al. 2016).

5.2.3.2 Salinity Tolerance

Increased alkalization and salinization of soil are realized threats to all crop plants across the globe, with alkaline–salt stress being more destructive than neutral salt stress. In such a scenario, deciphering the mechanisms that regulate plant tolerance to saline–alkaline stress has become an active field of plant research. Guo et al. (2013) analyzed the effect of these conditions on germination in flax plant. They reported that of all the 10 popular flax genotypes they studied the germination was found to decrease with an increase in the ionic concentrations in three experimental conditions. As expected, low concentration treatment of neutral salt and alkaline salt was found to have little effect on germination in all genotypes. Alkaline–salt stress was found to have the maximum effect on impeding germination across all genotypes, and increased concentration of this treatment inhibited germination altogether. Five linseed genotypes were found to have higher salt tolerance when grown after treating with neutral salt and alkaline salt (Guo et al. 2013). Yu et al. (2014) analyzed digital gene expression of flax plants under alkaline stress, alkaline–salt stress and neutral salt stress. It was found that under alkaline–salt stress photosynthesis and response to biotic stimulus was severely affected while under neutral salt stress carbohydrate metabolism was affected. Key regulators that are involved in abiotic stresses such as mitogen-activated protein kinase kinase (MAPKK), WRKY, abscisic acid (ABA), and ion channels were found differentially expressed. In comparison to neutral salt stress and alkaline stress, alkaline–salt stress triggered more of these differentially expressed genes, indicating that a lot more number of genes are involved in regulating alkaline–salt stress pathway. Molecular basis of salinity stress is not yet fully understood. Therefore, to understand the molecular basis of salinity tolerance in flax, Yu et al. (2016) analyzed small RNA and degradome via deep sequencing in samples that were treated with the aforesaid three stress conditions. It was found that small RNA target genes that regulate responses to stimuli were found induced. These results were validated by analyzing the expression of eight miRNAs via qRT-PCR. Degradome sequencing and transcriptome profiling revealed inverse expression patterns for 29

miRNA-target pairs under all stresses. Further, the role of two miRNAs, miR398a and miR530, was implicated in governing salinity stress in flax. In addition, Wei et al. (2013) studied biochemical changes in flax seedlings during salt stress, wherein they found that flax seedlings of YOI254, Tianxin3, HIZ019 had increased salt tolerance.

5.2.3.3 Heavy Metal Stress

Heavy metal contamination in soil leads to accumulation of these heavy metals in plants. This results in biomagnification of heavy metals in the food chain. Cadmium is one such heavy metal that is harmful to both plants and animals even in small amounts. Plants have developed specialized control mechanisms through which they can sequester metals form complexes with ligands such as metallothionein and phytochelatins (metallothionein group III). Many studies showed that flax pants tolerate cadmium contamination efficiently. Although cadmium toxicity prevents their use for both in food and pharmaceutical purposes, the use of flax cadmium (Cd)-accumulating plant for phytoremediation of contaminated soils opens up a novel and a promising avenue toward improving tolerance of its cultivars and varieties to Cd stress.

Mechanisms that determine cadmium tolerance was comprehensively studied in the last decade. (Belkadhi et al. 2013) assayed the effect of salicylic acid on antioxidant defense system in flax seedlings. High Cd concentrations resulted in the inhibition of root growth and enhanced hydrogen peroxide (H_2O_2) production, lipid peroxides, protein oxidation, and membrane permeability, to varying degrees. Further, it was found that the efficiency of scavenging and antioxidant enzymes was significantly altered. However, salicylic acid was found to ameliorate the toxic effects of Cd on membrane lipid content, antioxidant system, and root growth. Belkadhi et al. (2014) found that pretreatment with salicylic acid preferentially preserved the plastidial lipids by acquiring higher levels of polyunsaturated fatty acids. These results suggest that flax plantlets pretreated with salicylic acid exhibit more membrane stability under Cd-stress conditions. Belkadhi et al. (2015) elucidated that exogenous salicylic acid promotes defense in flax against cadmium stress by protecting phospholipids. These results indicated that salicylic acid is cardinal in triggering root antioxidant system and enhances cadmium tolerance in flax. Kaplan et al. (2015) studied the influence of cadmium stress in the presence of mycorrhizal fungi on fatty acid profile of flax. They found that seeds from plants that were grown with mycorrhizal fungi produced seeds with increased concentrations of unsaturated (18:1, 18:2, and 18:3) fatty acids. These effects were found more pronounced at 15 ppm of Cd (the concentrations of 18:1, 18:2, and 18:3 were augmented by 169, 370, and 150%, respectively). These results suggested that, after the Cd concentration reaches a certain threshold in seeds, this heavy metal enhances the efficiency of enzymes that regulate the conversion of saturated fatty acids to unsaturated fatty acids.

Zinc (Zn) is an essential element that is required for the normal growth and development; however, it can be toxic when present in excess. In flax, significant efforts have been made toward the understanding of plant tolerance to zinc. In a study by (Grant et al. 2000), higher translocation of Zn from shoot to seeds has been observed in flax varieties as compared to Cd. The translocation of metal ions to seed is the deciding factor for the use of plant species for the phytoremediation. Several biotechnological approaches are also being used in plants to restrict uptake or translocation of the metal ions in plant tissues. Smykalova et al. (2010) studied the ability of flax verities to accumulate and translocate Ca and Zn in an in vitro culture obtained from hypocotyl tissues. The flax genotypes identified by (Smykalova et al. 2010) those are tolerant to Zn, and accumulating more Zn and less Cd in seed will be important for improvement of the nutritive value of linseed. Another significant study conducted by Soudek et al. (2010), where the toxic effect of heavy metals like cadmium (cd), cobalt (Co), copper (Cu), zinc (Zn), nickel (Ni), lead (Pb), chromium (Cr), and arsenic (As) on seed germination in different cultivars of the flax have been evaluated. The different heavy metals showed the toxic effect in the order of As $\geq$ Cu $\geq$ Cd $\geq$ Co $\geq$ Cr $\geq$ Ni $\geq$ Pb $\geq$ Zn. Indeed, many of such reports suggest the efficient use of potential of flax for the phytoremediation, particularly when grown for the fiber production. In-depth understanding of heavy metal tolerance mechanism will also help to improve cultivation of flax on soils contaminated with heavy metals.

5.2.3.4 Cold Stress

Vernalization and photoperiodism are two important physiological processes related to the yield of many cool-season annual crops. In a growth chamber study, Darapuneni et al. (2014) have analyzed the flowering response of diverse flax genotypes under two photoperiod and vernalization regimes. The results suggest the significant effect of photoperiod, vernalization, and genotype on early flowering in flax. The early flowering trait is more critical for the cold weather areas like Upper US Midwest and Canada. However, some areas like Texas, flax is grown in the fall due to high spring and summer temperatures. Flax cultivars showed genotypic interaction with both vernalization and photoperiod. Specifically, flax genotypes from Texas (winter type) were sensitive to both vernalization and photoperiods for flowering. Texas genotypes delayed anthesis for 7 days or more in unvernalized seedlings, whereas flowering time of most other spring grown flax genotypes was unaffected by the vernalization treatments. Texas genotypes also delayed anthesis for 12 days or more under vernalized and short-day conditions, whereas most other genotypes were not influenced by photoperiodism in vernalized seedlings. The selection for vernalization and photoperiodic sensitivity in Texas genotypes and introgression of these traits into recently adapted spring grown genotypes is needed for development of high-yielding flax genotypes for production areas in southern Great Plains. Although flax is grown mainly in temperate climatic conditions, less effort has been made to study the genotypes which can withstand the cold stress.

5.2.3.5 Disease Resistance

Breeding is the most efficient and practical way to engineer disease resistance in crop plants. Early efforts in flax improvement were directed at breeding disease resistance (Kenaschuk and Rowland 1993). Based on the number of genes that control inheritance, three distinct forms of resistance have been identified—(i) monogenic: resistance is controlled by one gene, (ii) oligogenic: resistance is controlled by a few genes, and (iii) polygenic: resistance is controlled by many genes (Russell 2013). Fusarium wilt, caused by *Fusarium oxysporum,* is a common flax disease. Shortly after emergence, the seedlings are killed by the fungus, while delayed infections cause yellowing and wilting of leaves (Bailey et al. 2009). Agrawal et al. (1991) found that inheritance of Fusarium wilt resistance in flax is determined by recessive alleles as was indicated from F_2 segregation ratios from nine intervarietal crosses involving the flax wilt resistant cultivars RLC6 and R552, and four susceptible commercial varieties. (Spielmeyer et al. 1998b) studied the inheritance of resistance to Fusarium wilt in a recombinant doubled haploid (DH) population that was derived from F_2 seeds of a cross between a wilt resistant (twinning Linola TM) and a wilt susceptible (Australian flax cultivar Glenelg) line. The observed phenotypic variation was attributed to the segregation of two independent genes with additive effects that accounted for 38 and 26% of the genetic variability for Fusarium wilt resistance. (Spielmeyer et al. 1998a), mapped the QTLs to two linkage groups using amplified fragment length polymorphism (AFLP) marker. Portyankin and Karachan (1999) found that flax cultivars Torzhoksky 85, Kievsky, Rodnik, Kalininsky, Ustiensky, Niva, Nika, T-17, Mogilevsky-2, and K-65 were resistant to Fusarium wilt. Diederichsen et al. (2008) reported diversity in tolerance to Fusarium wilt wherein they reported that flax accessions from East Asia and North and South America were found to have higher than average and average resistance, respectively, while flax accessions from Europe and Indian subcontinent were found to have lower than average resistance to Fusarium wilt.

Powdery mildew in flax is another major disease, caused by the fungus *Oidium lini*, was first observed in Western Canada in 1997 (Bailey et al. 2009). Ashry et al. (2002) evaluated flax genotypes for resistance to powdery mildew resistance. They found that selection for fiber type flax results in an increase in powdery mildew resistance, while selection for seed type flax results in a decrease in resistance to powdery mildew. Rashid and Duguid (2005) studied the genetics of resistance to powdery mildew in flax plants and found that a single dominant gene (*PM1*) is responsible for resistance to powdery mildew.

Pasmo disease is also known to affect the production of flax crop. Pasmo disease, caused by *Septoria linicola* (Speg.) Garassini, is prevalent and widespread on flax in Western Canada (Rashid et al. 2010). Most Canadian flax cultivars are susceptible, and little is known about the pathogenicity of different *S. linicola* isolates. Rapid disease in development is exhibited in areas with high humidity and temperature. The yield of susceptible varieties infected during flowering is reported to be reduced by 75% (Saskston 1959). *S. linicola* survives from one season to the next on infected crop residues left in the field (Bailey et al. 2009).

5.2.4 *Genome Plasticity*

Flax has also attracted a lot of interest over the years owing to the capacity of a few varieties that display genome plasticity. Genome plasticity is broadly defined as the ability of the genotype to withstand diverse growing conditions and to carry forward heritable genomic alterations to future generations that were caused by environmental and (or) nutritional changes. Flax is a unique crop given its capacity of genome plasticity (Evans et al. 1966; Evans 1968; Durrant and Jones 1971), a characteristic that can be manipulated to address pertinent questions such as food security. The genomic changes that take place include variation in copy number of repetitive regions, total DNA content, and the appearance of Linum insertion sequence 1 (LIS-1) (Cullis 1976; Goldsbrough and Cullis 1981). Changes in nuclear C-value of up to 15% have been reported among first-generation progeny of self-pollinated individuals when subjected to different growing conditions (Evans et al. 1966). Moderate nutrient stress conditions result in plants that retain genomic plasticity, however, extreme stress conditions results in the development of genotrophs wherein the genomic changes are stable during future nutritional variation. Two flax varieties, Stormont cirrus and Lyral prince (plastic genomes) are the only varieties which have the potential to develop genotrophs. Among all stable changes that occur in flax, the best-understood genome change is the appearance of LIS-1 (Chen et al. 2005). LIS-1 sequence (5.7 kb) is inserted specifically into a single copy target sequence in the plastic genome while growing under low nutrient conditions (Bickel et al. 2012; Dmitriev et al. 2016). Plastic genome becomes homozygous for LIS-1 in the inducing generation, and LIS-1 is a stable part of the plant genome in subsequent generations. The lack of LIS-1 sequence in plastic plant genomes before nutrient stress and lack of LS-1 sequence homology to the transposable elements indicates a novel mechanism of their insertion in response to stress. However, this sequence has been optimized as a marker to assess the stress response of the flax varieties (Bickel et al. 2012). With the availability of increased flax genome sequence, understanding the roles of specific genes that control LIS-1 insertion shall aid in deciphering mechanisms regulated assembly and insertion of LIS-1. Further, identification of loci that control changes in flax will ease understanding and generating genetic variability in flax and also identify potential genotypes at the same time.

5.3 Crop Improvement in Flax for Climate-Smart Traits

One of the most fundamental concepts in achieving sustainability in agriculture is meticulous development, maintenance, and use of plant genetic resources. It is only with such approach objectives like increased crop productivity, and food security can be addressed. In light of climate change, a constant requirement of better crop varieties shall continuously challenge plant breeders to develop varieties that are not

only better adapted to the new ecoclimatic conditions but also have a high yield. Access to available genetic diversity is central to crop improvement and adaption of any crop in accordance with the changing environmental and market needs. There are chiefly three major germplasm resources: (i) commercial varieties, (ii) landraces, and (iii) wild relatives and weedy races. Likewise, flaxseed germplasm is available under these three classes. Globally, out of 46,513 flax accessions reported, only 279 accessions belong to *L. bienne* in gene banks (Diederichsen 2007).

The flax breeding was first started during 1816 in Europe. Initially, the mass selection was the approach used for breeding flax; later pedigree selection was adopted. During 1922, high-yielding fiber flax variety Fleischmann was developed through breeding. Gradually, the individual plant selection from landraces was practiced to identify superior genotypes which enhanced yield compared to local verities. In 1950s, flaxseed breeding and comprehensive agro-botanic variation (Vavilov 1951; Dillman 1953) displayed relevance of germplasm collections of the nineteenth century. During 1970s, increased flax germplasm resources provided an opportunity for breeders to undertake crossbreeding. Conventional breeding methods focused on stabilizing yield under different environments, increasing quantity and quality of fiber and oil, resistant to wilt and rust diseases, and resistance to lodging (Kenaschuk and Rowland 1993; Mpofu and Rashid 2001). Flaxseed germplasm diversity is well documented, curated and is routinely updated (Diederichsen 2007; Diederichsen and Fu 2008; Diederichsen et al. 2013).

Zhuchenko and Rozhmina (2000) illustrated the use of landraces for breeding of fiber flax. These germplasms are frequently referred while breeding traits of abiotic and biotic resistance in flax plants (Zhuchenko and Rozhmina 2000; Brutch 2002). However, wide genetic diversity in flaxseed germplasm offers an enormous potential source for breeding improved traits. In dynamically changing agro-climatic conditions it is imperative for the development of new crops to adapt swiftly concerning biotic (such as diseases) and abiotic stresses (temperature stress, drought stress, etc.). Flax germplasm accessions have been evaluated for disease resistance and environments (Kutuzova 1998; Zhuchenko and Rozhmina 2000; Rashid 2003; Singh 2004) and adaptation to both dry and warm conditions (Diederichsen et al. 2006). Initially, efforts to develop salinity and alkalinity resistant flax crops did not meet with a large-scale success. However, continued efforts of breeders fructified into the development of a salt-tolerant flax cultivar (McHughen 1987; El-Beltagi et al. 2008). Physiological drought is an unfortunate reality of rapid climate change (Gupta 2007).

Exponential growth is seen in understanding plant response to drought stress in many model and crop plants such as wheat, rice, and mustard. However, attempt to develop drought-tolerant flax cultivars are limited and only a few genome-wide studies in flax span this area of research so far (Dash et al. 2014). To develop flax crops tolerant to biotic stress traditional breeding and molecular approaches are in place (Gupta 2007). To aid this research, disease resistance traits have been investigated (Harper 1990; Keijzer and Metz 1992; Kutuzova 1998). High genetic variation for pathogen resistance is evident in wild flax relatives, and they have been found to coevolve. Such coevolved regions from pathogens and pathogen

resistance are called pathogen parks and are excellent sites for studying pathogen resistance in wild crop relatives. This expanding knowledge of disease patterns aids in the collection of resistant germplasm. Nizar and Mulani (2015) identified accessions with traits such as high seed yield, oil content, more capsules per plant, and also earliness to flower. However, the dilemma of linkage drag restricts flax breeders to use this information for breeding programs. To overcome this limitation, concepts of core collection and pre-breeding were introduced with the main purpose of providing access to the germplasm with maximum variation and minimum repetitiveness. The early phase of utilization of germplasm is pre-breeding that filters many characters to give an opportunity to breeders to pick the desirable traits. However, pre-breeding filters desirable characters along with the undesirable.

5.4 Flax Genetic Diversity

The evolutionary potential of crop plants to cope up with environmental changes is referred as Genetic diversity (Sundar 2011). Genetic diversity in flax was first analyzed using morphological parameters (Diederichsen 2001) and isozyme markers (Månsby et al. 2000). Later the DNA-based markers were employed to study flax diversity (Oh et al. 2000). The molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeats (ISSR) techniques were used to assess the genetic diversity among flax cultivars (Spielmeyer et al. 1998b; Everaert et al. 2001; Fu et al. 2002b, 2003; Rajwade et al. 2010; Uysal et al. 2010). Among different molecular markers, simple sequence repeats (SSRs) are known for high polymorphism, reproducibility, reliability, and wide distribution (Powell et al. 1996) making them suitable markers for genetic diversity analysis in crop plants. Cloutier et al. (2009) analyzed genetic diversity among different kinds of Canadian flax cultivars using SSRs. Later a study also used the retrotransposon-based markers to assess diversity among 708 flax accessions. Together all genetic analysis studies indicated presence narrow genetic base in cultivated flax compare to wild relatives and other crop plants, possibly resulting from a domestication process.

The low genetic diversity in flax hindered flax improvement through breeding. Conservation of the existing genetic diversity is important for successful crop improvement program. In this direction, measures were taken by different countries to preserve the existing flax accessions. The Centre for Genetic Resources at Wageningen, the Netherlands, maintains a flax core collection of 84 accessions from 506 fiber flax accessions (Van Soest and Bas 2002). In Canada, approximately 3500 accessions of flax were maintained by Plant Gene Resources of Canada (Diederichsen 2007). The Czech Republic maintains 2081 accessions (Smýkal et al. 2011); over 5282 accessions were collected by the Russian Federation. About 4000 flax accessions were maintained in China (Wang and Song 2008). Food and Agriculture Organization/The European Co-operative Research Network on Flax

and other Bast Plants (FAO/ESCORENA) established the International Flax Database (IFDB). The IFDB is involved in collecting all the European data concerning about 27,000 flax accessions (Pavelek and Lipman 2010).

5.5 Association Mapping Studies

Association mapping studies are significant for the plant as well as animal science since it helps to identify loci governing phenotypic variations and provide the basis for the identification of genes performing a pivotal role in observed variation. In the rapidly expanding genomic era, genome-wide association studies (GWAS) became a more routine exercise. Apparently, because of the early availability of whole genome sequence data in human, GWAS first frequently performed in Humans and then adopted in plant science. After the revolutionary improvements sequencing technology over the last decade, several plant genomes have been sequenced. The whole genome sequence availabilities in plants provided an opportunity to conduct efficient GWAS. Traditionally, mapping of quantitative trait loci (QTLs) in plants is performed using biparental crosses which are very easy to develop. However, biparental crosses have serval limitations. The most critical limitation is lack of allelic diversity since the population is derived from two parents, it deals with genetic variation only within the parental lines. In additions, biparental QTL mapping also suffers from low resolution. In contrast, GWAS provides several benefits over the QTL mapping approach including high resolution and use of genetic diversity existed in the accessible germplasm resources. The success of the GWAS is highly depended on the genetic structure of the population used for the study, phenotypic variation, and number and distribution of the molecular markers. In this regard, recent improvements in molecular marker technology provide several options to identify genome-wide markers and subsequent high-throughput genotyping. Marker system like single nucleotide polymorphism (SNP) has all the features required for the high-throughput genotyping and plenty availability uniformly distributed over the entire genome. There are thousands of published reports describing the use of SNP-based GWAS in several species including animals, microbes, birds, fishes, and plants. In plants, most of the GWAS are performed in model species like Arabidopsis and major food crops like rice, maize, and wheat. Compared to the major food crops and model species limited efforts using GWAS have been made in flax. Being an oilseed crop, Flax breeding program primarily targets the improvement of seed oil content and overall production. Secured production highly depends on climatic conditions imposing several biotic and abiotic stresses. The complex polygenetic mechanism governs plant tolerance and resilience over such stresses. To dissect such highly complex genetic regulation, GWAS is an affordable and efficient option (Fig. 5.1). Identification of significantly associated loci and subsequent candidate gene identification needs an in-depth understanding of the extent of linkage disequilibrium (LD), profile of LD variation across the genome, and at the significantly associated loci and functional annotations of predicted gene models.

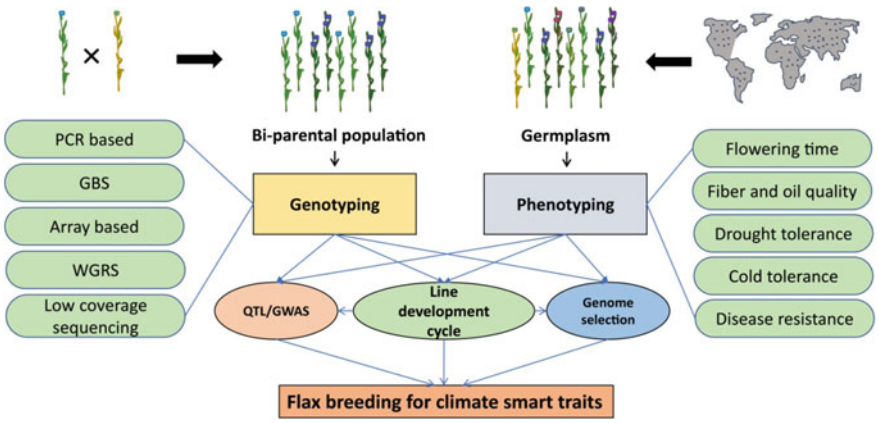

Fig. 5.1 Schematic representation of breeding for climate-smart traits using combined approach of QTL mapping, genome-wide association study (GWAS), and genomic selection (GS)

5.5.1 Extent of Linkage Disequilibrium

The extent of LD is not uniform among plant species, and even it varies drastically across different chromosomes and loci on the same chromosome. Factor affecting LD includes population drift, selection, admixture, inbreeding, and domestication. The LD difference at different genomic loci mostly depends on the recombination rate, transversion, translocation, and chromosomal duplications. Decays in LD have been extensive studies in several plant species. Overall species tend to have higher cross-pollination found to have very less LD decay. For instance, LD decays within 0.3–2 kb have been observed (Remington et al. 2001; Li et al. 2017). However, LD decays after very long segments have been observed in self-pollinated species like soybean (Sonah et al. 2015). In soybean, Sonah et al. (2015) reported varied LD decays at $r^2 < 0.2$ across different chromosomes ranging from 250 kb to 2.5 Mb with an average of 900 kb. Similarly, being a self-pollinated plant species Flax genome also shows relatively slower LD decays than the cross-pollinated species. In a genome-wide study performed by Soto-Cerda et al. (2013) have used genotyping data of 448 microsatellite markers and globally distributed 407 flax accessions. Soto-Cerda et al. (2013) observed genome-wide LD decayed within an average of 1.5 cM. Similarly, (Soto-Cerda et al. 2014a) performed two more genome-wide studies using 460 SSR markers and 390 accessions and 112 SSR and 407 accessions and observed LD decay up to 1.8 cM. However, these three studies have used almost the same genetic material, and the genotypic data and the difference in the LD decay are due to the slightly different number of lines used. Besides having whole genome sequence availability insufficient efforts have been made toward an efficient LD analysis and subsequent GWAS.

5.5.2 *Genetic Loci Identified by Genome-Wide Association Studies*

Improvement in linolenic acid (LIN) content is one of the prime targets for flax breeding programs. Because of the negative correlation of LIN content with palmitic acid (PAL), stearic acid (STE), oleic acid (OLE), and linoleic acid (LIO), LIN improvement without affecting other fatty acids (FA) is challenging for the breeder (Cullis 2007). Through extensive evaluation of diverse germplasm, some of the flax lines with high LIN have been identified, but those are not well adapted in a cropping system (Kenaschuk 2005; Zhang et al. 2016). In this regards, identification of genetic loci governing FA composition has great importance. An attempt to identify genetic loci governing different FA contents including LIN, Soto-Cerda et al. (2014b) has performed GWAS using a core set of 390 flax accessions genotyped with 460 SSR markers. The study has identified nine loci significantly associated with five seed quality traits. The loci with the largest effects have been reported on linkage group (LG) 8 for iodine index, on LG5 for LIN and for total oil content on LG9. The study failed to identify significantly associated loci for PAL and OLE, however, the markers on LG2 (Lu2046) and LG6 (Lu2555) explained around 8 and 4% of the phenotypic variation for PAL and OLE. In this study, several markers associated with different FA found to be collocated that may be the answer for why there is a negative correlation of LIN with other FAs. Interestingly, associated loci mapped by Soto-Cerda et al. (2014b) also collocate with genes predicted to be involved in FA biosynthesis. For instance, the significantly associated locus on LG3 has found to have the acyl-CoA:diacylglycerol acyltransferase A (dgatA) gene in close proximity. The flax dgatA was earlier identified using biparental quantitative trait locus (QTL) mapping approach. Therefore, the loci identified using GWAS approach in flax looks promising. However, the study has used very less number of markers, and therefore needs efforts to advance map and develop more tightly linked marker for the breeding. Apart from this, there is no significant GWAS reported in flax.

5.6 Brief Account of Molecular Mapping of QTLs

Genetic mapping was first demonstrated by Morgan (1911). In early nineteen century, while Morgan was working with mutant traits fruit flies, he observed co-segregation of Mendelian traits. Based on segregation data he could predict order and genetic distance among different traits. In animals and plant species, genetic mapping has been performed extensively after the discovery of DNA-based markers. There were very few efforts of genetic linkage mapping based on isozymes, but because of the limited availability and lengthy procedure, it becomes out

of use after the arrival of the DNA markers. After the first report of restriction fragment length polymorphism (RFLP) by Botstein et al. (1980) genetic mapping efforts increased, but still, those were suffering from the efficient analysis. In the early 1990s, several improvements in mapping analysis have been made that resulted into the availability of first genetic map covering the entire set of chromosome started reporting in human and some of the plant species.

In 1988, the first linkage map developed using the RFLP marker has been used for the QTL mapping in plants (Paterson et al. 1988). The successful demonstration of genetic linkage mapping and subsequent QTL identification by Paterson et al. (1988) paved the way for advanced QTL mapping. Apart from the RFLP, RAPD was the choice of the marker in the early 1990s. History of inheritance studies and genetic mapping flax is dated back to 1930 when Henry (1930) studied the inheritance of rust resistance in ottawa770B and Bombay cultivars. Another classic study by Flor (1965) in the early 1960s used tree way crosses to predict genetic distance between rust resistance genes. However, these mapping efforts were based on morphological characters. After the availability of DNA marker techniques, in 2000, a first genetic linkage map of Flax has been developed using RFLP and RAPD markers (Oh et al. 2000). The genetic linkage map covering 1000 cM developed by the Oh et al. (2000) have been composed of 15 distinct linkage groups harboring well distributed 94 markers. The study has used the F_2 mapping population developed from the cross between the cross, CI1303, and Stormont Cirrus. Apart from RAPD and RFLP, the map has a sequence tagged site (STS) marker. Subsequently, several studies have identified QTLs for different traits in flax. Cloutier et al. (2011) developed a genetic linkage map using 114 SSR markers and five SNPs using a DH population of 78 individuals obtained from a crossing SP2047 (yellow-seeded with low linolenic acid) and UGG5-5 (brown seeded with high linolenic acid). QTL analysis detected two major QTLs each for LIO, LIN, and iodine value (IOD), and one major QTL for palmitic acid was identified (Table 5.1). A mutant allele of fad3A found in QTL on linkage group 7 was accounted for approximately 34, 25, and 29% of the phenotypic variation observed in population for these LIO, LIN, and IOD, respectively. The QTL identified on linkage group 16 contributed approximately 20, 25, and 13% of the phenotypic variation for the same traits, respectively. For palmitic acid, the QTL QPal.crc-LG9 accounted for 42% of the phenotypic variation. In a similar study, Kumar et al. (2015) constructed a genetic map using 329 SNPs and 362 SSRs using a recombinant inbred line (RIL) population of 243 individuals from a cross between varieties CDC Bethune and Macbeth. A total of 20 QTLs were identified for 14 different traits (Table 5.1). Three QTL each for STE and OLE, two QTL each for iodine value and LIO and one each for LIN, PAL, oil content, cell wall, seed protein, thousand-seed weight, seeds per boll, straw weight, yield, and days to maturity were identified. Analysis of the QTL led to the identification of candidate genes involved in yield component traits, cell wall synthesis, fiber formation, and fatty acid biosynthesis.

Table 5.1 List significant QTLs identified for different traits in flax (*L. usitatissimum*)

Trait			LG	Marker	QTL	Mapping parent 1	Mapping parent 2	References
Fatty acid composition	Palmitic acid	PAL	7	Lu402/ Lu7-1820805	*QPal.BM.crc-LG7*	CDC Bethune	Macbeth	Kumar et al. (2015)
			9	Lu741-Lu675	*QPal.crc-LG9*	SP2047	UGG5-5	Cloutier et al. (2011)
	Stearic acid	STE	1	Lu2183a/ Lu1-2670961	*QSte.BM.crc-LG1*	CDC Bethune	Macbeth	Kumar et al. (2015)
			3	Lu3-8415336/ Lu2164	*QSte.BM.crc-LG3*	CDC Bethune	Macbeth	Kumar et al. (2015)
			11	Lu2128/ Lu11-19000928	*QSte.BM.crc-LG11*	CDC Bethune	Macbeth	Kumar et al. (2015)
	Oleic acid	OLE	3	Lu3-3979616/ Lu3-5950394	*QOle.BM.crc-LG3-1*	CDC Bethune	Macbeth	Kumar et al. (2015)
			3	Lu658/Lu3150	*QOle.BM.crc-LG3-2*	CDC Bethune	Macbeth	Kumar et al. (2015)
			5	Lu5-9728492	*QOle.BM.crc-LG5*	CDC Bethune	Macbeth	Kumar et al. (2015)
	Linoleic acid	LIO	7	FAD3A/Lu44E4	*QLio.crc-LG7*	SP2047	UGG5-5	Cloutier et al. (2011)
			16	Lu206-Lu765B	*QLio.crc-LG16*	SP2047	UGG5-5	Cloutier et al. (2011)
			3	Lu3-3979616/ Lu3-5950394	*QLio.BM.crc-LG3*	CDC Bethune	Macbeth	Kumar et al. (2015)
			6	Lu2545	*QLio.BM.crc-LG6*	CDC Bethune	Macbeth	Kumar et al. (2015)

(continued)

Table 5.1 (continued)

Trait			LG	Marker	QTL	Mapping parent 1	Mapping parent 2	References
	Linolenic acid	LIN	7	FAD3A/Lu44E4	*QLio.crc-LG7*	SP2047	UGG5-5	Cloutier et al. (2011)
			16	Lu206-Lu765B	*QLio.crc-LG16*	SP2047	UGG5-5	Cloutier et al. (2011)
			5	Lu5-9728492	*QLin.BM.crc-LG5*	CDC Bethune	Macbeth	Kumar et al. (2015)
Iodine value		IOD	7	FAD3A-Lu44E4	*QIod.crc-LG7*	SP2047	UGG5-5	Cloutier et al. (2011)
			16	Lu206-Lu765B	*QIod.crc-LG16*	SP2047	UGG5-5	Cloutier et al. (2011)
			5	Lu5-9728492	*QIod.BM.crc-LG5*	CDC Bethune	Macbeth	Kumar et al. (2015)
			6	Lu6-2260313/ Lu6-2330258	*QIod.BM.crc-LG6*	CDC Bethune	Macbeth	Kumar et al. (2015)
Oil content		OIL	8	Lu8-22516618/ Lu3189	*QOil.BM.crc-LG8*	CDC Bethune	Macbeth	Kumar et al. (2015)
Seed protein		PRO	11	Lu11-21716266/ Lu52	*QPro.BM.crc-LG11*	CDC Bethune	Macbeth	Kumar et al. (2015)
Fiber components	Cell wall	CW	4	Lu2031	*QCw.BM.crc-LG4*	CDC Bethune	Macbeth	Kumar et al. (2015)
	Straw weight	SW	4	Lu2031	*QSw.BM.crc-LG4*	CDC Bethune	Macbeth	Kumar et al. (2015)
Thousand-seed weight		TSW	15	Lu2010a/Lu2001	*QTsw.BM.crc-LG15*	CDC Bethune	Macbeth	Kumar et al. (2015)

(continued)

Table 5.1 (continued)

Trait			LG	Marker	QTL	Mapping parent 1	Mapping parent 2	References
Seeds per boll		SPB	4	Lu2031	*QSpb.BM.crc-LG4*	CDC Bethune	Macbeth	Kumar et al. (2015)
Seed yield		YLD	4	Lu2031	*QYld.BM.crc-LG4*	CDC Bethune	Macbeth	Kumar et al. (2015)
Days to maturity		DM	4	Lu2031	*QDm.BM.crc-LG4*	CDC Bethune	Macbeth	Kumar et al. (2015)
Resistance to powdery mildew			1	Lu2698-Lu2712	*QPM-crc-LG1*	NorMan	Linda	Asgarinia et al. 2013
			7	Lu2810-Lu2832	*QPM-crc-LG7*			
			9	Lu1125a-Lu932	*QPM-crc-LG9*			
Colour-L* (brightness)			22	Colour-Lu178	*QL*.crc-LG22*	SP2047	UGG5-5	Cloutier et al. (2011)
Colour-B* (yellow-blue chromacity)			22	Colour-Lu178	*QL*.crc-LG22*	SP2047	UGG5-5	Cloutier et al. (2011)

5.7 Genomic Selection

Genomic selection (GS) is a breeding method that efficiently explores information of phenotype and genetic markers to accelerate selection and breeding line development. The GS workflow includes six major steps (Fig. 5.2): (1) Selection of diverse genotypes to be used as training population (2) precise phenotyping of training population at multiple environments (3) genotyping of training population using marker covering entire genome; (4) developing statistical model by using the phenotypic and genotypic data of training population; (5) genotyping of breeding population (test individuals) with the markers used for optimized statistical model (GS model); and (6) finally employing the GS model for the estimation of genomic estimated breeding values (GEBVs) to proceed selection of individual lines. The GS method has several advantages over the conventional as well as marker-assisted breeding since it deals with the several minor effect loci governing the trait. The GS method becomes more promising with the recent technological developments in sequencing and subsequent genotyping platforms. Because of the reduced genotyping and phenotyping cost, GS become more convenient to accelerate breeding (Table 5.2).

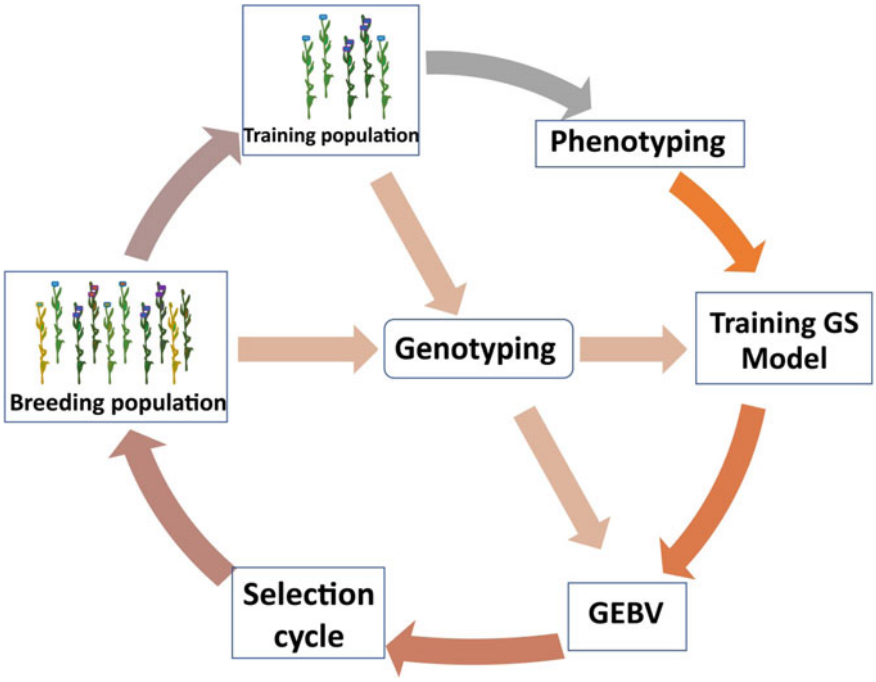

Fig. 5.2 Major steps involved in achieving maximum breeding gain in short time by using genomic estimated breeding value (GEBV) through genomic selection (GS) method

Table 5.2 Details of publicly available SNP genotyping facilities available at various institutes, expected cost, and time required

Genotyping platform	SNPs	Time	Price/ sample	Facility available
GBS (Hi-seq)-96 plex	30–80 K	2–6 months	$38	Cornell University, Laval University
GBS (Hi-seq)-384 plex	10–40 K	2–6 months	$25	Cornell University, Laval University
GBS (Ion-torrent)-96 plex	10–20 K	1 month	$35	Laval University
GBS (Hi-seq) 0.2×	1 M	2 months	$65	BGI
nextRAD (3×)	60 K	1 month	$55	SNPsaurus (nextRAD services)
Infinium 6 K	6 K	1 month	$80	IRRI
BeadXpress	384	1 month	$40	UC Davis
iScan	1536	1 month	$80	UC Davis
Fluidigm 96 × 96	96	1-month	$20	IRRI

In flax, besides having well-annotated genome and plenty of marker resource, relatively very less efforts have been made toward utilization of GS. Use of phenotyping and genotyping data previously developed for the QTL mapping and GWAS can be efficiently used as training population to optimize GS model (Deshmukh et al. 2014; Chaudhary et al. 2015). Use of QTL mapping, GWAS and GS all together have been proposed as cost and time efficient method for the identification of genes and simultaneous variety development particularly for the stress tolerance (Deshmukh et al. 2014; Vuong et al. 2015). In line of the principle, You et al. (2016) have used three different biparental populations used for the QTL mapping in flax: (1) the first population (recombinant inbred line, RIL) developed by using high-yielding Canadian linseed verities; (2) the second population (RIL) derived from a cross between E1747 (a low omega-3 fatty acids (LIN)) and Viking (fiber flax cultivar); and (3) the third population (doubled haploid) derived from a cross between breeding line SP2047 (contains only 2–3% LIN) and UGG5-5 (63–66% LIN). These three populations served as excellent material as a training population to optimize GS model in flax. You et al. (2016) evaluated three different GS models, namely, Bayesian LASSO (BL), ridge regression best linear unbiased prediction (RR-BLUP), and Bayesian ridge regression (BRR). They have performed GS for seed yield and oil content, linoleic and linolenic acid content and iodine value. You et al. (2016) observed similar accuracy and phenotypic selection however a higher coefficient of determination (R2) was observed with BRR as compared to RR-BLUP or BL. Varied prediction accuracy of GS and the efficiency of GS relative to phenotypic selection have been observed for different traits that seem to be affected by the genetic variation present in the populations. The You et al. (2016) study provides the first validation of GS in flax demonstrating an increase in genetic gain per unit time in flax breeding cycle. More extensive GS studies are anticipated in the near future with increases genomic resources in flax.

5.8 Genomic Resources for Flax

Presently, a huge amount of genomic resource is available at National Center for Biotechnology Information (NCBI) database for different oilseed crops including flax (Table 5.3). Genomic resource of flax comprises expressed sequence tags (ESTs), Genome Survey Sequence (GSS), genome, and transcriptome data. Additionally, several DNA-based markers have been developed in flax to analyze diversity and assist in molecular breeding. The diversity of flax genotypes were initially assessed by using morphological parameters (Diederichsen 2001) and isozyme markers (Månsby et al. 2000). Subsequently, several DNA-based markers including RAPD, RFLP, AFLP, ISSR, and SSR have been developed (Spielmeyer et al. 1998b; Oh et al. 2000; Everaert et al. 2001; Fu et al. 2002b, 2003; Diederichsen et al. 2006; Cloutier et al. 2009). The application of DNA-based markers to analyze flax diversity was first reported by Oh et al. 2000 through RAPD and RFLP techniques. In another study, RAPD-based diversity analysis of 61 Canadian cultivars and landraces of flax showed distinction among fiber and oil types, suggesting their distinct genetic makeups. Among different molecular markers, RAPD and AFLP showed less cross applicability, while markers such as RFLP and AFLP are quite labor intensive. Gradually the SSRs also known as microsatellites gained popularity because of their abundance, wide distribution, codominant, and highly polymorphic nature (Powell et al. 1996). SSR markers are derived from either genomic (nuclear or organel) sequences or ESTs (Bhati et al. 2010; Tomar et al. 2014) . Several SSR markers have been reported in flax, for instance, 23 (Roose-Amsaleg et al. 2006), 35 (Deng et al. 2010), 60 (Soto-Cerda et al. 2011a), 38 (Deng et al. 2011), 9 (Kale et al. 2012), 20 (Rachinskaya et al. 2011), 42 (Bickel et al. 2011), 248 (Cloutier et al. 2009), and 23 (Soto-Cerda et al. 2011b). ISSR primers from University of British Columbia (UBC) collection have been used widely in genetic diversity studies of *L. usitatissimum* L. or its wild progenitor L. bienne Mill. (Chen et al. 1998; Wiesnerova and Wiesner 2004; Rajwade et al. 2010; Uysal et al. 2010).

5.9 Flax Genome Sequencing and Genomics

After the genome sequencing of the model plants, many crop genomes were sequenced. Most of the genome assemblies are in draft stage and extensive work is going on to close the gaps and re-sequence. Introduction of next-generation DNA sequencing technologies hastens the genome sequencing process. In addition to the genome sequence, expression and transcriptome resources were generated in many crop plants to generate more information to augment crop breeding. Considering the importance of flax an effort is made to develop genomic tools needed for molecular breeding. Hence a project called Total Utilization Flax GENomics (TUFGEN) was undertaken. The physical map of flax containing 416 contigs covering ~368 Mb, assembled from 32,025 fingerprints. Bacterial artificial

Table 5.3 The genomic resources of the different oilseed crop available at NCBI database

Database name[a]	*L. usitatissimum*	*C. tinctorius*	*H. annuus*	*R. communis*	*B. juncea*	*S. indicum*	*G. Max*
Nucleotide	12,949	1258	139,884	86,923	155,643	209,033	217,285
Nucleotide EST	286,856	41,584	133,718	62,629	5483	44,905	1461,724
Nucleotide GSS	80,339	#N/A	662	#N/A	33	79	553,482
Protein	2811	539	138,530	60,228	1391	35,785	198,980
Genome	1	1	1	1	1	1	1
Popset	118	56	382	98	41	24	186
GEO datasets	114	5	554	15	75	#N/A	7024
PubMed central	849	453	1940	3336	1166	537	9604
SRA experiments	425	16	1382	27	239	1394	3926
Assembly	1	1	1	2	–	3	6
Bio Project	29	9	62	15	26	27	447
Bio Sample	340	25	850	40	243	1461	4449

[a]The database was accessed on November 2017

chromosome (BAC) end sequencing resulted in covering 54.6 Mb representing 8–14.8% of the genome. The genome-wide physical map of flax provided a framework for building whole genome shotgun assembly and development of molecular markers. Recently, the genome of flax variety CDC Bethune was sequenced using a whole genome shotgun (WGS) sequencing strategy on the Illumina sequencing platform (Wang et al. 2012). The flax genome size was estimated to be ~373 Mb based on flow cytometry. A de novo assembly of contigs comprised of 302 Mb of nonredundant sequence representing an estimated covering around 81% genome size. A total of 43,384 protein-coding genes were predicted in the assembled genome sequence, and 93% of known flax ESTs aligned to these predicted genes, showing excellent coverage and accuracy of the sequence at the gene level.

5.10 Flax Genetic Engineering

The flax has not got much attention in terms of genetic manipulation compared to other crop plants. However, flax is amenable to genetic transformation and some important agronomic traits, such as herbicide tolerances, fiber quality, oil quality improvement for flax have been undertaken.

5.10.1 Herbicide Tolerance

Herbicide tolerance is the first trait engineered into flax. The flax was engineered to withstand the herbicide glyphosate (Roundup), which inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in higher plants. Glyphosate-insensitive EPSP enzyme from Petunia hybrida was transformed into flax by *Agrobacterium*-mediated method of transformation to develop transgenic flax lines resistant to 5 mM glyphosate (Jordan and McHughen 1988). Subsequently, several other genes resistant to herbicides such as chlorsulfuron (Haughn et al. 1988) and glufosinate ammonium (McHughen and Holm 1995) were introduced into flax. The phosphinothricin acetyltransferase (PAT) gene from Streptomyces viridochromo was overexpressed under the control of cauliflower mosaic virus (CaMV) 35S promoter. The introduction of the PAT enzyme in flax plants imparted resistance to glufosinate ammonium herbicide (McHughen and Holm 1995).

5.10.2 Fiber Quality

Efforts were undertaken to improve fiber quality by genetic engineering approach. Wróbel et al. (2004) transformed flax with the three genes, 3-ketothiolase, acetoacetyl-CoA reductase, and polyhydroxybutyrate (PHB) synthase enzymes from *Ralstonia eutropha*. The transgenic plants have significantly high PHB than

nontransgenic flax and displayed significant modifications in stem mechanical properties. The lignin, pectin, and hemicellulose content were decreased in GE plants while the elastic properties of the fiber increased substantially (Wróbel-Kwiatkowska et al. 2007). Genetic modification overproduction of flavonoids in flax increased antioxidative properties of flax fiber, which increased its biomedical applications (Żuk et al. 2011; Zuk et al. 2012). The gene encoding a potato β-1,3-glucanase was transformed into flax resulted in decreased levels of lignin and increased levels of pectins and phenolics thereby improving the mechanical properties of the fiber (Wojtasik et al. 2013).

5.10.3 Oil Quality

Initially, attempts using genes which naturally synthesize long-chain PUFA by elongation and desaturation reactions were employed to improve oil quality in flax. Seed-specific expression of cDNAs encoding a fatty acid desaturase and a Δ6 fatty acid elongase (Abbadi et al. 2004) resulted in significant Δ6-desaturation of ALA to form stearidonic acid and further elongation and Δ5-desaturation to eicosapentaenoic acid (EPA). The efforts to improve oil quality of flax efforts were made to increase the antioxidant capacity of to protect PUFA from oxidation (Lorenc-Kukuła et al. 2005; Żuk et al. 2011; Zuk et al. 2012). Similarly, co-suppression of chalcone synthase encoding gene in flax led to the partial redirection of substrates from flavonoid biosynthesis to other phenylpropanoid pathway (Zuk et al. 2012). This resulted significantly increased antioxidant capacity in seeds from transgenic lines and the oil stability was increased.

5.10.4 Abiotic Stress Tolerance

Many crop plants are modified genetically to withstand abiotic stress conditions. The modification involved several candidate genes comprising functional genes and regulatory genes (Shinozaki et al. 2003). Functional genes are directly involved in the protection of cells from stresses, which includes those encoding water channel proteins, ion transporter, late embryogenesis abundant (LEA) protein and heat shock protein (HSP). The regulatory genes include those encoding transcription factors, protein phosphatases, and protein kinases which regulate signal transduction and gene expression during stress responses (Wang et al. 2016). Till date, such studies to genetically engineer flax for abiotic stress tolerance have not been reported. When plants are exposed to abiotic stress, initially the activity of the transporters gets affected resulting in hindered physiological activity of the plants (Young et al. 2004). Among plant transporters, aquaporins play an important role in transporting water and other small solutes such as urea, ammonia, glycerol, silicic acid, boric acid, CO_2, and H_2O_2 (Tyerman et al. 2002; Maurel et al. 2008; Bienert

and Chaumont 2014; Deshmukh et al. 2016). Recently, aquaporin (AQP) encoding genes were used successfully to demonstrate their role in combating abiotic stress in several plant species (Table 5.4). Considering the importance, aquaporins have been identified in different crop plants including flax (Deshmukh et al. 2013; Deokar and Tar'an 2016; Song et al. 2016; Kadam et al. 2017; Shivaraj et al. 2017a, b; Sonah et al. 2017). The genome-wide analysis of flax has revealed the presence of 51 AQPs belonging to different subfamilies. The better characterization of these AQPs in flax will provide an opportunity to develop climate-smart abiotic stress-tolerant cultivars.

Table 5.4 List of selected studies describing engineering of aquaporin encoding genes to enhance abiotic stress tolerance in different plant species

S. No.	AQP	Species	Transgenic plants	Effect of transgene	References
Drought tolerance					
1	*VfPIP1*	*Vicia faba*	Arabidopsis	Enhanced drought tolerance	Cui et al. (2008)
2	PIP1b	Arabidopsis	Tobacco	Under normal growth condition increased vigor but no effect under salt stress and deleterious effect during drought stress	Aharon et al. (2003)
3	*TaAQP7*	Wheat	Tobacco	Enhanced drought tolerance	Zhou et al. (2012)
4	*MaPIP1;1*	Banana	Arabidopsis	Enhanced tolerance to drought	Xu et al. (2014)
5	PgTIP1	*Panax ginseng*	Arabidopsis	Beneficial effect on salt stress tolerance, drought tolerance, and cold acclimation ability in transgenic Arabidopsis plants	Peng et al. (2007)
6	*TaTIP2;2*	Wheat	Arabidopsis	Enhanced tolerance to salt and drought	Xu et al. (2013)
7	TsTIP1;2	Thellungiella salsuginea	Arabidopsis	Enhanced tolerance to salt, drought, and oxidative stress	Wang et al. (2014)
Salt tolerance					
8	HvPIP2;1	Barley	Rice	Raised salt sensitivity in transgenic rice plants	Katsuhara et al. (2003)
9	*TaNIP*	Wheat	Arabidopsis	Enhanced salt tolerance	Gao et al. (2010)

(continued)

Table 5.4 (continued)

S. No.	AQP	Species	Transgenic plants	Effect of transgene	References
10	*Td*PIP1;1, *Td*PIP2;1	Durum wheat	Tobacco	Increased tolerance phenotype toward osmotic and salinity stress	Ayadi et al. (2011)
11	OsPIP2;1	Rice	Rice	Increased rice seed yield, salt resistance, root hydraulic conductivity, and seed germination rate	Liu et al. (2013)
12	OsPIP1-1, OsPIP2-2	Rice	Arabidopsis	Enhanced tolerance to salt and drought	Guo et al. (2006)
13	CfPIP2;1	Cucumber	Arabidopsis	Enhanced tolerance to salt and drought	Jang et al. (2007b)
14	*TaAQP8*	Wheat	Tobacco	Enhanced salt tolerance	Hu et al. (2012)
Other physiological traits					
15	OsPIP2;4, OsPIP2;6 and OsPIP2;7	Rice	Arabidopsis	Enhanced arsenite tolerance and higher biomass accumulation	Mosa et al. (2012)
16	HmVALT, HmPALT1	Hydrangea macrophylla	Arabidopsis	Enhanced aluminum tolerance	Negishi et al. (2012)
17	PIP1;4, PIP2;5	Arabidopsis	Arabidopsis and Tobacco	Enhanced cold tolerance and increased susceptibility to drought	Jang et al. (2007a)
18	*SlTIP2;2*	Tomato	Arabidopsis and tomato	Significant increase in fruit yield, harvest index, and plant mass relative to the control under both normal and water-stress conditions	Sade et al. (2009)
19	NtAQP1	Tobacco	Tobacco	Enhanced photosynthesis due to different leaf mesophyll conductances to CO_2	Flexas et al. (2006)
20	NtAQP1	Tobacco	Tobacco	Lowered the CO_2 permeability of the inner chloroplast membrane	Uehlein et al. (2008)
21	*RcPIP2;1* and *RcPIP2;2*,	*Rhododendron catawbiense*	Arabidopsis	Freezing tolerance and cold acclimation	Bots et al. (2005)

(continued)

Table 5.4 (continued)

S. No.	AQP	Species	Transgenic plants	Effect of transgene	References
22	NtAQP1	Tobacco	Tobacco	Increases membrane permeability for CO2 and water, and increases leaf growth	Uehlein et al. (2003)
23	OsNIP2;1 (Lsi1)	Rice	Rice	Enhanced silicon uptake that resulted in increased yield	Ma et al. (2006)
24	PgTIP1	*Panax ginseng*	Arabidopsis	Enhanced seed size and seed mass plus greatly increased the growth rate	Lin et al. (2007)
25	PIP2;5 and PIP1;4	Arabidopsis	Arabidopsis	Responded to high irradiance with an increase in transpiration rates	Lee et al. (2009)
26	HvPIP2;1	Barley	Rice	Increased CO_2 assimilation	Katsuhara and Hanba (2008)
27	PIP2;2	Arabidopsis	Arabidopsis	Enhanced water uptake	Javot et al. (2003)

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Chapter 6
Breeding *Brassica juncea* and *B. rapa* for Sustainable Oilseed Production in the Changing Climate: Progress and Prospects

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Abstract The uncertainties of climatic variability and global warming are leading to rising concerns towards ensuring global food security of an expanding population. Unfavorable climatic conditions, like extremes of temperature, drought, flood, and salinity, in addition to the elevated greenhouse gases adversely affect the physiology, and accordingly the quantitative and qualitative characteristics of plants. Mustard (*Brassica juncea*) and rape (*Brassica rapa*), the two important oilseed crops of the Indian subcontinent, are also cultivated in Eastern Europe, Russia, China, and Canada. These oilseed crops are affected by various biotic and abiotic stress during different growth and developmental stages, that severely influences agricultural productivity. Extensive breeding efforts toward the development of Brassica cultivars that can resist these climatic variabilities are under various stages of progress. The Brassica germplasm and the wild relatives of *B. juncea* and *B. rapa,* which constitute important genetic stocks, are also being utilized in these breeding programs. An integrated approach is required that will study plant–insect pest and disease–climate interactions for conceiving future strategies to develop disease-, insect-resistant, and climate-resilient plant varieties. Developing mustard varieties, efficient in the utilization of soil nutrients, are also required for improving productivity in impoverished soils and for better uptake/utilization of nutrients in soils rich in resources. Future research in oilseed mustard

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and rape should, therefore, involve examining the influence of climate-smart traits on yield/production in targeted environments, so that climate-resilient cultivars adapted to climate change conditions could be developed. This chapter summarizes the advances in breeding of climate-smart traits such as, tolerance to drought, heat, salinity, flooding and frost, and efficient nutrient utilization, in oilseed mustard and rape, that could assist in the genomic designing for climate-smart crops.

Keywords Climate resilient · *Brassica juncea* · *Brassica rapa* · Molecular markers · Genetic mapping · Quantitative trait loci · Genome sequencing

6.1 Challenges, Priorities, and Prospects of Recent Plant Breeding

6.1.1 Food, Nutrition, Energy, and Environment Security

Utilization of genetic resources and genomic approaches to address "food, nutrition, energy and environment (FNEE) security" is the pivotal theme of current plant breeding endeavors (Kole 2017). Climate change imposes a major challenge towards achieving a hunger-free world for the ever-increasing world population. However, the impact of the changing climatic conditions on the future of food availability are still poorly understood. The World Food Summit (1996) defined food security as the state of affairs when there is complete physical, social, and economic access to adequate, safe, and nutritious food which meets the dietary needs and food preferences of each and every person for an active and healthy life (FAO 1996). The changing climate is impacting food security by influencing crops, livestock, forestry, fisheries, and aquaculture production besides causing serious social and economic problems in the form of reduced incomes, decaying livelihoods, trade disruption, and adverse health impacts (FAO 2016). Efforts towards the judicious use of natural resources such as land, water, soil nutrients, and genetic resources (FAO 2013b), and retaining the natural ecosystems, would be crucial for sustainable production to achieve food security.

Oilseed Brassicas, such as rape (*B. rapa* L.), mustard [*B. juncea* (L.) Czern. & Cosson], rapeseed (*B. napus* L.), and Ethiopian mustard (*B. carinata* A. Braun.) (Raymer 2002; Rakow 2004) comprises the second largest oilseed crops group, following soybean (*Glycine max* L. Merr.) in the world (USDA 2018). Of these species, *B. juncea*, is important oilseed crop in Asia, while *B. napus*, is mostly grown in Europe and Canada. During 2016–17, the total production of oilseeds was 576.96 million metric tons, of which 71.33 million metric tons were contributed by rapeseed and mustard (USDA 2018). There is a large gap in the production and demand of edible oilseeds, leading to a growing dependency on oil import. Mustard and oilseed rape are grown largely on unirrigated soils, and therefore experience extremes of drought and cold/frost stress during the life cycle, especially in the

reproductive stages. Therefore, to reduce the huge gap between the production and demand, there is an urgent need for breeding climate-resilient, high-yielding Brassica oilseed varieties through improvised crop breeding endeavors and effective utilization of the diverse germplasm available.

6.2 Prioritizing Climate-Smart (CS) Traits in Mustard and Rape

It is predicted that by 2050, World's population will increase by one-third and majority of this, estimated to be over 2 billion, will be in the developing countries. Considering the present income and consumption trends, agricultural production has to be increased by 60% in 2050 to meet the requirements of the increasing population. As a consequence, the production of vegetable oils also needs a large quantum jump. However, the changing climatic conditions such as increased temperatures, drought, and CO_2 levels, are significantly influencing the yield and quality of oilseed Brassica crops. Therefore, several studies are being focused on the effects of high temperature and drought stress and increased CO_2 levels on the growth, development, quality and overall oilseed Brassica production (Canvin 1965; Mailer and Cornish 1987; Hocking and Stapper 1993; Walton et al. 1999; Aksouh et al. 2001; Qaderi et al. 2006), the details of which are highlighted in the following sections. Considerable success has been achieved with traditional plant breeding methods however, the production of improved varieties through these methods is a time-consuming and labor-intensive process, involving identification of segregant offsprings from crosses between parents with contrasting genotypes for the desired traits. These breeding endeavors have hugely benefited from the development of molecular markers, that enabled identification of markers linked with the gene of interest and facilitated enhanced precision in the transfer of desired traits from one genetic background into another. The recent advances in high-throughput genome sequencing technologies have greatly advanced precision breeding of crop plants for desired traits through genomics-aided selection. The inability to utilize sexual hybridization for tranfer of desirable alleles through interspecific and intergeneric crosses can be overcome by genetic engineering technology, thereby enabling development of crop varieties resistant to various pests, diseases, and erratic climatic factors (Kaur and Murphy 2012; Atif et al. 2013). Further, the genetic manipulation also excludes chances of linkage drag, and could, therefore, accelerate crop improvement endeavors. Brassica oilseed crops, especially Indian mustard and oilseed *B. rapa*, experience a wide variety of erratic climatic conditions during their growing season. Therefore, breeding climate-smart oilseed mustard and *B. rapa* crop is essential for ensuring sustainable production. This can be achieved by screening a large number of mustard and oilseed rapa germplasm for climate and other biotic stress resistant traits and utilizing them in

breeding program. The recently developed advanced technologies such as genomics-aided selection and genetic engineering technologies could be potent tools for alleviating the dejected agricultural productivity situation, thereby contributing towards reduction of poverty and ensuring better food security in the developing world (Qaim 2010; Sainger et al. 2015).

6.2.1 Flowering and Bolting Time

The onset of flowering signifies the shift from vegetative to reproductive phase in the life cycle of the plant. The timing of the onset of flowering is a major component of crop adaptation and grain yield in crop Brassicas. Early bolting or a complete inhibition of flowering interferes with the vegetative growth and can, therefore, severely decrease yield. Early flowering genotypes that quickly complete their reproductive phase are better adapted to drought since their short growth duration allows them to evade the drought conditions (Araus et al. 2002). The genotype of a plant interacts with the environment and determines the crop duration, and the potential to evade thermal and drought stress (Dingkuhn and Asch 1999).

Optimum sowing time has a significant role in determining the genetic potential of the plant as it promotes plant growth in most suitable conditions of temperature, light, humidity, and rainfall (Devi and Sharma 2017). It also determines the duration of the growing season and hence the yield. Any shift in the sowing time, exposes the crop to different environmental conditions, and thus modifies the duration of phenological phases, including flowering (Sharghi et al. 2011). Delayed planting and suboptimal or adverse weather conditions, especially during the onset of flowering have detrimental effects on the flower bud development. Delayed planting results in reduced pod maturity duration which adversely affects the pod (number and growth) and seed (in terms of both number and weight) formation, thereby reducing the overall plant yield. Temperature and photoperiod have been reported as the two most important environmental factors affecting the phenological development (Mendham et al. 1981; Zhang et al. 2016).

6.2.2 Heat Tolerance

Temperature significantly affects plant growth and development. All plants require certain amount of heat units during growth periods and the duration to receive these, depends upon the climatic conditions. Exposure to heat at seed filling stage directly impacts the yield, as accelerated senescence hampers seed setting, ultimately leading to weight reduction (Siddique et al. 1999). Incumbent heat stress forces plants to divert its resources at the expense of photosynthesis, which hampers normal growth and development (Hayat et al. 2009).

Reckoning to climate change weather extremes are increased during crop growing season (Qian et al. 2010) and warmer climate in the future is likely to be unfavorable to winter crops such as mustard (Qian et al. 2018). Studies show that plant development in Brassica species responds significantly to the environment, especially to increased temperatures and heat stress during flowering, leading to reduction in seed yields (Morrison et al. 1989; Nanda et al. 1996). High temperature stress results in flower abortion, reduction in siliques per plant, less seeds per silique and also reduced seed size thereby leading to low oil content and yield loss (Hall 1992; Rao et al. 1992). High temperature affects stress damage recovery and the chances to cope up decrease with progression of developmental stages, with the worst effects during pod development and oil filling stages. Indian mustard shows significant sensitivity to heat stress during early seedling stages. Although, early sowing has many advantages, the early sown crop encounters high temperature stress, which results in significant yield loss. Early sowing of mustard and an early harvest, assists in avoiding disease infestation and pest attack that normally coincides with the flowering stages and shattering of siliques at the time of harvest when the crop encounters high temperature. The short duration mustard varieties are efficient at evading the temperature stress exposure at terminal stages (Sahni et al. 2013), and hence are highly suitable for multiple cropping (Sharma and Sardana 2013).

Critical flowering period in canola and mustard is often juxtaposed with high temperatures of semiarid prairie. Higher temperatures (mean temperature) along with high wind velocity and water being a scarce commodity in the region further aggravate heat stress conditions in comparison to the traditional growing areas. However, mustard is a well-adapted oilseed crop for the semiarid prairie.

6.2.3 Cold Tolerance

Subzero temperatures prevail in two-third of the world's entire landmass (Larcher 2001). However, the success in achieving cold tolerance in plants is still limited to classical plant breeding or gene transfer techniques, both of which are slow, owing to the fact that cold tolerance is not a single gene governed trait, rather it has been recognized as a syndrome manifesting interplay of a complex network of cellular processes such as biomembrane's fluidity, synthesis, and aggregation of low and high molecular weight cryoprotectants (Beck et al. 1995; Hansen et al. 1997; Steponkus et al. 1998; Nanjo et al. 1999; Fowler and Thomashow 2002). The various tissues of a plant responds differently to cold stress and thereby complicate our understanding of this trait (Sakai and Larcher 1987). Low temperatures either impose stress on a plant alone, or in many cases cell sap freezing can lead to dehydration of the cells and tissues. This threat from winter cold not only results in ice formation in plant tissues, but also develops from freezing of water in and on the ground and by the load and duration of the snow cover (Sakai and Larcher 1987). Cold stress affects the flower and grain filling stage by impacting pollen viability,

grain development, and anthesis time. Low temperature affects crucial stages of reproductive cycles such as pollination, fertilization, gametogenesis, and embryogenesis (Singh et al. 2008). Furthermore, the agronomic and yield attributes of crops are affected as plant shows wilting of leaves, bleaching, or death in extreme cases of colds/frost.

6.2.4 Drought Tolerance

Drought can be defined as diminished water availability in plant's root zone that ceases down to sustain maximum growth and productivity (Deikman et al. 2012). Drought seriously impacts crop production, agro-morphological, and biochemical properties of crop plants, and is ranked among the most appalling abiotic stress (Micheletto et al. 2007). Drought stress affects photosynthesis and causes disorganization of thylakoid membranes, as a result, chlorophyll content and the pigments are reduced (Ashraf and Harris 2013). Furthermore, it also reduces/affects accumulation of dry matter, stomatal opening, and protein synthesis (Larcher 2003; Ohashi et al. 2006). The drought stress was also found to reduce significantly the number of seeds per silique, siliques per plant, seed weight, seed yield, seed oil content, and oil yield of five mustard cultivars (Sinaki et al. 2007; Nasri et al. 2008). Seed oil and protein contents are reported to be the two main qualitative traits strongly affected by drought (Istanbulluoglu et al. 2010). Among the major oilseed crops, Brassicas are the most affected by drought as they are principally grown in the arid and semiarid areas (Zhang et al. 2014a). A study conducted to understand the relative drought tolerance among different Brassica species found close relationship between water content and biomass production in *B. rapa*, *B. carinata*, *B. juncea*, and *B. napus* (Ashraf and Mehmood 1990).

6.2.5 Flooding

Flooding, waterlogging, or submergence is characterized by excessive soil water, which impedes normal crop growth by affecting the plant anatomy, morphology, and physiological functions (Maryam and Nasreen 2012). Waterlogging, wherein root and some portion of the shoot goes under water, and complete submergence, where the whole plant goes underwater, are two main types of flooding which significantly reduce the performance and yield of Brassica crops (Ahmed et al. 2013). Submerged plant shoots face asphyxiation owing to poor gas exchange (Stünzi and Kende 1989; Blom et al. 1993; Jackson and Armstrong 1999). When soil is waterlogged, oxygen exchange rapidly decreases due to the slow diffusion of gases in water (Jackson and Colmer 2005). Cellular and organ structures undergo multifaceted alterations to cope with low oxygen stress during development with the help of ethylene, gibberellin, and abscisic acids (Bailey-Serres and Voesenek

2008). Waterlogging reduces respiration and photosynthetic processes and causes chlorophyll degradation, accumulation of malondialdehyde (MDA), and lipid peroxidation (Jackson and Colmer 2005). As a result, anaerobic respiration takes place which generates ethanol and lactic acid, accumulates reactive oxygen species as balance of ROS production, and scavenging is disrupted. All these are harmful to the normal growth and development of plants (Ashraf 2012; Irfan et al. 2010). Due to the hypoxic and anoxic environments caused by waterlogging, plants develop morphological and physiological defense mechanisms by increasing soluble sugar content, enhancing the activity of fermentative enzymes, and employing the antioxidant defense system (Hossain and Uddin 2011). Some plants, i.e., *Melilotus siculus* (Verboven et al. 2012), *Oryza sativa* (Shiono et al. 2011), and Dendranthema (Yin et al. 2013), develop aerenchyma for surviving in hypoxic and anoxic conditions. The waterlogging experiments conducted on *B. napus, B. juncea, B. carinata,* and *B. rapa* found highest shoot fresh and dry biomass in *B. juncea* and *B. carinata* and the lowest in *B. napus*. Reduced chlorophyll content was observed in all species, but was relatively lower in *B. juncea* and *B. napus*. An increase in the soluble protein content *in B. juncea* and total amino acids in *B. carinata* was also observed. Furthermore, increased iron levels in shoot and roots were observed in all the species of Brassica in waterlogged condition (Ashraf and Mehmood 1990).

6.2.6 Salinity

Salinity, one of the major abiotic stress, affects around 7% of the total land area of the world, thereby reducing the crop productivity (Rozema and Flowers 2008). Worldwide, FAO estimates that more than 830 million hectares of land area are affected by salinity, due to either presence of saline water (403 million hectares) or by the conditions related with presence of disproportionately high concentration of sodium (434 million hectares; FAO 2008). These areas include all global drought and salt-affected lands that are essentially unproductive. Farming communities are losing arable, cultivable land to salinity stress, thereby raising major concerns to ensure sustainable food supply for the surging population. Saline conditions muddle up ion balance of plants leading to ion toxicity and osmotic stress and ultimately affect crop growth and productivity (Yamaguchi and Blumwald 2005; Kumar et al. 2013a, b, 2014). High salinity induces both hyperionic and hyperosmotic effects, and perturbs the ability of roots to absorb water, while their high concentrations within the plant itself become toxic, inhibiting many physiological and biochemical processes (Hasegawa et al. 2000). Plant roots facing salt vicissitude struggle for water uptake, which disrupt metabolic processes and impacts cell growth. However, maintaining low cytosolic balance by sodium exclusion, compartmentalization, and secretion are evolutionary strategies adopted by plants to combat salt stress (Purty et al. 2008), which to some extent is possible by plants. However, if affected for long during their life cycle, cellular and physiological

processes are drastically affected leading to the crop loss. Therefore, to combat salinity stress and to produce Brassica oilseed in a sustainable manner, there is a requirement of an urgent initiative to develop new varieties which will be able to give optimal yield under abiotic stress conditions such as salinity.

Several morphological, biochemical, and physiological selection parameters have been used to evaluate the salt tolerance capacity in crop plants, including photosynthetic rate, relative cell membrane permeability, antioxidant enzyme activity, shoot Na^+ content, etc. (Munir et al. 2013; Khayat et al. 2010). Significant interspecific variation exists among the Brassica species for salt tolerance (Ashraf and McNeilly 1990). The amphidiploid Brassica species (*B. napus*, *B. carinata,* and *B. juncea*) were observed to be more tolerant than the diploid ones (*B. rapa*, *B. nigra,* and *B. oleracea*) under salt stress (Nazir et al. 2001; Kumar et al. 2009). Several studies have screened the *B. rapa* and *B. juncea* germplasm to identify salt tolerant varieties (Siddiqui et al. 2009; Hayat et al. 2011; Jan et al. 2016). A strong correlation was observed between the physiological responses of the different species under salt stress with the transcript abundance for SOS pathway-related genes (Kumar et al. 2009). Of these, the *SOS2* gene was found to be upregulated in salt stress in all the Brassica species studied, except for *B. juncea,* wherein high levels of *SOS2* expression were found even in controlled conditions (Kumar et al. 2009). In another study, exogenous application of gibberellic acid was found to enhance salinity tolerance in the mustard plant (Ahmad 2010).

6.2.7 Disease Resistance

Among many objectives of crop improvement, the development of crop varieties for disease and insect pest resistance are two most important objectives. Plant breeders have been exploring diverse sources of resistance available in crop germplasm and utilizing those in the breeding programs to achieve durable disease resistance. However, pathogen populations keep on evolving thereby producing new virulent races (Bariana et al. 2013). Therefore, for successful development of disease resistant crop plants, knowledge of available genetic variation for resistance, evolutionary potential of the pathogen, and modern breeding methods are required. A large number of pathogens infect oilseed Brassicas and limit their production throughout the world. The major fungal diseases that affect the crop yield include Alternaria blight (caused by *Alternaria brassicae* (Berk) Sacc.), white rust (*Albugo candida* (Pers.) Kuntze), downy mildew (caused by *Peronospora parasitica* (Pers. ex Fr.), and clubroot disease (*Plasmodiophora brassicae* Woronin).

A. candida is an obligate parasite and produces white-colored pustules on leaves, stems, and pods. Spreading of the infection on the flower buds (Verma and Petrie 1980) can cause extensive distortion, hypertrophy, and even sterility of inflorescences leading to “stagheads formation”, a stage that majorly affects crop yield. The percent yield losses of up to 60% in *B. rapa* in Canada (Berkenkamp 1972; Harper

and Pittman 1974; Petrie and Vanterpool 1974), 89% in *B. juncea* in India (Bains and Jhooty 1979; Lakra and Saharan 1989), and up to 10% in Australia (Barbetti 1981; Barbetti and Carter 1986) have been reported.

Plants infected with clubroot causing phytopathogen *P. brassicae* elicits extensive growth and division of root cells that leads to the formation of galls and blocking of the vascular tissue (Hirai 2006). This restricts the movement of water and nutrients in the root that subsequently affects the overall plant growth and yield. Around 11 genetic loci, conferring resistance to clubroot has been reported in *B. rapa* and mapped to different chromosomes (details given in Sect. 4.3.1). However, no natural resistant source of clubroot has been reported in *B. juncea* (Jakir Hasan et al. 2012). Recently, Hasan and Rahman (2018) developed a clubroot resistant resynthesized *B. juncea* by interspecific crossing between a clubroot resistant *B. rapa* ssp. *rapifera* and two susceptible *B. nigra* lines.

6.2.8 Insect Resistance

In agriculture, a variety of phytophagous insect pests causes major crop yield losses. The severity of crop yield loss due to the insect infestation depends on several factors including the plant growth stage at the time of the infestation and duration and intensity of infestation. The management of insect pests is also one of the biggest challenges facing the cultivation of oilseed Brassicas. The crop is susceptible to several major insect pests that include large white or cabbage moth (*Pieris brassicae*), mustard aphid (*Lipaphis erysimi*), diamondback moth (*Plutella xylostella*), mustard sawfly (*Athalia proxima*), and leaf miner (*Bagrada cruciferarum*). The nymph and adult insects of mustard aphids cause damage by sucking the plant sap during flowering and seed formation, and thereby restricting the nutrient flow (Singh and Sachan 1994; Kumar 1999; Patel et al. 2004). As a result, the plant fails to produce healthy pods, consequently affecting the crop productivity. The diamondback moth is another worldwide pest of vegetable and oilseed Brassicas (Furlong et al. 2008; Zalucki et al. 2012; Furlong et al. 2013). It causes severe damage to the crucifer plants which includes cultivated vegetable crops *B. oleracea, B. rapa* and oilseeds *B. napus* and *B. juncea* (Eigenbrode et al. 1991; Talekar and Shelton 1993). An Integrated Pest Management (IPM) approach is considered to be more effective and sustainable for controlling insect pests (Panda and Khush 1995) as compared to the chemical control methods that were commonly employed in the past. Although, effective, the chemical control methods do not provide long-term, sustainable control strategy and also have adverse effects on the environment. Developing insect-resistant cultivars is a more effective pest management strategy, as it is both cost-effective and environment friendly.

6.2.9 Nutrient Use Efficiency (NUE)

The NUE of plants is based on the nutrient uptake, incorporation, and utilization efficiency of the plant (Baligar and Fageria 2015). The observed variability in the NUE among plants is due to the inherent natural genetic variability within the germplasm, which consecutively reflects on the nutrient uptake efficiency, absorption, translocation efficiency to shoots and leaves, production of dry matter, etc. and also affects the plant–environment interactions (Baligar et al. 2001). During the last few decades, global food production could be increased due to increased use of fertilizers such as nitrogen, phosphorus, and potassium. However, the changing climate can impose several challenges such as nutrient cycling, uptake, and availability which directly influences the NUE of plants, thereby affecting plant growth, development and eventually the overall yield (McDonald et al. 2013). Among the major nutrients, nitrogen is insufficient in most soils worldwide. Nitrogen plays an important role in growth of all plants including Brassica crops and is involved in the metabolism and transformation of energy, chlorophyll, and protein synthesis. It also affects uptake of other essential nutrients and helps in the optimal partitioning of photosynthates to reproductive parts which increases the seed: stover ratio (Singh and Meena 2004). In view of the fact that mustard crop is highly elastic, its plant architecture is bound to be modified by the availability of nutrients and may affect light interception and carbon dioxide assimilation, which could not only deteriorate the quality but also reduce the seed yield drastically.

Efforts toward improving the NUE in Brassica crops have involved either selection and breeding for improved genotypes or transgenic approaches to create varieties with altered gene expression (Good et al. 2007; Fischer et al. 2013). Significant genetic variation of NUE is found to exist among *Brassica* populations including the Indian mustard (Ahmad et al. 2008). The existing yield of modern cultivars, however, is still limited because the traditional breeding and selection experiments have mainly been conducted in near-optimal conditions and possibly miss the scope for exploring the potential of varieties under deficient nutrient availability (Ceccarelli 1996).

6.2.10 Water Use Efficiency (WUE)

A promising opportunity exists for improving yields under reduced availability of water, by influencing the association between photosynthesis and transpiration. Plants essentially act as hydraulic channels between the soil and atmosphere. The exchange through the soil–plant–atmosphere continuum is possible because the atmosphere is almost always drier than the sub-stomatal cavities of leaves. The water evaporates from the interstitial tissues into the atmosphere through the open

stomatal pores. WUE is the ratio between these two parameters, which describes how "efficient" the plant is at optimizing photosynthesis while simultaneously minimizing transpiration (Bramley et al. 2013). Drought-tolerant plants and plants adapted to limited water conditions exhibit higher WUE than plants growing in well-irrigated soils (Smith et al. 1989).

In oilseed Brassicas , like other crop plants, the overall yield, forms a part of the total biomass that is partitioned into seed, leaf, stem, root, or floral buds. The genetic potential of a crop, as reflected in terms of yield, is influenced by the associated negative environmental factors, which must be controlled to increase crop productivity. Thus, in the situation of declining water availability for agriculture, plants must be transformed with a built-in genetic capability so that they are able to use the available water more efficiently.

Mustard and rape are grown mainly in rainfed conditions and the water availability heavily influences the overall yield. The cultivation of these crops will have to be expanded so as to cover drought-prone areas with the objective to meet the increasing demand for Brassica oils. McVetty et al. (1989) compared WUE and stomatal conductance in Moricandia and Brassica species and attributed lower WUE in the latter species to higher stomatal conductance. Differential osmotic adjustments have also been associated with variation in tolerance to drought among different Brassica species (Ashraf and Mehmood 1990). The genotypic differences in response to drought stress were found to be related to oxalic acid (OA) (Ashraf and Mehmood 1990; Kumar and Singh 1998; Kumar and Elston 1992). These studies highlight a rich source of existing genetic variation for WUE in Brassica species that can be exploited in breeding for WUE in oilseed mustard and rape.

6.3 Genetic Resources for CS Genes

Endeavors toward crop improvement, apart from developing varieties with increased productivity, must also aim at identifying climate-resilient germplasm thereby ensuring long-term food security. Such efforts will be greatly benefitted by efficient utilization of the available genetic resources (Brozynska et al. 2016). The genetically diverse germplasm offers a useful resource to identify novel genes/alleles that can help to mitigate the effects of the changing environment. Expansion of the genetic resources by inclusion of Crop Wild Relatives (CWRs) is crucial since they are expected to have beneficial traits that can be utilized to develop varieties suited for the climate-change-affected production systems (FAO 2015). All crop plants have been domesticated from the CWRs, which represents a primary source of diversity for all breeding endeavors.

6.3.1 *Gene Pools and Genetic Diversity of* B. rapa *and* B. juncea

B. rapa containing the A genome includes Chinese cabbage, sarson, turnip, turnip greens, turnip, and turnip rape crops, which on a morphological basis are assigned, respectively, to the leafy, rapifera and oleifera types. The B genome is held by black mustard, while the genome-C is represented by *B. oleracea* diversified into several botanical varieties and related crops by domestication processes, i.e., cabbage, cauliflower, broccoli, kale, brussels sprout, etc. Nagaharu U as early as in 1935, demonstrated the cytogenetic relationship of diploid species *B. nigra* L. (n = 8), *B. oleracea* L. (n = 9), and *B. rapa* L. (n = 10) and polyploid species *B. carinata* (n = 17), *B. juncea* L. (n = 18), and *B. napus* L. (n = 19) which were formed by natural interspecific hybridisation between diploid species. The genetic boundaries limit the breeding process through sexual hybridization between Brassica species thereby confining them into primary, secondary, and tertiary gene pools and *B. oleracea* is considered to be primary gene pool (Harlan 1975). The secondary gene pool was investigated by studies on the pachytene chromosome morphology which permitted to identify the basic genomes of chromosome number of *Brassica* crops and secondary gene pool. The wild relatives, belonging to different species and genera in 36 cytodemes, namely, *Diplotaxis*, *Enarthrocarpus*, *Eruca*, *Erucastrum*, *Hirschfeldia*, *Rhynchosinapis*, *Sinapis*, *Sinapodendron*, *Trachystomagenera,* etc. form the tertiary gene pool (Harberd 1976). Many wild relatives of the tertiary gene pools contain genes for tolerance to biotic and abiotic stress and could be used as a source to transfer the trait into the cultivated *B. rapa* and *B. juncea* germplasm through specialized techniques of embryo rescue, somatic hybridization, etc. It is believed that *B. rapa* and *B. nigra* originated first compared to *B. oleracea*, similarly, *B. juncea* was formed by natural hybridization between *B. rapa* and *B. nigra* prior to *B. carinata* and *B. napus* (Branca and Cartea 2011).

Among the different *B. rapa*, turnip, with rustic, invasive, and easy growing traits, is believed to be the first Brassica domesticated for various purposes and is found in the form of weed in the natural habitat of Mediterranean to central Asia. This crop was found in Neolithic sites and proposed to be grown during the period between 2500 and 2000 BC (De Candolle 1886; Hyams 1971). Leafy vegetable *B. rapa,* such as Chinese cabbage, was first utilized in China and differentiated in west Asia from oilseed type *B. rapa* of central Asia (Li 1982; Branca and Cartea 2011). It is further believed that the European oleifera type was developed in Mediterranean basin and the Asian form originated in central Asia and Northwest India (Branca and Cartea 2011). Among the oleifera types, brown sarson is said to be the oldest form which is different from the toria, in morphology and plant types, and differed from yellow sarson with yellow seed coat color. These views were also supported by Restriction Fragment Length Polymorphism (RFLP) analysis (Song et al. 1990). However, an opposing view was put forward by McGrath and Quiros (1992), that East Asia lacks wild types and it was proposed that agriculture domestication in Europe resulted in an eastward migration of *B. rapa* during

Neolithic period. Studies using Amplified Fragment Length Polymorphism (AFLP) analysis reported the occurrence of several groups of *B. rapa* in Europe, India (South Asia), and East Asia (Zhao et al. 2005; Warwick et al. 2008). Furthermore, Annisa and Cowling (2013), based on the Simple Sequence Repeat (SSR) markers reported three genetic groups of oilseed *B. rapa*, i.e., South Asia (with two subgroups in South Asia), southeastern and in northern Europe. The proposal of Sun (1946) that *B. rapa* has two evolutionary lines, one western, in Europe and Central Asia in which turnip and oilseed forms are domesticated, and another eastern in East Asia, where diversification of several vegetable forms are found supported by many. These two independent origins are supported by morphological, geographical, and molecular data (Denford and Vaughan 1977; Song et al. 1988b).

B. rapa, being one of the progenitor species of the allotetraploid species of *B. napus* and *B. juncea*, has tremendous potential in oilseed Brassica breeding as this species is comparatively easy to hybridize with and hence can be used to transfer favorable alleles to *B. napus* and *B. juncea* through interspecific hybridisation (Qian et al. 2006; Chen et al. 2010; Jiang et al. 2011; Mei et al. 2011). Therefore, *B. rapa* has great potential to contribute to the genetic improvement of *B. napus* and *B. juncea.* In *B. juncea,* two major gene pools are reported, the east European gene pool and the Indian gene pool. The Indian gene pool lines possess high erucic acid and high glucosinolate contents, with larger seed size and comparatively shorter height and are early flowering, while east European gene pool lines are with yellow seed coat color, smaller seed size, low erucic acid content, and are of taller stature (Pradhan et al. 1993; Oram et al. 1999). East European germplasm is shown to be cold/frost tolerant. Apart from that, vegetable-type *B. juncea* is also found in China. Pradhan et al. (1993) in a study involving germplasm from east Europe and India, and resynthesized *B. juncea,* reported that maximum diversity was observed between the two gene pools. This genetic diversity study was based on morphological traits. Based on the nine agronomic and yield traits, the different accessions of *B. juncea* were grouped into two major groups. Phylogenetic analysis using molecular markers further substantiated the existence of the two gene pools (Jain et al. 1994; Srivastava et al. 2001; Burton et al. 2004). These studies revealed that the two gene pools are significantly divergent from each other, however, the Indian gene pool is constituted by accessions with limited variability (Pradhan and Pental 2011).

6.3.2 *Germplasm Resources, Access, and Benefit Sharing*

Crucifer genetic resources are maintained worldwide in gene banks of different countries such as China, India, United Kingdom, United States, Netherland, Spain, and Germany, and are constantly augmented by discoveries in plant exploration and introduced to new areas of the world. Collective representation of these countries gene banks accounts for more than 60% of global total collections (Singh and Sharma 2007). Brassica breeding work heavily relies on these genetic stocks of cultivated and wild relatives through interspecific crossing program in India and

various parts of world to create new genetic variability and diverse base populations (Gupta 2016).

Resource institutions around the world have the mandate to collect, document, evaluate, enhance, preserve, and distribute germplasm with diverse genetic backgrounds. These institutions procure seed germplasm through international and domestic exchanges, and develop in situ maintenance programs. Most institutions allow access to these genetic resources through Material Transfer Agreement (MTA), while private institutions may require an MTA or a contract that administers the transmission of tangible research materials between organizations, defining the rights of the provider and the recipient with respect to the materials and any derivatives (Knee et al. 2011).

The recently approved International Treaty on Plant Genetics Resources for Food and Agriculture established a multilateral system for having facilitated access of the germplasm of a number of crops. Brassica germplasm gene/resources banks are established since early 1970s. The Universidad Politécnica de Madrid (UPM), in Spain, established in 1966 has the world's largest collection of wild crucifer germplasm listing around 600 crucifer accessions which includes rare and endangered species mainly collected from the western Mediterranean area (Branca and Cartea 2011). In 1983, Global Base Collection of wild crucifer was initiated by International Bureau of Plant Genetic Resources (IBPGR) which later changed its name into International Plant Genetic Resources Institute (IPGRI), and finally to Bioversity International, Rome (Italy). In Europe, a working group of Brassicas was established under the aegis of European Cooperative Programme for Crop Genetic Resources Network (ECP/GR) in 1991. This initiative encouraged to develop European Brassica Database (Nras-EDB) by Center for Genetic Resources, the Netherlands (http://documents.plant.wur.nl/cgn/pgr/brasedb/) and has 19,600 collections of wild and cultivated species from 22 countries. Furthermore, other genetic resources/germplasm banks which have got wild and cultivated Brassica species are Kew Gardens and the Horticulture Research Institute in Wellesbourne (United Kingdom), and the Nordic Gene Bank (Sweden) (Gupta 2012), the Germplasm Resources Information Network (GRIN) in USA, and the Chinese Genetic Resources Information System (CGRIS) in China (Branca and Cartea 2011).

Germplasm Resources Unit (GRU) based at the John Innes Centre, Norwich, UK also host seed collections of Brassica crop and its wild relatives. Multilateral System (MLS) of the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA) fetches massive support from GRU with 35,000 accessions as of now (Horler et al. 2018).

India maintains plant genetic resources including mustard and rape at National Bureau of Plant Genetic Resources (NBPGR) in New Delhi. NBPGR was established by Government of India for governance and policy-making in area of PGR management. NBPGR ensures sustainable use of genetic resources of agri-horticultural crop plants, by coordinating with several institutions, and also conducting capacity building programs. NBPGR has introduced over 3950 accessions of rapeseed and mustard from more than 25 countries (Singh and Sharma 2007) since

last three decades. The gene bank at the NBPGR hosts a total of 7498 collections of *B. juncea*, 1136 of *B. napus* and *B. rapa*, and 179 of *B. campestris* (NBPGR 2018).

6.4 Genetic Mapping and QTL Analyses of CS Traits

Majority of agronomic traits such as morphology, yield, biotic and abiotic stress tolerance are governed by many genes quantitatively and are highly influenced by environment thereby, making conventional breeding endeavors (based on phenotypic selection) difficult and time consuming. However, recent advances in development of molecular markers and generation of high-density linkage maps have not only accelerated identification, tagging, and mapping of genomic regions underlying the desired traits but also facilitated precise introgression of these loci into relevant genetic backgrounds. For genetic analyses, mapping- and marker-assisted breeding of CS traits, various molecular markers, and mapping populations derived from the contrasting bi-parental crosses [F_2, F_3, backcross (BC), recombinant inbred (RIL), and doubled haploid (DH) lines] have been used, these have been listed in Tables 6.1 and 6.2.

6.4.1 Mapping of Flowering Time, Circadian Clock, and Bolting Traits

Genetic mapping of one of the major adaptive and agronomically important traits, flowering time and bolting, has been elucidated in several studies (Song et al. 1995; Teutonico and Osborn 1995; Nozaki et al. 1997; Osborn et al. 1997; Axelsson et al. 2001; Kole et al. 1997, 2001, 2002a; Schranz et al. 2002; Nishioka et al. 2005; Kim et al. 2006; Kakizaki et al. 2011; Lou et al. 2011; Kitamoto et al. 2014; Xiao et al. 2013a; Jing et al. 2016). Song et al. (1995) used F_2 plants genotyped with RFLP loci to analyze the inheritance of more than 20 morphological and quantitative traits in *B. rapa*, including leaf, stem, and flowering characteristics (days to bud, days to flower, and days from bud to flower).

Based on the requirement of cold treatment/vernalization to induce flowering, Brassica cultivars are classified as biennial or annual. Quantitative Trait Loci (QTLs) controlling the vernalization requirement were identified in a *B. rapa* F_2 population derived from a cross between an annual and a biennial oilseed cultivar (Teutonico and Osborn 1995). The study identified two major QTLs, for days to flowering time (DTF) on LG2 and LG8, the former was found to be homologous to LG9 QTL for flowering time of *B. napus* (Ferreira et al. 1995). Later, these two QTLs (on LG2 and LG8) of *B. rapa* were designated as *VFR1* and *VFR2*, respectively, and were shown to be homologous to two regions in *B. napus* which

Table 6.1 Overview of the genetic mapping and QTL studies for CS traits in *B. rapa*

CS Trait	Mapping population	Parental lines	Type of markers	Total markers	Genetic length (cM)	References
Erucic acid content	F_3	Per, R500	RFLP	139	1785	Teutonico and Osborn (1994)
Morphological traits	F_2	Michihiti, Spring broccoli	RFLP	220	1593	Song et al. (1995)
White rust resistance, Leaf pubescence	F_2, F_3	Per, R500	RFLP	12	146.9	Kole et al. (1996)
Morphological traits, self-incompatibility	F_2	Chinese cabbage, Mizu-na	RAPD, isozyme	52	733	Nozaki et al. (1997)
Clubroot resistance	DH	Homei P09, Siloga S2	RAPD	115	–	Kuginuki et al. (1997)
Clubroot resistance, flower color	DH	T136-8, Q5	RFLP	63	735	Matsumoto et al. (1998)
Bolting time	F_2	G309, DH27	RAPD	527	–	Ajisaka et al. (2001)
Flowering time	F_2	Rc50a7, late flowering *B. rapa* ssp. *chinensis*	RFLP, cDNA, FLC	31	–	Axelsson et al. (2001)
Flowering time	BC_3S_1	Per, R500	RFLP, cDNA, *CO*	12	–	Kole et al. (2001)
Flowering time	BC	Per, R500	RFLP, SSR, FLC	–	–	Schranz et al. (2002)
Linolenic acid content	F_2	93651–2, Sv3402	RAPD, RFLP (*Fad3*)	27	–	Tanhuanpää and Schulman (2002)
Heat tolerance	RILs	Chinese cabbage	AFLP, RAPD	352	2666	Yu et al. (2003a)

(continued)

Table 6.1 (continued)

CS Trait	Mapping population	Parental lines	Type of markers	Total markers	Genetic length (cM)	References
Bolting, plant height, and leaf-related traits	RILs	Chinese cabbage	AFLP, RAPD	352	2666	Yu et al. (2003b)
Characterization of dwarf gene (*dwf2*)	F_2, BC	CrGC1-21, CrGC1-33, R500	RFLP	92	–	Muangprom and Osborn (2004)
Clubroot resistance	F_2	A9709, G004	SSR	94	–	Suwabe et al. (2003)
Clubroot resistance	F_2	94SK, Shinki	AFLP, SCAR, CAPS	16	–	Piao et al. (2004)
Clubroot resistance	F_3	N-WMR-3, A9709	RAPD	–	–	Hirai et al. (2004)
Bolting time	DH	A9408, Homei P09	AFLP	248	1096.6	Nishioka et al. (2005)
Clubroot resistance	F_3, F_4	N-WMR-3, A9709	STS	23	–	Saito et al. (2006)
Clubroot resistance	F_2, F_3	G004, nou 7 (A9709)	RAPD RFLP SSR	262	1005.5	Suwabe et al. (2006)
Flowering time	F_2	Chinese cabbage F_1 cultivar Jangwon	RFLP, SSR	545	1287	Kim et al. (2006)
Black rot of crucifers	F_2	R-o-18, B162	AFLP, SSR	246	664	Soengas et al. (2007)
Anthocyanin pigment	F_2	Rapid cycling *B. rapa* (15-8812, 15-8804)	SSR	22	–	Burdzinski and Wendell (2007)

(continued)

Table 6.1 (continued)

CS Trait	Mapping population	Parental lines	Type of markers	Total markers	Genetic length (cM)	References
Seed coat color	F_2, F_3, BC	SPAN, BARI-6	SRAP, SCAR, SNP	48	–	Rahman et al. (2007)
Flowering, seed, growth, leaf, and turnip formation	F_2, F_3, BC, DH	RC-144, CC156, YS-143, PC-175, VT-115	Flowering time related genes (FLC, FTA, FCA, CO, LFY)	–	–	Lou et al. (2007)
Clubroot resistance	F_2, F_3	K10, Q5; C9, 6R	AFLP, RAPD, SSR, STS	236, 187	640.3, 485.9	Sakamoto et al. (2008)
Glucosinolate content	DH	YS-143, PC-175, VT-115	AFLP, SSR	321, 300	688.0, 763.0	Lou et al. (2008)
Leaf mineral accumulation and tolerance to Zn stress	DH	Y-177, Y-195	AFLP, SSR, SRAP, ESTP	287	1090.0	Wu et al. (2008)
Root morphological traits	F_2	Qishihai, AJH97-2	RFLP, RAPD	192	1837.1	Lu et al. (2008)
Flowering time and leaf morphological traits	F_2	C634, P11	EST, SSR, BAC sequence-based markers, SNP	241	1396	Li et al. (2009)
Bolting time	F_2	PL6, A9709	SSR	220	875.6	Kakizaki et al. (2011)
Gene expression under varying soil phosphorus supply	RILs	R500, IMB211	Gene expression markers (GEMs)	125	870.8	Hammond et al. (2011)
Circadian clock and flowering time	RILs	R500, IMB211	RFLP, SSR	225	1113.6	Lou et al. (2011)

(continued)

Table 6.1 (continued)

CS Trait	Mapping population	Parental lines	Type of markers	Total markers	Genetic length (cM)	References
Resistance to downy mildew	DH	91-112, T12-19	AFLP, RAPD, SSRs, SCARs, SRAPs, STSs, Isozyme markers	519	1070.5	Yu et al. (2011)
Leaf- and Heading related traits	F_2	Chinese cabbage lines 501, 601	UGM, SSR	238	915.4	Ge et al. (2011)
Ecophysiological and circadian traits	RILs	R500, IMB211	RFLP, SSR	225	1113.6	Edwards et al. (2011)
Plant architecture and seed characteristics	RILs	L58, R-o-18	SNPs, AFLP, InDel, SSR	270	1150.0	Bagheri et al. (2012)
Chlorophyll content	F_2	Chinese cabbage lines 501, 601	UGM, SSR	238	926.7	Ge et al. (2012)
Seed color	RILs	Sampad, 3-0026.027	SSR, AFLP	163	1369.3	Kebede et al. (2012)
Clubroot resistance	F_2	CR turnip (*B. rapa*) "CR Shinano"	SSR, InDel	33	–	Kato et al. (2013)
Yield-related traits	DH	FT' (f. *depressa* Li) "WZ" (f. *cylindrica* Li)	SRAP, SSR	233	1063.8	Liu et al. (2013)
Bolting time	F_2	Tsukena No. 2, Early (Sakata Co.)	SSR, FLC	62	1009.0	Kitamoto et al. (2014)
Br-or gene (carotenoid synthesis)	F_2S_4	11J16, 11S39-2	RAPD, SCAR, SSR, InDel	–	–	Zhang et al. (2013)
Secondary metabolites	RILs	L58, R-o-18	SNPs, AFLP, InDel, SSR	270	1150.0	Bagheri et al. (2013a)

(continued)

Table 6.1 (continued)

CS Trait	Mapping population	Parental lines	Type of markers	Total markers	Genetic length (cM)	References
Seed-related traits	F_2	L58, R-o-18	AFLP, SSR	118	757.0	Bagheri et al. (2013b)
Clubroot resistance	F_2	G004, A9709	InDel, BAC-end	–	–	Hatakeyama et al. (2013)
Flowering time	DH	YS-143, PC-175	AFLPs, SSRs, Ft, Intron-based polymorphism, CAPS	278	1234.0	Xiao et al. (2013a)
Tetralocular ovary	RILs	Chiifu, Tetralocular	SNP, IP, SSR	733	669.7	Paritosh et al. (2013)
Morphological and yield component Traits	F_2, F_3	Chiifu 401–42, rapid cycling *B. rapa*	BAC-SSRs, EST-SSRs and Intron Polymorphisms (IP)	444	1119.5	Li et al. (2013)
Leaf size and shape	DH	YS-143, PC-175	AFLP, SSR, SNPs	509	1328	Xiao et al. (2014)
Vegetative traits and onset of reproduction	RILs	R500, IMB211	RFLP, SSR	225	1113.6	Dechaine et al. (2014a)
Resistance to Turnip mosaic virus	DH, F_2, BC	VC1, VC40, SR5	SSR	–	–	Jin et al. (2014)
Reproductive fitness Characters	RILs	IMB211, R500	RFLP, SSR, Flower color	224	1113.6	Dechaine et al. (2014b)
Tetralocular ovary and silique width	F_2, RILs	Chiifu, Tetralocular, YSPB-24	IP, SNP, SSR	414 (CTF2), 224 (CTF6), 734 (CTF7), 217 (CYF2)	813.9 (CTF2), 851.8 (CTF6), 679.6 (CTF7), 869.8 (CYF2)	Yadava et al. (2014)

(continued)

Table 6.1 (continued)

CS Trait	Mapping population	Parental lines	Type of markers	Total markers	Genetic length (cM)	References
Flowering time	DH	Y177, Y195	SNP	887	958.6	Jing et al. (2016)
Clubroot resistance	BC	T19, ACDC	SNP	1584	4802.52	Yu et al. (2017)
Plant size, color, and leaf characteristics	F_2	Huangxiaoza, Bqq094-11	SNP	3985	1223.4	Huang et al. (2017a)

Table 6.2 Overview of the genetic mapping and QTL studies for CS traits in *B. juncea*

CS Trait	Mapping population	Parental lines	Type of markers	Total markers	Genetic length (cM)	References
Yield-related traits (plant height, days to flower, oil content, etc.)	F_2	Varuna, BEC-144	RFLP	15	173.9	Sharma et al. (1994)
Seed coat color and other quantitative traits	F_2	Varuna, BEC-144	RFLP	25	243.3	Upadhyay et al. (1996)
White rust resistance	DH	J90-4317 J90-2733	RFLP	22	106	Cheung et al. (1998)
Oleic acid content	RIL (F_6)	Varuna, BEC-144	RAPD	114	790.4	Sharma et al. (2002)
Seed fatty acid content	DH	BJ-99, BJ-70	AFLP, RAPD	273	1641	Lionneton et al. (2002)
Fatty acid profile	DH	RLM-514 (HEP), LEP	RFLP	316	1564	Mahmood et al. (2003)
Yield influencing traits	DH	Varuna, Heera	RFLP, AFLP, SSR	1448	1840.1	Ramchiary et al. (2007)
Blackleg disease	F_2 and Backcross	AC Vulcan, UM 3132	RFLP, SSR	325	1577.3	Christianson et al. (2006)
Yield-associated traits	DH	Varuna, Heera	IP, RFLP and gene markers		1922.2	Panjabi et al. (2008)
Yield-associated traits	DH	TM-4, Donskaja-IV	IP, AFLP, SSR	911	1629.9	Yadava et al. (2012)
Glucosinolates and erucic acid	DH	Varuna, Heera	SNP,IP	1708	1933.5	Paritosh et al. (2014)
Stem expansion traits	F_2	Tumida, Multiceps	SSR	116	2061.0	Li et al. (2016)

contain QTLs (*VFN1* and *VFN2*). The *VFR2* and *VFN2* of Brassicas were also shown to be homologous with the top of the chromosome 5 of *A. thaliana* which harbors flowering-time genes *CONSTANS* (*CO*), *EMBRYONIC FLOWER 1* (*EMF1*), *FY* and *FLOWERING LOCUS C* (*FLC*) (Osborn et al. 1997). Further, Kole et al. (2001) reported that *FLC* is the gene responsible for flowering time at *VRF2* (controlling flowering time) QTL detected by Teutonico and Osborn (1995)

and Osborn et al. (1997). Later, the allelic effects of *VFR2* were determined by backcrossing with an early flowering line and showed monogenic segregation in the BC_3S_1 population (Kole et al. 2001).

Schranz et al. (2002) cloned four *FLC* genes from the diploid *B. rapa* and three from *B. oleracea*, and compared the sequences of those genes with that of the *A. thaliana*. They further reported that polyploidy/replication of *FLC* gene has contributed to variation in flowering time of *B. rapa* and *B. oleracea* in a dosage-dependent manner.

Lou et al. (2011) observed wide variation of circadian clock and flowering time among the collected *B. rapa* accessions, wild populations, and RILs derived from a cross between a rapid cycling Chinese cabbage (ssp. *pekinensis*) and a yellow sarson oilseed (ssp. *trilocularis*). A significant correlation between circadian period and flowering time, and the co-localization of circadian period and flowering-time QTLs indicated that the two phenotypes shared common genetic loci besides having possibility of tight linkage of two distinct loci or epistasis of the genes of the two traits. These results provided some of the first evidences for a link between quantitative variation in the circadian clock and plant ecophysiology.

For identification of QTLs controlling late bolting in Chinese cabbage, Kakizaki et al. (2011) used a F_2 population derived from a cross between an extremely late-bolting parental line (PL6) and an early bolting parental line (A9709) of Chinese cabbage. The study identified three QTLs at the periphery of the well-known orthologous flowering time genes from Arabidopsis, including *FLC* and *FT*.

The influence of day length and/or temperature has been reported in *B. rapa* (Young et al. 2013). Recently, Jing et al. (2016) genotyped 172 DH lines derived from a cross between two Chinese cabbage (*B. rapa* ssp. pekinensis) DH lines, Y177 and Y195, using the next-generation sequencing platform and detected a high-density Single Nucleotide Polymorphism (SNP) markers, with a density varying between 32 and 46 SNPs in a 100 kb interval. Flowering time was investigated for the vernalized plants under long day (LD) or short day (SD) and mapped in the high-density linkage map. They further reported that vernalization is the most important factor regulating flowering time in *B. rapa*. This information can be applied for breeding oilseed *B. rapa*.

6.4.2 Mapping of Vegetative and Plant Architecture Traits

The canopy architecture determines to a large extent, how efficiently environmental resources are utilized by the plant organs. Song et al. (1995) through inheritance studies reported that plant height, leaf length, petiole thickness, and stem diameter are polygenic and are influenced by several genes with small and similar effects. They further observed high correlation among functionally related traits, such as petiole morphology and flowering time, which further were governed by QTLs located on the same linkage groups.

In another study, multiple populations generated by crosses between five morphologically diverse parents were used to identify QTLs governing flowering time variation, seed-related traits, growth-related leaf morphology, and turnip formation traits (Lou et al. 2007). The parental accessions belonged to three highly diverse morphotypes of *B. rapa*; oil, leafy, and turnip types. The use of multiple populations to dissect the traits allowed for simultaneous evaluation of allelic variability available across the different parental lines. Several loci harboring allelic variation for flowering time were identified in the study. QTLs for certain phenotypic traits were found to colocalize suggesting their significance in the overall plant growth and development.

Similarly, Li et al. (2013) also identified genomic regions harboring colocalized QTLs for several different morphological traits. These genomic regions might harbor a single gene exhibiting pleiotropy or might harbor several independent but tightly linked loci governing different traits (Lou et al. 2007; Ramchiary et al. 2007). The study identified QTL clusters in conserved QTL blocks that were predicted to harbor active genes that govern leaf traits, plant morphology, and seed traits.

More recently, Huang et al. (2017a) mapped QTLs for 16 complex morphological and agronomic traits in Chinese cabbage (*B. rapa* L.) by generating a high-density linkage map. The study identified single QTLs each for turnip width, weight, and length colocalizing with the major flowering time QTLs. This observation was attributed to either tight linkage exhibited by several independent loci or pleiotropy. Additionally, epistasis of flowering time over turnip formation could also result in this, since an early flowering plant allocates most of its energy toward flower and seed development, while during turnip formation most resources are re-directed toward the roots. Presence of dwarfing genes is a valuable trait in several crop plants as it reduces the lodging and thereby improves the harvestable yield. These genes have been associated with early maturity, improved fertility, and increased tillering in plants. "Green Revolution" was brought about by the introduction of dwarf cultivars in wheat and rice (Hedden 2003; Khush 2001). Many present cultivars of oilseed Brassica crops are susceptible to lodging that not only leads to yield loss but also affects the harvesting. Introduction of dwarfing genes in Brassica crops would, therefore, be beneficial. A dwarfing gene, *dwf2* from *B. rapa* was evaluated by Muangprom and Osborn (2004) for its phenotypic effect. The *dwf2* near-isogenic lines exhibited 47.4 or 30.0% reduction, respectively, in plant height and had the same or significantly higher numbers of primary branches than the wild type line.

In another study, the tetralocular trait was mapped in *B. rapa* using three mapping populations; F_2, F_6 and F_7 (Yadava et al. 2014) The mapping populations were derived from a cross between the bilocular (Chiifu) and tetralocular parental plants. Tetralocular yellow sarson has been shown to bear higher number of siliques on the primary raceme as compared to the bilocular plants and the number of seeds in the tetralocular siliques is also higher (Roy and Sinhamahapatra 2011). Three population-specific maps and an integrated map of *B. rapa* consisting of 1037

markers (595 SNPs, 230 SSR, 211 IP, and morphological marker *tet-o*) were developed. The map was used for the traits governing locule number (*tet-o*) and siliqua width (Fig. 6.1). A major QTL governing the siliqua width was identified on LG A4, colocalizing with the *tet-o* locus (Fig. 6.2). Saturation mapping of the *tet-o* region with SNP markers identified the candidate gene *Bra034340* for locule number based on its homology with *CLAVATA3* gene of *A. thaliana*.

6.4.3 Disease Resistance

Projected changes in temperature and rainfall will have profound effects on diseases of oilseed Brassicas as they affect both the pathogen and the resistance response of the host (Huang et al. 2006). The major diseases that cause severe yield losses in both *B. rapa* and *B. juncea* include diseases caused by *Alternaria* species, clubroot (caused by *P. brassicae*), white rust (caused by *A. candida*), and black rot (caused by *X. campestris*). *A. brassicae* preferentially infects the oil-yielding Brassicas and is the most invasive on all brassicaceous hosts (Rajarammohan et al. 2017).

6.4.3.1 Genetic Mapping of Clubroot Resistance Genes

Clubroot, caused by *P. brassicae*, has been reported widely in *B. rapa*, *B. oleracea*, and *B. napus*. Around eleven clubroot resistance (CR) genes have been mapped in *B. rapa* using bi-parental mapping populations. These include, a single locus each on linkage group A01 (*Crr2*), A06 (*Crr4*) and A08 (*Crr1*) (Suwabe et al. 2003, 2006), two loci (*Crr3* and *CRc*) on A02 (Hirai et al. 2004; Sakamoto et al. 2008) and six loci (*CRa*, *Crb*, *CRk*, *PbBa3.1*, *PbBa3.3,* and *Rcr1*) on A03 (Matsumoto et al. 1998; Piao et al. 2004; Sakamoto et al. 2008; Kato et al. 2012; Chen et al. 2013; Chu et al. 2014). To facilitate introgression of the trait into suitable cultivars, four of these loci (*CRb*, *Crr3*, *Rcr1,* and *Rcr2*) have been fine mapped. The *CRb* locus was localized to a 140-kb region flanked by markers KB59N07 and B1005, using a F_2 population of 2032 individuals (Kato et al. 2013). The fine mapping of *Crr3* locus was performed using a *B. rapa* population of 888 F_2 individuals and STS markers developed from Arabidopsis genome sequence information. The *Crr3* locus was delimited to a 4.74 cM genomic region (Saito et al. 2006). The other locus, *Rcr,* was initially fine mapped to a 0.28 cM interval using SSR markers (Chu et al. 2014) and in a subsequent study, tightly linked SNP markers were developed from the genes present in the candidate interval (Yu et al. 2016). Similarly, in another study the *Rcr2* locus was delimited to a 0.2 cM region using SNP markers (Huang et al. 2017b). Two of these genes, *CRa* and *Crr1*, have been isolated from *B. rapa* (Ueno et al. 2012). Both the genes were found to code for Toll Interleukin 1 receptor-nucleotide binding site-leucine-rich repeat (TIR-NBS-LRR) proteins.

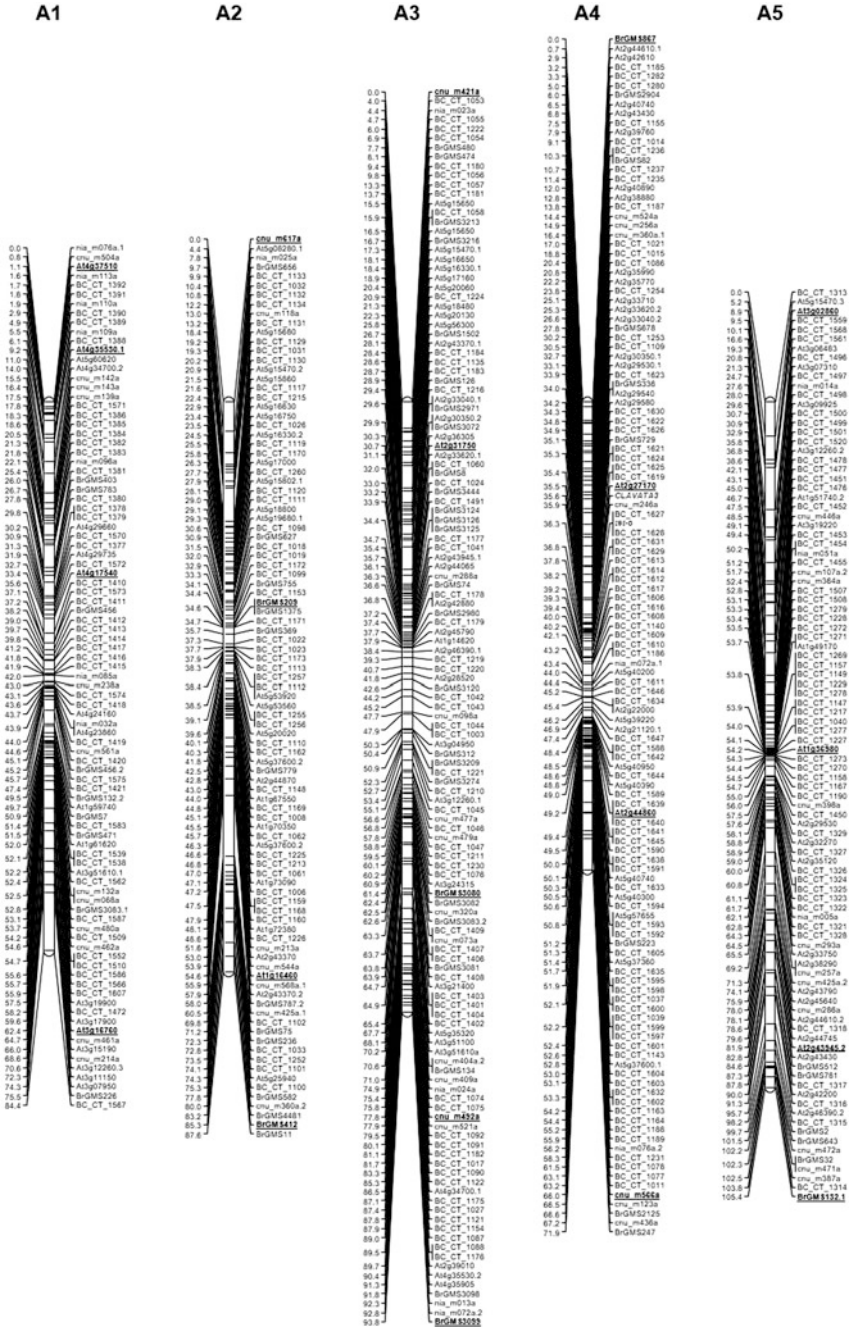

Fig. 6.1 The integrated map of *B. rapa* developed with 1037 markers (595 SNPs, 230 SSR, 211 IP and morphological marker *tet-o*) distributed over 925 intervals spanning a length of 831 cM. Adapted from Yadava et al. (2014)

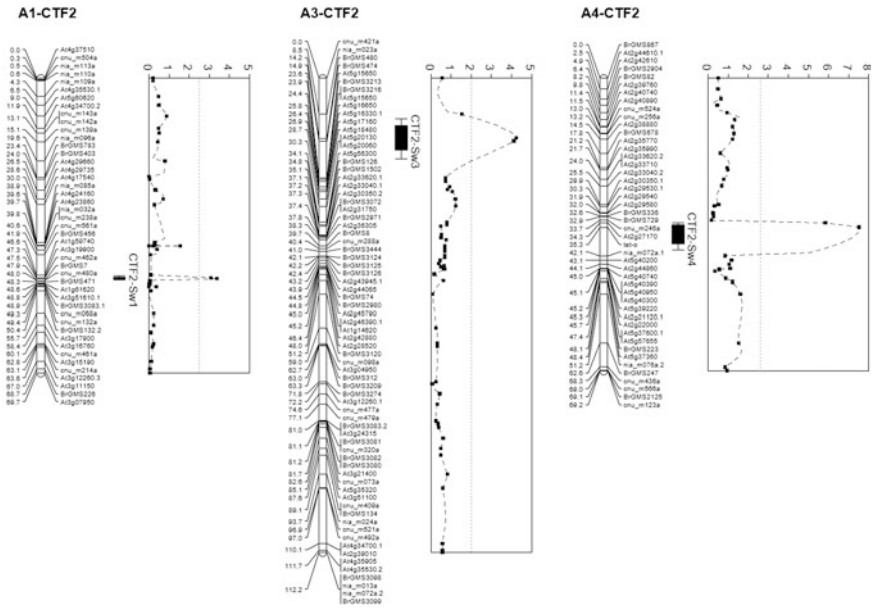

Fig. 6.2 QTL mapping for silique width in CTF_2 mapping population of *B. rapa*. Marker names are on the right of the linkage group bar and the positions in centiMorgans (cM) are on the left. The adjacent graphs show the logarithm of odds (LOD) profile for each linkage group. On the *X*-axis of the graph are the markers and on the *Y*-axis the corresponding LOD scores. The dotted line drawn parallel to the *X*-axis on each graph is the LOD threshold. The position of QTL (one LOD interval) is indicated by bars. The two LOD intervals on either side of the peak are represented by whiskers. Adapted from Yadava et al. (2014)

6.4.3.2 Genetic Mapping of White Rust Resistance

White rust disease, caused by the oomycete *A. candida*, is one of the major diseases to affect Brassica crops. Studies on inheritance of resistance to white rust in Brassica have found different number of genes which governs the trait, i.e., a single dominant locus governing resistance (Ebrahimi et al. 1976; Delwiche and Williams 1974; Tiwari et al. 1988; Kole et al. 1996), two dominant genes (Verma and Bhowmik 1989; Santos et al. 2006) or minor genes (Edwards and Williams 1987; Kole et al. 1996, 2002b). Most of the *B. juncea* accessions belonging to the Indian germplasm are highly susceptible to this disease. However, sources of resistance have been found in the east European germplasm of *B. juncea* (Prabhu et al. 1998; Tiwari et al. 1988; Mukherjee et al. 2001; Panjabi-Massand et al. 2010) and in *B. rapa* (Kole et al. 1996). Several other studies have identified markers linked to the trait governing locus. Prabhu et al. (1998), identified two RAPD markers linked to white rust resistance in *B. juncea*. Varshney et al. (2004) developed CAPs and AFLP markers linked to white rust resistance locus in *B. juncea*. In another study, Panjabi-Massand et al. (2010) mapped two independent loci of white rust in linkage

groups A4 and A5 of *B. juncea* using two doubled haploid mapping population derived from crossing Varuna X Heera and TM4 X Donskaja IV. Heera and Dosnkaja IV are partially and fully resistant European lines, while Varuna and TM4 are susceptible Indian cultivars. Heera and Donskaja IV contributed one independent white rust resistance loci each. They further developed tightly linked molecular markers for marker-assisted selection of white rust resistance traits in *B. juncea*. These markers were more recently validated in different mapping populations and germplasm of *B. juncea* (Singh et al. 2015)

6.4.3.3 Genetic Mapping of Black Rot Resistance

Black rot disease in Brassicas is caused by *X. campestris* pv. *campestris* (Xcc) (Pammel) Dowson. Worldwide six races of pathogens are reported of which, race 1 and race 4 are reported to be more virulent (Soengas et al. 2007). Very rare sources of resistance to this disease have been reported in *B. oleracea*. However, presence of resistance is reported in other Brassica species including *B. rapa* (Bradbury 1986; Soengas et al. 2007; Vicente and Holub 2013). Soengas et al. (2007) in a study detected one major QTL on A06, conferring resistance to both race 1 and race 4, and two QTLs on A02 and A09, conferring resistance to race 4 of the pathogen. The study identified tightly linked markers to the trait that can be used for black rot resistance breeding. These QTLs can be used for finding resistance genes in *B. rapa* and also in *B. juncea* through comparative mapping or candidate gene approach.

6.4.4 Temperature Stress

Stress due to temperature in plants includes; (a) chilling stress—which occurs at temperatures below freezing, (b) freezing stress—which occurs at low temperatures above freezing, and (c) high temperature stress (Źróbek-Sokolnik 2012). Cold stress can affect several physiological, biochemical, and developmental processes in plants, thereby affecting several parameters including seed germination, seedling growth, leaf development and may also result in death of the plant tissue. Cold stress has also been found to severely affect the reproductive development of plants (Humphreys and Gasior 2013).

The genetic control of freezing tolerance in oilseed *B. rapa* and *B. napus* was investigated by Teutonico et al. (1995). RFLP markers developed from cold-induced /plant stress response genes of *B. napus* or *A. thaliana* were mapped in *B. rapa* and QTL analysis was done to identify cosegregation of candidate loci with the variation for freezing tolerance. An RFLP locus from *DHS2* gene [which encodes 3-deoxy-D-arabino-heptulosonate 7-phosphate (*DAHP*) synthase] was mapped in close proximity to the *FTA*/*FTB* QTL on LG 7. This enzyme has been observed to be induced during various stress conditions in *A. thaliana*, such as physical wounding and pathogenic attack (Steponkus 1980; Keith et al. 1991).

Kole et al. (2002a) analyzed winter survival, non-acclimated and acclimated freezing tolerances, and flowering time in RIL populations of oilseed *B. rapa* and doubled haploid lines of *B. napus* derived from crosses between annual and biennial cultivars. A total of 16 QTLs for winter survival were detected, of which six were found in more than one winter season. QTL mapping also provided evidence of greater acclimated freezing tolerance and later flowering time alleles contributing towards enhanced winter survival. Furthermore, the study found a correspondence in the map positions of QTL between species suggesting that allelic variation exists at homologous loci in *B. rapa* and *B. napus*.

In a separate study using an F_2 population derived from a cross between Longyou6 and Tianyou 4, cold resistance and physiological indexes of *B. rapa* varieties were analyzed (Huang et al. 2017c). Physiological indicators were used to indirectly evaluate the effects of low temperatures in the plants. The study identified correlation of the degree of frost damage with the relative conductivity and malondialdehyde content of the plants. Previous studies had also shown that varieties with high malondialdehyde content exhibit reduced cold resistance owing to higher levels of membrane lipid peroxidation in them (Fechner et al. 1986; Lin et al. 2012).

Similarly, QTLs for heat stress tolerance were identified using an F_2 population derived by a cross between a heat-tolerant (line 92) and a heat-sensitive (line 93) Chinese cabbage (*B. rapa* L. ssp. *pekinensis*) lines (Ali and Chung 2013). A genetic linkage map, constructed with RAPD and SSR markers, was used for the QTL analysis, and identified three QTLs contributing 17.6, 18.5 and 41.1% towards the total observed phenotypic variation. The identified QTLs are a useful resource for aiding future MAS program for heat stress tolerance breeding of Chinese cabbage

6.4.5 Agronomic and Yield Traits

Most agronomic traits are governed by polygenes (quantitative traits) and their expression is heavily influenced by the environment. The recent molecular-marker-based genetic mapping and QTL analyses (Ramchiary et al. 2007; Yadava et al. 2012) have emphasized the scope to genetically dissect these complex traits into their component loci and locate their tentative positions in the genome (Table 6.2).

Ramchiary et al. (2007) performed QTL mapping of 12-yield-related traits using a *B. juncea* mapping population in three different environmental conditions. This study revealed that the A genome is the main contributor toward the domestication of the oilseed crop. Major yield QTLs were predominantly clustered on linkage groups A7 and A10 and few others of *B. juncea* genome. Yadava et al. (2012) also performed a genetic analysis of 12-yield-associated traits through meta-analysis and epistatic interaction studies in *B. juncea* using an integrated genetic map of *B. juncea*. This study also reaffirmed the presence of QTL clusters on LG A7, A8, and A10, and provided a detailed insight into the genetic architecture of the yield-associated traits in *B. juncea*.

6.5 Association Mapping Studies

Linkage-based methods to find markers tightly linked to the trait of interest utilize experimental populations that are both limited by the level of allelic diversity and the power of resolution. Association mapping or linkage disequilibrium (LD) mapping, on the other hand, exploits the much higher genetic variability available in the natural population to establish marker-trait associations (Flint-Garcia et al. 2003; Myles et al. 2009). An understanding of the LD structure of the population is crucial prior to any such endeavor, as this helps to decide the number of markers to be used for the study and also defines the power of resolution expected.

Selected studies have utilized natural population to establish marker-trait associations in *B. rapa* and *B. juncea* crop plants. Zhao et al. (2007) undertook association mapping of leaf traits, flowering time, phytate content, and phosphorus levels in a collection of 160 *B. rapa* accessions representing wide range of morphological types and geographical origins. The accessions were genotyped using 437 AFLP markers. The population was found to be structured into four subgroups that differed from each other for the trait values. LD analysis using a limited set of 76 mapped markers was found to be insufficient to estimate the LD decay. Marker-trait association analysis (without correction for population structure) identified a total of 170 markers associated with the scored traits. This number was reduced to 27 markers when the analysis was corrected for population structure. In another study, the same population was deployed to find markers associated with metabolites: tocopherols, carotenoids, chlorophylls, and folate (Carpio et al. 2011a). The population structure analysis (STRUCTURE) using genotypic data scored using 553 (AFLP, Myb, SSR) markers revealed the presence of four subpopulations. Several promising marker-trait associations were identified in the study, of which eight myb and AFLP markers and two SSR markers were found to be strong candidates for MAS in *B. rapa*. Subsequently, a much larger set of 239 *B. rapa* accessions having representation from a wide range of morphological types and geographic origin were developed by Zhao et al. (2010) for association mapping studies. The accessions were characterized for 21 morphological traits and genotyped with 13 SSR primer pairs to evaluate the diversity of the collection. Association mapping studies using two flowering time gene (*BrFLC1* and *BrFLC2*) specific markers exhibited significant association with the trait.

Artem'eva et al. (2013) used association mapping approach in *B. rapa*, to identify genetic determinants of various morphological, biochemical, and physiological traits that included resistance to three races of *Xanthomonas campestris* pv. *campestris* Pam. (Dow.). The study was performed using *B. rapa* VIR (Vavilov All-Russian Research Institute of Plant Industry) core collection of 96 cultivars and 258 SSR and S-SAP markers. In two other studies, the casual polymorphisms in the flowering g*enes, BrFLC1* and *BrFLC2,* associated with flowering time variation in *B. rapa* were identified through population-based association studies (Yuan et al. 2009; Wu et al. 2012). In both cases, the specific gene was initially amplified from a

subset of the total *B. rapa* accessions chosen for the study. Based on the comparative sequence alignments of the multiple alleles that were amplified, the most promising variant was identified and converted into a genetic marker. This marker was used to screen a larger collection of *B. rapa* accessions. For both the genes, the identified variant was found to be significantly associated with the flowering time trait variation seen in the population. An association mapping approach was deployed in *B. juncea* for identifying markers associated with 12 traits including grain yield components and root traits (Akhatar and Banga 2015). The mapping panel comprised of 48 accessions from a Diversity Fixed Foundation Set (BjDFFS). The panel comprising of breeding lines and genotypes selected from important *B. juncea* adoption zones were phenotyped for the 12 traits under irrigated and restricted moisture conditions. The accessions were genotyped using 158 polymorphic SSR markers. Marker-trait association analysis identified a total of 13 and six significant associations under irrigated and restricted moisture conditions, respectively. The genetic determinants of seed grain yield and root traits under variable moisture content identified in the study can be exploited for enhancing the crop productivity.

All these studies are, however, limited by the number of markers (SSRs, AFLPs) used. Next-Generation Sequencing (NGS) technologies have greatly benefited the population-based marker-trait association studies. Rapid, low-cost and high-density marker generations coupled with high-throughput genotyping have enabled Genome-wide Association Studies (GWAS) in *B. rapa* (Gao et al. 2017; Zhang et al. 2018a). GWAS will facilitate identification of novel genes that will expedite efforts toward breeding for climate-resilient traits.

6.6 Genomics-Aided Breeding for CS Traits

Climate change will adversely affect yield of most major crops as predicted by many simulation-based studies (Varshney et al. 2011; Zhao et al. 2017a). Maintaining sufficient global food production would, therefore, be a major challenge for agriculturist world over (Abberton et al. 2016). With the rate at which the population is growing, it is estimated that 1.1–1.3% increase in yield per year of major crops is required to produce enough food to feed the world population in 2050 (Fischer et al. 2014). Most efforts toward increasing crop production have majorly focused on cultivation of limited number of improved crop varieties, optimizing growth conditions, and the use of chemicals and fertilizers to minimize environmental affects (Edwards 2016). However, it is increasingly being realized that efforts need to intensify toward more sustainable agricultural approaches that can generate crops with enhanced environmental resilience.

The "Omics" tools can assist in developing a combinatorial approach toward dissecting plant responses in adverse conditions. Genomic, transcriptomic, proteomic, and metabolomic approaches enable discovery of genes, pathways, and their interactions during plant development and adaptation in adverse climatic

conditions. An understanding of these pathways would facilitate breeding for novel climate-resilient crops. There are two key steps underlying any successful crop breeding program, (a) discovery of the genetic variation or novel alleles present in the population (which is the source for crop enhancement) and (b) improving the efficiency of plant breeding. Recent advancements in plant genomics have revolutionized both these processes. Reference genomes for the major Brassica crops are now available (Wang et al. 2011a; Chalhoub et al. 2014; Liu et al. 2014; Yang et al. 2016) facilitating genetic and genomics studies. Moreover, low-cost genotype-by-sequencing (GBS) methods have made both large-scale, rapid discovery of markers and genotyping of huge populations a reality. GBS has revolutionized all population-based genomic endeavors, including high-resolution mapping, map-based cloning, assessment of genetic diversity, discovery of novel alleles, and Genome-wide Association Studies (GWAS). These advancements in the field of population-based genomic studies have provided unparalleled impetus to genomics-assisted breeding for crops adapted to changing climate (Scheben et al. 2017).

6.6.1 *Whole Genome Sequencing of* Brassica *Crops*

Sequencing and assembly of a reference crop genome lay the foundation for all crop genetic- and genomic-based studies. A reference genome apart from providing an insight into the gene content, genome architecture, and genetic variation, also reinforces functional and comparative genomic studies in the crop. It is a useful resource for developing genome-wide markers that can facilitate genetic and genomic endeavors.

Many *Brassica* genomes have been recently sequenced. This includes *B. rapa* (Wang et al. 2011a), *B. oleracea* (Liu et al. 2014), *B. napus* (Chalhoub et al. 2014), *B. nigra,* and *B. juncea* (Yang et al. 2016). The first Brassica genome to be sequenced was of *B. rapa* ssp. *pekinensis*,cv. Chiifu-401-42 (Chinese cabbage inbred line), as it has a smaller genome compared to the other cultivated Brassica species (Johnston et al. 2005; Lim et al. 2006). The sequencing was one of the first initiatives of a Multinational Brassica Genome Sequencing Project (MBPG) Consortium that was formulated in the early 2000s. The initiative started as a Bacterial Artificial Chromosome (BAC)-to-BAC sequencing strategy, which was soon aborted due to the arrival of the much faster and cost-effective second-generation sequencing technologies. Paired end (PE) read sequence data generated by Illumina GA II technology was integrated along with BAC sequencing information to assemble the draft sequence of the genome (version 1.5; Wang et al. 2011a). For anchoring the genome assembly onto the 10 pseudochromosomes, an integrated map was developed from several linkage maps, including *B. rapa* (Choi et al. 2007; Kim et al. 2009) and an A genome linkage group from *B. napus* map. Additionally, a new *B. rapa* linkage map was developed using 119 lines of a RCZ16_DH population (cross between a rapid cycling line

L144 and Chinese cabbage DH line Z16). The map, with a total length of 1244.3 cM, was constructed using 508 markers, of which 94 SSRs were publicly available. The rest, 414 InDel markers, were developed by resequencing the parental lines L144 and Z16. The assembled genome (283.8 Mb) represented more than 98% of the genic space and 58.52% of the total estimated genome size (485 Mb). A total of 41,174 protein-coding genes were identified based on the available Brassica EST data (NCBI) and the newly generated Illumina RNA-seq PE reads of Chiifu-401-42.

More recently, an improved version of the *B. rapa* genome has been assembled by incorporating additional data generated by Illumina PE reads, mate-paired reads, and PacBio sequencing (Cai et al. 2017). For assembling the scaffolds onto the 10 pseudochromosomes, two high-density genetic maps with a total length of 1316.731 and 1391.516 cM were constructed using 2063 and 1622 bin markers, respectively. The maps were constructed using already available RIL (Yu et al. 2013a) and F_1DH populations. A total of 96,589 and 6944 polymorphic SNPs were identified between parents of the two populations using either the resequencing data available (for RIL) or by performing SLAF-seq (in case of F_1DH). The maps have a much higher marker density as compared to the previous version used to assemble *B. rapa* genome V1.5. The newly assembled *B. rapa* genome V2.0 has a total size of 389.2 Mb representing 80.25% of the total expected size. As compared to V1.5, in the new assembly, an additional 7652 protein-coding genes were identified thereby increasing the total count to 48,826. The new version also provides updated gene models with added information regarding the UTRs and alternate splicing isoforms.

The allopolyploid, *B. juncea* (var. tumida) was sequenced more recently, with the draft sequence being released in 2016 (Yang et al. 2016). The genome was assembled de novo using Illumina HiSeq™ shotgun, PacBio, and BioNano sequencing. The Illumina shotgun sequence reads (176×) generated using 13 PE and mate-paired libraries were used for the initial de novo assembly. The gaps in the assembly were then filled using the 11.09 Gb PacBio single molecule read data (12× coverage). The draft assemblies were aligned with the Irys molecules to correct for scaffold errors, which were then anchored to the optical maps to generate an assembled genome with a total size of 955 Mb size and scaffold N50 of 1.5 Mb. The assembly was validated by mapping 10 random subreads from PacBio which were found to have on an average, >99.4% coverage and 92.3% identity. Additionally, 98.8% of the 458 conserved Core Eukaryotic Genes (CEG database) and 98.9% of the ESTs from NCBI were found to be represented in the genome.

A reference genetic map was constructed based on genotyping by whole genome resequencing of 100 individuals of an F_2 mapping population. PE reads from the two parents were aligned with the draft reference genome to identify 62,580 SNPs. Using the SNPs, a high-resolution genetic map with 18 linkage groups (10 A and 8 B subgenomes) and 5333 bin markers was constructed. Using this map, 91.5% of the A-subgenome-specific assembly sequences were anchored on the 10 pseudochromosomes and 72.3% of the B-subgenome-specific assembly sequences were anchored onto eight pseudochromosomes. The final assembled genome is represented

as 402.1 Mb BjuA and 547.5 Mb BjuB subgenomes. RNA-seq data (11.56 Gb) of RNA pooled from different tissues (root, stem, leaf, flower, and silique) was also generated using Illumina HiSeq^{TM} 2000 sequencing platform. This was used to validate the 80,050 protein-coding genes models that were annotated in the genome.

The genome sequences of the two A subgenomes belonging to the allopolyploids *B. juncea* (BjuA) and *B. napus* (BnaA) were compared with *B. rapa* A genome (BraA) to study their divergence from the common *B. rapa* ancestor. For this, A subgenomes from 18 *B. juncea* accessions, five *B. napus* accessions, and 27 *B. rapa* accessions (representing most subspecies) were resequenced. The SNPs generated were used to construct a neighbor-joining tree that identified divergent origin of BjuA and BnaA. While BjuA was found to be derived most likely from *B. rapa* ssp. *tricolaris*, BnaA appeared to have been derived from the *B. rapa* ssp. *rapa*. The A subgenome of all *B. juncea* accessions was found to have diversified from a common ancestor (monophyletic origin) into different oil- and vegetable-type subvarieties. Further, using phylogenetic and Bayesian methods (Drummond et al. 2012), *B. juncea* was estimated to have formed ~0.039–0.055 Mya.

The availability of the reference genomes for Brassica crop plants not only provide a deeper insight about the genome organization and evolution but have also greatly facilitated development of functional genomic approaches toward gene discovery. Together with this, the advancements made in the low-cost sequencing and genotyping platforms have ushered in an era of genomics-assisted breeding.

6.6.2 Advances in Gene Discovery and Marker Development

6.6.2.1 Transcriptomic Approaches

Analysis of the transcriptome is an efficient way to decipher the mechanisms underlying the plant response to different environmental conditions, biotic and abiotic stresses, or during different developmental stages. Transcriptomic technologies aim toward characterization and the relative/absolute quantification of RNA. High-throughput transcriptomics started with the development of microarray technologies, which detect/quantify RNA present in the test sample by hybridization with a set of probe sequences present on microchips. DNA microarrays comprise a set of known DNA transcript sequences that are present on a solid support at fixed locations. The earlier versions were built by immobilizing transcripts (cDNA microarray), while in the more recent ones (oligonucleotide array), 20–70 nt long oligonucleotides designed from the transcript are attached to the chip (Knudsen 2004). However, as the technique relies on a set of predetermined probe sequences, it is unable to detect for novel transcripts and also the background hybridization signals interfere with detection of RNA expressed at very low levels.

Two of the earliest versions of *B. rapa*-specific microarrays were KBGP-24K (Lee et al. 2008) and KBGP-50K, developed using the Nimble-Gen platform. The 24 K chip was developed using sequence data of 24,000 unigenes that were represented as 60mer long probes, with six probes per gene. The 50 K chip was developed with an additional 8500 unigenes and about 17,500 annotated genes from the BAC sequencing data. An advanced version of this, Br300 K (version 2) was developed using 47,584 unigenes, that were available upon completion of the *B. rapa* genome sequencing project (Dong et al. 2013). Of the total genes utilized for the construction, 17,458 genes were predicted from BAC sequences with no cDNA/EST support. More recently, version 3 of the microarray, Br135 K, representing all the 41,173 genes of the sequenced *B. rapa* accession Chiifu-401-42 was developed (Jung et al. 2014). The chip was constructed using 60mer long probes with three probes developed from each gene. Some of other microarray resources, developed to enable wider applicability across Brassica species, utilized gene sequence information from more than one Brassica species. One such array was developed using 810,254 EST sequences belonging to *B. napus* (567,240), *B. rapa* (180,611), and *B. oleracea* (59,696) genomes (Trick et al. 2009). Using this information a total of 90,864 unique sequences were assembled. A 60-mer oligo microarray was constructed with 94,558 probes representing the identified unigenes. An improvised version of this, 135 K Brassica Exon array platform, was developed using sequence information from 135,201 gene models. This Affymetrix GeneChip® array contains 2.4 million 25-base oligonucleotide probes with 15 probes per gene (Love et al. 2010). The microarray resources developed have been used extensively to understand gene expression in Brassica crops under various conditions including heat stress (Lee et al. 2013a; Dong et al. 2015), cold stress (Yang et al. 2005; Jung et al. 2014; Hwang et al. 2014), and abiotic and biotic stresses (Kayum et al. 2015, 2017a).

Over the past few years, NGS-based RNA sequencing (RNA-seq) technologies have revolutionized the transcriptomic studies. RNA-seq analysis that aims at capturing all the expressed transcripts has gained immense popularity owing to no prerequisite of a prior sequence information, low cost of sequencing, ease of data generation, and power of computational analysis (Wang et al. 2009). Moreover, RNA-seq can be used to detect quantitative and qualitative expression of all kinds of RNA transcripts including mRNAs, microRNAs, LncRNAs (long non-coding RNAs), and small interfering RNAs (Marioni et al. 2008; ‘t Hoen et al. 2008). The technique allows for qualitative and quantitative comparative profiling in different tissues, developmental stages, environmental conditions, and across individuals. Apart from the expression profiling, the RNA-seq data also assists in annotation of reference genomes and is a useful resource for development of genetic markers.

6.6.2.2 Genotype by Sequencing

The conventional marker systems, including RFLP, AFLP, and SSRs, are limited by their number and distribution or coverage of the genome and involve huge time

and effort in genotyping. The high-throughput NGS technologies have greatly decreased the time and cost of generating large number of markers which represent much wider genomic regions and can be deployed for very high-resolution genetic maps. Genotype by sequencing (GBS) enables rapid and low-cost genotyping of large populations

Several types of GBS methods have been developed that can be broadly divided into two categories; Whole Genome Resequencing (WGR) and Reduced-Representation Sequencing (RRS) methods (reviewed extensively by Scheben et al. 2017). WGR is mostly performed at low coverage of <1× (skim genotyping-by-sequencing or SkimGBS). This low coverage is adequate for genotyping large recombinant populations with high-quality parental reference genome (Golicz et al. 2015). However, WGR approaches for populations with large genomes remain costly. In such cases, RRS is advisable which represents fraction of the total genetic diversity present in the genome. The RRS approach usually involves digestion of genomic DNA with restriction enzymes (1–2 depending on the method) before sequencing, thereby reducing the genome complexity (Miller et al. 2007; Baird et al. 2008). There are at least 13 different RRS approaches available that includes, Restriction Enzyme-Associated DNA Sequencing (RADseq), Elshire GBS, Two-enzyme GBS, Sequence-Based Genotyping (SLAF-seq), RAD capture methods, etc. (Baird et al. 2008; Elshire et al. 2011; Poland et al. 2012; Sun et al. 2013; Ali et al. 2016). The various approaches differ in the number and type of enzymes used, average genome coverage, and the cost of genotyping (Scheben et al. 2017). Although, prior reference genome information is beneficial for the RRS approach, it is not a prerequisite. This makes RRS approaches a method of choice for non-model species and crop plants having complex genomes (Poland et al. 2012).

GBS allows for rapid identification of large number of genome-wide SNPs which could be used further for development of dense genetic maps, refinement of the target genome regions, diversity assessment, GWAS, genomic selection approaches, phylogenetic, and evolutionary studies in crop plants. Combining GBS along with the phenotypic data of the population enables for rapid, high-resolution mapping and identification of genes underlying major agronomic traits (Edwards et al. 2013). Examples of use of GBS approaches to facilitate breeding are known in several important crop plants (Huang et al. 2009; Elshire et al. 2011; Morris et al. 2013; Uitdewilligen et al. 2013) including Brassica (Yu et al. 2013a, 2017; Huang et al. 2017a; Jing et al. 2016). The GBS generated data are invaluable resources for breeding for climate-resilient crops.

6.6.2.3 Pangenomics

Apart from SNPs, structural variants also contribute significantly toward the genetic diversity within a species. These structural variants including Copy Number Variants (CNVs) and Presence/Absence Variants (PAVs) lead to variable amount of total genomic sequence found in individuals (Saxena et al. 2014). Sequences that

are present in variable copy number between individuals are referred to as CNVs, while those that are present in only some individuals are called PAVs. Multiple studies have reported the presence of structural variants even within individuals of the same species (Springer et al. 2009; Tan et al. 2012; Zhang et al. 2014b). Thus, a single crop reference genome is not a sufficient representation of that species. In order to get complete genomic content of a species, construction of a pangenome is essential. A pangenome represents all the genes in a given species including the core genes (present in all individuals) and the variable genes (found in only some individuals). Being first reported in bacteria (Tettelin et al. 2005), similar studies have now been reported in several plants including, rice (Schatz et al. 2014; Yao et al. 2015), maize (Gore et al. 2009; Hirsch et al. 2014), soybean (Lam et al. 2010; Li et al. 2014), *B. oleracea* (Golicz et al. 2016), and *B. rapa* (Lin et al. 2014).

An initial *B. rapa* pangenome has been developed by Lin et al. (2014). The group assembled and annotated two new reference genomes in *B. rapa* by resequencing representatives from morphologically very distinct subspecies; vegetable turnip (DH-VT117) and annual oilseed (RC-144). This study was initiated to develop an insight into the genetic basis of the huge morphological variation observed between the two *B. rapa* subspecies. The two newly assembled genomes were integrated with the preexisting *B. rapa* (Chiifu) reference genome (Wang et al. 2011a) for pangenome studies. The pangenome was analyzed to identify common (present in all accessions) and unique (morphotype-specific) genes. Genes in the pangenome were also compared with *A. thaliana* and *Thellungiella halophila* to define orthology and identify orthologs missing or retained in only one of the three *B. rapa* genomes. Using orthologous genes, the estimated date of divergence among the three morphotypes was found to be about 250,000 YA that is much earlier than the Brassica domestication. The *B. rapa* pangenome provides a better representation of the genetic diversity available for the crop and will be a useful resource for molecular breeding of climate-resilient Brassica crops.

6.6.3 Applications of Structural and Functional Genomics in Genomics-Assisted Breeding

6.6.3.1 Transcriptome Analysis and Gene Discovery

Plants have evolved different mechanisms to adapt to or deal with the various abiotic and biotic stresses. Genome-wide gene expression profiling provides useful insights into the complex molecular mechanisms involved in providing tolerance/resistance to abiotic/biotic stresses. Most of these profiling studies rely on analyzing Differentially Expressed Genes (DEGs) in stress versus non-stress conditions to identify and characterize genes and gene networks that underlie the plant response in a particular stress condition. Functional genomic approaches generate an invaluable resource to identify novel genes that can be used to develop climate-resilient crops.

Abiotic Stresses

Initial studies to decipher genome-wide gene expression of *B. rapa* were done using microarrays. In one such study, a *B. rapa*-specific microarray developed from 6233 unique cDNAs, identified cold-responsive genes in Chinese cabbage (Yang et al. 2005). Heat Shock Proteins (HSPs) were found to be predominantly upregulated under prolonged cold stress. A subsequent study used the first high-density *B. rapa*-specific microarray, KBGP-24K, to identify a total of 417, 202 and 738 DEGs under cold, salt, and drought treatments, respectively (Lee et al. 2008). Two differently thermo-tolerant *B. rapa* varieties Chiifu and Kenshin were compared for their gene expression profiles in response to heat shock using KBGP-24K microarray (Lee et al. 2013a, b) and later with the improved version, Br135 K microarray (Dong et al. 2015). Both varieties were found to respond to heat temperature by induction of genes encoding small Heat Shock Proteins (HSPs) and Heat Shock Factor (Hsf)-like proteins. Br135 K microarray was also used for genome-wide transcription analysis of the *B. rapa* lines (Chiifu and Kenshin) under cold treatments. Apart from the DEGs and unique transcripts, common signaling pathways and various transcriptional regulatory mechanisms involved during the cold stress were also identified (Jung et al. 2014).

This low temperature 135 k microarray dataset generated has been used extensively in independent studies to identify cold-responsive *B. rapa* genes belonging to specific gene families. In all these studies, initially, gene members belonging to the specific gene family, including transcription factors (*bZIP*, MADS-box, *TIFY*, *WRKY)* and genes coding for aquaporin (*AQPs*) and protein disulfide isomerase (*PDI*) were identified from the *B. rapa* reference genome based on *in silico* sequence analysis and characterization (Hwang et al. 2014; Saha et al. 2015, 2016a; Kayum et al. 2015, 2017a, b). Using this sequence information, cold-responsive gene members from each of the gene families were identified from the low temperature 135 k microarray dataset. The identified genes were further characterized for their role in different abiotic and biotic stresses by qPCR analysis of suitably treated tissue samples.

Arsenic (As), a highly toxic contaminant, is another major abiotic stress for crop plants. High levels of As affect both plant root and shoot growths, and thereby reduce the overall yield of most crop plants (Chaurasia et al. 2012; Kumar et al. 2015). Transcriptome profiling of *B. juncea* subjected to As stress was performed to unravel the mechanisms involved in the plant response to this stress (Srivastava et al. 2015). For the study, a microarray developed using 26,881 unigenes from different Brassica species including *B. juncea, B. napus,* and *B. rapa* was used. RNA profiling of root and shoot samples from plants exposed to As stress for different time durations identified a total of 1285 arsenic responsive genes belonging to various signaling pathways. A set of genes were validated through RT-PCR for further use as indicators of As stress.

With the recent advancements in the NGS technologies, transcriptome profiling is predominantly being performed using RNA-seq. In Brassica crops also, several studies have used RNA-seq to identify genes and mechanisms involved in abiotic

stresses. Genome-wide transcription analysis using tag sequencing with Solexa Illumina array was performed to capture the gene expression of *B. rapa* in drought conditions (Yu et al. 2012a). The study identified 1092 dehydration response genes highlighting the complexity of the molecular mechanisms involved in the response to drought stress. RNA-seq (using Illumina HiSeq 2000) analysis of *B. rapa* plants subjected to heat stress, identified 625 DEGs which included transcription factors, kinases/phosphatases, and effectors of homeostasis (Wang et al. 2016a). In a similar study in *B. juncea,* a combinatorial approach of next-generation sequencing and *de novo* assembly was used to unravel the transcriptome under high temperature and drought stress (Bhardwaj et al. 2015). The study identified 77,750 unique and 19,110 differentially expressed transcripts. Most of the upregulated transcription factors in both stress conditions belonged to HSF and Dehydration-Responsive Element Binding (DREB) families. A total of 239 metabolic pathways affected during the two treatments were also identified. Sinha et al. 2015, performed de novo transcriptome profiling of cold-stressed *B. juncea* siliques at different developmental stages. *B. juncea* siliques are sensitive to freezing stress during seed development and severe stress results in seed abortion. Siliques from six different developmental stages were subjected to cold stress at two different time intervals (short and long durations). In total 18 RNA-seq libraries (including controls) were constructed and sequenced using Illumina NGS platform. Pair-wise comparison of the different libraries led to identification of a total of 13,342 DEGs. These were segregated into two clusters based on the stage of silique development; early (5–15 days after pollination) and late stages (20–30 days after pollination). Based on the analysis of the two groups, 283 "core cold-inducible" transcripts (common to both stages), 689 transcripts upregulated in early stages, and 100 transcripts upregulated in late stages of development were identified.

Salt stress is another major abiotic stress affecting *B. rapa* (Qiu et al. 2015). Digital Gene Expression Profiling (DGE) of *B. rapa* plants subjected to salt stress for 12 h identified a total of 1235 DEGs that included transcription factors, antioxidant proteins, and proteins belonging to signal transduction and osmolyte synthesis pathways (Qiu et al. 2017). Similarly, RNA-seq analysis of control and salt-stressed *B. juncea* identified 1469 differentially expressed unigenes. Genes found upregulated belonged to calcium signaling, ROS detoxification, and sulfur assimilation pathways (Sharma et al. 2015). One of these, a transcription factor (*RITF1* homolog), regulating *SOS1* and several other ROS scavenging genes, was found to be upregulated by >100 folds.

RNA-seq analysis has also been used to elucidate mechanisms underlying drought tolerance in Brassica crops. Guo et al. (2017) performed comparative transcriptome analysis of two *B. rapa* genotypes; a drought-tolerant wild type variety (CR2355) and a drought-sensitive variety ATC92037 (yellow sarson type), under drought and control conditions. During the first 12 h of treatment, significant differences in the stress response were observed between the two genotypes. In the drought-tolerant plants, a rapid upregulation of jasmonic acid and salicylic acid metabolism genes was observed. Genes involved in endoplasmic reticulum stress and programmed cell death was also upregulated. In another study, transcriptomic

profile of root and leaf samples from control and drought stressed Chinese cabbages identified stress-induced activation of several transcription factor genes (Eom et al. 2018). These included AP2/ERFs, bHLHs, NACs, and bZIPs. Analysis of the DEGs indicated differences in the drought response mechanisms in the root and leaves. Greeham et al. (2017) performed temporal network analysis to identify early signals of drought in *B. rapa*. The temporal transcriptomic profile and the physiological response of *B. rapa* plants subjected to mild stress was measured over a period of 2 days. The physiological parameters measured included photosynthetic rate, stomatal conductance, and efficiency of photosystem II in light conditions. A co-expression network approach was used to co-relate the changes in the physiological parameters with that of the gene expression profile. Based on the study stomatal conductance and efficiency of photosystem II were found to be reliable indicators of mild drought in *B. rapa*.

Apart from DEGs, stress-responsive small non-coding RNAs (smRNAs) have also been identified and are found to be involved in regulating plant responses toward stress conditions. Heat-responsive miRNA and nat-siRNA were identified through RNA-seq analysis in *B. rapa* (Yu et al. 2012b, 2013b). High temperature was found to suppress the production of another class of non-coding smRNA derived from the chloroplast; csRNA (Wang et al. 2011b). More recently, cold and heat treatments identified 9687 novel LncRNAs apart from 14,329 DEGs in *B. rapa*. A co-expression network analysis for LncRNAs and miRNA led to the identification of LncRNA specific genes regulated under cold and heat treatments (Song et al. 2016). A comprehensive abiotic stress influenced miRNAome of *B. juncea* was generated by ultra-deep parallel sequencing using Illumina Genome Analyzer IIx (Bhardwaj et al. 2015). Three smRNA libraries were prepared from seedling of *B. juncea* that were subjected to different conditions; high temperature, high salt, and drought. These, along with an untreated sample, were subjected to deep sequencing and the data generated was pooled for downstream processing. In the absence of a reference genome, genome sequences were also generated and a reference map was assembled at low coverage. Using this and the publicly available Brassica genomic/transcriptomic resources, a set of 126 novel and 51 conserved miRNAs were identified. The expression profile of a few selected members from both categories was reconfirmed under different stress conditions using RT-PCR.

Cold-responsive miRNAs were identified by Zeng et al. (2018) in two *B. rapa* varieties, "Longyou 7" (cold-tolerant) and "Tianyou 4" (cold sensitive). Small RNA libraries prepared from leaves and roots of cold-stressed plants were subjected to high throughput sequencing. In total, from the two varieties 353 cold-responsive miRNAs were identified of which 84 were found to be novel. Differentially expressed miRNAs between the two varieties, included both novel miRNAs and miR166 and miR319 family members. Most of the target genes predicted for a set of known and novel miRNAs were found to belong to metabolic and stress-responsive pathways.

Bilichak et al. (2015) explored the role of smRNA in providing transgenerational inheritance of heat-shock-induced response (stress memory) in *B. rapa*. The somatic and reproductive tissues from heat-shock-treated plants and their progeny

were subjected to sequencing based transcriptome and small RNA profiling. Both the transcriptome and the smRNAome were found to contribute toward the stress memory in *B. rapa* with maximum perturbations found in the smRNAome of the progeny. The study discovered the heat-induced small RNA derived from tRNA that has the ability to get transmitted into the next generation.

Biotic Stress

Transcriptome profile of Brassica plants in response to various biotic stresses has also been characterized to identify the genes and gene mechanisms underlying host–pathogen interactions. Narusaka et al. (2006) developed a *B. rapa* microarray by spotting 1820 cDNA clones. The microarray was used to compare the expression profile of *B. rapa* plants infected with the fungal pathogen *Colletotrichum higginsianum* with those treated with the signaling molecules, salicylic acid, methyl jasmonate, or ethephon. The two expression profiles were found to be highly correlated. In most other studies, a comparative RNA-seq analysis of the resistant and susceptible lines has been performed to gain insight into the plant–pathogen interactome. The *B. rapa-Plasmodiophora brassicae* pathosystem was studied using comparative RNA-seq analysis of pathogen-infected resistant and susceptible near-isogenic lines (Chen et al. 2016). *P. brassicae* causes clubroot disease in Brassica crops. A total of 3978 DEGs were identified. The resistant line had higher expression of the effector receptors and PR genes of the salicylic acid (SA) pathway, while most of the PAMP receptors were suppressed, suggesting a strong Effector-Triggered Immunity (ETI) response against the pathogen. A similar approach was used to analyze the plant response toward another major disease of *B. rapa*, Fusarium yellows, caused by *Fusarium oxysporum* f. sp. *conglutinans* (Miyaji et al. 2017). Comparative transcriptome analysis of the pathogen inoculated and uninoculated resistant and susceptible lines of *B. rapa* plants revealed several DEGs. qRT-PCR analysis revealed that most genes were upregulated 24 h after infection. Comparative analysis with infected *A. thaliana* plants identified common DEGs between the two species. Another study utilized RNA-seq to explore the mechanisms involved in the biocontrol of *P. brassicae* pathogen by the antagonistic bacteria *Zhihengliuell aaestuarii* in *B. juncea* var. *tumida* Tsen (Luo et al. 2018). Three transcriptome analysis was conducted, one with the plant inoculated with the pathogen, second of plant simultaneously infected with both the pathogen and the biocontrol and the third of an uninoculated plant sample. A total of 19.94 Gb data generated was de novo assembled. Between the pathogen-infected and the biocontrol-treated samples, a total of 5629 DEGs belonging to 126 KEGG pathways were identified. The analysis enabled identification of biocontrol-induced genes and pathways in the plant against the pathogen.

Stress-responsive miRNAs and miRNA-mediated regulatory networks involved during the *B. rapa-P. brassicae* interaction were identified using deep sequencing and degradome analysis (Wei et al. 2016). Transcriptome analysis of pathogen inoculated and control *B. rapa* root samples identified 221 known and 93

potentially novel miRNAs. Between the two samples, 14 known and 10 novel miRNAs were differentially expressed. Most of the miRNA putative target genes belonged to the selenocompound metabolism and hormone signal transduction. In another study, *B. rapa* plants infected with bacterial soft rot caused by *Erwinia carotovora* were found to have increased expression of host 28-nt smRNAs. The infected tissue had also accumulated certain host smRNAs homologous to the microbe genome (Sun 2014).

These studies on characterization of the transcriptomic profiles of Brassica crops under various biotic and abiotic stresses have generated useful resources for identifying novel genes or alleles that can be used to breed for climate-resilient *Brassica* crops.

6.6.3.2 Sequencing Based Trait Mapping

Most of the earlier QTL mapping studies using traditional markers was unable to generate effective markers for trait introgression. Further, in very few cases, the candidate genes could be identified. The present repertoire of sequencing information available for Brassica crops like the availability of reference genomes, BAC sequences, ESTs, and RNA-seq data, provide valuable resources for generation of large number of genetic markers that have been utilized to construct high-density linkage maps and also to refine mapped trait intervals leading to candidate gene identification in both *B. rapa* and *B. juncea*. Moreover, GBS platforms have further revolutionized such studies.

Trait Mapping Using GBS of Parents and Individuals of Population

Several studies in *Brassica* crops have deployed GBS of both the parents and the individuals of the entire mapping population for trait mapping. In one such study, WGR approach was successfully deployed to map QTLs for leafy heads in *B. rapa* using 150 RILs derived from crossing heading and non-heading Chinese cabbage (Yu et al. 2013a). The WGR of the parents (33–45×) and low coverage sequencing (0.2×) of 150 RIL individuals enabled development of a genetic map with 2209 high-quality SNP markers. In this study, 18 QTL for six head traits were identified. Similarly, six QTLs controlling flowering time were mapped in *B. rapa* using resequencing of the parental lines (5× coverage) and 172 individuals (0.5× coverage) of a DH population (Jing et al. 2016). A total of 22,747 identified SNPs were used to construct a high-density linkage map (with 887 bin markers) to facilitate the QTL mapping. Putative candidate genes were identified based on *B. rapa* genome annotation. Another high-resolution genetic map was developed in *B. rapa* L.syn. *B. campestris* through RAD-seq of the parental and 120 F_2 individual lines (Huang et al. 2017a). The linkage map developed using 3985 SNP markers was used to identify QTLs for 16 morphological and agronomic traits in *B. rapa*. All these studies of trait mapping with high-density genetic maps are useful resources for

marker-assisted selection and introgression of the trait and the subsequent map-based cloning of the underlying gene.

Trait Mapping Through Sequencing of Pooled Samples

Several studies have combined GBS with Bulk Segregant Analysis (BSA) to facilitate trait mapping. In one such study, GBS was used to identify QTLs for clubroot resistance in *B. rapa* (Yu et al. 2017). Clubroot disease, caused by *P. brassicae,* is an important disease affecting *Brassica* crops. In the study, a total of 1584 SNPs were identified through GBS of the resistant (T19) and susceptible (ACDC) *B. rapa* parental lines and the 92 BC_1 lines. QTL mapping identified a single major QTL conferring resistance to six different pathotypes and contributing toward 85–95% of the total phenotypic variation. Another two QTLs imparting resistance to a novel pathotype 5× were also identified. From the BC_1 population, resistant, and susceptible individuals were selected based on both their response to the pathogen and the genotyping profile (presence of SNP allele in the identified QTL). DNA short-read sequences from the identified resistant and susceptible individuals were pooled to generate two bulks. The two bulked sequences were aligned to the reference genome. TIR-NBS-LRR proteins were identified from each of the three QTL and DNA poly variants for each gene were analyzed from the two bulks to shortlist putative candidate genes.

Sequencing of bulked samples has also been used in several studies to reduce the candidate intervals of previously mapped QTL, thereby facilitating identification and in certain cases cloning of the candidate gene. In one such study, Whole Genome Resequencing (WGR) with Bulk Segregant Analysis (BSA) was used for fine mapping of a previously identified *BjPC2* loci that controls flower color in *B. juncea* (Zhang et al. 2018b). WGR of the parents of the BC_4 population and the two contrasting bulks (yellow and white flowers) were performed to identify polymorphic SNPs. Based on the SNP profile of the two bulks, a candidate-interval spanning 2.45 Mb and housing 371 annotated genes was identified. New SSR markers developed from the candidate interval were used to develop a linkage map further narrowing the region to 31 kb. Of the six annotated genes found in this region, *BjuB027334* was identified as the most promising candidate gene based on expression profiling and RNA-seq analysis. In another study by Wang et al. (2016b), a 2.8 Mb interval on A09 of *B. rapa*, harboring a QTL for seed coat color gene (*Brsc1*; Xiao et al. 2012) was initially narrowed to 1.04 Mb by using region-specific SSRs that were designed from the *B. rapa* reference genome. WGR of the parents and the two bulks (yellow-seeded and brown-seeded individuals) from a BC_4 mapping population identified three candidate intervals for *Brsc1*. One of these three intervals was found to overlap with a region that was identified through construction of linkage map of the region surrounding the *Brsc1* gene. Based on the two approaches, the candidate region was reduced to 678 kb. Of the total of 46 predicted genes within this region, *Bra028067* (*BrTT1*) was identified as the most likely candidate gene based on its similarity with Arabidopsis *TT1* gene

involved in flavonoid synthesis. *BrTT1* was found to be mainly expressed in Brassica seeds and had sequence polymorphisms between the yellow- and brown-seeded plants further strengthening its candidature.

WGR with BSA also facilitated fine mapping of *BjPl1* gene responsible for purple leaf color in *B. juncea* (Zhao et al. 2017b). Using SNPs derived from WGR of the parents and the two DNA pools (of purple- and green-leaved individuals) from the BC_4 population, the initial mapped region (225 Mb) was further delimited to two smaller regions of 65 kb in total. Four homologous anthocyanin biosynthetic genes were identified within one of the intervals spanning 27 kb.

Bulk Segregant RNA Sequencing (BSR-seq) approach was deployed to identify variants present in the *Rcr1* gene imparting clubroot resistance in *B. rapa* (Yu et al. 2016). The gene was earlier mapped to a 0.28 cM interval on A03 using SSR markers (Chu et al. 2014). Using BSR-seq of the resistant and susceptible pools, variants were identified within the 21 annotated genes present in the *Rcr1* region. Four genes having highest polymorphic variants were selected as the most probable candidate genes. Fourteen SNP markers spanning ~42 kb, developed from these four genes were genotyped using KASP method and found to be completely associated with the *Rcr1* gene. The *Rcr1* locus was subsequently introgressed in *B. napus* using marker-assisted introgression using 7 of the 14 SNPs identified. A similar approach was used to map another locus (*Rcr2*) imparting clubroot resistance (Huang et al. 2017b). The locus was mapped onto chromosome A03 of *B. rapa* using 173,383 SNPs identified through BSR-seq of the resistant and susceptible pools. For fine mapping, 15 SNPs of the total of 500 identified within the *Rcr2* interval (4 Mb) were used to genotype a larger set of segregating F_1 individuals (675 plants) using KASP assay thereby reducing the interval to 0.2 cM. Six genes were identified as the most likely candidate genes as they had high number of polymorphic variants present.

6.6.3.3 Genome-Wide Association Studies (GWAS)

Genome-wide association based studies involve population-based approaches to identify the genetic determinants of any trait. Success of such endeavors, apart from other prerequisites, requires genotyping of a large population with high-density, genome-wide markers. Large-scale genotyping has always been a major limitation. However, in the last decade, the advancements in cost-effective NGS technologies, availability of newer genotyping methods (GBS) and improved data processing has made GWAS a possibility even in crop plants with larger genome size and higher complexities.

In one such study in *B. rapa*, a total of 116 accessions were subjected to GWAS to identify genetic determinants for flowering time regulation (Gao et al. 2017). The accessions were resequenced at 1.2× sequencing depth and also phenotypically characterized for flowering time variations in both greenhouse and open-field conditions. LD analysis with the identified 2000 high-quality SNPs revealed a LD decay at 2.3 kb. Through GWAS, 33 SNPs exhibiting significant association with

flowering time were identified, 13 of these were common in both the growth conditions. Based on comparative analysis of syntenic regions in *A. thaliana* regions, 14 candidate genes involved in flowering time were identified.

A combinatorial approach of bi-parental mapping and GWAS was used to identify the functional SNP affecting leaf hair (trichome) number in *B. rapa* (Zhang et al. 2018a). Trichomes, present on plant surfaces provide physical barrier against several biotic and abiotic stresses and hence are an important trait. A major QTL for hairy leaves was previously identified using bi-parental mapping approach and *Bra02511* was found to be the most probable candidate gene based on comparisons with the *B. rapa* reference genome and *A. thaliana* (Yu et al. 2013a). Cloning (based on available genomic resequencing data) and sequence comparison of the allelic counterparts of the gene from the two parents was not informative as several polymorphic SNPs were found to be present. Hence, the gene was cloned from 13 representative *B. rapa* accessions and the sequences obtained were compared to identify variants. None of the identified SNPs exhibited strong association with the hairy phenotype. Therefore, the search for the causal SNP was extended to a larger population comprising of 210 *B. rapa* accessions which were also resequenced. Based on marker-trait association analysis one SNP was shortlisted. The functional SNP was subsequently identified though mutagenesis and functional complementation of an Arabidopsis null mutant line.

6.6.3.4 Genetic Diversity and Population Structure Analysis

Assessment of the genetic variation available within a crop is an essential component of any breeding endeavor. Genetic variation for most traits, including tolerance to abiotic and biotic stresses exists in the natural population but due to selection and domestication only a portion of this gets incorporated into the cultivars. It is, therefore, crucial to identify and preserve the available genetic diversity to facilitate targeted crop breeding programs. NGS-based technologies have revolutionized population-level high-density genotyping to identify the widespread genomic variation within species (Huang and Han 2014). High-throughput discovery of variation powered with ability of sequencing by multiplexing of samples (as many as 384) has led to SNPs becoming a marker of choice for most studies.

Earlier studies to assess the genetic diversity in *B. rapa* and *B. juncea* were based on conventional marker systems, mostly RAPD, AFLP, and SSRs (Zhao et al. 2005, 2009; Khan et al. 2008; Carpio et al. 2011b; Tahira et al. 2013; Guo et al. 2014). More recently, SNPs developed from the available genomic resources for Brassica crops, through NGS-based resequencing of accessions and GBS have been utilized for such studies. Zhang et al. (2014c), performed resequencing of four genotypes of *B. rapa* to develop SNP markers that were used for characterization of 56 accessions of *B. rapa* subsp. *rapa*. The resequencing data from the four genotypes, line L144 (subsp. *oleifera*), line R-0-18 (subsp. *trilocularis*), Wutacai (subsp. *narinosa*), and DH_VT_117 (subsp. *rapa*) were compared with the Chiifu reference genome (Wang et al. 2011a) to generate genome-wide SNPs. Population

structure analysis using genotyping data generated from 280 SNP markers identified two subpopulations. The accessions were also characterized for 16 leaf traits, 12 tuber traits, and flowering time. In another study, a set of 61 *B. rapa* accessions, mostly oil type, were genetically characterized using 209 SNP markers discovered through amplicon resequencing (Tanhuanpää et al. 2015). The SNP markers were used to genotype 893 individuals from the collection using Illumina BeadXpress. Population structure analysis divided the accessions into three diverse STRUCTURE groups. The three groups partially correlated with the morphology and flowering habit, but had no correlation with the geographic origin. GBS of a subset of 12 accessions was performed to identify 5727 GBS-SNPs. Neighbor-joining trees of the 12 accessions built from GBS-SNPs was highly correlated with that built using BeadXpress derived SNPs.

Utilizing the advances made in the DNA sequencing technologies, Cheng et al. (2016a, b) resequenced a large set of accessions of both *B. rapa* and *B. oleracea* to identify the genetic basis of diversification and domestication in the two crops (Cheng et al. 2016a, b). The Brassica crops display extreme morphological characteristics. During the process of domestication, similar morphotypes have got selected for, in different species of the crop. A total of 199 accessions representing 12 morphotypes of *B. rapa* and 119 accessions representing 9 morphotypes of *B. oleracea* were subjected to whole genome resequencing using Illumina HiSeq 2000 platforms. The species-specific resequencing reads generated were aligned to their respective reference genomes, i.e., *B. rapa* genome version 1.5 (Wang et al. 2011a) and *B. oleracea* V1.0 (Liu et al. 2014). This comparison identified 2,249,473 SNPs and 303,617 InDels in *B. rapa* populations while 3,852,169 SNPs and 417,004 InDels were identified from the *B. oleracea* populations, respectively. Using a set of 6707 SNPs common, a phylogenetic tree was generated to understand the relationship shared between the similar morphotypes (leaf heading and tuber forming) present in both the species. The analysis revealed that post divergence of the two species from a common ancestor; similar morphotypes had independently developed (convergent domestication). Genomic regions under positive selection exhibit reduced genetic diversity. Using nucleotide diversity and Reduction of Diversity (ROD) metrics regions of selective sweeps were defined. A Population-Based Integrated Haplotype Score (PiHS) was developed to identify such regions. The study identified five subgenome loci for the leaf-heading trait that are under selection and are shared between the two species. Similarly, strong signals of parallel selection of subgenomes were observed for domestication of the tuberous morphotypes within the two species. Using the genomics approach, the role of genome polyploidization toward the diversification of the two extreme morphotypes was established. The variome datasets, developed by resequencing of the large accessions of the two crops, is a valuable resource for future studies.

In another study, GBS approach was used to analyze the population structure and phylogenetic relationships of a globally diverse panel of 333 *B. rapa* accessions (Bird et al. 2017). A total of 18,272 SNP markers were scored in the accessions which represented 10 subspecies of *B. rapa*. Population genetic and phylogenetic analyses revealed presence of five subpopulations, which correlated well with the

morphological and geographic differentiations. Improved population sampling (inclusion of accessions that were missing from previous studies) use of a large number of diverse accessions and the high-density SNP markers in this study provided much improved and novel insight into the genetic relationships among the *B. rapa* subspecies.

Global genetic diversity analysis provides a useful resource for identification of novel genes/alleles conferring climate resilience. Availability of an extended reservoir of genetic diversity coupled with the advancements in the field of genomics will empower breeders to implement effective strategies for developing climate-resilient crops. This will enable sustainable crop production that can keep pace with the increasing world population.

6.7 Map-Based Cloning of CS Genes

With the availability of genomic resources for Brassica crops and the NGS-based low-cost and high-throughput genotyping platforms available, map-based cloning of some important traits has been successful in both *B. rapa* and *B. juncea*. The cloning of the genes underlying the traits was achieved by initially limiting the candidate region to <1 cM in most cases. Comparative analysis between syntenic regions in *A. thaliana* and *B. rapa* reference genome helped to identify candidate genes which were subsequently validated through functional complementation using either Arabidopsis mutants or Brassica.

The *BrTT8* gene involved in imparting seed coat color was isolated from *B. rapa* (Li et al. 2012). Yellow seed coat is a desirable trait as these seeds are found to produce more oil. Two populations, BC_1 (202 individuals) and BC_5 (1183 individuals), were developed by crossing black-seeded 3H219 (donor parent) to *Yellow sarson* (yellow-seeded parent). Genotyping the BC_1 individuals with AFLP markers identified markers tightly linked to the trait. The marker sequences were used to identify the corresponding BAC clone from BRAD (http://brassicadb.org/brad/). A set of 22 SSR primers developed from the BAC further reduced the interval to 1. 4 cM. A similar region in Arabidopsis, identified through blast analysis, was found to harbor 22 genes. Of these, *TT8* (*At4g09820*), involved in regulation of flavonoid biosynthesis in Arabidopsis appeared as a strong candidate gene. Moreover, an Arabidopsis line (*tt8-1*) having a mutant *TT8* gene has transparent testa (Nesi et al. 2000) further strengthening the candidature. Amplification of the *TT8* orthologs (*BrTT8*) from the two contrasting parents identified a large insertion of the transposable element *Helitron* in the yellow-seeded allele that led to disruption of transcription. The *BrTT8* allele from the black-seeded plant along with the 1.3 kb promoter region was able to functionally complement Arabidopsis *tt8-1* mutant, thereby confirming its role in development of the seed coat color.

In another report *Crr1a* gene conferring resistance to clubroot disease in *B. rapa* was cloned and characterized (Hatakeyama et al. 2013). The locus had been previously fine mapped using 1920 F_2 individuals from a cross between the resistant

and susceptible plants. Three BAC end markers from the BAC spanning this region were used to genotype a larger population of 3700 plants. Based on the phenotype of the recombinants lines, the candidate interval was limited to 8-kb. Sequencing of the BAC clone revealed the presence of a TIR-NB-LRR type of R gene. Expression of the full-length cDNA of the resistant allele (*Crr1a*^{G004}) was found to confer resistance to the clubroot-susceptible Arabidopsis Col-0 confirming the candidature. The susceptible allele was found to have three large insertions, one of which was similar to *Ty1-copia* LTR retrotransposon elements.

Using a different approach, a miR319a-targeted *BrpTCP* gene that modulates the head shape was cloned from *B. rapa* (Mao et al. 2013). In a previous study, using resequencing of 150 individuals of a RIL population (cross between heading and non-heading Chinese cabbage), three QTLs were identified for the head shape indices (Yu et al. 2013a). As dissection of the three loci would be a time-consuming effort, an alternate approach was used. The individuals of the population were grouped based on the head morphology; 58 RILs had compact heads, while the rest formed small or no head. The compact heads could be grouped into four types: round, conical, cylindrical, and oblong. Each of the four head types were strongly correlated with a particular type of rosette leaf morphology. As an example, majority of RILs with cylindrical heads had rosette leaves with wavy margins. This correlation was similar to one observed in *cin* mutants of *Antirrhinum majus* and *jaw-D* mutants of Arabidopsis (Nath et al. 2003; Palatnik et al. 2003), wherein silencing of *CIN* or miR319-targeted genes, respectively, caused the wavy margins. Using the sequences of the single miR319 gene and five miR319-targeted *TCP* genes present in Arabidopsis, all the Brassica homologs were identified from the Chiifu reference genome. The combined information was used to identify homologs and transcripts from the genome resequencing data of one of the parents (heading type). A single base substitution was identified at the fourteenth nucleotide of miRNA binding site in *BrpTCP4*-3C resulting in an enhanced sequence complementarity with miR319. The *BrpTCP4*-1 expression levels were highly variable across the individuals of the RIL population. *B. rapa* lines transformed with p35S::*BrpMIR319a2* exhibited a transition from round to cylindrical leafy head morphology, confirming that the decreased expression of *BrpTCP4* (target gene for *BrpMIR319a2*) leads to the cylindrical shape of leafy head.

The gene underlying a major QTL responsible for circadian period length in *B. rapa* was isolated using map-based cloning (Xie et al. 2015). The QTL, *PERIODA9a* (*PERA9a*) was previously mapped on chromosome A09, using a RIL population derived from a cross between oilseed R500 and rapid cycling IMB211 (Lou et al. 2011). Using an additional 13 SSR markers, generated based on region-specific BAC clone, the candidate interval was narrowed to lie between two genes, *Bra024534* and *Bra024560*. Of the total of 27 genes found within this region, *GI* (*Bra024536*) was identified as a strong candidate gene based on its homology with *A. thaliana*. *GI* (*GIGANTEA*) has been shown to be involved in circadian period determination, response to different abiotic stresses, and inhibition of hypocotyl elongation in light. Progeny of a F_4 RIL line, heterozygous for the candidate locus was genotyped using SSR markers that further reduced the region

to ~10 cM. Of the multiple polymorphic SNPs between the parental *G1* alleles, three were found to cause amino acid differences. Functionality of the two alleles was checked by transforming them to Arabidopsis *gi-201* (loss of function mutant) line. Both the alleles could fully rescue the Arabidopsis mutant for its photoperiodic flowering defect. However, they were found to have variable functionality in terms of their response to freezing and salt tolerance and also hypocotyl elongation. With the help of several site-directed mutagenesis, chimeric alleles were developed that were subjected to transgenic mutant rescue experiments in Arabidopsis *gi-201* to identify the casual nucleotide polymorphism.

In two separate studies, map-based cloning was also used to clone the gene for multilocular trait in *B. juncea*. The trilocular silique in *B. juncea* was found to be due to an insertion of copia-LTR retrotransposon in *CLAVATA1* gene homologue (*BjMc1*), the wild type allele results in bilocular siliques (Xu et al. 2017). The trait locus was previously mapped to a 2.7 cM interval (Xu et al. 2014). A NIL population comprising of 9300 individuals was screened with SSR markers developed from the BAC clone overlapping the region, to further limit this region 1.14 cM. The candidate interval had 25 predicted ORFs that were annotated using Arabidopsis genes and among them a *CLAVATA1* (*CLV1*) homologue (*BrCLV1*) was identified as a strong candidate. *CLV1* has been found responsible for multilocular trait in Arabidopsis. For each of the 25 ORFs, the genomic region (promoter and CDS) was sequenced from both bilocular and trilocular plants from the NIL population. Sequence variation was observed only in the *BrCLV1* sequences. Two copies of the gene were found in *B. juncea* (*BjCLV1a* and *BjCLV1b*), of which *BjCLV1a* was found to be similar in both the bilocular and trilocular plants. However, *BjCLV1b* allele of the trilocular plant was found to have an insertion of a LTR retrotransposon. The candidature of *BjCLV1b* for the *BjMc1* locus was confirmed through functional complementation of the *B. juncea* trilocular parental plant and an Arabidopsis multilocular mutant *clv1-1*. In both cases, the bilocular phenotype was found to be restored.

In a subsequent study, another gene *BjLn1* governing the siliqua locule number was successfully isolated using map-based cloning from *B. juncea* cultivar Duoshi. In a previous study, the tetralocular trait was found to be governed by two recessive genes (*BjLn1* and *BjLn2*) and the *Bjln1* was mapped to a 2.4 cM interval (Xiao et al. 2013b). This region was further fine mapped by genotyping 1325 individuals of a BC_3 population with 50 intron polymorphism (IP) primers developed from syntenic region in Arabidopsis. The fine mapping reduced the interval to 0.7 cM. The candidate-interval flanking markers were used to screen 4387 individuals of a newly generated BC_3 population and 16 recombinants. These 16 recombinants were then genotyped with SSR markers developed from syntenic *B. rapa* sequence, further limiting the interval to 87 kb. The syntenic region in Arabidopsis was found to contain 22 genes in this region including *CLV1* (*At1g75780*), known to be involved in increased carpels, was shortlisted as a candidate gene. Functional complementation of the multilocular parent, Duoshi with the cloned Brassica homolog of CLV1 (*BjuA07.CLV1*) could restore the bilocular phenotype confirming the results.

With the recent advancements made in sequencing technologies, map-based cloning of genes underlying mapped QTL would become a more routine approach. Earlier these efforts were hindered due to the time, cost, and efforts involved in genotyping large populations. Cloning the casual gene for important traits will not only enable precision breeding of climate-resilient crops but also facilitate opportunities for genetic engineering.

6.8 Brief Review of Genetic Engineering of Climate-Resilient Traits

Apart from conventional breeding and molecular breeding using advanced genetics and genomics technologies, transgenic and cisgenics also played major role in the understanding and development of biotic and abiotic stress tolerant/resistant mechanisms in Brassica plants. Furthermore, brassica crops have been exploited as an alternative source of energy since most reserved fossil fuels are fast diminishing. Several independent studies have reported that Brassica species including *B. rapa, B. napus*, *B. juncea*, and *B. carinata* could be exploited as a source of biodiesel. Ahmed et al. (2014) found that mustard biodiesel gives better engine performance and results in lesser emission of HC, CO, and also noise. Blackshaw et al. (2011) reported that oilseed crops, camelina, flax, *B. rapa* canola, and oriental mustard, have high potential for use as source of biodiesel production since their oils can be easily converted to biodiesel. It has been observed that high content of fatty acids especially erucic acids has high potential for biodiesel production, and are preferred in biodegradable plastic, cosmetic, and emollient industries (Nath et al. 2016). The biosynthetic pathway of fatty acids in Brassica has been well characterized and fatty acid elongase (*FAE*1 and *FAE2*) and fatty acid desaturase (*FAD1* and *FAD2*) genes are shown to be important in producing different fatty acids such as erucic acid, linolenic acid, linoleic acid, and oleic acids (Sivaraman et al. 2004; Nath et al. 2009). Silencing and overexpression of these genes have shown that metabolic engineering of fatty acid biosynthesis could be done (Sivaraman et al. 2004; Nath et al. 2009, 2016). Therefore, Indian *B. juncea* and *B. rapa* (both oilseed crops) being high in erucic acid and other fatty acids are useful for biodiesel production. Furthermore, overexpression of those genes would enhance production of fatty acids suitable for biodiesel production.

Abiotic stresses are major problem hampering crop production. Several studies using foreign genes showed that transgenic *B. juncea* plants are tolerant to cold, drought, and salinity stress (Table 6.3). Glucosinolates, sulfur-containing secondary metabolites, are reported to be involved in imparting stress tolerance mechanism in Brassica crops (Martínez-Ballesta et al. 2013). Few studies have identified genes involved in glucosinolate biosynthesis pathway and validated them through silencing and/or overexpression (Augustine et al. 2013; Augustine and Bisht 2015). In natural conditions, *B. juncea* plants are grown in dry areas in winter season and

these plants can also grow in soils with comparatively higher levels of toxic metals as compared to other Brassica species, thereby making this crop plant to be more preferred in soils with high metal and salinity that might have resulted due to excessive use of fertilizers and other chemicals (Park et al. 2005; Gasic and Korban 2007). Kumar et al. (2013a, b) observed that transgenics *B. juncea* plants developed by introduction of the chickpea lectin genes exhibited resistance to salinity and drought stress. Saha et al. (2016b) by overexpressing Arabidopsis Late Embryogenesis Abundant protein group 4 gene (*AtLEA4-1*) in *B. juncea* plants showed that transgenic plants alleviated the osmotic stress, protected cytosolic structures, and cell by increasing the membrane and protein stability, thereby resulting in enhanced drought and salinity tolerance. Transgenic *B. juncea* lines transformed with lectin and other genes also exhibited resistance to biotic stress (Mondal et al. 2007; Kumar et al. 2015). These results encourage us to combine transgenic technology along with molecular breeding methods for development of climate-resilient *Brassica* crop plants. Furthermore, using *barnase* and *barstar* genes male sterile *Brassica* lines were developed and this technology is being successfully used by the group at University of Delhi, South Campus, led by Prof. Deepak Pental, to develop heterotic F_1 *B. juncea* hybrid DMH-11 which gives higher performance and yield compared to the parental lines and the national check varieties. This hybrid is being tested for biosafety and yield trials being conducted in different locations in India. The details of transgenic plants developed for biotic and abiotic stress tolerances/resistance and gene and metabolite engineering are listed in Table 6.3.

Table 6.3 Details of transgenic plants developed with biotic and abiotic stress tolerance, gene discovery, and metabolic engineering in oilseed *B. rapa* and *B. juncea*

Serial no	Crop	Gene	Encoding protein	Traits	References
Abiotic stress					
1	*B. juncea*	*codA*	Choline oxidase	Tolerance to salinity stress, enhanced growth	Prasad et al. (2000)
2	*B. rapa*	*Lea*	Group 3 Late embryogenesis abundant	Salinity and drought tolerance	Park et al. (2005)
3	*B. juncea*	*AtPCS1*	Arabidopsis *phytochelatin synthase*	Enhanced cadmium (Cd) and arsenic (As) tolerance	Gasic and Korban (2007)
4	*B. juncea*	*PgNHX1*	Vacuolar Na^+/H^+ antiporter	Tolerance to salinity stress, exhibited normal growth	Rajagopal et al. (2007)

(continued)

Table 6.3 (continued)

Serial no	Crop	Gene	Encoding protein	Traits	References
5	*B. juncea*	*CGS*	cystathionine-*c*-synthase	Enhanced capacity to remove Se from contaminated sites in the form of low-toxic volatile dimethyl selenide.	Huysen et al. (2003)
Biotic stress					
6	*B. juncea*	Tomato *glucanase* gene	Pathogenesis related protein	Arrested growth of *Alternaria brassicae*, and reduction/ delayed infection	Mondal et al. (2007)
7	*B. juncea*	*α-tocopherol*	Vitamin E	Tolerance against salt, heavy metal, and osmotic stresses	Kumar et al. (2013a, b)
8		Chickpea *Lectin* gene	Lectin	Salt and drought stress tolerance	Kumar et al. (2015)
9	*B. juncea*	*MsrA1*	a Synthetic Cationic Antimicrobial Peptide	*A. brassicae* and *Sclerotinia sclerotiorum* resistance	Rustagi et al. (2014)
10	*B. juncea*	Chickpea *Lectin gene*	Lectin	Resistance to *A. brassicae*	Kumar et al. (2015)
11	*B. juncea*	*BjNPR1*	Non-expressor of pathogen-related gene 1	Enhanced resistance to *A. brassicae* and *Erysiphe cruciferarum*	Ali et al. (2017)
Gene discovery and metabolic engineering					
12	*B. juncea*	*PiD6*	Δ6 desaturase from *Pythium irregulare*	Enhanced production of γ-Linolenic acid	Hong et al. (2002)
13	*B. juncea*	*fad2*	Suppression of "Fatty acid desaturase 2" gene involved in fatty acid biosynthesis pathway	High oleic acid and low linolenic acid	
14	*B. rapa*	*BrTT8*	*B. rapa* TRANSPARENT TESTA 8	Insertion causes yellow seed coat color	Li et al. (2012)

(continued)

Table 6.3 (continued)

Serial no	Crop	Gene	Encoding protein	Traits	References
15	*B. juncea*	ORF288	Male sterility associated cytotoxic protein ORF288	Make sterility due to aborted pollen development	Jing et al. (2012)
16	*B. juncea*	*fad2*	Silencing of fatty acid desaturase gene, involved in fatty acid biosynthesis pathway.	Increased oleic acid and reduced linolenic acid	Sivaraman et al. (2004)
17	*B. juncea*	*BjMYB28*	*B. juncea MYB28* gene	Silencing results low glucosinolates contents in *B. juncea*	Augustine et al. (2013)
18	*B. juncea*	*GSL ALK*	Suppression of glucosnilate gene *GSL-ALK* involved in glucosinolate biosynthesis pathway.	High glucoraphanin content, anticancer glucosinolate compound.	Augustine and Bisht (2015)
19	*B. juncea*	Silencing of *SGT* and *SCT* genes	*UDP- glucose: sinapate glucosyltransferase* and *sinapoylglucose: choline sinapoyltransferase*	Reduction of sinalpine content of *B. juncea* seeds	Kajla et al. (2017)
Male sterility and heterotic hybrid F1 production					
20	*B. juncea*	*ALS* gene	Acetolactate synthase gene	*Barnase/ barstar B. juncea* male sterile transgenics for hybrid seed production with *ALS* gene conferring resistance to imidazolinone based herbicide "Pursuit"	Ray et al. (2007)
21	*B. juncea*	*Barnase/ barstar*	*Barnase/barstar* male sterility system for heterotic hybrid seed production	High vigor and yield of F_1 hybrid (DMH-11) compared to parents	Pental (2010, personal communication)
22	*B. juncea*	*Cre/lox*, lox linked with *ALS* gene	Cre/Lox, *Allium sativum* leaf agglutinin	Marker free, insect (*Lipaphis erysimi*) resistance transgenics plants	Bala et al. (2013)

6.9 Brief Account on Role of Bioinformatics as a Tool

Databases handle datasets of varied complexity, with the aid of tools specialized for data retrieval and filtering and the data content is presented to the user in visual formats. These are crude representation of information and could be presented in any likable manner. Bioinformatics has revolutionized the way biological information is organized, stored, and curated in terms of accessibility and organization. Primary genomic data repositories, such as NCBI (Benson et al. 2013), EBI (Leinonen et al. 2011), and DDBJ (Tateno et al. 2002), overwhelm naive user with their complexity. For the Brassica community, plethora of analysis tools and specialized databases are being reported and made publicly available to keep pace with surging multiomics datasets.

6.9.1 Germplasm Resources

Bras-EDB (Bas and Menting 2007), is an example of one of the earliest Brassica database, which was conceptualized in 1993 and is being constantly updated. This database had started as a standalone service distributing offline copies. Nowadays, online access is also possible at "http://ecpgr.cgn.wur.nl/Brasedb". It is a very useful resource for plant breeders and helps to identify Brassica germplasm, at taxon, species, or genus levels, besides giving information on the place of origin. The germplasm is classified using passport descriptors and information available is fully navigable through lists and spreadsheets. Brassibase, (Kiefer et al. 2014) is another service, which also facilitates germplasm-related queries and offers a more polished interface and extends taxonomic information through phylogeny viewers. There is an additional layer of cytological information which gives details of cytologically verified chromosome counts and related information. This database contains information of the entire Brassicaceae family and the data can be explored by multiple parameters including morphological attributes. Brassibase weblink is available at "https://brassibase.cos.uni-heidelberg.de/".

6.9.2 Genome and Gene Regulation Databases

6.9.2.1 Genomic Resources

BRAD (Brassica database), one of the first genome database for Brassica crops, was developed following the first release schedule of *B. rapa* whole genome sequence. For many years, only members could access it, however, it is now in the public domain, with the genome information being integrated with the NCBI genome database. Another important resource for genome sequence information can be

accessed at "http://www.brassicagenome.net/". It compiles all published Brassica genome resources and pairs them with the Gbrowse service for enabling genome browsing. Gbrowse, as an independent utility, offers several tools to help the users navigate through chromosomes and also allows retrieval of locus-wise information and sequences (Stein et al. 2002). TAGdb (Marshall et al. 2010) and AutoSNPdb (Duran et al. 2009) databases help to identify SNPs between different Brassica cultivars and also against the reference genome (*Arabidopsis*). AutoSNPdb is accessible at "http://autosnpdb.appliedbioinformatics.com.au/". The gene and genome databases available for Brassica crops have been compiled in Table 6.4.

Table 6.4 Gene and genome databases

Name	Synopsis	Data interface	Available tools
BRAD (Cheng et al. 2011); http://brassicadb.org	Database of predicted genes and associated annotations, non-coding RNAs, transposable elements (TE)	**Browse:** Markers and maps, gene families, phenotype, species info, important labs and people; **Search:** Annotation, syntenic genes, syntenic figures, non-syntenic orthologs, flanking regions	BLAST and Gbrowse; GbrowseSYN
Phytozome (Goodstein et al. 2012); https://phytozome.jgi.doe.gov/pz/portal.html	The plant genomics resource and annotations	**Browse:** Species-wise records leading to many visual tools; **Search:** Species, keyword, gene sequence	Phytomine, BioMART, Jbrowse, BLAST
TagDB (Marshall et al. 2010); http://sequencetagdb.info/tagdb/cgi-bin/index	Service performs BLAST alignment between a single query and short pair reads of selected species	**Browse:** It is a web service, no direct browsing allowed; **Search:** Sequence-based search enabled using blast tool	BLAST
PGML BACMan (Pterson 2007); http://www.plantgenome.uga.edu/bacman/brassica/BACManwww.php	The Brassica BACMan database is a product of the Plant Genome Mapping Lab at the University of Georgia. *B. juncea* BACs are also listed here.	**Browse:** Data supplied as tables, access as whole or filter using search query; **Search:** Locus name, genbank id	Designed over BACMan platform (Estill 2005); Keyword based table search
PTGBase (Yu et al. 2015); http://ocri-genomics.org/PTGBase/	Plant tandem repeat genes database	**Browse:** Tandem repeat gene clusters in various species; **Search:** Search by id, keywords, clusters, functional annotation class	BLAST

6.9.2.2 Functional Regulation

Functional regulations involves (a) direct regulators such as enzymes, transcription factors, etc. and (b) miscellaneous indirect regulators. There are several enzyme databases available to study functional regulation. Furthermore, curated datasets of these web services are available for offline usage, which are useful in annotation workflows as well. Brenda, an important database in this regard, is a generic resource for many organisms, wherein all presented data is literature mined and curated. The database is in active development stage and is repeatedly updated with modern tools to allow efficient browsing and mining of the information. Brenda extends its role to analysis of enzymes involved in metabolic biosynthesis pathways in plants, including Brassicas. Brenda is highly recommended database as all data entries are cross mapped to curated literature sources. Many other databases (enlisted in Table 6.5) also provide information on the Brassica enzymes.

To study the indirect gene regulators of gene expression, users can refer to transposable elements (TEs) and various non-coding RNA species. Transposable elements have been known since long, but only recently their mechanisms are being studied in Brassica crops. The role of TEs in functional and epigenetic regulation of genomic regions has been well studied in Arabidopsis (Bucher et al. 2012). Availability of complete Brassica crop genome sequences and the several databases and tools provide a useful resource for mining of TE sequences and also for information on other functional regulators including the various non-coding RNA such as small RNAs, miRNAs, and long non-coding RNAs. These resources enable not only the basic understanding of the functional gene regulation but also have practical utility in designing transgenics (Table 6.5).

6.9.2.3 Gene Expression Databases/Workbench

Expression datasets are very complicated to store and handle, as they hold lots of meta-information associated with them, details such as specimen details, experimental conditions, attributes, etc. Web-based workbenches and databases are immensely useful tools to offer analysis and management service to end users. Madmax (Lin et al. 2011) is a management and analysis database for multi-platform microArray experiments; it offers solution for storage and analysis of complex omics datasets. Madmax database and workbench is accessible at "https://madmax.bioinformatics.nl". Login account is mandatory to access this service. Rapa-net (Kim et al. 2017) is another web tool, to analyze co-expression of genes, and gene functions are assigned based on microarray datasets of 143 tissues of *B. rapa* compiled from various organs collected at distinct developmental stages and environmental/stress conditions. Data is presented as correlation networks, phylogenetic trees, histograms, and other means as applicable. Rapa-net URL, "http://bioinfo.mju.ac.kr/arraynet/brassica300k/query/" can be used to access the service.

Table 6.5 Protein resources

Name and URL	Synopsis	Data interface	Available tools
PlantCAZyme (Ekstrom et al. 2014); http://cys.bios.niu.edu/plantcazyme/	Database for plant carbohydrate active enzymes	**Browse:** Plant species, Enzyme class and enzyme families; following datasets such as dbCAN HMM profile, proteins from three different CAZyme datasets are available for offline downloaded; **Search:** Search categories include: ID, family, species, domain, domain description, CDD ID, EST ID, gene ontology ID, NR ID, NR description, PDB ID, PDB description, length, molecular Wt., isoelectric point	BLAST and annotation service for user sequences
BRENDA (Placzek et al. 2017); http://www.brenda-enzymes.org/	BRaunschweig ENzyme DAtabase; collection of enzyme and metabolic information, based on primary literature	**Browse:** Enzyme classes, Taxtree, protein folding, ontologies; **Search:** Enzyme structure, metabolic pathways, ligand substructure, enzymes and diseases, keywords	Enzyme detector, word maps, genomes, functional params, pathways
PathoPlant (Bolívar et al. 2014); http://www.pathoplant.de/	Database on plant–pathogen interactions and components of signal transduction pathways related to plant pathogenesis	**Browse:** cis-elements (8mer, 9mer, 10mer) responsive to a given stimulus; Signaling pathways; **Search:** Search by Accession number, plant, pathogen, molecule and interaction type	Validate potential cis-sequences; access microarray expression datasets in tabular format with all associated statistics.
PlantP (Gribskov et al. 2001); http://plantsp.genomics.purdue.edu/	Functional genomics of plant phosphorylation	**Browse:** Protein families; **Search:** Keywords, accession numbers and PlantP IDs, Blast search against Plantx family of databases	Gene lexicon generator; GeneGC GC content estimation of genomic, cDNA, mRNA; Motif/Domain scan
PlantTFDB 4.0 (Jin et al. 2017); http://planttfdb.cbi.pku.edu.cn/	Plant transcription factor (TF) database; annotation, domain features, GO, expression pattern and	**Browse:** Majority of datasets are available for download and Browse: offline browsing; **Search:**	BLAST; Transcription site prediction: binding site, regulation, GO enrichment, TF enrichment;

(continued)

Table 6.5 (continued)

Name and URL	Synopsis	Data interface	Available tools
	ortholog groups, database cross-references and TFs literature citations	Keyword based search e.g. *MYB*, leads to full TF synopsis page with relevant out links to external services and TF binding motif's cartoon renderings	transcription regulatory map
BrassicaTED (Murukarthick et al. 2014); http://im-crop.snu.ac.kr/BrassicaTED/index.php	Brassica transposable elements database for miniature transposable elements in Brassica species	**Browse:** MITE, TRIM and SINE families; gene lists and major repeats in A, C & AC genome are also available to download; **Search:** MITE, TRIM and SINE members; Expression of paralogs and Insertion polymorphism(IP)	BLAST, sequence extractor, Karyotype BLAST
P-MITE (Chen et al. 2014); http://pmite.hzau.edu.cn/	Plant miniature inverted-repeat transposable elements	**Browse:** 41 plant species-wise MITEs; Alphabetically organized MITE families(3527) list; **Search:** Sequences, sequence IDs; MITE family name	BLAST, alignment viewer, tables
PNRD (Yi et al. 2015); http://structuralbiology.cau.edu.cn/PNRD/index.php	Plant Non-coding RNA database	**Browse:** By species, by category and by ex-profile; **Search:** ID search, literature search, miRNA target search, miRNA epigenomics search	miRNA predict; Coding potential predict; BLAST; Gbrowser
PMRD (Zhang et al. 2010); http://bioinformatics.cau.edu.cn/PMRD/	Plant microRNA database; hosts miRNA genes, target genes, and expression profiling datasets	**Browse:** Browse option appears dead; stem loop sequence and mature sequences are download offline; **Search:** Search tab appears dead at time of writing	Basic tabular navigation, genome browser
Mirbase (Kozomara and Griffiths-Jones 2014); http://www.mirbase.org/index.shtml	microRNA database; hosts published miRNA sequences and annotation	**Browse:** Taxonomy wise drill down list, ends up at relevant tables of miRNA sequences; **Search:** miRNA keyword, name and accession; genomic location; miRNA clusters; tissue expression	BLASTN; SSEARCH; alignment viewer

6.9.3 Annotation Services and Workbenches

Brassica-specific ontology project crop ontology (Shrestha et al. 2012) at "http://www.cropontology.org/ontology/CO_348/Brassica" can be accessed for local use while, AgriGO (Tian et al. 2017) and Planteome (Cooper et al. 2018) offer a wide range of high-end computing analysis for downstream applications as useful web-toolkit. AgriGO and Planteome allows viewing of complex gene sets at higher level, and the genes and annotation features can be visualized in functional or organizational terms. AgriGO interface can be queried with "Species + gene identifier; GO term + gene identifier"; with limitation of 100 in a single session. AgriGO offers a range of analysis provision such as (a) Singular Enrichment Analysis (SEA), (b) Parametric Analysis of Gene Set enrichment (PAGE), (c) Transfer ID by BLAST (BLAST4ID), (d). Cross comparison of SEA (SEACOMPARE), and (e) Customized comparison. The service can be accessed at "http://bioinfo.cau.edu.cn/agriGO/". On the other hand, planteome, integrates reference ontologies, plant genomics, and phenomics into a single service. Data can be browsed by taxon, data type, e.g., tRNA, miRNA, QTL, protein, etc. and drill down ontology listings. Search can be performed for ontology terms, bioentities and annotations. Apparently overlapping in nature, both web services maintain their niche, attributing to their distinct visual presentation and user interaction styles. AgriGO allows data to be ported to external service called REVIGO (Supek et al. 2011), which is an GO slimming (summarizing) service to trim down excess "GO descriptions" based on their confidence score. Planteome depends upon, well-established generic service called AmiGO (Carbon et al. 2009), which modularize GO terms access and also with loss of useful visualization and analysis provisions.

6.9.4 Brassica Comparative Genomics Resources

Comparative genomics relies on understanding the segmental migration and integration patterns of genomic sections. Two or more genomes with homology are aligned to find out regions where possible duplications, segmental migrations, and deletions have occurred in the past. Closely related species share lot of orthology among gene and genome sequences, it gives a vantage point to assess time events to establish evolutionary time scale. These tools are indispensable resources to visualize synteny across species since many biosynthetic pathways and gene families are highly conserved among them. Direct gene models can be adapted to understand gene regulation and related genomic resources can be reliably deployed to understand from lesser known species. Many popular Synteny analysis platforms such as CoGe with its range of tools (Tang et al. 2015) such as McScan (Wang et al. 2012) have been used as back-end service to precompute these datasets. On the other hand, visualizations such as dot plot, parallel maps, and simple sequence alignment

are available as options. Circos (Krzywinski et al. 2009) visualization tools are recently catching attention and are offered by many analysis tools. The various comparative genomics databases available for Brassica crops have been compiled in Table 6.6.

Table 6.6 Comparative genomics databases

Name and URL	Synopsis	Data interface	Available tools
BOLBASE (Yu et al. 2013c); http://ocri-genomics.org/bolbase/	A comprehensive genomics database for *B. oleracea*; *B. oleracea* genome data and comparative genomics information with *B. rapa*	**Browse:** Phenotype, genome components, gene families, pathways, genome browse; **Search:** Putative gene, repeat element, non-coding RNA, orthologous genes, syntenic region, similarity search	Gbrowse
BrGDB (Dong et al. 2004); http://www.plantgdb.org/BrGDB/	Comparative plant genomics	**Browse:** Genbank or uniprot datasets; Custom transcript assemblies; Genome Survey Sequences (GSS) data and many other crop specific datasets; data is also available for download; **Search:** Search by gene ID, keyword; List based search retrieval	GenomeThreader for spliced alignment of genes; GeneSeqer; BLAST service for brGDB and all GDB databases; community annotation service; GAEVAL analysis
Narcisse (Courcelle et al. 2008); http://narcisse.toulouse.inra.fr/	A mirror view of conserved synteny	**Browse:** Most of the interface is navigation based, leading user to select option for guiding the visual exploration; **Search:** Search available for locus info and genomic coordinates	Several visualization services, such as alignment viewer, map-based synteny viewer, dot plot visualization, circos visualizations
PGDD (Lee et al. 2013b); http://chibba.agtec.uga.edu/duplication/	Plant genome duplication database; intragenome or cross-genome syntenic relationships	**Browse:** Synteny blocks across 47 plant species(in all possible pairs) are available for download; **Search:** Synteny regions in multiple species by locus identifier	Map viewer, dot plot, locus search and synteny tools(CoGe, VISTA, phytozome, Plaza and Ensemble); tool to calculate Significance of segmental duplication

6.9.5 Data Integration and Gateway Services

Most of the time any analysis workflow involves lot of data integration from heterogeneous resources, unfortunately there are no simple solutions to accomplish that easily. There are few services and data handling approaches to steer users in the right direction. Many diverse datasets reside in various databases; however, despite significant overlap of information among them, it is difficult to mine and cross compare information. Data integration allows modular access across all data repositories; there are workbenches for screening information for further usage. Data contexts could be genomic, literature, and other metadata regarding experiments. Modern data services rely on Application Programming Interface (API) level access to enable desktop usage with help of various programming languages, and it allows better integration in user's custom workflows. BioMART (Kinsella et al. 2011) service is an integral component of many databases, e.g., "Ensembl Plants", it allows transparent ID conversion, sequence retrieval, and other tasks to interface across services. It defines datasets in terms of XML markings, which enable them readily processable into other web services and desktop tools. Standard file formats such as FASTA, GFF, FASTQ, VCF, BAM, SAM, etc. also make data access modular across services, majority of desktop softwares understand or generate such files, and many are also understandable by web services. Services to assist in Brassica data integration and mining are listed in Table 6.7.

Table 6.7 Data integration services

Name and URL	Synopsis	Data interface	Available tools
PGP repository (Arend et al. 2016); http://edal-pgp.ipk-gatersleben.de/	Plant Genomics and Phenomics Research Data Repository; research data submission service	**Browse:** Deposit, explore and cite datasets, each dataset gets its own DOI number; **Search:** Keyword based generic search	Elixir data submission toolkit, IPK data-submission toolkit, Several stats generation and reporting tools
BIP; https://bip.earlham.ac.uk/	Brassica information portal; population and trait scoring information	**Browse:** Populations, traits, trials, linkage maps, QTLs, marker assays; **Search:** All browse-able datasets can be searched as well	Data submission tool; GWASSER analysis tool
Ensembl Plants (Kersey et al. 2018); http://plants.ensembl.org/index.html	Plant genomes repository with range of mining tools	**Browse:** Genome assembly, comparative genomics, regulation, variation and gene annotation; **Search:** Species for gene information, sequence-based BLAST search	BLAST, BLAT; BIOMART; variant effect predictor; IDs history converter; assembly converter; HMMER

6.9.6 *Brassica Community Resources*

Some websites serve as gateway to all Brassica related information, and they help to integrate resources in their own manner. Brassicainfo, is a useful integration service for community and hosts three different resources such as Genome, phenome, and infome. The Genome module, offers completed genomes, comparative genomics (BMAP), cytological maps, and linkage maps. Phenome module incorporates links to ionome, metabolome, proteome, transcriptome, and trait ontology services. The third module, infome, is actually a broad class, outlining Brassica databases, data standards, and reference information to important publications in the field and roadmap for future. It is amongst the most updated resource on Brassica news, publications, and all other related information that is suitable in context of this site. It can be accessed at http://www.brassica.info/.

6.10 Social, Political, and Regulatory Issues

The lack of adequate infrastructure and very limited technology intervention in developing countries often leave agriculture vulnerable to environmental calamities. The expertise and the required machinery are generally in short supply, while techniques are inefficiently implemented. More importantly, the crop faces continuous danger posed by crop pests and extremes of weather conditions. Oilseed mustard and rape are mostly cultivated in unirrigated lands during the winter season in India when the rainfall is negligible and the crop faces extremes of low temperature during the months of January and February thereby, hampering crop production. However, most farmers nowadays, preserve and cultivate only few well-known, high-yielding varieties with narrow genetic base and therefore limited climate-resilient traits. Alternative mustard and rape varieties therefore need to be developed that can withstand drought and extreme cold conditions besides, being tolerant to biotic stress. High-throughput marker- or genomics-assisted breeding, utilizing climate-resilient germplasm, would be very beneficial for accomplishing such endeavors. The landraces of mustard and rape preserved by farmers over several years, could also be used in such breeding programs. Through the use of genetic engineering technology, genetically modified (GM) mustard has been developed, the details of which have been discussed later in the text. However, the concept of cultivation of GM crops on millions of hectares of lands and their introduction into our food chain has started a debate amongst the consumers, farmers, and policy-makers worldwide, with no concensus on the acceptance of GM crops. The emergence of agricultural biotechnology has also generated several social and ethical concerns. There exists widespread debate on the use of biotechnology for developing improved quality and higher yielding crops without any adverse effects on the ecosystem and human health (Maghari and Ardekani 2011). The primary concern while growing transgenic crops and incorporating them

into the food chain, is towards protecting humans and the environment from any adverse affects. Several critics have put forth arguments against the use of GM crops, including concern that GM crops produces more greenhouse gases, they may jeopardize biodiversity in the long-term, insect-resistant GM crops may cause harm to the non-target species and/or accelerate resistance in insect populations, cross-pollination and transfer of genetic materials to autochthonous plant varieties may cause "gene-pollution", especially in centers of origin and diversification (Vezzani 2018). Development of the first GM crops with the traits such as tolerance/resistance to herbicide, insects, or pathogens and various stress conditions such as drought (Halford 2006), proved very promising and significantly beneficial to farmers by enhancing agricultural productivity and reducing poverty in developing countries (Christou and Twyman 2004; Farre'et al. 2010, 2011; Rommens 2010; Halford 2012; Chen and Lin 2013). Insect-resistant and herbicide-tolerant GM crops have also reduced the production costs as farmers/growers will have to spend less on insecticides or herbicides which in turn helps in generating higher income and thus incur direct gain to farmers in productivity and economic returns from the technology (Sainger et al. 2015).

6.10.1 GM Mustard in India

A new generation high-yielding herbicide-resistant mustard hybrid Dhara Mustard Hybrid-11(DMH-11) has been developed using GM technology by the research group headed by Prof. Deepak Pental at the Centre for Genetic Manipulation of Crop Plants (CGMCP), University of Delhi South Campus, New Delhi. This GM mustard hybrid was developed by introducing *barnase/barstar* genes for pollination control mechanism in Indian mustard Varuna and the east European mustard EH2 and has been shown to give 25–30% higher yield compared to the best national check mustard varieties. Prof. Pental's group had sought approval for environmental release of this genetically engineered (GE) oilseed mustard by submitting an application to the Ministry of Environment, Forest, and Climate Change (MoEF & CC), Government of India (MoEF & CC-GEAC 2018). The GM mustard which was awaiting approval since 2015 was granted the initial permission by Genetic Engineering Appraisal Committee (GEAC) in 2017. However, the government decided to stay the release of transgenic mustard and kept the matter pending for further review, owing to the receipt of various representations from different stakeholders. In March 2018, the GEAC advised the applicants to undertake field demonstration for the purpose of generating additional data on effects of GM mustard on honey bees, other pollinators, and on soil microbial diversity. These events and issues related to the commercial release of GM mustard in India clearly show that even when the government did not receive any adverse report regarding destruction of local varieties or health hazards due to genetically modified crops, GM mustard still remains a very contentious issue, both socially and politically.

6.10.2 Patent, IPR Issues, and Farmers' Rights

Farmers are interested in increasing their income by methods that enhance the agricultural productivity of the crops, with less labor, time, and resources, and with the practices that are safe for themselves and the environment. A developing country like India has a policy on agricultural patency that adheres to the principle of common heritage or the promotion of free exchange based on the notion that no one has ownership over the chief food plants of the world, which are our common heritage (Ramanna and Smale 2004). India's 2001 *sui generis* plant variety protection bill, Protection of Plant Varieties and Farmers' Rights (PPV & FR) upholds the farmers' rights to save, share, exchange, and sell seeds except branded seeds of a protected variety if they are labeled as such (Peschard 2014). The 2002 amendment of the '1970 Patent Act' also excludes seeds from being patented. Farmers' rights are an unambiguous recognition of the abilities of farmers to innovate. Historically, farmers have contributed seeds for breeding high-yielding varieties which benefited the global society (Kloppenburg 1988). These rights enable the farmers to save, use, and exchange but not to sell seed without penalty (UPOV, 1961 revised 1978; modified UPOV, 1991). They also include "benefit sharing", a concept that evolves from the Convention on Biological Diversity (1992) which views genetic resources as common heritage (shared by all). Benefit sharing refers to compensation to farmers or communities who contribute to the creation of a new variety or the development and conservation of existing varieties. The farmers' rights also recognize the ownership over their varieties as breeders and extend the concept of IPRs to farmers' varieties, in order to promote and reward farmer innovation (Ramanna and Smale 2004).

The major concerns of farmers while planting genetically modified crops are related to suitability of seeds for the agroecological conditions of their particular fields (Kershen 2010), and the associated stringent laws governing the use of GM seeds. The public will be able to make an informed decision about the choice of consumption of GM food if they are provided with enough information on the safety tests conducted on such products. Furthermore, it is factually difficult to compare the nutritional contents of GM crops with their conventional counterparts grown over different areas as the composition of crops grown in different areas might vary depending on the growth and agronomic conditions (Maghari and Ardekani 2011). Even if the information is shared reasonably, there are people who would still resist consuming GM foods, even after thorough testing for safety, on account of their personal or religious beliefs. Furthermore, one of the arguments against the IP protection of GM crops is that the GM seeds barge high price and are more expensive than conventional seeds and consequently the price of food is high. Also, the farmers have to be in agreement with the policies of the seed company for not replanting the harvested seeds.

6.11 Strategies to Enhance Oilseed Production

The current levels of mustard oilseed production and other attributes of global oil availability are far less than the required figures. Two broad strategies to enhance production of oilseeds are indicated below.

6.11.1 Potential for Expansion of Productivity

The major opportunities for increasing oilseed production exist in adopting strategies that enhance the productivity of oilseed crops. The lack of nutrient management systems and the neglected use of mechanization have been implicated in lower yields. The key strategies to enhance productivity must focus on sources of improved and efficient use of agricultural inputs and methods, timely availability of seeds of improved varieties, promotion of water use efficient cultivars, effective crop management techniques, adoption of integrated pest and nutrient management, use of resource conservation technologies, precision farming, and crop contingency planning. Farmers should also be provided better prices of their crops so that they can endure the higher adaptation costs of cultivation under changing climatic scenarios. Furthermore, screening of a large number of mustard and rape germplasm for biotic and abiotic stress resistance and identification of important genetic stocks and utilizing them in breeding program will help in development of varieties which will give higher productivity in changing climate.

6.11.2 Potential for Expansion into Non-traditional Areas

B. juncea is an important oilseed crop in India, China, and in southwestern areas of the former Soviet Union. Because of its drought and heat tolerance, disease-resistant and shattering-resistant phenotypes, *B. juncea* is considered as an alternate oilseed crop to *B. napus* for semi arid regions of Western Canada, Australia, and the United States (Burton et al. 1999; Banga et al. 2009; Potter 2011). The oil from canola quality *B. juncea* can replace *B. napus*, or the two products can be blended (GRDC 2009).

In India, *B. juncea* is largely cultivated in the states of Assam, Rajasthan, Uttar Pradesh, Haryana, and Madhya Pradesh (Directorate of Economics and Statistics, Government of India 2016). Its cultivation is also being extended to non-traditional areas of southern states like Karnataka, Tamil Nadu, and Andhra Pradesh. The expansion of oilseed growing areas could alleviate the problems associated with increased demand.

Crop intensification in the underutilized farming locations like rice fallows can also contribute to an increase in the area under mustard oilseed crops, without sacrificing the yield or area under other crops. The current acreage covered by

oilseed mustard crops could be expanded by the inclusion of less fertile lands receiving lower rainfall along with modifying existing cropping patterns (Lu et al. 2011; Rahman and de Jiménez 2016). Most of these areas experiencing such drought-like conditions are left fallow, and can be utilized for cultivation of oilseed crops, which can outperform (in terms of overall yield) other input-intensive crops. Significant cultivable area gains can also be acheived by growing oilseed crops in an intercropping sequence and by their inclusion in crop diversification plans.

6.12 Future Perspectives

Global consumption of oilseeds has increased drastically in the last decade due to an increased demand for edible oils along with an expanding demand for biodiesel and other non-food based industrial products (Lu et al. 2011). The major challenge for the oilseed industry is to meet this rising demand for oil by producing inexpensive and sufficient quantities of plant-derived oils. To achieve and maintain a sustainable production of oilseeds, classical breeding efforts need to be strengthened with the modern biotechnological approaches.

Due to climatic changes, there has been an increase in the frequency of weather extremes that are likely to decrease crop yields further, and will simultaneously affect all dimensions of crop production (Singh et al. 2015). Human activities are also immeasurably accelerating these variations in global climate. The constantly increasing human population is further making the situation worse and is expected to peak before the end of the century, with an expected population size of 10 billion people before the begining of 2100 (Lutz et al. 2001; Duhamel and Vandenkoornhuyse 2013).

Genetic improvements in oilseed crops during the last century, have contributed significantly toward enhanced yields. It is, however, increasingly being realized that crop improvement through the use of conventional breeding approaches is tending towards stagnation, due to the fact that these breeding methods do not utilize sufficient amount of genetic variation. The breeding of mustard oilseed crops should, therefore, intensify its approches towards incorporation of superior climate-smart traits thereby developing climate-resilient varieties that can withstand and adapt to the changing climatic conditions.

For surpassing the yield barrier in oilseed mustard, crop improvement programs could be designed involving diverse parents. Yield-associated QTLs will have to be identified, which can be introgressed in genetically adapted backgrounds using marker-assisted selection (MAS). High temperature stress at the seedling stage influences actual yield potential in a timely sown crop, and hence impels for the development of heat stress tolerant genotypes. Furthermore, as the mustard crop is mostly grown on marginal lands, plant genotypes with enhanced water use efficiency are required that could maximize the yield even in water-deficient conditions.

Abiotic stresses including drought, frost, and heat also impacts seed development at the flowering stage, and consequently reduces the number of siliques per plant. The cumulative effect of these stress factors, results in reduction of seed weight and deteriorates the quality of oil by influencing the fatty acid composition. Biotic stress, such as various phytopathies (white rust, blackleg, etc.) and aphids present in high density or pathogens, can also lead to impaired seed development or even seed abortion (Edwards and Hertel 2011). Sources of resistance to biotic stress have been identified through screening of the available germplasm of *B. napus* and *B. juncea*, and have subsequently been utilized to transfer the identified loci into the commercial varieties through conventional breeding (Sharma et al. 2009; Somers et al. 2002). White rust and alternaria resistance conferring loci identified from other Brassica species (*B. carinata*), have been transferred via ovule culture, into *B. juncea* (Gupta et al. 2010), highlighting the potential of related species and/or gene pools in genetic manipulation for disease resistance in oilseed mustard.

Climate change is also expected to influence the plant nutrient-use efficiency, as there would be direct consequences on the growth and yield of plants. The change will also have damaging effects on the plants' nutrient demand, soil nutrient cycling, nutrient availability, and uptake, thereby necessitating the development of high fertilizer-use efficient lines. The loss of arable land due to salinization is becoming one of the limiting factors in Brassica oilseed production and therefore, continuous breeding efforts are required to develop newer varieties that can adapt to the changing soil conditions and thereby minimize yield losses.

A survey of stress-responsive genes, that have been utilized to develop transgenic plants tolerant to climate-change-related extreme climatic conditions, has shown that these genes belong to different signaling pathways. These pathways, that are involved in imparting stress tolerance in plants are not exclusively restricted to either abiotic or biotic stress(Bauddh et al. 2015). Most of these plant metabolic pathways, that result in enhanced accumulation of compatible solutes and free radical-scavenging systems during stress conditions, involve a large number of genes. It will therefore, be practically impossible to pyramid these multiple genes and develop stress tolerant varieties. A more amneable approach might be the identification of regulatory genes, e.g., transcription factors, which play important roles in the cascade of events related to signaling and biosynthesis of stress-related metabolites. These regulate multiple loci and therefore are promising candidate genes that can be targetted for mitigating climate-change-related stress in plants.

The innate phenotypic plasticity of mustard oilseed crops constitutes a promising escape route for evading the associated changes of climate change in terms of shrinking natural resources. The innate plasticity of the crop plant harbors potential for rapid adaptation during changing environmental conditions. An understanding of this plastic response, is therefore crucial for predicting and managing the impacts of climate change. Understanding the role of historical breeding methods in the adaptive plasticity exhibited by present cultivars will definitely be useful, and will be a key factor in designing breeding programs for incorporating plasticity in the major traits such as phenology, flowering time, and reproduction. It will also be

worthwhile to investigate and classify the hierarchies among the adaptabilities of different plant characters associated with single or multiple abiotic stress.

Meeting the demands of the rapidly changing climatic conditions, will impose additional demands on the available genetic resources, including plants related to the cultivated varieties, the semi-domesticated types, and wild species. The crop wild relatives, due to genetic barriers, have remained relatively untapped and harbor immense potential for utilization in crop improvement programs. They still share genetic affinities to cultivated oilseed mustard crops, and their "genetic memory" of tolerance/resistance to abiotic/biotic stress can be employed in breeding climate-resilient oilseed crops. The adaptive significance of these largely undocumented, locally rare, or widespread alleles of the underutilized genetic resources, in breeding for CS traits will therefore, certainly be very significant.

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Chapter 7
Potential for Adaptation to Climate Change Through Genomic Breeding in Sesame

Haiyang Zhang, Hongmei Miao and Ming Ju

Abstract Sesame is an important oilseed crop with high oil content and oil quality. Abundant unsaturated fatty acids, proteins, and antioxidants in sesame seeds attract the worldwide consumption of sesame products. Sesame is highly tolerant of drought and poor soil condition, even though it is readily affected by diseases and waterlogging stress, thereby leading to reduced seed yield and quality. For sesame, increasing the high and stable yield is requisite and urgent. Meanwhile, it is necessary to increase the mechanization level of its harvest for the world's sesame production. Sesame, *S. indicum,* is the sole cultivated species in *Sesamum* genus. The relatively low genetic diversity limits sesame breeding for new and substantial improved varieties. In this section, we present a review of the key agronomic traits and the breeding methods currently used in the species. We also pinpoint the achievement of the Sesame Genome Project (SGP) and the potential for the genomics-assisted breeding in sesame.

Keywords Sesame (Sesamum indicum L.) · Climate change · Yield · Seed quality · Resistance · Breeding · Genomics-assisted selection

7.1 Introduction

7.1.1 An Ancient Oilseed Crop

Sesame (*Sesamum indicum* L., $2n = 2x = 26$) is one of the worldwide annual oilseed crops (Fig. 7.1). It is widely grown in the tropic and subtropic regions. Sesame is

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the sole cultivated species in *Sesamum* genus with the long cultivation history (Kenoyer 1991; Fuller 2003). The sesame seed remnants were found in an excavation at Harappa, Pakistan, in South Asia between 1920–1921 and 1933–1934 (Fuller 2003). Archaeological analysis indicated that sesame has been cultivated in Pakistan and Syria at least since the Early Bronze Age (3000 B.C.) (Bedigian 2004).

Sesame seeds are abundant in oil (50–55%, average in varieties), proteins (18–20%), carbohydrate (13.4–25.0%), and digestible fiber (high to 9.8%) (Jimoh and Aroyehun 2011; Zhang et al. 2012b; Makinde and Akinoso 2013; Prakash and Naik 2014; Sene et al. 2017). Meanwhile, they are also rich in vitamins and minerals, such as pantothenic acid (0.60%), calcium (1.35%), and potassium (0.67%) (Zhang et al. 2012b; Yuan et al. 2018). Particularly, sesame seeds are rich in the specific molecules—antioxidants—such as lignans (Ram et al. 1990; Baydar 2005; Suwimol et al. 2012). Sesame lignans are comprised of sesamin, sesamolin, and small amounts of sesaminol, piperitol, sesamolinnol, pinoresinol, (+)-episesaminone, hydroxymatairesinol, allohydroxymatairesinol, and larisiresinol (Shittu et al. 2007). Sesamin and sesamolin are the main lignans in sesame seeds, ranging from 0.02 to 1.06% and 0.002 to 0.75%, respectively. Sesame seeds are regarded as "the queen of the plant oil crop seeds," probably for its resistance to oxidation and rancidity (Bedigian and Harlan 1986).

Fig. 7.1 Sesame plants with flowers and capsules. The image was taken from the Chinese sesame variety, Zhengzhi 15, planted in field. Supplied by H. Zhang

In addition, sesame leaves also have high contents of minerals such as calcium (1.92%), magnesium (0.14%), and ferrum (0.03%) (data kindly supplied by Zhigang Liu). Till now, sesame seeds, leaves, buds, and flowers are being applied for developing more new sesame products. Sesame really supplies the healthy nutrition for food and medicine industries (Anilakumar et al. 2010; Abuja and Albertini 2002).

Sesame belongs to the *Sesamum* genus of the Pedaliaceae family of the order Lamiales, which is located on the asterids clade of the core eudicots in the angiosperm phylogeny. It is close to the Solanaceae and Phrymaceae families in the outer branch of eudicots, based on the chloroplast genome sequences (Zhang et al. 2013a, b). Therefore, sesame has an important phylogenetic position in eudicots. Sesame was named "*S. indicum*" by Carl Linnaeus for the first time in 1753 (Bedigian 2010a). Even though another name, "*S. orientale*", was also given, *S. indicum* is more broadly accepted by sesame scientists. According to Index Kewensis, there are 36 species in *Sesamum* (Kobayashi 1991); nevertheless, *S. indicum* is the sole cultivated species in the genus and evolved from wild populations (Joshi 1961; Weiss 1971; Zhang et al. 2012a).

As 29 of its 36 species have been found in Africa (Kobayashi 1991), *Sesamum* was inferred to be originated in Africa (Joshi 1961; Kobayashi 1991). However, the geographic origin and domestication of sesame remain debated. The existence of an overwhelming number of wild species in Africa confirms that this continent is the place of sesame's origin (Joshi 1961; Weiss 1983; Sharma et al. 2014). Fuller (2003) proposed that sesame originated in the northwestern South Asia by the time of the Harappan civilization, based on the archaeological findings of charred sesame seeds. This is supported by several wild species growing in India. Moreover, a wild species, *S. malabaricum* Burm., presented in India can readily cross with the cultivated sesame, *S. indicum*, and produce fertile hybrid seeds, which suggests its close relationship with the cultivated sesame (Bedigian 2010b). Comparative analysis of the chloroplast DNA genes, *ndhF* and *trnLF* showed that *S. indicum* is closely related to *S. malabaricum* (Bedigian 2010b). In addition, many other analyses, from morphological traits to molecular markers, suggested that sesame was domesticated on the Indian subcontinent, including the western Indian peninsula and parts of Pakistan (Bedigian et al. 1985; Bedigian 1988; Bedigian and Harlan 1986; Powell 1991; Bhat et al. 1999; Fuller and Madella 2001; Fuller 2002).

7.1.2 World Sesame Production

Sesame is a traditional crop that is mainly cultivated in about 75 countries in Africa, Asia, Central America, and Latin America. Because it has long been cultivated in tropical and subtropical regions, sesame is generally highly tolerant to high temperature and drought environments. In 2016, the world's total harvested area of sesame was 10.58 million hectare (ha), with an annual production of 6.11 million tons (FAO data). The top five sesame production countries ranked by harvested area

included Sudan (2,134,860 ha), India (1,900,000 ha), Myanmar (1,495,250 ha), Tanzania (900,000 ha), and South Sudan (611,644 ha). However, Tanzania was the largest producer, with a sesame production of 0.94 million tons, followed by Myanmar (0.81 million tons) and India (0.80 million tons). China was the top country, with the highest sesame unit area yield of 1056.9 kg per hectare. Nevertheless, the unit area yield of the world sesame remained low, with only 577.9 kg per hectare.

All the ten top sesame production countries currently are developing countries in Asian and African continents, as sesame is a low-profit crop. In the past decade, the world sesame production has increased from 3.62 million tons in 2007 to 6.11 million tons in 2016, with an average annual increase of 6.85% (Fig. 7.2). Meanwhile, the world harvested area of sesame has also increased by 4.84% annually. The world average yield of sesame has gone up with an annual increase of 1.36%. Thus, the increase of the harvested area has been the main contributor to the increased world sesame production.

7.1.3 World Sesame Trade

The increased sesame production has stimulated the world sesame trade market. As shown in Table 7.1, the total trade amount of the main export countries was ranged from 1155 kilotons in 2011–2012 to 1519 kilotons in 2015–2016 (Table 7.2). In 2015–2016, Ethiopia became the biggest exporter of sesame in the world, with its sesame export amount being 27.9% of the total world trade.

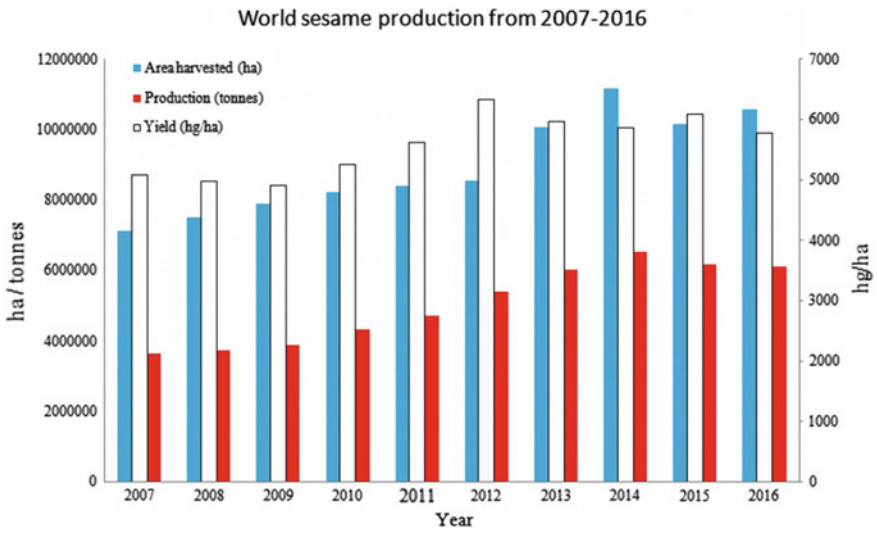

Fig. 7.2 Tendency of the world sesame production from 2007 to 2016 (FAO data)

Table 7.1 Global trade of sesame product from 2011 to 2016

Country	Annual export amount (kiloton)				
	2011–2012	2012–2013	2013–2014	2014–2015	2015–2016
India	360	250	300	330	300
Ethiopia	320	245	290	285	424
Nigeria	180	175	180	200	210
Sudan	90	145	110	190	170
Burkina Faso/ Western Africa	90	90	120	180	200
Tanzania	80	95	115	145	140
Mozambique	25	30	55	50	45
Uganda	10	20	40	40	30
Total	1155	1050	1210	1420	1519

[a]Data supplied by Olam International Company. 75% trade involves crude sesame products, and 25% is processed sesame products

Table 7.2 The world import of sesame products in the past decade (kilotons)

Country	Year								
	2008–2009	2009–2010	2010–2011	2011–2012	2012–2013	2013–2014	2014–2015	2015–2016	2016–2017
China	311	391	389	396	443	487	815	929	820
Japan	129	161	164	173	152	141	192	164	162
Turkey	90	102	101	117	103	107	128	134	140
South Korea	73	78	77	69	76	78	82	74	72
Syria	58	52	40	32	30	24	18	15	15
Israel	36	43	47	52	54	54	59	65	50
USA	36	37	37	37	35	33	32	32	33
Saudi Arabia	24	30	35	40	40	44	48	46	44
Vietnam	13	38	78	81	31	42	61	88	75
Others	361	361	385	468	529	564	502	511	551
Total	1133	1292	1353	1462	1492	1572	1936	2058	1962

Data are collected from Oil World database

Correspondingly, the world imported amount has continuously increased, ranging from 1133 kilotons in 2008–2009 to 1962 kilotons in 2016–2017 (Table 7.2). In 2015–2016, the world total trade amount of the sesame products touched 2058 kilotons, approximately 33.7% of the world annual production in 2016. Of the sesame export countries, Africa supplied approximately 85% of its sesame products to the international market. Asia is the biggest sesame consumer and consumes about 80% of the world total sesame production. In 2015–2016, China and Japan were the two largest importers of sesame products, having

imported 53% of the world total annual sesame trade. The other main importers were Turkey, Vietnam, South Korea, Israel, Saudi Arabia, the USA, and Syria in a descending order.

China is not only the largest sesame importer but also the main sesame producer. The annual sesame production of China currently retains about 650 kilotons. As Chinese sesame varieties usually have high oil contents and pure appearance with high flavor values, China is in an important position in the international sesame market. However, Chinese sesame production has been experiencing a significant change since 2003. Contrary to the world increased trend of sesame production, the sesame harvested area of China has gradually declined in the past decade. In 2015–2016, the amount of sesame products that China imported approached the historic peak of 929 kilotons (Table 7.2). In fact, the status and variation of sesame production in China reflect the development of Chinese agriculture in the last decade. The decrease of the harvested sesame area directly resulted from the massive labor transfer and the high competition of cereals and cash crops in the country.

According to the above status of the world sesame production and trade, we consider that the world sesame product demand will stimulate the development of sesame production. Sesame traditionally is a manual crop with high adaption potential to various environments. Fortunately, in 2008, the first improved non-dehiscent (IND) sesame variety was released by Ray Langham, the USA (Langham 2008). At present, Brazil, the USA, Paraguay, Venezuela, and several other countries in the Central America have realized the mechanization in sesame production. We believe that more new sesame varieties with high yield potential and high adaption to harvest mechanization will be bred, thus improving the world sesame production in the future.

7.2 Sesame Agronomic Characters and Genetic Diversity

7.2.1 Sesame Growth and Development

Sesame well grows and is cultivated worldwide. There are approximately 20,000 worldwide sesame germplasm accessions that are archived in India, Korea, China, and African countries (Gong et al. 2016). Sesame is an ideal crop for genetics research for its great genetic variability (Banerjee and Kole 2009a). Its abundant germplasm accessions show the high capability and potential of adaption of sesame for various environments.

Sesame has a life cycle, from planting to maturity (for normal harvest), varying from 77 days (d) to 144 d (Langham 2018). The life cycle of sesame includes four phases in phenology: vegetative stage (or seedling stage), reproductive stage (flowering stage), ripening stage (flower termination to physical maturity), and drying stage (from physiological maturity to direct harvest) (Langham 2007, 2008, 2018). For most varieties adaptable for manual harvest, the traits of “days to first

flowering," "days to 50% flowering," and "days to maturity" represent the important phenology characters of sesame.

The days to first flowering is an important trait that usually decides the sesame's whole lifetime. Sumathi and Muralidharan (2014) investigated the days to first flowering and other nine important traits of sesame using the six generations derived from six crosses. The results indicated that the days to first flowering varied from 40.2 to 51.1 d among the parents of these crosses. We grew 763 worldwide sesame germplasm accessions at the Pingyu Experimental Station, Pingyu County (114.62° E, 32.97° N), the largest sesame production area, China, in early June 2017 and found that nine foreign varieties did not blossom until the end of August. The days to first flowering of the remaining 754 accessions varied from 22.3 d to 59.3 d, with an average of 32.8 d (unpublished data, H. Zhang).

The "days to 50% flowering" refers to the days to flowering stage when 50% of the plants have opened flowers (Zhang and Feng 2006; Langham 2018). The days to flowering is determined by both genotypes and environments. Satish (2013) investigated 10 sesame varieties and found that the days to 50% flowering varied from 30.4 d (in TKG 314) to 83.0 d (in CST-2001-05).

The "days to maturity" trait is related to plant growth and development, and is one of the most important traits for sesame breeding and production. However, the definition of "maturity" for sesame is inconsistent in the literature (Langham 2018). In the International Plant Genetic Resources Institute (IPGRI), sesame maturity was defined as the number of days from planting or first irrigation to 75% of plants reaching physiological maturity (IPGRI 2004). In China, sesame maturity is referred to the physiological maturity stage by which most of the leaves have shed and the lower capsules have become yellowish. In the USA, sesame maturity is the date when 50% of the plants have reached physiological maturity, and the seeds in 75% of the capsules on the way up the capsule zone appear in the seed line and black tip (Langham 2017). UPOV (International Union for the Protection of New Varieties of Plants) (2013) defined that sesame maturity was the date when approximately 50% of plants show dehiscence of capsules on the middle third of the main stem.

Genetic analysis showed that the three traits of sesame, "days to first flowering," "days to 50% flowering," and "days to maturity," are controlled by genotype × environment interactions. The gene additive, dominant, and dominance × dominance effects, as well as several other effects of non-additive gene action, including significant additive and non-additive genetic effects, non-additive gene action, duplicate epistasis, magnitude additive gene effects, and additive gene action, had been observed in dozens of sesame crosses (Satish 2013).

7.2.2 Yield and Yield Component Traits

Yield is the most important trait for crops, and it is the consequence of interaction of numerous yield component traits. In sesame, many agronomic traits, such as plant height (PH), height to the first capsule (HFC), branch number (BN), leaf number

per plant (LN), capsule number per plant (CN), capsule number per stem (CNS), seed number per capsule (SNC), thousand seed weight (TSW), and seed number per plant (SN), are positively correlated with seed yield (Muhamman et al. 2010; Azeez and Morakinyo 2011; Daniya 2013). Biabani and Pakniyat (2008) analyzed 15 yield-related traits and the seed quality traits in 15 sesame genotypes and found that five indexes, i.e., biological yield (*p*-value, 0.647**), harvest index (0.637**), TSW (0.197*), CNS (0.147*), and HFC, were the most important for yield formation. CN, TSW, and SN always were significantly positively correlated with the seed yield per plant; therefore, they were defined as the principal yield components in sesame (Liu et al. 1980; Gnanasekaran et al. 2008; Banerjee and Kole 2009b; Sumathi and Muralidharah 2010; Ibrahim and Khidir 2012; Gangadhara et al. 2012). Here, we present seven main yield component traits, plant height, height to first capsule, capsule zone length, capsule number per plant, flower number per axil or capsule number per leaf axil, capsule size, and thousand seed weight, to show the characters of the seed yield traits in sesame.

7.2.2.1 Plant Height

Sesame is a crop with long stem or branches. For a sesame variety or line, plant height is often positively correlated with seed yield. However, a higher plant height is often susceptible to lodging due to a strong wind or hurricane. Therefore, to increase the sesame lodging resistance and seed yield, breeders often set up reducing plant height as a sesame breeding objective. For the 763 worldwide sesame germplasm accessions that we grew at Pingyu Experimental Station (114.62° E, 32.97° N, China) in 2017, their plant heights dramatically varied from 71.7 to 200.0 cm, with a mean plant height of 132.0 cm (Table 7.3) (unpublished data, H. Zhang). More than 50% of the accessions with uniculm stems grew to 120–150 cm in height. It was observed that the plant height of sesame was substantially influenced by environments, such as moisture, heat, fertility, light, and plant density.

Table 7.3 Statistics of stem-related traits of 763 sesame germplasm accessions in 2017[a]

Trait	Maximum	Minimum	Mean value	Standard deviation
Plant height (PH) (cm)	200.0	71.7	132.0	20.60
Height to first capsule (HFC)	160.0	15.6	48.6	15.97
Capsule zone length (CZL) (cm)	129.1	13.5	79.8	14.60
Tip length without capsule (TL) (cm)	26.5	0.0	3.7	2.33

[a]All 763 sesame germplasm accessions are cultivated at the Pingyu Experimental Station (114.62° E, 32.97° N, China), with three replicates. Five plants per entry are measured. The data of each trait listed in the table are the average value of five plants (Supplied by H. Zhang)

7.2.2.2 Height to First Capsule

The height to the first capsule node is referred as to the height from the first leaf node or the ground to the lowest capsule node on the main stem. Langham (2018) reported that the height to the first branch of sesame ranged from 1 to 135 cm. As shown in Table 7.3, for the 763 worldwide sesame germplasm accessions, the height to the first capsule node (from the first leaf node to the first capsule node) varied from 15.6 to 160.0 cm, but most of them had a medium height to the first capsule of 30–60 cm. A few accessions from Africa or Latin America presented the highest height to the first capsule of ≥ 100 cm because of their inadaptability to the changed environments.

For sesame, a slow developmental rhythm is always accompanied with late flowering for the inadaptability to the relative long day. Kobayashi (1986) studied the genetics of the sesame height to the first capsule and found that the high height to the first flower or capsule was dominant over the low height to the first capsule. The height to the first capsule was affected by both genotype and environments (such as moisture, fertility, time of planting, light quality, and population). In addition, the height to the first capsule is also an important trait for mechanical harvest in sesame production. It has been reported that 15–40 cm of the height to the first capsule is desirable for mechanized harvest (Van Zanten 2001; Langham et al. 2002).

7.2.2.3 Capsule Zone Length

Capsule zone length is referred as to the length of the main stem from the first capsule note to the last capsule node. Of the 763 sesame germplasm accessions investigated (Table 7.3), the capsule zone length varied from 13.5 to 129.13 cm, while 387 (50.7%) had a capsule zone length of 70–90 cm. For the indeterminate accessions, the tip zone continuously grows, with no mature capsules. The results showed that the tip zone lengths of the 763 accessions ranged from 0.0 cm to 26.5 cm, while their capsule node numbers ranged from 4.4 to 32.1.

7.2.2.4 Capsule Number Per Plant

Capsule number per plant (CN) is an important quantitative trait in sesame. For most sesame varieties, their inflorescences are indeterminate. Thus, the capsule number per plant can be affected by environments and the rhythm of plant growth and development. Of the 763 worldwide sesame germplasm accessions grown at the Pingyu Experimental Station, China in 2017, their CNs varied from 9.2 to 96.2, with a standard deviation of 16.5 (Table 7.3) (unpublished data, H. Zhang). Many scientists reported that the CN was significantly influenced by gene $\times$ gene interaction and its effects on CN varied across crosses and environments (Sharmila et al. 2007; Satish 2013; Sumathi and Muralidharan 2014).

Sumathi and Muralidharan (2014) investigated CN genetics using six crosses. Among the parents used for the six crosses, TMV5 had the highest average CN (77.3), while KS990813 presented the lowest average CN (41.63). For the TMV4 × ls99153 cross, the gene effects of CN coincided with the additive-dominant model. For the crosses of TMV4 × KS99037, TMV5 × KS99153, TMV4 × KS99153, and TMV5 × KS99037, the dominance effect was significant and positive for CN. However, both additive and dominance gene effects were observed for the TMV 4 × KS99037 cross.

7.2.2.5 Flower Number Per Axil and Capsule Number Per Leaf Axil

Flower number per axil is the same as the capsule number per axil and is always substituted by the capsule number per axil during the field investigation. Capsule number per axil is one of the yield components. The capsule number per axil is often divided into single (1) and triple (3) capsules. Nevertheless, the plant lines with 7–8 capsules per axil were reported (Weiss 2000; Langham 2017). The predominant capsule number per axil in the middle of the capsule zone is often used as the capsule number per axil of a variety or line. Langham (2007) found that the axillary capsules of triple capsule lines formed fewer seeds and lower 1000-seed weight than the central capsules. The central flowers of a triple capsule line always form 3–5 days earlier and get more nutrition than the axillary flowers. Studies showed that the formation ratio of sesame central and axillary capsules varied from 61.4 to 82.8% (Langham 2007).

7.2.2.6 Capsule Size

Capsule size is one of the major yield components. Capsule length is referred as to the distance measured from the tip to the bottom of a capsule. Of the 763 germplasm accessions planted with three replicates in 2017, the ranges of capsule length, width, and thickness varied from 1.87–4.98, 0.61–2.20, and 0.57–1.46 cm, respectively (Fig. 7.3 a–c). Their seed numbers per capsule varied from 17.4 to 117.7 (Fig. 7.3 d), of which 450 (59.0%) accessions had 50–70 seeds per capsule (unpublished data, H. Zhang).

7.2.2.7 Thousand Seed Weight

Sesame is a small-seed-size crop. Langham (2017) classified the sesame seed size with six scales using 100-seed weight: 0 = segregating, 3 = small (<0.25 g); 4 = (0.25–0.29 g); 5 = medium (0.30–0.34 g); 6 = (0.35–0.39 g); and 7 = large seeds (>0.39 g). Of the 763 germplasm accessions studied in 2017, the 1000-seed weight ranged from 0.79–4.47 g (unpublished data, H. Zhang). About 5% of them were categorized into large-seed accession category (TSW > 3.5 g). Early studies proved

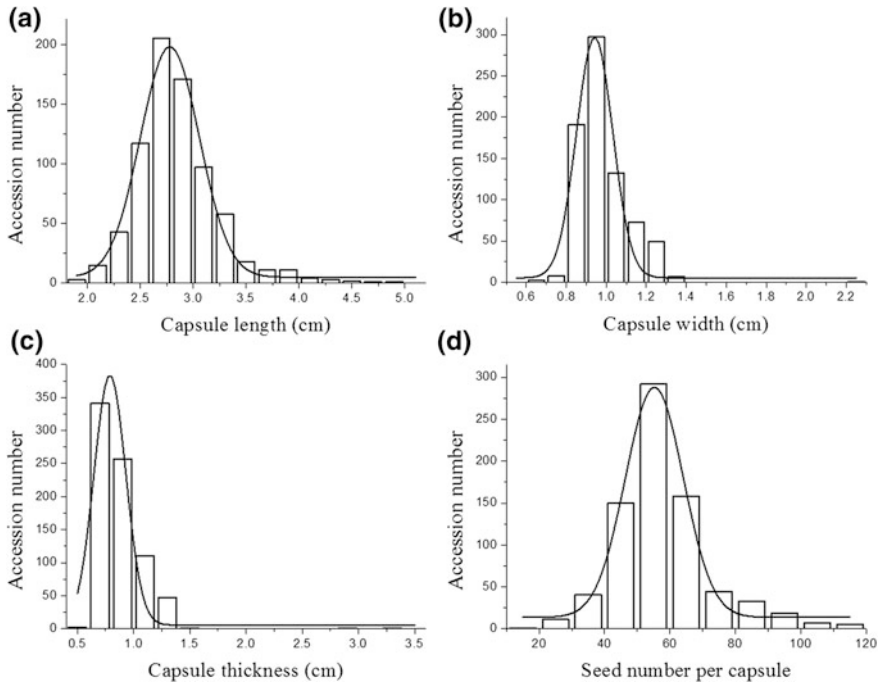

Fig. 7.3 Variation of capsule size among 763 sesame germplasm accessions. **a** Capsule length. **b** Capsule width. **c** Capsule thickness. **d** Seed number per capsule. Supplied by H. Zhang

that 1000-seed weight was controlled by substantial genotype × environment interactions. For example, Sharmila et al. (2007) found that TSW was additive and complementary for the "Si 1115/1" × "TMV 3" cross, while additive, dominance × dominance, and additive × dominance effects and duplicate epistasis are predominant in three other crosses.

7.2.3 Seed Quality Traits

Sesame seeds contain abundant unsaturated fatty acids and proteins, and antioxidants (such as lignan and vitamin E). It is still a query why sesame seeds have a higher than oil content than those of other annual oilseed crops. In the past decades, scientists carried out extensive analyses of the genetics basis and inheritance of the sesame seed oil and protein contents and their correlations, as well as the related genes and associated molecular markers, to clarify the genetic basis of the high quality of sesame seeds.

7.2.3.1 Oil Content

In sesame, the oil content (OC) and protein content (PO) are complicated quantitative traits (Jin et al. 2001; Chun et al. 2003; Leduc et al. 2006; Magni et al. 2010; Wei et al. 2013; Li et al. 2014). Yermanos et al. (1972) assessed 721 sesame accessions collected worldwide and found that the oil and protein contents of sesame seeds ranged from 40.4 to 59.8% and 19 to 31%, respectively. Li et al. (2014) evaluated 369 worldwide sesame germplasm accessions in five environments. The oil contents of seeds varied from 27.9 to 58.7%, with an average of 51.3%, while their protein content varied from 16.7 to 27.8%, with an average of 21.2% (Table 7.4). These results indicated that the oil and protein contents of sesame seeds evidently varied among its germplasm accessions. Both OC and PC were significantly influenced by genotype, and genotype × environment, and their broad-sense heritability (H^2) reached up to 76.2 and 50.0%, respectively (Li et al. 2014). Moreover, the results also suggested that OC was more determined by genotype, as its broad-sense heritability was much higher than those of PC. OC and PC were shown to be significantly negatively correlated ($r = -0.66$).

Sesame oil is composed of polyunsaturated fatty acids (Table 7.5) (unpublished data, H. Zhang), of which approximately 86% are oleic acid (18:1) (~40%) and linoleic acid (18:2) (~46%). Differing from those of peanut, soybean, oilseed rape, sunflower, and other oilseed crops, the ratio of sesame oil oleic acid content to linoleic acid content is nearly 1:1.

Culp (1959) studied the N124 × K8 cross to estimate the inheritance of OC and PC. For this cross, OC and PC had a broad-sense heritability of 50 and 60%,

Table 7.4 Variation of the seed oil and protein contents in the assayed population under various environments

Trait	Year	Location	Min.	Max.	Mean	Std. DEV.	H^2 (%)	
							Per year	Under five environments
Oil content	2011	Pingyu	27.89	55.03	49.59	4.61	83.06[a]	76.2[a]
	2011	Yuanyang	34.28	57.53	51.94	3.72		
	2012	Pingyu	31.95	54.80	49.82	3.43	79.2[a]	
	2012	Yuanyang	30.77	57.88	52.23	4.22		
	2012	Xinyang	32.74	58.73	53.14	4.59		
Protein content	2011	Pingyu	18.74	26.49	21.65	1.30	65.65[a]	50[a]
	2011	Yuanyang	17.60	25.10	20.28	1.16		
	2012	Pingyu	20.22	27.79	22.51	1.04	51.4[a]	
	2012	Yuanyang	16.72	27.44	20.94	1.25		
	2012	Xinyang	17.24	26.84	20.59	1.57		

H^2 denotes broad-sense heritability

[a]The two-tailed significance at $P \leq 0.01$. (Cited from Li et al. 2014)

Table 7.5 Fatty acid components of sesame oil

Sesame seed type[a]	Fatty acid component (mg/g)							Total
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	
White seed	46.35	0.82	23.81	215.14	246.50	1.63	3.03	537.28
Black seed	46.07	0.69	26.76	205.86	262.19	1.79	3.28	546.64

[a]White sesame seeds (var. Yuzhi 11) and black sesame seeds (var. Ji9014) harvested from Yuanyang Experimental Station in 2009 are evaluated using the standard chemical assay method by Henan Sesame Research Center (HSRC), Henan Academy of Agricultural Sciences (HAAS), China

respectively. One or two genes were estimated to be responsible for the inheritance of OC, while one gene was estimated to be involved in determination of PC. Banerjee and Kole (2009a) investigated the genetic architecture of five physiological parameters, OC, and oil yield (OY) per plant using seven sesame genotypes and their F_1 and F_2 progenies. The results suggested that additive gene action for OC was predominant in F_1, while non-additive gene action played a role in F_2. In addition, El-Bramawy and Amin Shaban (2008) reported the major contribution of non-additive gene action to OC in sesame 6 × 6 half-diallel progenies (F_1). In a short, these studies showed that additive gene, non-additive gene, or additive and non-additive gene actions determined the OC in various sesame crosses (Balasnse et al. 1991; Reddy et al. 1992; Backiyarani et al. 1997; Mansouri and Ahmadi 1998; Supriya 2007; Kumar et al. 2012).

7.2.3.2 Protein Content

Insoluble 11S globulin and soluble 2S albumin, conventionally known as α-globulin and β-globulin, are the two major storage proteins in sesame seeds. The two proteins constitute 80–90% of the sesame total seed proteins. Amino acid composition analysis indicated that insoluble 11S globulin and soluble 2S globulin were substantially less hydrophobic than those of the known oleosins, and thus should not be aggregated into multimers of oleosins. The functional properties of globulin in sesame could be influenced by interactions with food components and processing treatments (Anilakumar et al. 2010).

As to amino acid components, sesame seed contains 18 types of amino acids (Table 7.6) (unpublished data, H. Zhang). In sesame seeds, the main amino acids are glutamic acid (4.21%) and arginine (2.79%). Sesame seeds are rich in sulfur-containing amino acids, but limited in tryptophan (Kapadia et al. 2002; Anilakumar et al. 2010; Gao et al. 2011; Prakash and Naik 2014).

Table 7.6 Amino acid components and contents of sesame seeds

Amino acid component	Content (%)	
	White seed[a]	Black seed[a]
Glutamic acid	4.21	4.02
Arginine	2.79	2.60
Aspartic acid	1.66	1.62
Leucine	1.41	1.33
Phenylalanine	1.02	0.96
Valine	1.01	0.97
Glycine	1.00	0.98
Alanine	0.90	0.88
Tyrosine	0.90	0.84
Threonine	0.89	0.86
Methionine	0.84	0.61
Isoleucine	0.77	0.72
Serine	0.73	0.71
Lysine	0.67	0.69
Proline	0.58	0.54
Histidine	0.47	0.47
Cystine	0.37	0.36
Tryptophan	0.21	0.20
Total	20.42	19.33

[a]White sesame seeds (var. Yuzhi 11) and black sesame seeds (var. Ji9014) harvested from Yuanyang Experimental Station in 2009 are evaluated using standard chemical method by HSRC, HAAS, China

7.2.3.3 Sesamin and Sesamolin Content

Being the major lignan components, sesamin and sesamolin have multiple benefits to human health (Wang et al. 2012). Among the 1380 worldwide sesame germplasm accessions analyzed, the total lignan content varied from 0.05 to 1.58% (Fig. 7.4) (unpublished data, H. Zhang). Most of the accessions had approximately 0.5% lignans in their seeds. The average sesamin content (0.3%) of sesame seeds was a little higher than that (0.2%) of sesamolin.

As to their genetic basis, Ogata and Kato (2016) reported that the sesamin and sesamolin contents of sesame seeds were fit to an additive-dominant model, without significant effects of epistasis. Since no major differences were observed between the F_1 and F_2 plants in the sesamin and sesamolin contents, and a statistically significant positive correlation was observed between the two in each generation, the contents of both sesamin and sesamolin would be controlled by a common polygenic system.

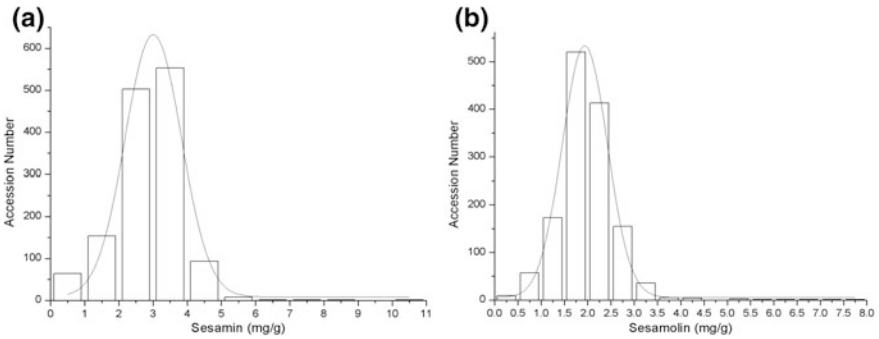

Fig. 7.4 Variation of sesamin and sesamolin contents in the seeds of the 1380 world sesame germplasm accessions. One thousand three hundred eighty accessions selected from the world sesame germplasm collection are used for this study. **a** Sesamin content variation. **b** Sesamolin content variation (unpublished data). All the material accessions were grown at the Yuanyang Experimental Station, HSRC, HAAS, China in 2015

7.2.3.4 Seed Coat Color

Seed coat color is also an important agronomic trait in sesame. The high polymorphism can be observed for seed coat color, ranging from white to black through all intermediate colors. The natural colors of mature sesame seeds vary from black, intermediate colors (e.g., gray, brown, golden, yellow, and light white) to white (Zhang et al. 2013c; Pandey et al. 2013). In the IPGRI (2004), the seed colors of sesame are categorized into white, cream, beige, light brown, medium brown, dark brown, brick red, tan, olive, gray, dull black, and bright black. Based on the variation of RGB (red, green, and blue color) values of seed image, Zhang et al. (2013c) classified the seed coat color of sesame into 14 groups, with an RGB range from 20 to 150 (Fig. 7.5).

Compared with the white sesame seeds, black sesame seeds usually have higher ash and carbohydrate contents, but lower protein content, oil content, and moisture ratios (Kanu 2011). The seed coat color was associated with seed quality traits, disease resistance, or even the species evolution trace (Shahidi et al. 2006; El-Bramawy et al. 2008; Zhang et al. 2013c).

Recently, the color value of sesame seeds was represented by L^*, a^*, and b^* values, respectively (Yuan et al. 2018). All the color values of seed coats were directly collected using a seed color monitor (ColorFlex EZ, USA). L^* represents the color brightness, a^* represents the color red and green, and b^* represents yellow and blue (Imtiaz et al. 2008). The equation of $\Delta E = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}}$ has been used to evaluate the seed coat color variation between two sesame samples or treatments (Yuan et al. 2018) (Table 7.7).

The inheritance analyses of sesame seed coat color indicated that the black seed coat was invariably dominant in the F_1 generation of the crosses between black and dirty white sesame seed types. The seed coat color was regulated by two genes (Sikka and Gupta 1947).

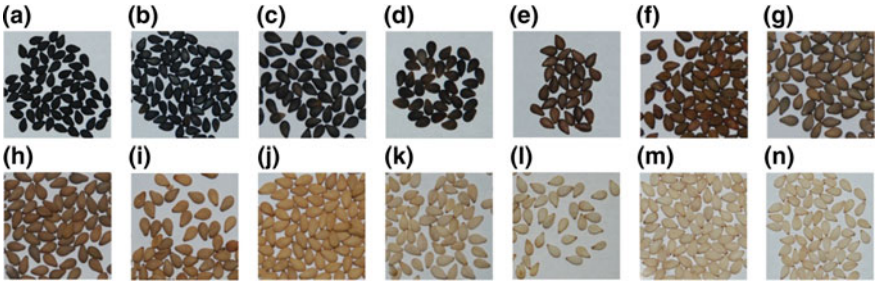

Fig. 7.5 Seed coat color variation in sesame populations. The 14 seed color pictures (**a**–**n**) represent the RGB (red, green and blue) values corresponding to 14 grades of 20–150. The black-seeded parent is presented in black (**a**) and the white-seeded parent is presented in white (**n**). **a** RGB value of 20–30; **b** 30–40; **c** 40–50; **d** 50–60; **e** 60–70; **f** 70–80; **g** 80–90; **h**: 90–100; **i** 100–110; **j** 110–120; **k** 120–130; **l** 130–140; **m** 140–150; **n** 150. Cited from Zhang et al. (2013c)

Table 7.7 Evaluation standard of seed coat color variation in sesame[a]

Scale	ΔE^*ab value	Color variation type
1	$0.0 < \Delta E^*ab \leq 0.5$	Faint difference
2	$0.5 < \Delta E^*ab \leq 3.0$	Difference
3	$3.0 < \Delta E^*ab \leq 6.0$	Significant difference
4	$\Delta E^*ab > 12.0$	Different colors

[a]Evaluation standard of seed coat color variation in sesame is determined according to the standard of Li (2010). Cited from Yuan et al. (2018)

7.2.3.5 Seed Flavor

Seed flavor is an extremely subjective trait in sesame. Flavor is important and decides the final quality grade of sesame seeds. The seed flavor is comprised of sweetness, astringency, bitterness, sourness, dusty taste, first taste, aftertaste, accent of taste, and total taste for raw and roasting samples (Takada and Uno 2001). Langham, R. listed the values of its four seed flavor ratings: 0 = Segregating or mixed; 3 = Bitter; 5 = Neutral; and 7 = Sweet (personal communication). Ashri and Singh (2007) failed to identify information on the genetic control of the taste components for its complicated evaluation and genetic background. Moreover, the seed flavor can be affected by environment. The level of rainfall, dews, and humidity can result in the seed taste a bit musky (Langham 2017). Obviously, sesame breeding for sweetness flavor currently is too difficult and too complicated. Takada and Uno (2001) rated various sesame seeds on a −2 to +2 scale on their taste. Some scientists believed that the taste of sesame seeds is associated with the level of phenols, carbohydrate, or oxalic acid in the seed testa.

7.2.3.6 Shattering Resistance of Capsule

Sesame inflorescence is indeterminate. The flowering stage of some varieties can last more than 1 month. Therefore, the shattering resistance of capsules is a key trait and affects seed harvest time, yield, and even seed quality. The indehiscent or non-dehiscent varieties with high shattering resistance can reduce the harvest loss of mature seeds and adapt to mechanized harvest. However, for most sesame germplasm lines and varieties, capsules are dehiscent, with a low shattering resistance. Langham D. and Langham R. identified eight indicators for sesame capsule shattering resistance, including capsule opening, capsule split, capsule constriction, membrane completeness, placenta attachment, tip roll back, capsule position on stem, and seed blocks (Langham 2017). To assay the capsule shattering resistance, Langham (2017) developed an evaluation method and quantified the capsule shattering resistance with 11 rating scales, i.e., 0 = Segregating or mixed, 1 = SUS (Super-shattering) (visual seed retention <25%), 2 = SHA (Shattering) (visual seed retention >25% but <50%), 3 = SSH (Semi-shattering) (visual seed retention at 50–75%), 4 = SR (Shatter resistant) (a visual seed retention of >75%, but without *id* or *gs* alleles), 5 = ID (Indehiscent) (presence of *id/id* with capsule closed), 6 = IDO (Indehiscent) (presence of *id/id* with capsule open at tip), 7 = GS (Seamless) (presence of *gs/gs* with capsule closed), 8 = GSO (Seamless) (presence of *gs/gs* with capsule open at tip), 9 = C1 (presence of C+1 alleles with closed capsule), 10 = C1O (presence of C+1 alleles with capsule open at tip), and 99 = Other.

In the sesame germplasm library, a natural mutant, *cl1*, with indehiscent capsules and curly leaves, was found in 1940s (Langham 1946; Langham and Rodriguez 1946). Investigation showed that the shattering resistance level of the *cl1* mutant was complete; therefore, it was not suitable for sesame production. Compared with the naturally dehiscent capsule, the carpels of the indehiscent capsule of the mutant cannot split naturally after matured or desiccated. The indehiscent trait of the *cl1* mutant was shown to be controlled by a single gene allele and not affected by environments. The *cl1* gene has been now cloned by Chinese scientists using the genome association method (data not shown). In the past years, Langham R. adhered to non-dehiscent variety breeding and bred dozens of sesame varieties with high shattering resistance. His first improved non-dehiscent sesame variety was registered and released in 2008 (Langham 2008). Studies showed that the shattering resistance of the improved non-dehiscent (IND) variety S29 reached a shattering resistant level of more than 65% (Langham 2017). When cultivated with higher moisture/fertility or in a lower density, the shattering resistance level of a non-dehiscent variety often increases. Therefore, it is necessary for improved sesame harvest mechanization to breed new and more non-dehiscent varieties with an adaption to various sesame production regions.

7.3 Sesame Adaption to Environmental Variation

7.3.1 Adaption to Abiotic Factors

At present, sesame distributes widely in tropic and subtropic regions for the high tolerance and adaption to high temperature, drought, and other abiotic environments. Meanwhile, to respond to the change of the environments and growing conditions, the plant type, leaf number, and type, biomass biosynthesis and even development rhythm would be changed in sesame (Rahman and Das 1994; Mensah et al. 2006; Bor et al. 2009; Sun et al. 2010; Ozkan and Kulak 2013).

7.3.1.1 High Drought Tolerance

Sesame has high tolerance to drought and infertility, as the tap root system of sesame can penetrate into deeper soil layers to find moisture and nutrients. Moreover, the surface structure of leaves and the components of the trichome secretions also vary accordingly (Su et al. 2016). Treated with 40% PEG (polyethylene glycol) solution for 4 h, the varieties with high drought tolerance presented the low damage rate (0.0088–5.3447%). For sesame, the adaxial stoma is surrounded by surface cells. There are four types of leaf hairs on the sesame leaves surface, i.e., nonglandular hairs, long stalk glandular hairs, short stalk glandular hairs, and mucilage hairs (Su et al. 2016). The secretions of glandular hairs contain a variety of components. Under drought conditions, the secreted components of Ji 9014 with high drought tolerance included C36 alkanes and C34 alkanes. Thus, Su et al. (2016) put forward to use the glandular trichome structure and the secretion components to evaluate the drought tolerance level of sesame varieties. Meanwhile, Kim et al. (2007a) found that the wax amount on leaves reflected the response of the drought stress and was negatively correlated with seed yield ($r = 0.466$*).

In addition, Sun et al. (2010) analyzed 43 characteristic indices from 5 sesame varieties using principal component analysis and subordinate function. Heizhi 09-1 and Jinhuangma presented the high tolerance to drought. Fazeli et al. (2007) studied the effects of drought on growth, protein content, lipid peroxidation, superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and polyphenol oxidase (PPO) in leaves and roots of two sesame accessions under the water-deficit treatment of 100, 75, 50, and 25% field capacity for next 4 weeks. Aside from the decrease of the fresh and the dry masses, the protein content in leaves and roots and the lipid peroxidation also reduced. Meanwhile, the activities of SOD, POX, CAT, and PPO enzymes in leaves and roots increased and reflected the response to drought stress (Fazeli et al. 2007). To reflect the characters of drought tolerance in sesame, Ozkan and Kulak (2013) analyzed the growth parameters, lipid composition, and mineral contents in drought-treated seeds for the first time. Under the severe water-deficit (1/4 of field capacity) treatment, plant growth parameters significantly declined. However, the oil yield and the linoleic acid percentage in sesame seeds were not influenced.

7.3.1.2 High Adaption to Low Input Condition

Sesame is a survivor crop, as sesame is mainly grown under low input conditions (Langham 2007). At present, the planting regions for sesame cover the irrigated and rainfed regions. There are two types of the world sesame according to the levels of moisture and fertility (Langham 2007). One is cultivated in dry areas or seasons with low input (such as in US, Venezuela, Ethiopia, and Sudan). The leaves are smaller with short internode length and plant height. The other is mainly cultivated under the conditions with high moisture and fertilizer input (such as in China and Korea). The leaves are larger and the plant height is higher with long internode length and long stems. The same variety develops differently under different conditions (Langham 2007). As the input architecture changes from high to low, the plants with large leaves and high plant height will drop the lower leaves and the new leaves will be smaller with small capsules in the life cycle (Langham 2007). On the other hand, the same variety also presents the same development characteristics under various input levels. Zhao et al. (2014a, b) compared the dry matter accumulation and distribution in two sesame varieties (i.e., Wanzhi 1 and Yuzhi 4) under three fertilizer application levels, i.e., (1) high nutrition level with 150 kg/hm^2 nitrogen, 75 kg/hm^2 P_2O_5 and 150 kg/hm^2 K_2O; (2) middle level with 90 kg/hm^2 nitrogen, 45 kg/hm^2 P_2O_5, and 90 kg/hm^2 K_2O; and (3) low level with 45 kg/hm^2 nitrogen, 15 kg/hm^2 P_2O_5, and 45 kg/hm^2 K_2O. The results showed that the tendency of the total dry matters accumulation under the treatments is same with the rhythm of "slow-fast-slow." The distribution center of the dry matter gradually changes from stem and leaves to capsules and seeds during the plant development (Zhao et al. 2014a, b).

7.3.1.3 Waterlogging Tolerance

Waterlogging can affect the growth and development of sesame plants through the stress of oxygen lack on plant tissues and reduces the final seed yield (Liu et al. 1993; Wang et al. 2000; Sun et al. 2008, 2010; Wang et al. 2012). Sun et al. (2010) investigated 13 morphological and agronomic traits within 66 sesame germplasms after waterlogging treatment at full flowering stage. During the investigation, the waterlogging index was calculated according to the following formula: Waterlogging index (%) = $(1 - V_t/V_c) \times 100$, where V_t indicated the trait value of treatment and V_c referred to the trait value of control. Of the 13 traits, the seed weight per plant and the capsule number per plant presented the most significant effects. The average waterlogging indexes of seed weight per plant and the capsule number per plant were 69.18 and 67.48%, respectively. The following were capsule node number, capsule zone length, and capsule number per node. The results showed that 5 (7.6%) accessions of the 66 germplasm retained the high tolerance to waterlogging (Sun et al. 2010). Early experiments showed that the waterlogging tolerance level of sesame was related to pubescence amount on stem, seed coat color, and root vigority, and was controlled by multiple genes (Liu et al. 1993; Wang et al. 1999, 2000). Some improved cultivars (or lines) and native varieties,

such as Yuzhi No. 1, Henan No. 1, Yiyang Bai, and Zhongzhi 13, expressed the high tolerance and met the demand of breeding objectives (Liu et al. 1993; Ding et al. 2012). On the other hand, to reflect the genetic characters of the waterlogging tolerance in sesame, Zhang et al. (2014) investigated the tolerance variation of the progeny of a RIL (recombinant inbred lines) population derived from the cross between Zhongzhi No. 13 (tolerant to waterlogging) and Yiyangbai (susceptible to waterlogging). Six QTLs related to waterlogging tolerance were detected, with the phenotype explanation of 5.67–17.19%. Eight of 186 sesame core collections were screened and determined with high waterlogging tolerance.

7.3.1.4 Salinity and Alkalinity Stress Tolerance

Salinity and alkalinity stress are caused by the salts (such as NaCl and $CaCl_2$) and alkaline salts (such as Na_2CO_3 and $NaHCO_3$) in the soil. Sesame is cultivated in semi-arid regions and is generally affected by the presence of calcium chloride for its competition with various nutrients and specific toxicities (Ramírez et al. 2005). Xu et al. (2016) analyzed the effects of salinity and alkaline stresses on sesame seed germination. Under the 1–3 g l^{-1} NaCl and $NaHCO_3$ for 24–84 h at 23 °C, the germination rate of Yuzhi 11 retained highly to 96–99%. However, the root length and fresh weight are affected under long time ($\geq$ 72 h). Ramírez et al. (2005) measured the effects of calcium chloride on the seed germination of 50 sesame germplasm accessions and further compared the tolerance level of the four worldwide sesame genotypes with high salt tolerance using a sandy loam soil with pH of 6.4 (soil to water, V:V = 1:2.5), electric conductivity 0.51 dSm^{-1} (saturated soil paste), and rich in calcium (1110 mg kg^{-1}). Dry matter accumulation assay at 90 days after planting (DAP) showed that two genotypes (G41 and G42) produced the highest level of dry matter accumulation. Salinity treatments ranging from 0.5 to 5.0 dSm^{-1} did not affect the shoot dry matter accumulation at 90 DAP, while above 3.5 dSm^{-1} highly affected the average seed yield. The results showed that the salt-susceptible cultivar (G5) during germination was the most sensitive during the growth stage. Koca et al. (2007) found that the root and shoot dry weight maintained highly in cv. Cumhuriyet under NaCl concentration of 100 mM after treated for 21d. The activities of the anti-oxidative enzymes in Cumhuriyet were induced more efficiently. The results indicated that the growth parameters, lipid peroxidation, and proline accumulation are correlated with the relative tolerance to salinity in sesame.

Alkaline stress is more destructive to plants than salt stress, as the increased pH environment also causes direct toxicity effects. Bekhrad (2016) found that sesame seeds can tolerate the effects of 10 mM $NaHCO_3$ during germination. Under the treatment of 60 mM $NaHCO_3$, the germination rate reduced to 38.65%. With the increase of the alkaline stress, the potassium content increased, while the sodium concentration, Na^+/K^+ ratio, and the amount of malondialdehyde (MDA), total soluble sugars, and proline decreased.

7.3.2 Adaption to Biotic Factors

7.3.2.1 *Fusarium* Wilt Disease Resistance

Sesame *Fusarium* wilt (SFW) disease is a worldwide and common disease and is induced by *Fusarium oxysporum* f. sp. *sesami* (FOS) (Verma et al. 2005; Su 2011; Li et al. 2012a, b). SFW disease was primarily found in Egypt, India, the Soviet Union, and other main sesame production countries since 1920s (Armstrong and Armstrong 1950; Cho and Choi 1987; El-Shakhess and Khalifa 2007). As the hypha, conidium, and chlamydospore of FOS pathogen can grow on the plant remains, the ordinary disease control solutions such as chemical fungicide and cultivation regulation would not give the radical cure of the SFW disease (Alves-Santos et al. 1999; Fall et al. 2001). Improving the disease resistance of the host plants to the pathogens is the principal approach to reduce the yield loss caused by diseases.

Differing from other agronomic traits, the level disease resistance can be affected by the pathogenicity of pathogen strains or even races, and also the genotypes of the host and environmental factors. To assay the disease resistance of sesame germplasm lines to FOS, El-Bramawy (2006) employed the "infection percent" of the plants as the resistance evaluation indicator with the five rating scales under the natural infection conditions. In China, sesame scientists usually evaluate the resistance of sesame germplasm lines to FOS infection under the artificial SFW nursery. The disease index (DI) is calculated according to the following formula:

$$\mathrm{DI} = \frac{\sum (s_\mathrm{i} \times n_\mathrm{i})}{2N} \times 100$$

where s_i indicates symptom grade, n_i indicates plants infected by *Fusarium* wilt pathogen (FOS), and N indicates the total number of host plants assayed.

The resistance level to the disease was classified into five rating scales, based on the DI value (Table 7.8) (Qiu et al. 2014).

The FW resistance analysis based on hundreds of worldwide sesame accessions under the artificial SFW nursery indicated that about less than 10% of the germplasm accessions showed high resistance to high pathogenic FOS strains (data not shown, H. Zhang). Under the above evaluation condition, the FW resistance in an F_2 population was shown to be controlled by recessive dominant genes (data not shown). Meanwhile, two QTL loci were determined in the sesame super-dense SNP (single nucleotide polymorphisms) map, with high R^2 values of 0.11 and 0.15, respectively (Unpublished data, H. Zhang).

Some other researchers also performed the *Fusarium* wilt resistance assay of sesame germplasm lines and cross-derived lines under the natural field conditions or artificial nurseries (El-Bramawy 2006; Silme and Çarğirgan 2010). Wang et al. (1993) found that the resistance to FOS in some germplasm was controlled by 1–2 of dominant genes, while other lines presented the effects of recessive genes and the

Table 7.8 Resistance scale of sesame plants to *Fusarium* wilt

Grade	Disease index (DI)	Category
1	$0 \leq DI \leq 15$	Highly resistant (HR)
3	$15 < DI \leq 30$	Resistant (R)
5	$30 < DI \leq 55$	Moderately resistant (MR)
7	$55 < DI \leq 70$	Susceptible (S)
9	$70 < DI \leq 100$	Highly susceptible (HS)

Cited from Qiu et al. (2014)

interaction of multiple genes. El-Bramawy (2006) investigated two generations (F_3 and F_4) of 15 crosses in two successive seasons and found that the broad-sense heritability of disease resistance was very high (more than 95%) in both generations during the two seasons. Subsequently, El-Bramawy and Amin Shaban (2007) investigated the disease resistance of 15 F_1 and six parents. They found that the *Fusarium* wilt infection percent of the 21 groups of samples varied from 6.12 to 30.10%. Both additive and non-additive components of the genetic variance were shown to be involved in the inheritance behavior of the *Fusarium* wilt resistance, with a predominance of dominance components (El-Bramawy and Amin Shaban 2007). For some crosses, the additive, dominance, and epistatic components of genetic variation might control the *Fusarium* wilt resistance (Bakheit et al. 2000).

7.3.2.2 Charcoal Stem Rot Disease Resistance

Charcoal stem rot disease, caused by *Macrophomina phaseolina* (Tassi.) Goid., is one of the most important diseases for sesame (Rajput et al. 1998; El-Bramawy and Abdul Wahid 2006). Charcoal rot disease is destructive at all stages of sesame life cycle and can cause a loss of about 5–100% of the seed yield (Vyas 1981). The most common symptom of the charcoal rot disease is the sudden wilting mainly at the flowering stage. In addition, plant stem and root get black rapidly, due to severe infection of *Macrophomina* pathogen.

Considering that the infection and development of charcoal rot disease in sesame are also related to the host genotypes and environments, Zhang and Feng (2006) developed the charcoal rot resistance phenotype standard for sesame with five ratings, i.e., 0 = normal plant without disease spots, 1 = less than 1/3 of the plant and less than 1/4 of its capsules on plant exhibit charcoal rot, 3 = 1/3 to 2/3 of the plant and 1/4 to 1/2 of its capsules on plantlet exhibiting charcoal rot, 5 = over 2/3 of the plant and 1/2 to 3/4 of its capsules on plantlet exhibiting charcoal rot, and 7 = plant dead due to charcoal rot (Zhang and Feng 2006; Wang et al. 2017a). The disease index (DI) is calculated based on the following formula:

$$DIN = \sum ni = 1(Xi \times I) / \left(7 \times \sum ni = 1Xi\right)$$

where i = 0, 1, 3, 5, and 7, and *Xi* represents the number of plants with a disease resistance rank of I.

When over 50% of the plants in a population showed the charcoal rot symptoms at the end of flowering stage, the disease resistance ranks of all plants can be recorded. Thiyagu et al. (2007) established the artificial screening and sick plot methods to evaluate the disease resistance of different sesame genotypes. The disease resistance was classified into five grades based on the host infection percent, i.e., resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible (Dinakaran and Mohammed 2001). To our knowledge, there is no sesame germplasm accession that is immune to charcoal rot pathogen, *Macrophomina phaseolina*. Compared with the cultivated sesame, the resistance level of some wild species to charcoal rot disease is higher. Therefore, applying the wild species in interspecies breeding is requisite and urgent to improve the resistance of sesame varieties. In addition, Sinhamahapatra and Das (1992) analyzed the combining ability of the disease resistance using a 9 × 9 diallel set of sesame genotypes under artificial infection and showed that the dominance component functioned on the charcoal rot resistance. On the other hand, the charcoal rot disease resistance was controlled by additive and non-additive components or dominant and additive gene actions (El-Bramawy and Amin Shaban 2007).

7.3.2.3 Leaf Diseases Resistance

Leaf diseases are very common in sesame. Leaf blight disease usually causes 30–40% of the plants infected and consequently reduces the seed yield (Zhao et al. 2014a, b). Some leaf diseases such as *Alternaria* leaf spot can cause seed rot, pre- and post-emergence death of seedlings, and infect all the above-ground parts resulting in considerable yield loss both qualitatively and quantitatively. There are five very common fungal leaf diseases in sesame around the world, i.e., sesame leaf blight caused by *Helminthosporium sesami* Miyake, *Alternaria* leaf spot (or black spot) caused by *Alternaria sesami* (Kawamura) Mohanty et Behera, *Nigrospora* leaf blight caused by *Nigrospora sphaerica*, brown spot caused by *Ascochyta sesami* Miura, and *Corynespora* blight (or Corynespora blight spot) caused by *Corynespora cassiicola* (Berk and Curt) (Sun 2006; Zhao et al. 2014a, b).

In sesame field, two or more leaf diseases often occur simultaneously in a population. The occurrence of leaf diseases is affected by both genotype and environments. Till now, only several leaf diseases are applied for the genetic inheritance. Previous studies indicated that the seed color was associated with the plant resistance to pathogens (El-Bramawy et al. 2008; Li et al. 2014). El-Bramawy and Amin Shaban (2007) performed the *Alternaria* leaf spot resistance experiments under greenhouse conditions in 2005 and 2006. 45-d-old sesame plants were sprayed with *Alternaria* leaf spore suspension (2×10^3 spore per mL) and covered with plastic bags to keep humidity for 72 h. The disease syndrome was assessed after 1 week when the pathogen was inoculated according to Karunanithi (1996). The leaf spot percent of *Alternaria* infection with the tested samples varied from

1.32 to 18.54%. The results determined that the resistance to *Alternaria* leaf spot presented mostly additive variance. In order to study inheritance of *Alternaria* blight resistance in sesame, Eshwarappa (2010) investigated the crosses of RT-273 (resistant) and Gulbarga local black (susceptible). The results showed that *Alternaria* blight resistance was controlled by single dominant gene. The F_3 and F_4 families under field condition screened confirmed the single dominance of gene action (Goudappagoudar et al. 2014).

7.3.3 High Adaption Potential of Wild Species to Environmental Factors

In *Sesamum*, most wild species exhibit the high tolerance and resistance to different diseases, pests and abiotic stresses (Joshi 1961; Liu et al. 1993; Uzo 1985; Nimmakayala et al. 2011). As shown in Table 7.9, eight wild species, i.e., *S. alatum*, *S. malabaricum*, *S. mulayanum*, *S. prostatum*, *S. laciniatum*, *S. occidentale*, *S. radiatum*, and *S. shinzianum* individually present the specific tolerance and (or) resistance to drought, waterlogging, pests and diseases.

Recently, we evaluated the resistance level of four species, *S. indicum* (var. Yuzhi 11 and Jiangxi Yezhi), *S. radiatum*, *S. angustifolium*, and *S. calycinum* using the inoculation method of Qiu et al. (2014). Two FOS strains with high pathogenicity, HSFO 08027 and HSFO 09018, which were isolated by HSRC and HAAS, were applied to inoculate the tested sesame seeds and seedlings (Fig. 7.6). The results showed that *S. radiatum* has high resistance to *Fusarium oxysporum* isolates, followed by *S. calycinum*. Under the artificial inoculation condition, *S. angustifolium* and the two cultivated sesame accessions presented highly susceptible to FOS pathogens (Fig. 7.6) (unpublished data, H. Zhang).

Table 7.9 Wild *Sesamum* species with high resistance to environmental stresses

Sesamum species	Chromosome number	Characters
S. alatum	26	Resistant to phyllody and *antigastra*
S. malabaricum	26	Resistant to waterlogging
S. mulayanum	26	Resistant to *Fusarium* wilt and gall fly
S. prostatum	32	Resistant to antigastra, and salinity
S. laciniatum	32	Resistant to antigastra and drought
S. occidentale	32	Resistant to drought
S. radiatum	64	[a]Resistant to *Fusarium* wilt, charcoal rot, and drought
S. shinzianum	64	[a]Resistant to *Fusarium* wilt, charcoal rot, and drought

[a]The disease resistance has been evaluated using disease nursery and laboratory evaluation methods in China. Modified from Nimmakayala et al. (2011)

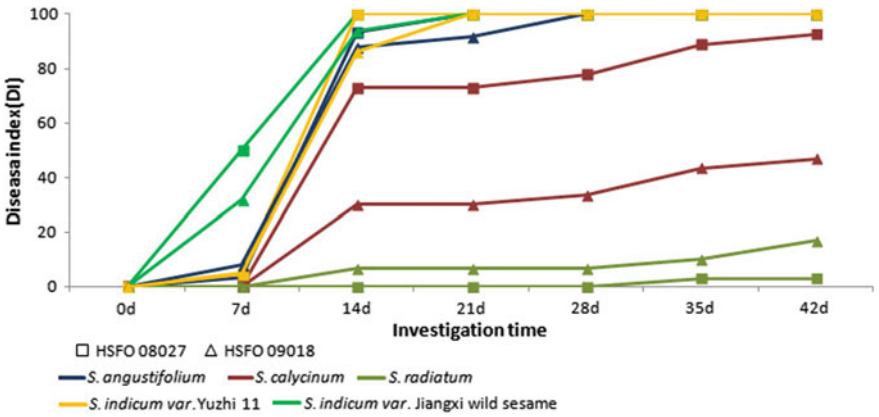

Fig. 7.6 Assessment of the resistance level of several *Sesamum* species to *Fusarium* wilt disease. Four species of *S. indicum* (var. Yuzhi 11 and Jiangxi Yezhi), *S. radiatum*, *S. angustifolium*, and *S. calycinum* are inoculated with two highly pathogenic FOS strains HSFO 08027 and HSFO 09018 according to the method of Qiu et al. (2014). Supplied by H. Zhang

7.3.4 Environmental Stresses on Seed Yield in Sesame

7.3.4.1 Disease Effect on Sesame Yield

Previous results reflected that the seed yield, branch number, and seed color were significantly correlated with the infection percent of *Fusarium* wilt and charcoal rot diseases in sesame (El-Bramawy and Abdul Wahid 2006; El-Bramawy and Amin Shaban 2007). Recently, Li (2017) investigated the plant height (PH), capsule zone length (CZL), capsule node number (CNN), and the capsule number per plant (CN) of three sesame varieties (i.e., TP4816-0, JDP12-0, and JDP21) under the artificial FW nursery (Yuanyang Experimental Station, China) (Table 7.10). The nursery was inoculated with five FOS strains with high pathogenicity. The results showed that there was no difference in the above four traits between Grade 0 (the control) and Grade 2. Compared with the control, the values of the four yield component traits in Grade 3 and Grade 4 treatments decreased significantly ($p < 0.05$). For example, in JDP21, the plant height of Grade 4 disease plants decreased by 45.38%, and the capsule zone length decreased by 57.17%; meanwhile, the capsule node and capsule number per plant reduced by 58.82 and 55.32%, respectively. The significant inhibition of yield component traits caused by *Fusarium oxysporum* infection also presented in TP4816-0 and JDP12-0. With the aggravation of *Fusarium* wilt syndrome, the growth and the seed yield of sesame plants decline significantly.

Table 7.10 Variation of the yield components traits under FOS inoculation condition

Sesame variety	FW grade	Plant height (cm)	Capsule zone length (cm)	Capsule node number	Capsule number per plant
TP4816-0	0	81.35 ± 0.17^{a}	52.19 ± 0.18^{a}	13 ± 0.22^{a}	50 ± 0.32^{a}
	1	78.83 ± 0.18^{a}	52.44 ± 0.18^{a}	12 ± 0.20^{a}	47 ± 0.30^{a}
	2	76.50 ± 0.14^{a}	47.90 ± 0.19^{ab}	10 ± 0.25^{ab}	39 ± 0.30^{b}
	3	73.39 ± 0.18^{b}	42.13 ± 0.17^{b}	9 ± 0.36^{b}	38 ± 0.42^{b}
	4	58.20 ± 0.17^{c}	31.29 ± 0.42^{c}	7 ± 0.40^{c}	22 ± 0.58^{c}
JDP12-0	0	149.82 ± 0.11^{a}	92.74 ± 0.13^{a}	22 ± 0.24^{a}	66 ± 0.53^{a}
	1	142.15 ± 0.15^{a}	89.35 ± 0.18^{a}	20 ± 0.32^{a}	61 ± 0.53^{a}
	2	141.27 ± 0.12^{a}	74.26 ± 0.22^{b}	20 ± 0.26^{a}	56 ± 0.48^{a}
	3	121.28 ± 0.13^{b}	58.19 ± 0.25^{c}	14 ± 0.27^{b}	30 ± 0.42^{b}
	4	102.08 ± 0.18^{c}	37.33 ± 0.34^{d}	8 ± 0.36^{c}	15 ± 0.49^{c}
JDP21	0	134.92 ± 0.15^{a}	77.59 ± 0.24^{a}	19 ± 0.27^{a}	50 ± 0.43^{a}
	1	128.95 ± 0.16^{a}	71.00 ± 0.13^{ab}	17 ± 0.30^{a}	47 ± 0.45^{a}
	2	116.40 ± 0.13^{a}	57.00 ± 0.31^{bc}	13 ± 0.46^{ab}	37 ± 0.21^{ab}
	3	116.43 ± 0.16^{a}	47.57 ± 0.48^{c}	12 ± 0.52^{b}	31 ± 0.21^{b}
	4	76.40 ± 0.27^{b}	37.00 ± 0.48^{c}	9 ± 0.28^{c}	24 ± 0.46^{c}

All the varieties are cultured in Yuanyang *Fusarium* wilt disease nursery (E 113.97°, N 35.05°). The nursery is inoculated with five FOS strains with high pathogenicity. The significance level is $p < 0.05$. Modified from Li (2017)

7.3.4.2 Waterlogging Effect on Sesame Yield

Many reports proved that the continuous drown treatment (36 h) would inhibit the growth of sesame root and stem and lead the serious yield loss of 51.67–58.24% (Wang et al. 1999; Sarkar et al. 2016). Yuan (2018) investigated the effects of 24–60 h waterlogging time on the four yield-related traits (i.e., plant height, capsule zone length, capsule number per plant, and capsule node number) within six sesame varieties (Table 7.11). As the waterlogging time prolonged from 24 to 60 h, the values of the yield-related traits decreased significantly. Compared with the control, the plant height of the six varieties reduced 19.50–46.76%. Meanwhile, the capsule zone length of the six varieties decreased 24.02–67.03%. After the 60 h waterlogging treatment, the plant yield of the six varieties decreased to 2.27, 5.05, 0.94, 2.45, 1.09, and 0.74 g, respectively, of which Zhengzhou 98N09 and Yuzhi 11 retained the relatively high yield potential, while Rongxian black sesame presented the maximum reduction of the plant yield (88.2%). Waterlogging caused the yield loss, while the genotype with high resistance level would determine the final yield level (Feng and Zhang 1991).

Table 7.11 Effects of 24–60 h waterlogging treatments on the yield-related traits within six sesame varieties

Variety	Treat (h)	Plant height (cm)	Capsule zone length (cm)	Capsule number per plant	Capsule node number	Seed yield per pant (g)
Yuzhi 11	0	122.80 ± 16.92^{a}	91.10 ± 18.77^{a}	62.00 ± 8.61^{a}	19.00 ± 4.50^{a}	8.52 ± 5.42^{a}
	24	118.10 ± 24.67^{a}	80.80 ± 7.84^{ab}	62.00 ± 4.59^{a}	17.00 ± 7.40^{ab}	7.30 ± 2.49^{a}
	36	115.20 ± 12.73^{a}	82.30 ± 10.83^{a}	51.00 ± 5.13^{a}	14.00 ± 4.40^{bc}	6.04 ± 2.31^{ab}
	48	94.20 ± 14.15^{b}	63.70 ± 17.17^{bc}	43.00 ± 7.1^{a}	10.00 ± 2.50^{c}	4.32 ± 3.64^{ab}
	60	82.20 ± 15.54^{b}	53.90 ± 16.79^{c}	20.00 ± 6.75^{b}	8.00 ± 1.60^{d}	2.27 ± 1.84^{b}
Zhengzhou 98N09	0	115.60 ± 15.74^{a}	84.50 ± 16.00^{a}	60.00 ± 3.06^{a}	18.00 ± 5.33^{a}	13.10 ± 5.59^{a}
	24	110.30 ± 10.78^{a}	75.90 ± 16.04^{ab}	58.00 ± 5.93^{a}	17.00 ± 3.26^{a}	7.33 ± 3.64^{b}
	36	105.40 ± 13.71^{ab}	71.00 ± 7.37^{bc}	45.00 ± 7.57^{a}	17.00 ± 4.10^{a}	7.51 ± 2.67^{b}
	48	88.50 ± 8.35^{b}	61.80 ± 12.38 ^{cd}	42.00 ± 7.63^{ab}	14.00 ± 4.42^{a}	5.32 ± 3.24^{c}
	60	96.00 ± 15.90^{c}	51.50 ± 7.42^{d}	23.00 ± 5.82^{b}	9.00 ± 3.67^{b}	5.05 ± 1.87^{c}
Ji9014	0	105.00 ± 7.74^{a}	64.30 ± 6.69^{a}	20.00 ± 10.24^{a}	13.00 ± 2.59^{a}	11.47 ± 5.35^{a}
	24	105.00 ± 15.44^{a}	63.20 ± 10.85^{a}	17.00 ± 7.34^{a}	11.00 ± 2.12^{a}	7.43 ± 1.57^{b}
	36	96.70 ± 6.83^{b}	55.60 ± 7.70^{a}	13.00 ± 13.29^{a}	11.00 ± 2.80^{a}	7.24 ± 2.64^{b}
	48	81.00 ± 6.05^{b}	36.70 ± 8.14^{b}	10.00 ± 15.68^{b}	5.00 ± 0.80^{b}	1.94 ± 1.89^{c}
	60	55.90 ± 8.07^{b}	21.20 ± 7.77^{c}	7.00 ± 7.13^{c}	3.00 ± 0.59^{c}	0.94 ± 0.20^{c}
Yuzhi 28 (determinate variety)	0	122.00 ± 10.43^{a}	73.40 ± 2.77^{a}	60.00 ± 3.31^{a}	15.00 ± 3.43^{a}	7.34 ± 2.85^{a}
	24	113.00 ± 5.87^{a}	63.10 ± 5.45^{a}	40.00 ± 6.30^{a}	13.00 ± 5.31^{a}	6.04 ± 1.67^{ab}
	36	109.90 ± 7.70^{a}	59.80 ± 2.24^{ab}	53.00 ± 4.68^{b}	13.00 ± 3.79^{a}	6.00 ± 1.77^{ab}
	48	86.60 ± 8.53^{b}	46.50 ± 9.66^{c}	39.00 ± 9.13^{b}	12.00 ± 2.83^{ab}	3.47 ± 1.27^{bc}
	60	81.60 ± 7.27^{b}	33.50 ± 5.14^{c}	25.00 ± 3.18^{b}	8.00 ± 1.74^{b}	2.45 ± 1.52^{c}

(continued)

Table 7.11 (continued)

Variety	Treat (h)	Plant height (cm)	Capsule zone length (cm)	Capsule number per plant	Capsule node number	Seed yield per pant (g)
Xincai Xuankang	0	90.60 ± 8.43^{a}	59.40 ± 9.75^{a}	61.00 ± 6.97^{a}	12.00 ± 3.60^{a}	7.48 ± 1.76^{a}
	24	80.20 ± 11.08^{ab}	50.60 ± 10.94^{ab}	50.00 ± 6.17^{ab}	12.00 ± 3.23^{a}	5.94 ± 1.49^{a}
	36	77.80 ± 6.28^{b}	50.80 ± 6.67^{ab}	42.00 ± 5.62^{bc}	10.00 ± 3.06^{ab}	2.48 ± 1.04^{b}
	48	77.50 ± 12.14^{b}	45.60 ± 10.42^{bc}	28.00 ± 4.79^{cd}	7.00 ± 2.64^{b}	1.57 ± 0.68^{b}
	60	58.50 ± 9.47^{c}	35.70 ± 6.80^{c}	18.00 ± 6.67^{d}	6.00 ± 1.64^{b}	1.09 ± 0.54^{b}
Rongxian black sesame	0	124.10 ± 15.94^{a}	58.70 ± 12.00^{a}	58.00 ± 7.75^{a}	14.00 ± 6.72^{a}	6.16 ± 2.48^{a}
	24	111.10 ± 9.68^{ab}	46.90 ± 6.72^{ab}	23.00 ± 8.87^{b}	6.00 ± 4.47^{b}	1.55 ± 1.87^{b}
	36	109.80 ± 10.97^{ab}	39.00 ± 4.05^{ab}	27.00 ± 6.24^{b}	8.00 ± 2.05^{b}	1.37 ± 0.74^{b}
	48	103.00 ± 13.40^{ab}	38.90 ± 6.10^{b}	22.00 ± 8.99^{b}	6.00 ± 3.24^{b}	1.31 ± 1.01^{b}
	60	99.90 ± 8.77^{b}	44.60 ± 6.46^{b}	23.00 ± 6.88^{b}	7.00 ± 0.71^{b}	0.74 ± 0.55^{b}

Lowercase letter a–d represents the significance level ($p < 05$). Modified from Yuan (2018)

7.3.5 Environmental Effects on Sesame Seed Quality

7.3.5.1 Disease Effects on Sesame Quality

The infection of fungal pathogens on sesame caused the reduction of growth and development and eventually the yield (Li 2017). As to the sesame seed quality, tens of the characters related to seed appearance, seed nutrition components, and oil quality should be considered (Wei et al. 2018; Yuan et al. 2018). To explore the change patterns of the seed quality caused by disease stress, Li (2017) evaluated dozens of indexes related to seed and oil qualities using three varieties, i.e., 4816, P12-0, and 21 under the *Fusarium* wilt artificial infection conditions. The plants of all the treatments were classified according to the 0–4 rating standard (Qiu et al. 2014). For Grade 4 plants, no mature seeds formed. For seed appearance traits, seed weight, seed length, seed width, the L value, a^* value, and b^* value were measured, and the ΔE^*ab value was calculated (Table 7.12). The results showed that the values of L, a^*, and b^* varied among the three varieties with a change of grades from 0 to 3. The L value of variety P21 decreased by 7.02%. As the *Fusarium* wilt disease grade increased, the values of ΔE^*ab for all three varieties gradually raised, with a range of 0.59–3.01, 2.24–4.29, and 0.52–4.14, respectively. For the treatments with FW Grade 3 or higher, the values of E^*ab significantly differed from those of the controls (Grade 0). However, the values of the seed length and width of the three varieties were relatively stable and were not affected by the disease inoculation.

The contents of oil, proteins, polysaccharide, and ash in the samples were also measured (Table 7.13) (Wei et al. 2018). Compared with those of the controls (Grade 0), the contents of oil, proteins, and polysaccharide of the treatment samples varied insignificantly. However, the disease occurrence significantly affected the polysaccharide content. Especially for Variety 4816, the polysaccharide content raised from the 9.74% of the control (Grade 0) to 11.23% of the treatment (Grade 3), and probably was related to the metabolism response to environmental stresses. Similarly, the ash content, mainly Ca^{2+}, K^+, Na^+, and Mg^{2+}, in all the treatments obviously changed (data not shown).

In addition, the quality of the sesame oil produced from the Grade 3 FW-infected seeds was evaluated (Table 7.14) (Wei et al. 2018). Even though the fatty acid component and contents did not obviously vary, the acid value, peroxide value, saponification value, and refractive index changed evidently. Compared with those of the controls, the acid values of the treatments increased from 36.48 to 68.27%, while the peroxide value reduced by 15.04–51.92%. The increase of the acid value usually reflects the low quality of the sesame oil with a short shelf life. Therefore, healthy seeds are essential for high-quality oil production.

Table 7.12 Variation of seed appearance quality among three sesame varieties with various FW grades

Variety	FW grade	Thousand seed weight (g)	Seed length (mm)	Seed width (mm)	L^* value	a^* value	b^* value	ΔE^*ab value
4816	1	2.24 ± 0.01^{a}	3.30 ± 0.001^{a}	1.66 ± 0.02^{a}	63.27 ± 0.05^{a}	4.17 ± 0.03^{bc}	17.61 ± 0.28^{ab}	0.59
	2	2.07 ± 0.02^{ab}	2.99 ± 0.02^{b}	1.61 ± 0.02^{ab}	61.47 ± 0.14^{b}	4.10 ± 0.06^{bc}	18.43 ± 0.15^{ab}	2.42
	3	1.63 ± 0.03^{b}	3.00 ± 0.02^{ab}	1.58 ± 0.03^{b}	60.71 ± 0.01^{c}	4.01 ± 0.05^{c}	18.19 ± 0.41^{a}	3.10
	0	2.30 ± 0.01^{a}	2.97 ± 0.01^{b}	1.57 ± 0.04^{b}	63.79 ± 0.05^{a}	3.96 ± 0.11^{bcde}	17.80 ± 0.20^{ab}	0
P12-0	1	1.55 ± 0.01^{a}	3.02 ± 0.03^{ab}	1.62 ± 0.02^{ab}	58.78 ± 0.12^{b}	3.84 ± 0.04^{e}	18.20 ± 0.15^{ab}	2.24
	2	1.49 ± 0.02^{a}	3.02 ± 0.02^{ab}	1.62 ± 0.01^{ab}	58.37 ± 0.40^{c}	4.05 ± 0.04^{bcd}	18.23 ± 0.13^{ab}	2.65
	3	1.41 ± 0.01^{b}	3.03 ± 0.04^{ab}	1.62 ± 0.03^{ab}	56.72 ± 0.51^{d}	3.83 ± 0.04^{de}	17.67 ± 0.11^{b}	4.29
	0	1.58 ± 0.00^{a}	3.01 ± 0.03^{ab}	1.61 ± 0.02^{ab}	61.00 ± 0.77^{a}	3.90 ± 00.06^{cde}	17.92 ± 0.15^{ab}	0
P21	1	1.98 ± 0.06^{b}	3.02 ± 0.01^{ab}	1.60 ± 0.04^{ab}	62.80 ± 0.11^{ab}	3.94 ± 0.07^{bcde}	17.93 ± 0.33^{ab}	0.52
	2	1.80 ± 0.05^{b}	3.00 ± 0.02^{ab}	1.60 ± 0.04^{ab}	62.30 ± 0.12^{b}	4.02 ± 0.10^{bcd}	17.68 ± 0.09^{b}	1.00
	3	1.47 ± 0.03^{c}	3.07 ± 0.00^{ab}	1.64 ± 0.02^{ab}	58.89 ± 0.38^{c}	4.23 ± 0.05a	18.57 ± 0.27^{a}	4.14
	0	2.39 ± 0.08^{a}	2.96 ± 0.01^{b}	1.58 ± 0.01^{b}	63.02 ± 0.17^{a}	4.16 ± 0.02ab	18.35 ± 0.14^{a}	0

L^* indicates bright index; a^* and b^* indicate color indexes. Lowercase letter represents the significant difference ($P \leq 0.5$). Modified from Wei et al. (2018)

Table 7.13 Main nutrition in sesame seeds of three sesame varieties with various FW grades (%)

Variety	FW grade	Moisture	Oil content	Protein content	Polysaccharide content	Ash content
4816	1	3.87 ± 0.04^{ab}	50.62 ± 0.30^{a}	20.58 ± 0.08^{bc}	11.23 ± 0.04^{b}	4.70 ± 0.02^{b}
	2	3.93 ± 0.05^{a}	50.83 ± 0.13^{a}	21.51 ± 0.36^{a}	12.56 ± 0.21^{a}	4.76 ± 0.01^{b}
	3	4.02 ± 0.02^{a}	49.93 ± 0.48^{b}	20.86 ± 0.37^{b}	9.23 ± 0.62^{c}	5.28 ± 0.06^{a}
	0	3.60 ± 0.08^{b}	50.05 ± 0.16^{ab}	20.47 ± 0.31^{c}	9.74 ± 0.37^{c}	4.55 ± 0.01^{c}
P12-0	1	3.80 ± 0.05^{b}	47.48 ± 0.27^{ab}	22.32 ± 0.27^{b}	13.13 ± 0.38^{a}	5.09 ± 0.03^{b}
	2	3.86 ± 0.02^{ab}	47.93 ± 0.24^{a}	22.81 ± 0.13^{a}	9.67 ± 0.56^{d}	5.31 ± 0.02^{ab}
	3	4.01 ± 0.03^{a}	47.12 ± 0.28^{b}	22.47 ± 0.49^{ab}	11.60 ± 0.49^{b}	5.58 ± 0.06^{a}
	0	3.77 ± 0.02^{b}	47.73 ± 0.24^{a}	22.05 ± 0.26^{b}	10.91 ± 0.23^{c}	4.98 ± 0.07^{c}
P21	1	4.08 ± 0.09^{a}	48.93 ± 0.21^{a}	20.72 ± 0.32^{a}	9.64 ± 0.63^{c}	5.07 ± 0.05^{b}
	2	3.89 ± 0.10^{b}	48.80 ± 0.27^{ab}	20.39 ± 0.10^{ab}	11.14 ± 0.09^{ab}	5.51 ± 0.01^{ab}
	3	3.90 ± 0.12^{b}	49.26 ± 0.30^{a}	20.96 ± 0.42^{a}	10.26 ± 0.27^{b}	5.71 ± 0.15^{a}
	0	3.91 ± 0.05^{ab}	48.48 ± 0.18^{b}	20.08 ± 0.24^{b}	11.17 ± 0.40^{a}	4.79 ± 0.10^{c}

Lowercase letter represents the significant difference ($P < 0.5$). Modified from Wei et al. (2018)

Table 7.14 Basic physiological and chemical indexes of sesame oil under disease stress

Sample		Acid value (mg/ g)	Peroxide value (mmol/ kg)	Saponification value (mg/ g)	Refractive index (20 °C)
4816	Control (Grade 0)	1.71 ± 0.04	0.67 ± 0.01	184.59 ± 0.12	1.4732 ± 0.00
	Grade 3	5.39 ± 0.20	0.57 ± 0.02	186.18 ± 0.24	1.4699 ± 0.01
P12-0	Control (Grade 0)	3.78 ± 0.05	1.04 ± 0.06	186.68 ± 0.08	1.4729 ± 0.01
	Grade 3	6.80 ± 0.12	0.50 ± 0.02	189.70 ± 0.27	1.4739 ± 0.02
P21	Control (Grade 0)	1.95 ± 0.03	0.83 ± 0.01	182.91 ± 1.26	1.4739 ± 0.00
	Grade 3	3.07 ± 0.12	0.70 ± 0.03	187.67 ± 0.82	1.4741 ± 0.03

Modified from Wei et al. (2018)

7.3.5.2 Waterlogging Effect on Sesame Quality

Studies have documented that waterlogging stress could cause the change of fatty acid components in oilseed crops (Xu et al. 2015). Recently, Yuan et al. (2018) systematically analyzed the variation of the seed appearance quality, seed nutrient components, and oil quality, and determined the effects of waterlogging on the sesame seed quality. Under a 36-h waterlogging treatment, the ΔE^*ab values of the six Chinese domestic varieties (i.e., Yuzhi 11, Zhengzhou 98N09, Ji9014, Yuzhi 28 (determinate type), Xincai Xuankang, and Rongxian black sesame) significantly changed, with a range of 0.72 (var. Yuzhi 11)—5.03 (var. Yuzhi 28) (Yuan et al. 2018).

The three main nutrition components, the contents of oil, proteins, and polysaccharide, varied from 51.99 to 58.61%, 19.08 to 22.05%, and 9.12 to 13.68%, respectively, within the six varieties studied (Table 7.15). The contents of fiber, polysaccharide, and ash varied from 2.83 to 12.43%, 12.21 to 15.06%, and 5.20 to 7.09%, respectively, within the six varieties. Under the 36-h waterlogging treatments, the oil content variation of the six varieties ranged from −3.73 to 2.03%. The protein contents of the varieties reduced ranging from −0.1 to −2.16%. The polysaccharide content varied differently in different genotypes. In addition, waterlogging also affected the ash accumulation. For Yuzhi 11, the increase of ash content was the highest, approaching 11.97%. Further analysis indicated that the above five traits were controlled by genotype and environment ($P \leq 0.01$). The contents of the main minerals, i.e., calcium (Ca^{2+}), potassium (K^{+}), magnesium (Mg^{2+}), and sodium (Na^{+}), significantly increased in most of the varieties. The results were similar with the plant response to drought in sesame (Ozkan and Kulak 2013). The protection and reasonable response system should give rise to sesame with a potential of adaption to the changing climates and environments.

Yuan et al. (2018) also evaluated the quality of the sesame oil crushed from the seeds under the 36-h waterlogging stress. For all the six varieties, the contents of the oleic acid and linoleic acid between the treatments and the controls remained consistently. However, the acid value and peroxide value of the oil significantly

Table 7.15 Variation of the main components of sesame seeds under waterlogging stress

Seed quality	Genotype					
	Yuzhi 11	Zhengzhou 98N09	Ji9014	Yuzhi 28	Xincai Xuankang	Rongxian black sesame
Oil content (OC) (%)						
Waterlogging	58.13 ± 0.14^{a}	$56.90. \pm 0.21^{a}$	48.67 ± 0.14^{b}	57.37 ± 0.11^{a}	57.23 ± 0.34^{a}	49.35 ± 0.30^{b}
Control	56.80 ± 0.38^{a}	57.15 ± 2.25^{a}	52.40 ± 0.29^{a}	58.61 ± 0.35^{a}	55.20 ± 0.20^{b}	51.99 ± 0.78^{a}
OC_t–OC_c	1.33 ± 0.52^{b}	-0.62 ± 0.89^{c}	-3.73 ± 0.14^{f}	-1.24 ± 0.14^{d}	2.03 ± 0.13^{a}	-2.64 ± 0.22^{e}
Protein content (PC) (%)						
Waterlogging	20.95 ± 0.21^{a}	20.65 ± 0.13^{a}	19.89 ± 0.13^{b}	19.89 ± 0.13^{b}	19.29 ± 0.14^{b}	18.98 ± 0.32^{a}
Control	21.43 ± 0.45^{a}	21.91 ± 0.18^{a}	22.05 ± 0.18^{a}	20.14 ± 0.09^{a}	21.02 ± 0.21^{a}	19.08 ± 0.28^{a}
PC_t–PC_c	-0.48 ± 0.66^{a}	-1.26 ± 0.31^{b}	-2.16 ± 0.50^{c}	-0.25 ± 0.21^{a}	-1.73 ± 0.35^{b}	-0.10 ± 0.62^{a}
Fiber content (FC) (%)						
Waterlogging	3.21 ± 0.12^{a}	3.11 ± 0.23^{a}	9.53 ± 0.45^{a}	3.11 ± 0.22^{a}	3.09 ± 0.28^{a}	13.54 ± 0.89^{a}
Control	3.19 ± 0.14^{a}	2.97 ± 0.17^{a}	8.45 ± 0.67^{b}	2.90 ± 0.19^{a}	2.83 ± 0.32^{b}	12.43 ± 0.96^{b}
FC_t–FC_c	0.02 ± 0.15^{c}	0.14 ± 0.56^{bc}	1.08 ± 0.13^{a}	0.21 ± 0.01^{b}	0.26 ± 0.15^{b}	1.11 ± 0.05^{a}
Polysaccharide content (PC) (%)						
Waterlogging	12.21 ± 0.82^{a}	12.40 ± 0.78^{a}	14.92 ± 0.96^{a}	15.06 ± 0.47^{a}	12.75 ± 0.79^{a}	12.96 ± 1.34^{a}
Control	12.90 ± 0.37^{a}	11.42 ± 0.03^{a}	9.12 ± 0.65^{b}	11.75 ± 0.68^{b}	13.68 ± 0.75^{a}	10.41 ± 0.44^{b}
SC_t–SC_c	-0.69 ± 0.59^{e}	0.98 ± 0.51^{d}	5.80 ± 0.82^{a}	3.31 ± 0.61^{b}	-0.93 ± 0.51^{e}	2.55 ± 0.38^{c}
Ash content (AC) (%)						
Waterlogging	6.08 ± 0.42^{a}	6.18 ± 0.07^{a}	6.58 ± 0.11^{a}	6.33 ± 0.78^{a}	7.18 ± 0.42^{a}	5.61 ± 0.28^{a}
Control	5.43 ± 0.13^{b}	5.65 ± 0.12^{b}	6.13 ± 0.11^{b}	6.09 ± 0.12^{b}	7.09 ± 0.64^{a}	5.20 ± 0.64^{b}
AC_t–AC_c	0.65 ± 0.85^{a}	0.53 ± 0.19^{a}	0.45 ± 0.42^{b}	0.24 ± 0.11^{c}	0.09 ± 0.11^{d}	0.41 ± 0.35^{b}

Lowercase letter indicates the significance level (F-test) at 0.05 level. Modified from Yuan et al. (2018)

varied in most of the tested varieties. For example, the acid value of var. Yuzhi 28 after the treatment increased from 2.03 mg KOH per g oil to 3.51 mg KOH per g oil, having exceeded the national standard of 3 mg KOH/g of the first grade sesame oil. In Ji9014 susceptible to waterlogging, the peroxide value increased from 0.509 to 1.343 mmol/kg. Furthermore, waterlogging stress led to the variation of sesamin and sesamolin contents in some of the varieties. Especially in var. Xincai Xuankang, the sesamin and sesamolin contents increased to 34.01%. However, the sesamin + sesamolin content of Zhengzhi 98N09 (tolerant to waterlogging) fluctuated insignificantly.

7.4 Sesame Breeding Techniques and New Germplasm Creation

7.4.1 Sesame Germplasm Creation Techniques

S. indicum is the sole cultivated species in the *Sesamum* genus. Pedigree selection and mutation induction are the two most widely used breeding techniques in sesame (Ashri 2001). To obtain sufficient genetic variations and breeding materials, sesame scientists began physical or chemical mutagenesis studies in 1950s (Shi 1991). In particular, the coordinated research project (CRP) organized by the Plant Breeding and Genetics Section of the Joint FAO/IAEA Division has impelled the sesame induction and improvement since 1993. Approximately, 140 useful sesame mutants related to plant architecture, leaf, flower, capsule, maturation, male sterility, disease resistance, and other agronomic traits were created (Ashri 1998, 2001; Van Zanten 2001). In recent years, more mutants with excellent phenotypes were reported (Sorour et al. 1999; Ramadoss et al. 2014; Wang et al. 2017b). Since the 1980s, the method that combined space mutagenesis, chemical mutagenesis, physical mutagenesis, and ion implantation followed by hybridization has been introduced to sesame breeding. Here, we introduce the main mutagenesis approaches and the characters of some important mutants induced with them.

7.4.1.1 Physical Mutagenesis

In 1979, Ashri, A. created a well-known sesame determinate mutant using the γ-ray method and demonstrated the high affect of physical mutagenesis on sesame breeding (Shi 1991; Melzer et al. 2008; Uzun and Çağırgan 2009). Further analysis proved that the γ-ray method caused loss of a large DNA fragment including the *SiDt* gene, and thus, formed the determinate mutant (*dt2* type) (Zhang et al. 2016). Physical mutation methods include electric field, magnetic field, magnetized water, laser, microwave, ultraviolet, plasma, high pressure, and other physical approaches (Yu et al. 1996; Melzer et al. 2008; Ashri 1998, 2001). For sesame mutation, the

common physical mutagens are X-rays, gamma rays, and fast neutrons (Ashri 1998; Sorour 1999; Muhammad et al. 2013). Murty and Bhatia (1990) isolated a mutant with free corolla lobes (N29), designated as polypetalous corolla mutant, from the M_2 generation of CV.N.62-32 after irradiation of 1.6KR fast neutrons.

Radiation sensitivity studies showed that sesame seeds were highly resistant to the relative high doses of gamma radiation. More than 80% of the seeds treated could germinate after 1500 Gy radiation. Ramadoss et al. (2014) studied the mutation efficiency of gamma rays using two varieties, TTVS 51 and TTVS 19, and obtained a large number of mutants involving branching habit, plant height, phyllotaxy, flower morphology, internode length, and other botanic traits under the doses of 250 and 350 Gy. For fast neutron irradiation (FNI) mutation, 12 and 16 μ Sv were proved to be the most efficient doses to induce viable mutations in sesame (Muhammad et al. 2013).

7.4.1.2 Chemical Mutation Techniques

Chemical mutagens used for plant mutagenesis include ethyl methanesulphonate (EMS), sodium azide, diethyl sulfate, and colchicines (Kodym and Afza 2003). EMS mutagenesis is the most widely used to induce plant mutagenesis. Furthermore, compared with other genetic modification methods, including interspecific hybridization, the EMS mutation technique is simple and easy to perform. So far, a great number of new germplasm lines with high yield, quality, early maturing, high resistance (tolerance) to environments and other new agronomic traits have been achieved through EMS mutagenesis in rice, corn, soybean, oilseed rape, other crops, and vegetables (Till et al. 2004; Chen and Wang 2005; Li et al. 2012a, b; Liu 2014; Tsuda et al. 2015).

The EMS mutagenesis technique was applied in the induction of sesame mutants in the late twentieth century (Shi 1991). Studies showed that 0.5% EMS was the optimal concentration for sesame mutagenesis with seeds (Begum and Dasgupta 2010; Kumar et al. 2012; Wang et al. 2017b). Wang et al. (2017b) analyzed the effects of EMS concentration and the treatment time on sesame mutagenesis and found that the treatments of 0.1%/24 h, 0.5%/24 h, 1.0%/12 h, and 1.5%/6 h were appropriate for sesame mutagenesis. Under the optimal induction conditions of 1.0% EMS and 12 h, the mutation frequency of sesame line 91-0 was 0.80% or higher (Wang et al. 2017b). As shown in Table 7.16, a total of 22,855 (32.65%) of the treated seeds grew into plants after EMS treatment. To determine the characters mutated with EMS, 24 agronomic traits related to five groups of traits, i.e., "leaf type," "plant type," "flower and fertility," "capsule and seed characters," and "other physical traits," were investigated. Results indicated that the mutation frequencies at the M_1 and M_2 generations were 7.60 and 2.78%, respectively. For the M_1 and M_2 populations, "flower and fertility" was the top mutated trait type, with a high mutation ratio of 4.08 and 0.93%, respectively. Of the 831 M_2 and M_3 mutants randomly selected from the sesame EMS mutant library, 191 (23.71%) stably descended the mutated traits to their progenies. The top mutated trait of this

Table 7.16 Types and efficiencies of sesame mutants induced by EMS

Year	Mutant generation	Number of seeds treated	Number of plant lines obtained	number of mutant lines	Mutation frequency (%)	Mutation type (ratio, %)				
						Leaf type	Plant type	Flower and fertility	Capsule and seed characters	Other physical characters[a]
2008–2010	M_1	70,000	22,855	1736	7.60	117 (0.51)	89 (0.39)	932 (4.08)	363 (1.59)	235 (1.03)
2009–2012	M_2	–	7978	222	2.78	69 (0.86)	55 (0.69)	74 (0.93)	23 (0.29)	1 (0.01)
2012	M_2–M_3	–	831[b]	197	23.71	36 (4.33)	12 (1.44)	14 (1.68)	123 (14.80)	12 (1.44)

[a]Other physical traits include early and late development, and irregular and uncoordinated metabolism syndromes

[b]The number is referred to the mutant lines randomly chosen from the M_1 and M_2 generations. Modified from Wang et al. (2017b)

mutation type was "capsule and seed characters," with a mutation frequency of 14.80%.

Recent studies aimed to the comparison and combination of various physical and chemical mutation approaches (Boranayaka et al. 2010; Begum and Dasgupta 2010; Anbarasan et al. 2015; Ravichandran and Jayakumar 2015). Begum and Dasgupta (2010) compared the induction efficiency between γ-rays (200, 400, and 600 Gy) and EMS (0.5, 1.0, 1.5, and 2.0%) based on the M_3 generation mutants of three sesame genotypes (i.e., Rama, SI 1666, and IC 21706). The results indicated that the average mutation efficiency of EMS was much higher than that of the γ-ray treatment. 200 Gy γ-rays and 0.5% EMS were shown to induce the largest change for branch number per plant, with a change percentage of 244.16% and for capsule number per plant, with a change percentage of 207.78%, respectively. A combined analysis of different parameters suggested that 0.5% EMS was the best mutagenic treatment (Begum and Dasgupta 2010). Boranayaka et al. (2010) studied the influence of both gamma rays and EMS on sesame germination and seedling survival. Under the treatment of gamma rays of ^{60}Co source with doses of 10, 20, 30, 40, and 50 krad, followed by EMS induction with concentrations of 0.8, 1.0, 1.2, 1.4, and 1.6%, seed germination reduced and shoot and root growth gradually decreased.

7.4.1.3 Sesame Mutant Library and Key Mutants

A great number of sesame mutants, including determinate flowering habit, short internode, branch density, short stalk, small capsule, small seed size, short flowering period, resistance to diseases and waterlogging, male sterility, and other agronomic traits, have been so far obtained through the EMS mutagenesis (Murty 1988; Kang et al. 1995; Wang et al. 2017b). For example, Murty and Oropeza (1989) identified a narrow leaf mutant with only vestiges of leaf around the veins from the EMS-induced M4 progeny of var. "Ven-52". Murty (1988) reported the inheritance of three new mutant phenotypes induced by EMS, i.e., fascinated cotyledons, tall seedling, and dotted corolla tubes. Rajput et al. (1994) reported the creation of mutants with early flowering date, short plant height, branching type, and high yield traits using gamma rays. Cagirgan (2001) reported the characters of the important mutants with indehiscent capsule, determinate growth habit, wilt tolerance, chlorophyll deficiency, capsule type, sterility, flowering time, and other agronomic traits under the irradiation of 150–750 Gy gamma rays. Furthermore, a sesame mutant library containing over 60,000 lines has been recently constructed by EMS mutagenesis in China. More than 1000 mutants have been identified that showed significant mutation and inheritance for dozens of agronomic and botanic traits (unpublished data, H. Zhang). In particular, two mutants, i.e., the determinate mutant, DS899 (*dt1* type), and the short internode mutant, Dw607, have been already used for sesame production and also exhibit the potential used for mechanization harvest in sesame.

Differing from the indeterminate sesame varieties and the determinate mutant (*dt1* type) induced by an Israeli scientist, Ashri, A., using γ-rays (5000 Gray) (Ashri 1998), the determinate mutant (*dt2* type), named DS899, was obtained from a sibling line of Yuzhi 11 (used for the Sesame Genome Project) by EMS mutagenesis in 2009 (China Patent No. ZL2015108760163). This DS899 mutant line has been used to develop the first determinate variety, Yuzhi DS899, that is worthy for production application using the pedigree selection in 2015. Since their shoot apical meristems were converted from continuous differentiation to the terminal meristems, both determinate mutants, *dt1* and *dt2*, can form a terminal flower cluster and consequently form a capsule cluster (Melzer et al. 2008; Zhang et al. 2016). Interestingly, the *dt1* mutant developed 8–20 capsule nodes and the capsule node number was affected by altitude and sowing time. However, the *dt2* mutant only produced 3–5 capsule nodes per stem (Fig. 7.7). All their morphological differences were shown to be controlled by the *SiDt* gene alleles (Zhang et al. 2016). Compared with the indeterminate varieties, such as Yuzhi DS899, the determinate varieties derived from the DS899 mutant demonstrated population consistency in plant growth and capsule mature, thus increasing the potential of their adaption for mechanization harvest accordingly.

Plant height and internode length are important for sesame production. A dwarf mutant with a short internode length, named Dw607, was also induced from var. Yuzhi 11 by EMS mutagenesis in 2009 (Fig. 7.8). The internode lengths of the mutant plants decreased from 6.0–8.4 to 3.5–4.0 cm, the plant height of the mutant plants reduced from 170 to 110 cm (unpublished data, H. Zhang). In addition, the height of the first capsule of the mutant was also lower. Therefore, its capability of high-density cultivation has been greatly increased. As a result, a dwarf sesame variety, Yuzhi Dw607, was developed from the Dw607 progeny using the pedigree selection in 2015.

7.4.2 Sesame Tissue Culture and Gene Transformation Techniques

Plant tissue culture and gene transformation are powerful bio-techniques for plant genetic improvement. In the past decades, many researchers performed the technical optimization of tissue culture and plantlet regeneration in sesame. Moreover, both *Agrobacterium*-mediated transformation and particle bombarding methods via adventitious shoot formation have been explored (Chen et al. 1996; Yadav et al. 2010; Chowdhury et al. 2014).

7.4.2.1 Sesame Tissue Culture and Regeneration

Sesame is one of the most recalcitrant crops on *in vitro* tissue culture and plantlet regeneration (Were et al. 2006; Yadav et al. 2010; Raja and Jayabalan 2011). Early

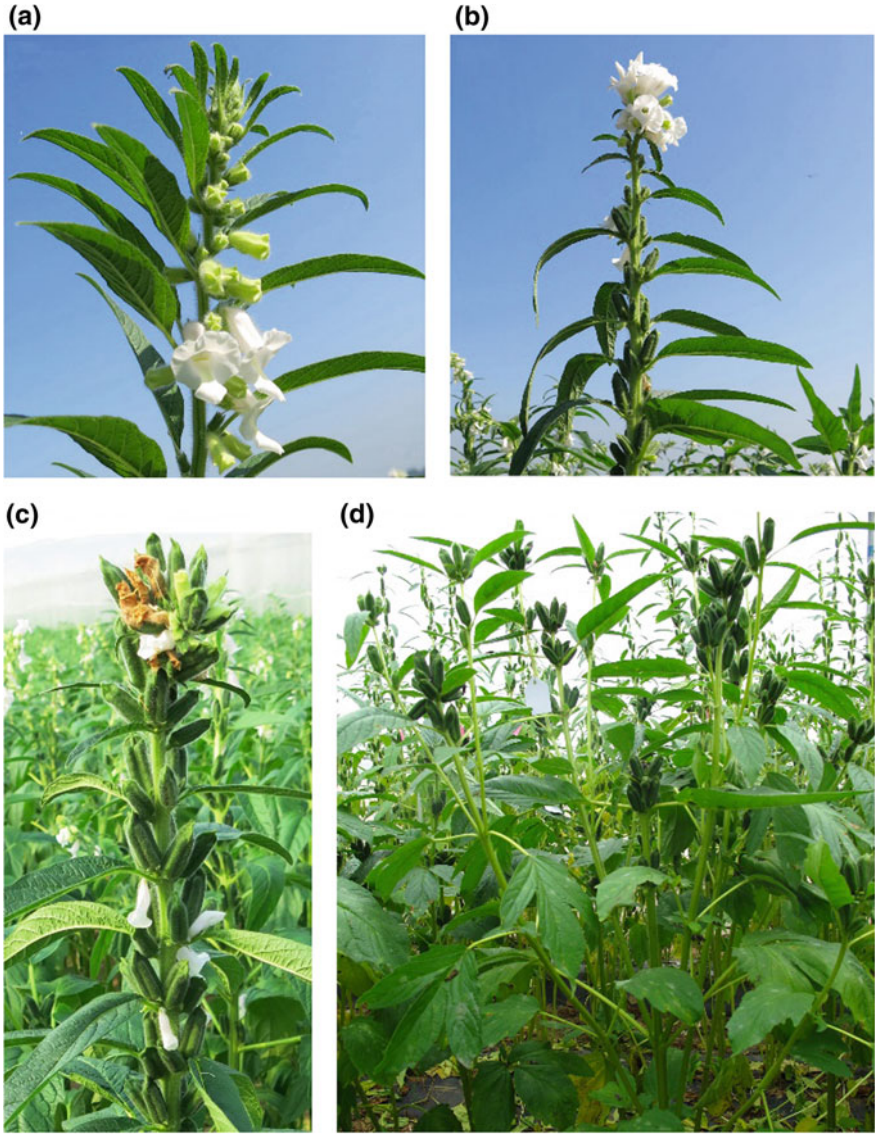

Fig. 7.7 Inflorescence meristem types of sesame. **a** Image of indeterminate inflorescence meristem of non-mutated varieties (var. Yuzhi 11). **b** Image of the determinate mutant DS 899 (*dt2* type). **c** The terminal capsule cluster of inflorescence meristem apex of the determinate mutant, DS 899. **d** Image of the determinate line, 08TP092 (*dt1* type). The line was derived from the determinate mutant induced by γ-rays (Ashri 1998). Photographs supplied by H. Miao

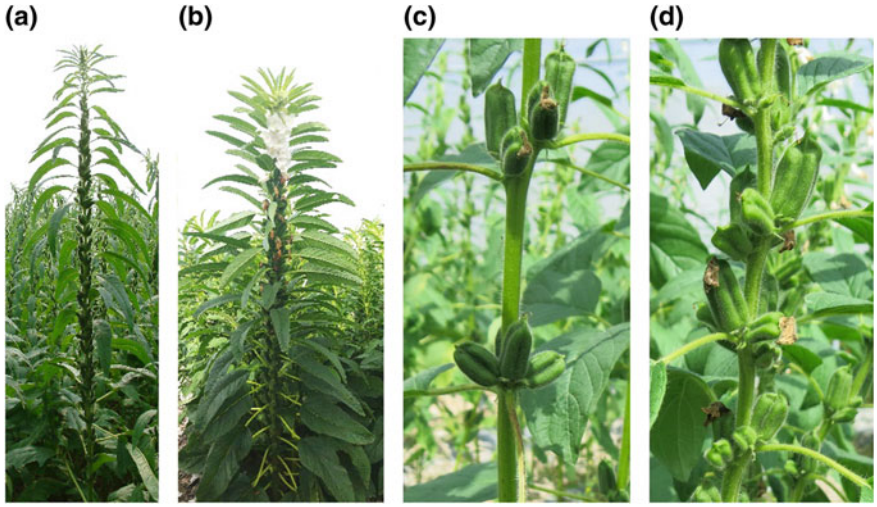

Fig. 7.8 Comparison of the dwarf mutant with short internode length with the non-mutated variety. **a** Plant image of the sesame variety, Yuzhi 11 with a normal internode length and plant height. **b** Plant image of the dwarf variety, Dw607. **c** Comparison of the internode length between Yuzhi 11 (left) and *Dw607* (right). Photographs supplied by H. Zhang

studies of sesame tissue culture were focused on the direct induction of adventitious shoot multiplication from various explants (George et al. 1987; Chen et al. 1994; Zhi et al. 1998; Saravanan and Nadarajan 2005; Seo et al. 2007; Chattopadhyaya et al. 2010; An 2009; Wei et al. 2011a, b). Several factors, including explant type and genotype, the pretreat of explants, cultivation condition, basic medium, and growth hormones and their concentration, were taken as the effects on multiple shoot formation and plantlet regeneration (Venkatachalam et al. 1999; An 2009).

In recent years, the techniques of callus induction and differentiation, followed by plantlet regeneration, from various explants have been established in sesame (Shi and Cai 1986, 1989; Yi et al. 1997; Were et al. 2006; Miao et al. 2012). Even though the frequency of the callus differentiation varied from 105 to 63%, the low reproducibility of the successful examples is still a limit for transgene application in sesame (Mary and Jayabalan 1997; Were et al. 2006; Chakraborti and Ghosh 2009).

Systematical studies indicated that the main factors of genotype, explant type, medium, and/or hormone concentration and combination affected sesame callus induction, differentiation, and plantlet regeneration (George et al. 1987; Yi et al. 1997; Were et al. 2006; Singh et al. 2006; Baskaran and Jayabalan 2006; Ahmed 2008; Chakraborti and Ghosh 2009; Raja and Jayabalan 2011; Miao et al. 2012). Miao et al. (2012) analyzed the effects of genotype, explant type, and hormone type and concentration on callus induction and plantlet regeneration and established a callus induction, differentiation, and plantlet regeneration technical system for *Sesamum* tissue culture (Fig. 7.9). In the wild species of *S. radiatum* ($2n = 64$) and *S. schinzianum* ($2n = 64$), the ratio of callus induction and differentiation

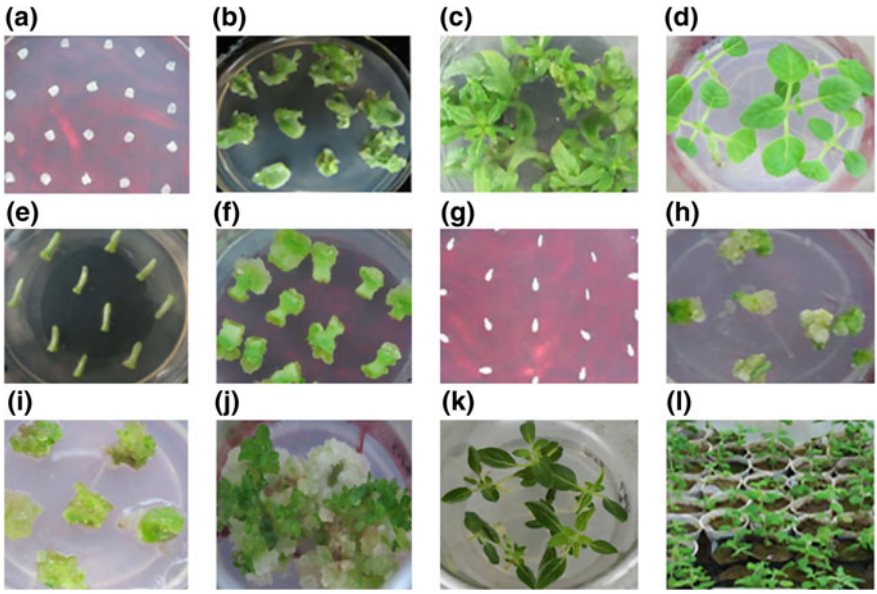

Fig. 7.9 Sesame tissue culture and regeneration system of sesame. **a** Cotyledon explants cultured on multiple shoot induction medium. **b** Expanding cotyledons. **c** Adventitious shoot regeneration. **d** Regenerated shoots. **e** Hypocotyl explants cultured on callus induction medium. **f** Expanding hypocotyl explants. **g** Immature embryo explants cultured on callus induction medium; **h** Calli induced from immature embryos. **i** Differentiation of calli. **j** Axillary shoot; **k** Regenerated seedling. **l** Plantlets cultured in paper cup for transplanting. Modified from Wei et al. (2011a, b) and Miao et al. (2012)

approached to 97.50 and 94.02%, respectively. However, the callus induction and differentiation ratio of the cultivated species were only 40.60 and 8.16%, respectively. For the immature embryo explants of the *S. schinzianum* × *S. indicum* hybrids, the frequency of callus induction (46.67%) and differentiation (89.29%) was much higher than that of the cultivated sesame (Miao et al. 2012). The results suggested that the genotype was the key factor to limit the callus induction and plantlet regeneration in sesame (Mary and Jayabalan 1997; Were et al. 2006; Chakraborti and Ghosh 2009). Increasing the narrow genetic basis of cultivated sesame is still an important objective for sesame breeding.

7.4.2.2 Genetic Modification and Transformation

Compared with other crops such as wheat, rice, cotton, and oilseed rape, the development of genetic transformation techniques in sesame is lagging for the arduous development of its tissue culture system. Fortunately, the recent success of tissue induction and plantlet regeneration techniques provides technical supports for gene transformation in sesame (Wei et al. 2011a, b; Yadav et al. 2010).

To develop an optimal gene transformation system in sesame, Chen et al. (1996), for the first time, transformed sesame using the particle bombardment with an aid of direct multiple shoot induction. Bhattacharyya et al. (2015) also reported a particle bombardment transformation technique with 5-day-old apical tissues in sesame. PCR (polymerase chain reaction), RT-PCR (real time PCR), western blot, and enzymatic assay showed that four transformants were integrated with the exogenous *bar* (bialaphos resistance) gene into a single site of the sesame genome. Yadav et al. (2010), for the first time, reported the establishment of the *Agrobacterium*-mediated sesame transformation via adventitious shoot induction. The transformation frequency reached 1.01%. Subsequently, Chowdhury et al. (2014) reported a high-frequency *Agrobacterium*-mediated transformation technique for sesame. The transformation was performed using the de-embryonated cotyledons at shoot development stage. The transgenic adventitious shoots formed from somatic embryos. The transgenic lines were selected on the medium containing 50 mg L^{-1} kanamycin and 500 mg L^{-1} cefotaxime. Under these transformation and selection conditions, the sesame plantlet regeneration frequency and transformation efficiency reached 52.00 and 42.66%, respectively. Both GUS (β-glucuronidase gene) histochemical activity and RT-PCR assays demonstrated that the obtained transgenic lines were positive. The assayed 10 T0 lines each had a single copy of the exogenous *nptII* (neomycin phosphotransferase II) gene.

To our knowledge, the two successful examples in application of gene transformation for sesame genetic improvement have been reported to date by Mitsuma et al. (2004) and Chowdhury et al. (2017), respectively. Mitsuma et al. (2004) transformed a carrot calmodulin gene (*cam-4*) into the sesame wild species, *S. schinzianum* ($2n = 64$), through the *A. tumefaciens* infection on the stems of seedlings. Both southern hybridization and RT-PCR showed that the *cam-4* gene was successfully transformed and expressed in the transformed plants. Chowdhury et al. (2017) reported that the sesame *OLP* (osmotin-like protein) gene (*SindOLP*) was transformed and integrated into the cultivated sesame, cv. VR1-1, using the above-optimized transformation approach described by Chowdhury et al. (2014). A total of 13 T0 lines, with each line having one copy of *SindOLP*, were verified by the kanamycin and RT-PCR screening. Chinese scientists from the Henan Sesame Research Center, Henan Academy of Agricultural Sciences (HSRC, HAAS), China, recently have also established and optimized the *Agrobacterium*-mediated transformation in sesame via the developed callus induction and differentiation techniques (Miao et al. 2012) and the adventitious shoot induction technique (Wei et al. 2011a, b), respectively (data not shown, H. Zhang). These developed genetic transformation techniques will allow exploring and applying unknown genes of agronomic importance for sesame genetic improvement.

7.4.3 Interspecific Hybridization

To improve the biotic and abiotic resistance of the cultivated sesame, sesame scientists have paid extensive attention to interspecific hybridization since 1950s

(Joshi 1961). However, the reproductive isolation and incompatibility between the cultivated sesame and most of the *Sesamum* wild species have limited the application of interspecific hybridization in sesame breeding (Joshi 1961; Subramanian 2003; Nimmakayala et al. 2011; Zhang et al. 2013d; Yang et al. 2017a). Till now, the crosses only between *S. malabaricum* and *S. indicum* could yield fertile F_1 progenies under the natural conditions (Nimmakayala et al. 2011). Nevertheless, hybrids were also achieved from the crosses between *S. indicum* and other *Sesamum* wild species, such as *S. schinzianum*, *S. radiatum*, and *S. indicatum* with the aid of tissue culture and young hybrid embryo rescue techniques (Nimmakayala et al. 2011; Miao et al. 2012; Zhang et al. 2013d; Yang et al. 2017a). Specific SSR markers have been applied to assess the interspecific hybrids obtained (Chinese Patent No. ZL.201210318496.8). Therefore, the applications of interspecific hybridization and elite interspecific hybrids will greatly enhance sesame breeding.

7.5 Sesame Genome Sequencing and Genomic Analysis

7.5.1 The Sesame Genome Project

7.5.1.1 Sesame Genome Project Initiation

Since sesame is a sole cultivated species in the *Sesamum* genus and also a self-pollinated crop, the genetic base of sesame is relatively narrow (Yue et al. 2012; Zhang et al. 2012c; Wu et al. 2014a; Uncu et al. 2015). Therefore, exploring and incorporating the elite genetic variations related to the key quantitative agronomic traits into elite cultivars seems more arduous through the conventional phenotypic breeding method. Sesame is an orphan species, having an ancient position in the phylogeny of angiosperms (Zhang et al. 2013b; Wang et al. 2014). Hence, it is requisite and urgent to initiate a genome project for sesame to enhance the sesame breeding.

By 2009, only 3328 ESTs of sesame had been deposited at NCBI. In recent years, the high-throughput next-generation sequencing technology (NGS) has been applied to explore DNA molecular markers and QTLs (genes) associated with the key agronomic traits in sesame and other crops (Ganal et al. 2011; Wei et al. 2011a, b; Sim et al. 2012; Zhang et al. 2012d, 2016; Zhao et al. 2015; Wang et al. 2015). To enhance the sesame genomics and genetics research and breeding, a blueprint for the Sesame Genome Project (SGP) was conceived and developed by the Sesame Genome Working Group (SGWG) in 2009 (Zhang et al. 2013b). Then, the SGP was initiated in 2010. The SGP includes three objectives: I. Sesame genome sequencing and its framework map and fine map construction; II. Sesame genome bioinformatics analysis; and III. Sesame functional genomics research and database construction. All these tasks have been basically completed by 2015 (Miao and Zhang 2016).

7.5.1.2 Genome Sequencing and Assembly

In order to realize the high-quality assembly of the sesame genome, the Sesame Genome Working Group (SGWG) developed the complicated "ABI3730xL+ Roche/454+ Illumina/ Solexa + PacBio SMRT" sequencing platform and the "SNP genetic map + BAC-FISH physical map + Optical map + Hi-C library" assisted assembly strategies. Therefore, the fine genome map for sesame (var. Yuzhi11) has been assembled at a chromosomal level. The assembled genome presently is 335.19 Mb, spanning 94.7% of the 354-Mb estimated genome of sesame (Zhang et al. 2013b). To differentiate this final fine genome assembly from the previously published sesame genomes, such as Version 1 (Zhang et al. 2013b, www.sesamum.org), and Version 2 (Zhang et al. 2016), the fine genome of *S. indicum* (var. Yuzhi 11) is herein defined as Sesame Genome Version 3 and will be uploaded to NCBI soon.

With the aid of the super-dense SNP genetic map (Zhang et al. 2016), BAC-FISH cytogenetic map, and Hi-C (High-through chromosome conformation capture) library, the sesame fine genome (unpublished data) has been assembled (Table 7.17). It is 335.19 Mb long, with the largest scaffold of 29.5 Mb. This genome assembly corresponds to 94.7% of the sesame estimated genome size (354 Mb). The numbers of N50 (the size of the contig or scaffold above which the collective size of the contigs or scaffolds was larger or equal to 50% of the total length of the sequence assembly) or larger contigs and scaffolds are 63 and 7, respectively. The lengths of the N50 contig and scaffold are 1.32 and 23.50 Mb, respectively.

Table 7.17 Statistics of the genome assembly of *S. indicum* (var. Yuzhi 11)

Description	S. indicum
Large scaffolds (>1000 bp)	
Length of the largest scaffold	29,511,934
Number of large scaffolds	2205
Bases in the large scaffolds	335,188,638
N50 or larger scaffolds	7
N50 length	23,500,156
All scaffolds	
Number of scaffolds	2210
Total bases	335,184,649
Large contigs (>1000 bps)	
Length of the largest contig	6,723,258
Number of large contigs	3927
N50 or larger contigs	63
N50 length	1,320,381
All contigs	
Number of contigs	3927
Length of the largest contig	6,723,258

Of the 335,184,649 bp sequences, approximately 303.63 Mb (90.58%) was assembled onto the 13 chromosomes (unpublished data, H. Zhang) and 31.56 Mb (9.41%) was present in unanchored scaffolds. The assembly results proved that the sequencing strategy described above was necessary to assemble a fine sesame genome map with high completeness and accuracy (Miao 2014; Miao and Zhang 2016).

7.5.1.3 Other Data of *S. Indicum* Genome

Aside from the Sesame Genome Project and the fine genome assembly, in addition, Wang et al. (2014) published a draft sesame genome (var. Zhongzhi No. 13) assembled at scaffold level using the Illumina sequencing platform. The draft genome size was 274 Mb (accession no.: SRA122008). Uncu et al. (2015) sequenced the genome of "MMuganli 57" using the Roche 454 GS-FLX. The assembly size was about 65 Mb, with a genome coverage of 19.3%. All the genome data are currently being applied for genomic and genetic analysis, genetic map construction, and MAS breeding in sesame.

7.5.1.4 Gene Function Analysis

In the sesame, fine genome Version 3 has been annotated into a total of 31,462 genes. The average gene length was 2620 bp and the gene sequences span 24.6% of the sesame genome (unpublished data, H. Zhang). Gene ontology (GO) analysis (Gene Ontology Consortium 2004) (http://www.geneontology.org/) categorized the 31,462 sesame genes into all three primary categories, i.e., "Molecular function," "Biological process," and "Cellular component." Of the sesame genes, 28,415 (41.53%) were categorized into the "Biological process" category, of which 21.9% participate in "metabolic process," followed by "cellular process" (21.5%); 31.8% were categorized into "Molecular function" that were further classified into 15 subcategories at Level 2, with the largest subcategory being "binding" (15.32%); and the "Biological process" category contains 26.7% genes, of which the "cell" subcategory being ranked the first with 5.2% genes.

7.5.2 QTLs of Key Traits Mapped in Sesame

7.5.2.1 Molecular Marker Development in Sesame

Since the late last century, DNA molecular markers, such as randomly amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), inter-simple sequence repeats (ISSR), amplified fragment length polymorphism (AFLP), and sequence-related amplified polymorphisms (SRAP), have been applied for genetic diversity analysis in sesame (Bhat et al. 1999; Kim et al. 2002;

Ercan et al. 2004; Laurentin and Petr 2006). Subsequently, species-specific markers, such as simple sequence repeats (SSRs) and expressed sequence tag-SSR (EST-SSR), have been developed and applied in sesame genetic analysis for their high abundance, high polymorphism, and high reproducibility in the sesame genome (Powell et al. 1996; Gupta and Varshney 2000; Dixit et al. 2005). In the genome of var. Zhongzhi No. 13, 110,495 genomic SSRs were identified. Of them, 39.1% were mono-nucleotides, followed by 34.3% di-nucleotides (Wang et al. 2017a). Uncu et al. (2015) identified 19,816 SSRs from the 65-Mb genome sequence of "MMuganli 57". Recently, Dossa et al. (2017) developed thousands of polymorphic SSRs and constructed an online database for the SSRs, SisatBase (http://www.sesame-bioinfo.org/SisatBase/). In Version 3 of the var. Yuzhi 11 genome, 153,488 perfect and compound SSRs were identified. The total length of the SSR loci spans 0.96% of the sesame genome (unpublished data, H. Zhang).

Furthermore, the recent accomplishment of the sesame reference genome has dramatically promoted the development of SNP and insertion/deletion (InDel) markers in sesame (Wei et al. 2014; Wu et al. 2014a; Zhang et al. 2016). Wei et al. (2014) combined three RNA-Seq datasets and constructed a reference transcriptome for sesame by *de-novo* assembly. Approximately, 10,950 SNPs were identified in 4660 contigs and 590 InDels were identified in 524 contigs between "Rongxian black sesame" (RXBS) and "Zhongzhi 11". The SNP primer pairs were designed for the SNPs and applied for PCR to screen sesame accessions. Of the 40 SNP primer pairs analyzed, 24 (60.0%) were polymorphic in three accessions. In addition, of 20 InDel primer pairs (InDel lengths of >3 bp), 17 showed evident polymorphisms among the accessions (Wei et al. 2014). Sesame genome re-sequencing indicated that millions of SNPs exist among cultivated sesame varieties (Zhang et al. 2016). Therefore, SNP markers can be useful for exploring sesame genetic diversity and genome evolution.

7.5.2.2 Molecular Linkage Map Construction in Sesame

A linkage map is a prerequisite to identify and characterize the genes controlling the traits important to agriculture at a genome-wide level (Sim et al. 2012; Verma et al. 2015). The first linkage map for sesame was constructed using 96 individuals of an F_2 population derived from "COI1134" and "RXBS" in 2009 (Wei et al. 2009). The linkage map contained a total of 220 DNA markers, including 8 EST-SSR markers, 25 AFLP markers, and 187 RSAMPL (random selective amplification of microsatellite polymorphic loci) markers. The 220 markers were grouped into 30 linkage groups (LGs) and spanned a total of 936.72 cM (Wei et al. 2009). In 2012, this map was improved using 260 plants of an F_2 population derived from the same cross (Zhang et al. 2013c). Moreover, four QTLs for sesame seed coat color were mapped for the first time (Zhang et al. 2013c). Subsequently, two SSR genetic maps were constructed using a recombinant inbred line (RIL) population (Zhang et al. 2013e; Wang et al. 2017a). Both maps were used to map the QTLs controlling waterlogging tolerance and charcoal rot disease resistance in sesame.

The recent developments of the reduced representation genome sequencing (RRGS) and NGS techniques have stimulated construction of high-density SNP genetic maps for sesame. Six dense SNP genetic maps have been so far constructed for sesame (Zhang et al. 2013e, 2016; Wu et al. 2014b; Wang et al. 2016; Uncu et al. 2016; Mei et al. 2017). Most of these six genetic maps have been applied for QTL mapping, while only the ultra-density SNP genetic map that has more than 30,000 SNP markers has been used for the sesame genome assembly (Zhang et al. 2016).

To construct the ultra-dense SNP genetic map, the genomes of both parents, "Yuzhi DS899" and "JS012", and 120 progeny of their derived F_2 population were re-sequenced using the whole genome sequencing approach (Zhang et al. 2016). The draft genome assembly of var. Yuzhi 11 (PRJNA315784, Version 2) was used as the genome reference for SNP discovery. The sequence depths of the two parents were 18.03× and 9.79×, respectively. The genome coverage of each F_2 progeny was 10.00×. A total of 192,744 SNPs/InDels were identified between "Yuzhi DS899" and the reference genome, Yuzhi 11, while 781,528 SNPs/InDels were identified between "JS012" and the reference genome, Yuzhi 11. As a result, the ultra-dense SNP genetic map was constructed for the population. The map was comprised of 3041 bins including 30,193 SNPs on 13 linkage groups (LGs), spanning a total length of 2981.28 cM. The average marker density of the map was approximately one SNP per 0.10 cM or one bin per 0.98 cM. Considering the LD (linkage disequilibrium) decay distance (150 kb) of the population and the genome size (369 Mb by flow cytometry or 354 Mb by k-mer) of sesame (Zhang et al. 2013b), the ultra-dense SNP linkage map was highly saturated.

7.5.2.3 QTL Mapping of Key Agronomic Traits in Sesame

To explore the inheritance of the sesame key agronomic traits, most of the developed molecular genetic maps have been used for identifying and mapping QTLs controlling seed yield and yield component traits, seed quality and related traits, and other important botanic traits in sesame. To our knowledge, 96 QTLs controlling 27 traits have been thus far identified and mapped, with each explaining more than 5% of the phenotypic variance of the targeted trait (Table 7.18).

Moreover, the ultra-dense SNP genetic map has been also used to clone the *SiDt* gene controlling the sesame determinate growth habit for the first time (Zhang et al. 2016). Based on the phenotype and SNP marker segregation, a locus linked to the determinate growth habit was located in the 16.7–22.2 cM interval of LG8 using WinQTLcart (R^2 = 70.2%). Meanwhile, QTLNetwork also identified a locus for the trait in the 18.0–19.2 cM interval of LG8 ($P \leq$ 1e-06). The identification of the genetic interval and physical distance containing the *QDt1* has greatly facilitated the *SiDt* gene cloning. The *dt2* line had the same recessive gene locus for the determinacy trait as the *dt1* mutant line. By screening the population and the *Sidt1* and *Sidt2* allelic test, the *SiDt* gene that encodes a terminal flower (TFL)-like protein (GenBank accession no. KU240042) was finally identified (Zhang et al. 2016).

Table 7.18 Information of QTL (gene) mapping of agronomic traits in sesame

Trait type	Trait	QTL locus	LG position	Marker interval (QTL region, cM)	Explanation ($\geq 5\%$) or *P*-value	References
Yield-related traits	Thousand seed weight	Qtgw-11	LG11	SBN1798-SBN1765 (17.9–21.2)	7.7–12.3	Wu et al. (2014b)
	Grain number per capsule	Qgn-6	LG6	SBN1261-SBI043 (74.4–99.0)	8.0–18.3	Wu et al. (2014b)
	Plant height	qPH-3.1	SLG3	SLG3_bin98-SLG3_bin99 (80.91)	6	Wang et al. (2016a)
		qPH-3.2	SLG3	SLG3_bin114-SLG3_bin115 (92.41)_	15	
		qPH-3.3	SLG3	SLG3_bin126-SLG3_bin127 (98.01)	18	
		qPH-4.1	SLG4	SLG4_bin49-SLG4_bin50 (34.11)	8	
		qPH-4.2	SLG4	SLG4_bin56-SLG4_bin57 (42.61)	6	
		qPH-8.1	SLG8	SLG8_bin107-SLG8_bin108 (65.61)	9	
		qPH-8.2	SLG8	SLG8_bin111-SLG8_bin112 (70.51)	23	
		qPH-9.1	SLG9	SLG9_bin102-SLG9_bin103 (78.81)	9	
		qPH-9.2	SLG9	SLG9_bin105-SLG9_bin106 (84.71)	10	
	Capsule zone length	qCZL-3.1	SLG3	SLG3_bin126-SLG3_bin127 (99.00)	9	
		qCZL-4.1	SLG4	SLG4_bin48-SLG4_bin49 (34.00)	7	
		qCZL-8.1	SLG8	SLG8_bin111-SLG8_bin112 (71.50)	24	
		qCZL-9.1	SLG9	SLG9_bin103-SLG9_bin104 (79.60)	6	
		qCZL-13.1	SLG13	SLG13_bin32-SLG13_bin33 (24.40)	7	
	Height of the first capsule node	qHFC-3.1	SLG3	SLG3_bin112-SLG3_bin113 (92.21)	9	
		qHFC-3.2	SLG3	SLG3_bin126-SLG3_bin127 (97.01)	22	
		qHFC-4.1	SLG4	SLG4_bin45-SLG4_bin46 (32.41)	5	
		qHFC-8.1	SLG8	SLG8_bin107-SLG8_bin108 (65.71)	5	
		qHFC-8.2	SLG8	SLG8_bin111-SLG8_bin112 (69.51)	9	
		qHFC-9.1	SLG9	SLG9_bin102-SLG9_bin103 (78.81)	10	
		qHFC-9.2	SLG9	SLG9_bin105-SLG9_bin106 (84.71)	11	
		qHFC-9.3	SLG9	SLG9_bin112-SLG9_bin113 (91.01)		

(continued)

Table 7.18 (continued)

Trait type	Trait	QTL locus	LG position	Marker interval (QTL region, cM)	Explanation (≥5%) or *P*-value	References
	Internode length	qIL-3.1	SLG3	SLG3_bin53-SLG3_bin54 (41.10)	10	
		qIL-8.1	SLG8	SLG8_bin111-SLG8_bin112 (71.50)	9	
		qIL-9.1	SLG9	SLG9_bin105-SLG9_bin106 (83.70)	10	
	Node number	qNN-3.1	SLG3	SLG3_bin111-SLG3_bin112 (91.80)	5	
		qNN-3.2	SLG3	SLG3_bin126-SLG3_bin127 (100.10)	10	
		qNN-8.1	SLG8	SLG8_bin110-SLG8_bin111 (66.50)	7	
		qNN-13.1	SLG13	SLG13_bin32-SLG13_bin33 (24.40)	9	
	Tip length without the capsule	qTL-3.1	SLG3	SLG3_bin124-SLG3_bin125 (94.40)	8	
		qTL-3.2	SLG3	SLG3_bin131-SLG3_bin132 (103.60)	8	
	Capsule length	Qcl-12	LG12	ZM1466-SBI005 (14.0-18.0)	52.2–75.6	Wu et al. (2014b)
	Length of the central capsule	q MCL9.1	SLG9	SLG9_bin105—bin106 (82.7)	7.74	Yang et al. (2017b)
		qMCL10.1	SLG10	SLG10_bin23—bin24 (19.3)	5.19	
		qMCL12.1	SLG12	SLG12_bin74—bin75 (54.4)	8.43	
		qMCL13.1	SLG13	SLG13_bin78—bin79 (50. 3)	13.69	
	Width of the central capsule	qMCW9.1	SLG9	SLG9_bin104—bin105 (81.1)	13.77	
		qMCW13.1	SLG13	SLG13_bin80—bin81 (50.8)	12.26	
	Thickness of the central capsule	q MCT9.1	SLG9	SLG9_bin112—bin113 (90.8)	8.44	
		q MCT13.1	SLG13	SLG13_bin80—bin81 (50.8)	8.93	
	Length of the lateral capsule	q FCL3.1	SLG3	SLG3_bin103—bin104 (84.5)	5.48	
		q FCL11.1	SLG11	SLG11_bin105—bin106 (69.6)	9.31	
	Width of the lateral capsule	q FCW3.1	SLG3	SLG3_bin110—bin111 (91.5)	7.05	
		q FCW9.1	SLG9	SLG9_bin105—bin106 (83.7)	19.65	
		q FCW13.1	SLG13	SLG13_bin78—bin79 (50.3)	8.48	
	Thickness of the lateral capsule	q FCT9.1	SLG9	SLG9_bin105—bin106 (83.7)	9.41	
		q FCT10.1	SLG10	SLG10_bin22—bin23 (18.2)	5.29	

(continued)

Table 7.18 (continued)

Trait type	Trait	QTL locus	LG position	Marker interval (QTL region, cM)	Explanation (≥ 5%) or *P*-value	References
	Determinate growth habit	SiDt	LG8	GenBank accession no. KU240042	100	Zhang et al. (2016)
	Branching habit	qBH-LG5	LG5	Marker41538—Marker31462 (1.75–2.75)	78.64	Mei et al. (2017)
	Flowers per leaf axil	SiFA	LG11	marker34507 (co-segregated)	100	
Seed quality	Oil content	Qoc-1	LG1	SBN2389–SBN2485 (37.0–40.6)	10.1	Wu et al. (2017)
		Qoc-2	LG2	SBN2776–SBN1045 (13.5–15.2)	6.5	
		Qoc-5	LG5	SBN3585–SBN1490 (41.5–52.1)	7.7	
		Qoc-9	LG9	SBN1388–SBN1459 (6.9–9.7)	8.6	
		Qoc-16	LG16	SBN1927–SBN3232 (22.6–26.6)	7.6	
	Protein content	Qpc-1	LG1	SBN741–SBN2389 (20.0–28.7)	7.6	
		Qpc-2	LG2	SBN2776–SBN2749 (13.5–15.2)	6.2	
		Qpc-3	LG3	HS184–SBI013 (6.3–9.7)	5.7	
		Qpc-5	LG5	SBI007–SBI057 (62.8–68.4)	10.9	
		Qpc-6	LG6	SBN3210–SBN636 (94.0–102.4)	6.4	
	Sesamin content	Qsc-4	LG4	SBI050–SBN1100 (70.7–73.7)	5.4	
		Qsc-5	LG5	ZHY01–SBN1548 (49.4–56.2)	11.1	
		Qsc-5	LG5	SBN3568–SBI007 (40.5–62.9)	18.6	
		Qsc-5	LG5	SBN3568–SBI007 (40.5–62.9)	14.1	
		Qsc-8	LG8	SBN1735–SBN2668 (35.4–44.6)	8.9	
		Qsc-8	LG8	SBN1407–SBN1140 (37.0–38.2)	5.2	
Seed coat color	RGB value	QTL1-1	LG1	Y1991F/R—Hs1015F/E9 (139.6–141.9)	23.32–39.95	Zhang et al. (2013c)
		QTL11-1	LG11	Hs1125R/E11—Y2017F/M11 (18.6–30.8)	9.72–20.61	

(continued)

Table 7.18 (continued)

Trait type	Trait	QTL locus	LG position	Marker interval (QTL region, cM)	Explanation (≥ 5%) or *P*-value	References
		QTL11-2	LG11	Y2017F/M11—Y640/ E16 (32.2–51.2)	9.6–31.86	
		QTL13-1	LG13	Hs1097F/E1—Y2632F/M7 (34.7–43.7)	12.8–30.56	
	*a** color value	qSCa-4.1	SLG4	SLG4_bin63-SLG4_bin64 (50.90)	13	Wang et al. 2016
		qSCa-8.1	SLG8	SLG8_bin114-SLG8_bin115 (73.40)	25	
		qSCa-8.2	SLG8	SLG8_bin105-SLG8_bin106 (62.60)	9	
	*b** color value	qSCb-4.1	SLG4	SLG4_bin63-SLG4_bin64 (50.90)	39	
		qSCb-8.1	SLG8	SLG8_bin114-SLG8_bin115 (72.40)	21	
	*L** color value	qSCl-4.1	SLG4	SLG4_bin63-SLG4_bin64 (50.90)	21	
		qSCl-8.1	SLG8	SLG8_bin114-SLG8_bin115 (73.40)	46	
		qSCl-11.1	SLG11	SLG11_bin1-SLG11_bin2 (0.00)	14	
Disease resistance	Charcoal rot	qCRR3.2	LG3	ZMM5636–ZMM5775 (39.30)	12	Wang et al. (2017a)
		qCRR3.3	LG3	ZMM2218–ZMM4682 (52.30)	10	
		qCRR3.4	LG3	ZMM4682–ZMM5444 (58.40)	9	
		qCRR8.1	LG8	ZMM5060–ZMM5061 (10.50)	5	
		qCRR8.2	LG8	ID0041–ZM638 (115.70)	5	
		qCRR8.3	LG8	ZM638–ZMM1682 (123.70)	5	
		qCRR9.1	LG9	ZMM2323–ZMM0205 (104.70)	8	
		qCRR12.1	LG12	ID0046–ID0133 (53.80)	6	
		qCRR12.2	LG12	ZMM0913–ZMM3752 (89.80)	14	
		qCRR13.2	LG13	ZMM2344–ZMM2343 (73.50)	8	

(continued)

Table 7.18 (continued)

Trait type	Trait	QTL locus	LG position	Marker interval (QTL region, cM)	Explanation ($\geq 5\%$) or *P*-value	References
Stress tolerance	Waterlogging tolerance	qEZ09ZCL13	LG13	ZM22-ZM92 (0.0)	10.20	
		qWH09CHL15	LG15	E16M19-314M14a (8.0)	7.55	
		qEZ10ZCL07	LG7	E5M12a-ZM351 (4.5)	8.14	
		qWH10ZCL09	LG9	M20E10-ZM428 (7.0)	5.67	
		qEZ10CHL07	LG7	E5M12a-ZM351 (4.5)	6.69	
		Qwh10CHL09	LG9	M20E10-ZM428 (7.0)	17.19	

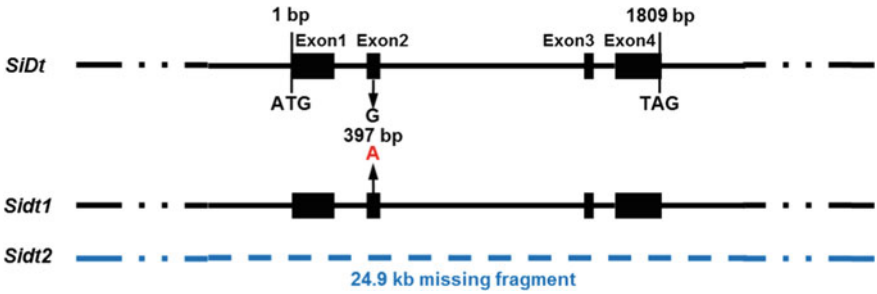

Fig. 7.10 Map-based cloning of the *SiDt* gene and its alleles in sesame (modified from Zhang et al. 2016). The *SiDt* (DS899s00170.023) gene, which is the wild type of the gene, is 1809 bp long and comprised of four exons (black blocks) and three introns. For the *Sidt1* allele mutant, a nucleotide substitution (the G397A SNP site in red) took place within the second exon of the *SiDt* gene. For the *Sidt2* allele in the *dt2* genotype, a 24.9 kb fragment including the entire *SiDt* gene sequence (in blue broken line) in Scaffold 00170 was deleted, according to the 08TP092 genomic data (PRJNA316751)

Interestingly, the alleles of the *SiDt* gene in various genotypes of the determinate growth habit have different gene structures (Fig. 7.10).

For the indeterminate parent (JS012, *Dt* type) of the population, shoot apexes grow indeterminately throughout the whole life cycle. The *SiDt* gene, the wild type of the gene, is 1809 bp long and comprised of four exons and three introns (Fig. 7.10). In the determinate parent (Yuzhi DS899, *dt1*), apical meristems show the determinate character, and the shoot apexes grow into a capsule cluster. For the growth habit change, only a single base in the *Sidt1* gene was substituted from 397G in the wild-type *SiDt* gene to 397A in the *Sidt1* gene mutant (Fig. 7.10). For the *dt2*-type mutant (described a) induced by gamma-ray (5000 Gray) mutagenesis, the shoot apexes are determinate, and the number of capsule nodes is limited and not affected by environmental factors (Melzer et al. 2008; Uzun and Çağırgan 2009). Genome comparison showed that the entire *SiDt* gene and flanking regions of 24.5 kb are deleted from the genome of the *dt2* mutant (Fig. 7.10). It appeared that the loss of the *SiDt* gene corresponds with the complete determinacy of inflorescence meristem development in the *dt2*-type mutant.

7.5.3 Genome-Wide Association Study and DNA Marker Identification

7.5.3.1 DNA Markers Associated with the Sesame Key Agronomic Traits

Compared with the traditional linkage analysis using artificial biparental mapping populations, genome-wide association study (GWAS) can be applied to identify the

DNA markers more closely linked with the target traits using natural populations because of their higher genetic recombination (Abdurakhmonov et al. 2009; Zhao et al. 2010). Wei et al. (2012) performed GWAS of seed oil content using 216 Chinese sesame accessions and 79 DNA marker primer pairs (including SSRs, SRAPs, and AFLPs). However, only three SRAP and five SSR markers were associated with the sesame oil content in two environments that each explained more than 5% of the phenotypic variance ($P \leq 0.01$) (Wei et al. 2012). To further explore the inheritance of the oil and protein contents in sesame seeds, 369 worldwide sesame germplasm accessions were applied to phenotype the traits under five environments (Li et al. 2014). One hundred twelve polymorphic SSR markers were applied to genotype these accessions. Nineteen markers were associated with the oil content (R^2 = 4–29%) and 24 markers were associated with the protein content (R^2 = 3–29%). When aligned to the Yuzhi 11 genome, 17 of the 19 SSR markers associated with the oil content were found near the locations of the plant lipid pathway genes, and 2 were located just next to a fatty acid elongation gene and a stearoyl-ACP desaturase gene, respectively. In total, 36 candidate genes related to lipid pathway were identified (Li et al. 2014).

Furthermore, to explore the genomic and genetic characters of the key agronomic traits, Wei et al. (2015b) performed GWAS with 705 sesame accessions under four environments using the genome re-sequencing techniques (Wang et al. 2014). As a result, a total of 549 associated loci were identified for 56 agronomic traits. Forty-six candidate genes related to oil content, fatty acid biosynthesis, and yield traits were screened. To clarify the genetic variants associated with the salinity and drought tolerances at sesame seed germination stage, Li et al. (2018) performed GWAS using 490 sesame accessions. A total of 13 and 27 candidate genes were uncovered for drought and salt tolerance indexes, respectively (Li et al. 2018). The GWAS performed so far have already included those for seed yield-related, seed quality, plant growth and development, and some key botanic traits in sesame (Table 7.19). These results have supplied enhanced sesame breeding assisted by MAS (molecular marker assisted selection) with useful DNA markers.

Table 7.19 Association mapping of agronomic traits in sesame

Population size	Marker type	Number of markers	Traits	References
216	SSRs SRAPs AFLPs	79	Oil content, protein content, oleic acid concentration, linoleic acid concentration, charcoal rot resistance, and drought tolerance	Zhang et al. (2012c), Wei et al. (2012, 2013), Li et al. (2012)
369	SSR	112	Oil content and protein content	Li et al. (2014)
705	SNP	1,800,000	56 agronomic traits involving seed yield, seed quality, and biotic and abiotic resistance traits	Wei et al. (2015a)
33	SSR	18	Drought resistance	Liu et al. (2017)
490	SNP	1,005,413	Salinity and drought tolerances	Li et al. (2018)

7.5.3.2 Comparative Genomics and Gene Detection

Comparative genomics has revealed the molecular mechanisms of the species-specific characters in sesame. In the sesame genome, some gene families (such as LTP1) related to lipid molecule transferring have been expanded, while other gene families (such as LOX and LAH genes) related to lipid degradation have been contracted (Wang et al. 2014). These variations of gene family sizes might result in the high content of fatty acids in the sesame seeds. Moreover, comparative genomics of *S. indicum* with several wild species of the *Sesamum* genus indicated that some biotic and abiotic stress resistance gene families have been also expanded or constraint. The loss of genes from these resistance gene families might cause the relatively lower resistance to diseases and abiotic stresses in the cultivated sesame (data now shown). Thus, introducing new genes from the wild *Sesamum* species as well as utilization of the modern breeding methods is recommended for future sesame breeding.

On the other hand, some sesame genes had been also identified through the orthologous gene analysis. Recently, the families of MADS-box genes, AP2/ERF transcription factors, and heat shock transcription factors (HSFs) genes in the sesame genome were analyzed (Wei et al. 2015b; Dossa et al. 2016a, b). These gene families probably play important roles in plant flower and seed development, drought tolerance, and disease resistance. For instance, Wei et al. (2015b) performed a genome-wide analysis of the MADS-box gene family in sesame, based on both SRF (type I) and MEF2 (type II) MADS-box domain sequences. They identified 57 MADS-box genes and mapped them to the 14 linkage groups of the cv. Zhongzhi No. 13 genome. These 57 MADS-box genes were classified into four groups: 28 $MIKC^C$-type, 5 MIKC*-type, 14 Mα-type, and 10 Mγ-type. $MIKC^C$-type MADS-box genes are likely responsible for sesame flower and seed development. Previous studies showed that the transcription factor family of AP2/ERF played significant roles in various plant processes, including in response to drought, high salt content, temperature change, and disease resistance (Wessler 2005; Fujita et al. 2006). Based on the roles of the known AP2/ERF genes in other plant species and their AP2/ERF domain, Dossa et al. (2016b) screened 132 AP2/ERF genes contained in var. Zhongzhi No. 13. Expression analysis showed that the sesame AP2/ERF genes were significantly up-/down-regulated under the drought stress and might be the candidate genes for sesame drought tolerance (Dossa et al. 2016b).

Numerous other genes, such as the *SeFAD2*, *SebHLH*, and *SeFAD2* genes involved in fatty acid synthesis, the *CYP81Q1* gene catalyzing sesamin biosynthesis, and the *SeMIPS1* gene taking part in myoinositol biosynthetic pathways, have been also identified in sesame using the homologous gene cloning method (Chun et al. 2003; Kim et al. 2006; Ono et al. 2006; Hata et al. 2010, 2012; Pathak et al. 2015). The identification of the above functional genes has provided a foundation for the molecular mechanism research of the key biological processes and traits in sesame.

7.6 Potential of Genomics-Assisted Breeding in Sesame

7.6.1 GWAS and Genome Application for Sesame Breeding

The main objective of sesame breeding always is to breed new varieties with high quality, high yield potential, and resistance to pathogens (including *Fusarium* wilt and Charcoal rot), insect pests, waterlogging, drought, and low temperature stress (Ashri 2006). Before the initiation of the SGP project, only a few functional genes, mainly involved in the formation and regulation of fatty acids, seed storage proteins, secondary metabolites, and salt stress response, have been cloned (Yukawa et al. 1996; Chen et al. 1997; Jin et al. 2001; Lee et al. 2005; Chyan et al. 2005; Hsiao et al. 2006; Kim et al. 2007b, 2010; Hata et al. 2010). Cloning of a *SiDt* gene controlling the determinacy of the inflorescence meristem development has indicated the high potential of the sesame genome information for sesame molecular genetics and functional genomics research. In 2013, the Sesame Genome Working Group (SGWG) released the first draft assembly of the *S. indicum* var. Yuzhi 11 genome (www.sesamum.org) (Zhang et al. 2013b). The fine genome assembly of the sesame genotype will be soon released in near future. Meanwhile, Wang et al. (2014) reported a draft genome of cultivar "Zhongzhi No. 13". The completion of the SGP with a high quality has provided a reference genome and laid a foundation essential for enhanced exploration of the genetic architecture of and efficient discovery of the genes related to yield, quality, and resistance traits in this oilseed crop.

Based on the reference genome information, large-scale GWAS of the 705 sesame germplasm accessions has allowed determination of the association between genome sequences and phenotypic traits (Wei et al. 2015a). Approximately, 549 loci have been so far identified that are associated with the phenotypic variations of 56 sesame agronomic traits. A total of 46 candidate causative genes, including those related to oil content, fatty acid biosynthesis, and yield traits, were determined. For example, two genes encoding lipases, CXE17 (SIN_1003248) and GDSL-like lipase (SIN_1013005), and two genes encoding lipid transfer proteins (SIN_1019167 and SIN_1009923) were associated with the sesame seed oil content. To our knowledge, more than 2000 worldwide cultivated sesame accessions have been re-sequenced to date using the NGS techniques. More genome re-sequencing data will be applied for sesame GWAS in near future.

7.6.2 Potential of Genomics-Assisted Breeding in Sesame

Genome-assisted breeding is referred to the implementation of molecular markers associated with important traits and genomic tools to predict trait phenotypes and to assist breeding (Varshney et al. 2013). To establish the relationships between genotype and phenotype, the genomic and genetic analyses of the target agronomic traits are necessary. It is very important for plant breeders to precisely select

desirable and elite traits, even though most of the agronomic traits are controlled by numerous genes with small and complex non-allelic quantitative effects.

As described in the above sections, with the aid of NGS and the third sequencing technologies, sesame genetics and genomics research has been extremely improved in recent years (Zhang et al. 2013a, b; Miao 2014; Wang et al. 2014; Wei et al. 2015b; Miao and Zhang 2016). Dossa (2016) gathered 151 published QTLs, candidate genes, and markers in 16 linkage groups of sesame and clustered them into 15 clusters. Based on the ultra-density SNP genetic map (Zhang et al. 2016) and the latest sesame genome sequence, 13 elite gene clusters closely linked to 58 markers ($P < 10^{-8}$) and 52 QTLs ($R^2 > 10\%$) that are associated with yield, seed quality, and *Fusarium* wilt disease resistance traits were identified in eight of the *S. indicum* chromosomes (Miao and Zhang 2016) (partially unpublished, H. Zhang). The findings of the sesame genetic mapping and association mapping related to the key agronomic traits will impel molecular breeding in sesame (Zhang et al. 2013b, 2016; Wei et al. 2015b; Wang et al. 2016).

DNA-based molecular markers have genetic stability and several other advantages for genotypic variation selection in crop breeding (Gupta et al. 2010; Kumpatla et al. 2012). Therefore, it is necessary to introduce and apply the modern molecular breeding methods to improve sesame breeding efficiency. Till now, several traits of agronomic importance still remain to be explored in sesame, and only a small number of genes and molecular markers have been developed and applied in MAS breeding. It is believed that the recent development of the advanced genomic resources and tools for sesame will promote the efficient genetic analysis of and cloning of the genes controlling the key agronomic traits in sesame.

7.6.3 *Future Genomics-Assisted Breeding in Sesame*

In recent years, molecular genetics and genomics research in sesame has been accelerated. However, there have been no reports about genomics-assisted breeding in sesame. GWAS revealed the lower nucleotide diversity of the sesame modern cultivars than landraces, indicating the narrow genetic basis of the cultivated sesame species (Wei et al. 2016). According to the molecular marker-assisted breeding in other crops, a molecular design breeding routine for sesame is supplied breeding. There are five major steps for molecular design breeding in sesame. (1) To identify the new elite breeding materials with multiple elite traits especially for quantitative traits. (2) With the aid of the molecular genetic characters of the receptor and donor parents, to determine the breeding objectives and design hybridization crosses. (3) To draw up the breeding pipeline and selection strategy for the breeding objectives and to predict the breeding results. All molecular markers, including QTL associated markers and genes, are applied for the breeding targets. (4) To screen the offspring of the crosses using the molecular markers and to evaluate the elite traits in the field under various environments. (5) To identify the new varieties containing the targeted genotypes and phenotypes. For sesame, it

seems remote to explain the regulatory networks of yield, resistance traits, and important biological processes. This is because it is still arduous to identify the genes, gene families and loci that are associated with seed yield, seed quality, and resistance to biotic and abiotic stresses. However, it is possible and practical to genome-wide high-throughput rapidly clone the genes controlling these traits and dramatically promote functional genome research in sesame with the aid of the NGS techniques and the quality sesame genome sequence (Zhang H., personal communication). It is strongly believed that genomics-assisted breeding as well as new germplasm lines and breeding material creation will be dramatically promoted in sesame in near future.

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Chapter 8
Breeding for Climate Resilience in Castor: Current Status, Challenges, and Opportunities

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Abstract Climate change and fossil fuel reserve depletion both pose challenges for energy security and well-being. Renewable bioenergy is considered as one of the practical alternative and castor plant is a potential choice due to the wide uses of castor oil in the industry. As a nonedible crop, castor plant is suitable for planting in marginal lands, without competition with food crops because of its strong tolerance to drought, high concentration of salt, and the adaptability to climate warming. Additionally, castor plant can be planted in heavy metal contaminated soils for phytoremediation. Lower genetic diversity, poor characterization and exploitation of germplasm and relative lag in genetic research limited castor breeding, resulting in low improvement of castor and the lack of high-yielding varieties with strong resistance to diseases and pests. With the development of the global economy, the increasing labor costs made it a great challenge for developing varieties suitable for machine harvesting. In this chapter, the challenges, priorities, and prospects of castor breeding were reviewed. The climate-smart (CS) traits, the genetic resources of CS genes, and the classical genetics and traditional breeding for CS traits are described; The achievements of molecular mapping of CS genes/QTLs, marker-assisted breeding, genomics-aided breeding, and genetic engineering for CS traits are summarized. These contents are expected to facilitate castor plant research and breeding for CS traits.

Keywords Castor plant · Climate-smart traits · Germplasm · High productivity · Adaptability · Resistance to diseases and insect pests · Genetics and breeding

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8.1 Introduction

8.1.1 Energy and Environment Security

Climate change and fossil fuel reserve depletion both pose challenges for energy security and for well-being in general. From global perspectives, one-third of oil reserves, half of gas reserves, and over 80% of current coal reserves should be reduced from 2010 to 2050 in order to meet the target of keeping global warming below 2 °C according to the Paris Agreement (IPCC 2013). Climate change mitigation requires a shift from fossil energy resources to renewables, and bioenergy is considered as one of the major potential resources (Bentsen and Moller 2017). Renewable bioenergy is viewed as one of the ways to alleviate the current global warming crisis. There is a growing scientific consensus on a large scale for deployment of bioenergy with CO_2 capture and storage in the actions and the strategies of organizations and governments for a sustainable future.

8.1.2 Effects on Global Warming and Climate Change

Anthropogenic climate change is increasingly threatening ecosystems and species worldwide (Rosenzweig et al. 2008; IPCC 2013). Evidence from a wide range of taxa and ecological systems suggests that climate change has already started to affect biodiversity at a global scale (Carnaval and Moritz 2008), for instance, by modifying species distributions, altering their habitats, or increasing extinction risk due to rapid shifts in abiotic conditions (Parmesan and Yohe 2003; Chen et al. 2011). Among terrestrial organisms, high-elevation cold-adapted species seem to be particularly vulnerable to climate change (Dirnböck et al. 2011; Scridel et al. 2018), with their future distribution being either expected to contract towards higher elevations due to ambient temperature warming (Sekercioglu et al. 2008; La Sorte and Jetz 2010; Chamberlain et al. 2013; Pernollet et al. 2015), or to vary in a complex way in response to shifts in precipitation regimes that remain difficult to forecast (Tingley et al. 2012). Mountain areas are indeed subject to higher rates of warming compared to the global average (Böhm et al. 2001; Brunetti et al. 2009), yet at the same time, they are also experiencing strong changes in landscape and land use (e.g., forest encroachment in abandoned pastures, upward treeline shift, or loss of areas permanently covered by snow). Various studies have shown that climate change will affect the intensity and distribution of diseases and pests on plants, and will lead to drought, heat, soil salinization, flood, etc., become the growing problems in crop production. Apart from energy saving and emission reduction to develop decarbonized economy, breeding, cultivating, and expanding climate-smart crops are realistic choice to maintain sustainable development.

8.1.3 Limitations of Traditional Breeding

Castor (*Ricinus communis* L.) is a diploid ($2n = 2x = 20$) species belonging to the family Euphorbiaceae, which is widely distributed throughout the tropics and subtropics and is well adapted to the temperate regions of the world. It is an important industrial crop because its seeds contain an oil with unique chemical and physical properties for industrial uses. As the only commercial source of a hydroxylated fatty acid, castor oil is very important to the global industry of specialty chemicals (Severino et al. 2012). Castor oil is widely used in engineering industry, medicine, textile, material, cosmetics, and many other fields covering from lower molecular weight aviation fuels, fuel additives, biopolymers, nano composites, biodiesel, phytoremediation to lubricant (Bauddh and Singh 2012; Halek et al. 2013; Madankar et al. 2013; Silva et al. 2013; Yang et al. 2013; Campos et al. 2014; Rui 2014; Alaa et al. 2015), known as green renewable petroleum. The major castor producing countries of the world include India, China, Brazil, Mozambique, Ethiopia, Paraguay, and Thailand. India ranks first in production, producing about 88% (1.64 million ton seeds) of the world's castor seed (1.86 million ton seeds) (FAO 2013). The demand for castor oil in the world is rising at 3–5% per annum (Anjani 2012). However, low seed productivity of castor varieties has been the bottleneck of castor industry. Breeding high-yielding hybrid varieties using pistillate lines has been the main direction of castor breeding but progressed slowly due to the lack of profound understanding of the genetic mechanism in pistillate character. Apart from the costly and laborious work in breeding pistillate lines, castor heterosis application has been plagued for a long time by the contradiction between the nonhomogeneity of hybrid due to the low homozygosity of the pistillate line and the low restorability of hybrid due to the high homozygosity of the pistillate line. Just like the pistillate character, very little of the genetic mechanism on other important traits is known, including yield traits, plant type related traits, quality traits, resistance to disease and pests, photoperiod sensibility and adaptability to the environment.

First of all, the research investment on castor biology is not enough in the world. As an ancient crop, the publications on castor genetics and physiology are too little to be commensurate with its status. Castor plant possesses a small genome size of ~350 Mb but its genome sequencing depth remained 4.6 folds (Chan et al. 2010). Not until 2016 the first genetic map of castor was published (Liu et al. 2016). Second, low genetic diversity of castor was found at molecular level by many researches (Allan et al. 2008; Bajay et al. 2009; Foster et al. 2010; Thatikunta et al. 2016). Third, the application of modern breeding methods, especially biotechnology, in castor breeding was less popular than the main crops. Fourth, as one of the most adaptable crop, different ecological conditions require different varieties and cultivation methods to get good planting benefits, which needs the collaboration between the institutes all over the world such as sharing the germplasm and performing shuttle breeding. Take China, for example, the most popular varieties were developed in the north but expressed premature senility, weak resistance to disease,

and poor tolerance to heat and moisture in South China, while wild castor plants in South China lack effective improvement.

With the development of economy and change of climate, requirements for varieties with high yield, better quality, resistance to biotic stress, and endurance to abiotic stress are increasing, which is difficult to realize by conventional breeding alone. Although castor has good variability for most of the morphological traits except presence of toxins (Auld et al. 2001), and this variability has also been exploited for crop improvement (Morris et al. 2011; Anjani 2012), there is a constant need of new variability for its genetic improvement because breeders have used most of the natural sources of tolerance/resistance to biotic and abiotic stresses due to its intensive cultivation over the years. Today, successful breeding is severely hampered by limited genetic diversity for productivity traits and sources of resistance to pests and diseases (Severino et al. 2012).

8.1.4 Breeding Objectives in Castor Bean

Because the three main countries producing castor are experiencing fast economic development, the manual labor necessary for conventional castor production has become scarce and expensive. Mechanized castor production is rapidly becoming mandatory to sustain or increase global castor production. Currently, only limited areas of castor production are fully mechanized because of the lack of nonshattering, short internode, and commercial cultivars (Baldanzi et al. 2003). With a view to address the challenges to reach greater productivity in castor, the main challenge is developing cultivars with adaptation to combine harvesting (Severino et al. 2012). Both cultivars and machines require further improvisation in order to obtain more efficiency in the process of combine harvesting. Nowadays, breeders look for plants that could be adapted for variety environmental conditions including increased ambient temperature caused by global climate change. The research for indehiscent cultivars with shorter plants to facilitate mechanical harvesting operations and earlier genotypes to reduce the retention time of the crop in the field has been focused on in Brazil. Development of these cultivars with additional improvements in machinery and agronomic practices will allow the rapid transition of castor to mechanized cultivation.

Although ricin has not been perceived as a limiting factor in castor cultivation in historic production regions, the development of low ricin or ricin-free castor cultivars is needed to allow commercial castor production in the United States. Nevertheless, research to develop low ricin, low ricinine, and low allergen cultivars should include parallel studies of increased potential resistance to pests and diseases. The development of low toxin castor cultivars will facilitate the use of castor meal in animal rations.

The dwarfing and dense cultivation is expected to facilitate increase in yield, which is now being promoted in Northwest China. This method can increase the number of spikes per unit area and allow increased density by removing the top of

main stem at ~5-leaf stage to promote the growth of the primary branches and by spraying cycocel to check the plant height. It promotes early maturation by only harvesting primary branch spikes. Further, ratoon cultivation with the perennial nature of castor is expected to realize the goal of harvesting multiple years by sowing once and harvesting 2–3 times a year in the tropical and subtropical regions, to reduce the cost and increase planting area in South China.

With the availability of genomic information in public databases, new vistas in genetic improvement have opened. However, the successful application of these information for the development of DNA-based markers and understanding metabolic pathways of oil biosynthesis, toxins, biotic stress resistance, etc., in castor still need to be elucidated. Demonstration of molecular markers for the development of newer lines/hybrids is needed. Efforts on physical mapping of genes using modern cytogenetic techniques also need to be explored. The methods for stable transformation from meristematic tissues could lead to the recovery of chimaeras. There is an urgent need for refining the established transformation protocol for better reproducibility and efficiency for successful genetic modification in castor bean for its wider cultivation and use. Efforts in basic research on oil biosynthesis pathways in castor and its applications in improving the oil composition and yield need to be intensified. Concentrated efforts for developing nontoxic and nonallergenic castor lines based on information on the presence, expression, and distinct regulation of ricin, RCA (*R. communis* agglutin), and 2S albumin genes during seed development are needed (Singh et al. 2015).

Castor has tremendous future potential as a bioenergy and industrial feedstock because of its high oil content, potential modifications in fatty acid composition, very high oil yields, wide range of adaptation, and the ability to be grown on marginal sites subject to drought, saline, and other environmental conditions. Modern biotechnology offers great promise in reducing noxious compounds, enhancing seed oil content, improving seed quality, and increasing stress tolerance. Recent researches revealed that castor plants are highly resistant to heavy metals and can be used for phytoremediation of soil contaminated by heavy metals. Consequently, most of the international scientific community working on castor believe that this crop will become a major crop for production of plant lipids for both energy and industrial applications (Severino et al. 2012).

8.2 Prioritizing Climate-Smart Traits

8.2.1 Flowering Time

It is generally acknowledged that castor is a short-sunshine plant. This is the top priority to be considered in breeding for adaptability, the introduction of better varieties and in determining of sowing time in cultivation. However, accessions from different ecological conditions differ greatly in sensitivity to short-sunshine.

Castor accessions are either sensitive or insensitive or intermediate to short-sunshine, which determines the flowering time. The flowering period of most accessions introduced from the world ranged from 45 to 200 days when sown at Zhanjiang in Southern China in March, but a few did not blossom until the next year. Some accessions did not bloom until December whether sowed in March or in September. The insensitive varieties can be planted more widely.

Early maturity is an important trait for castor cultivation in regions with short growing seasons or in tropical areas that produce multiple crops each year. The negative correlation between early maturity and high seed yield is the major impediment in developing very early maturing genotypes. However, Anjani and Reddy (2003) developed early maturing accessions with high seed yield potential using random crosses between extra-early accessions, one of the accessions had 50% of the plants flowered at 26 days after planting, and the early maturity trait in these accessions appeared to be only marginally impacted by environment (Anjani 2010a).

Although the flowering time of castor can be affected by the temperature and cultivation factors such as water, nutrients, etc., it is mainly determined by heredity.

8.2.2 Root Characters

The root system of castor plants is tap root system, composed of a developed principal root and all levels of lateral roots. The principal root can reach a depth of 2–4 m in the soil, from which 3–6 primary lateral roots are sent forth. Secondary lateral roots are sent forth from the primary lateral roots, and then come the ternary and quaternary lateral roots. The lateral roots of castor plants are also well developed and can extend 1–2 m horizontally. Fine roots and root hairs grow out of the principal and lateral roots to form a well-developed coniform root system. Most of the castor roots distribute within 10–50 cm from the Earth's surface, and they grow into deeper soil when it rains less and the water table is lower. Under moist conditions, exposed lateral roots can be visible. The root characters of castor are the basis of its tolerance to drought.

8.2.3 Cold and Heat Tolerance

Castor plant is a typical thermophilic crop. However, an issue that deserves attention is the slow, irregular, and cold-sensitive germination of castor seed. The minimum temperature required for germination is 14–15 °C, the optimum temperature being 31 °C, and the maximum temperature being 36 °C (Severino et al. 2012). Within this range, the germination and seedling emergence are accelerated with the increase of temperature. Often low soil temperatures delay germination and seedling emergence resulting in irregular stands (Moshkin 1986).

The castor seedling is frigolabile, it will suffer damage even death when meets the frost of −0.8–1 °C. When the temperature lowers below 5 °C, castor plants will suffer from cold. The castor seedling grows quickly when the daily mean temperature is over 15 °C. 20–28 °C is the optimum temperature for whole growth period. The flowering period and mature period will be shortened to some extent with the increase of temperature. The female and male flowers bloom only when the daily mean temperature is above 18 and 20 °C, respectively.

The heat tolerance varies with the ecological types in castor. The varieties from temperate region often exhibit premature aging even death in the tropical and subtropical regions. In South China, autumn sowing (September–October) can prolong the growth period, and lead to increase in capsule number and seed yield of the photoperiod-insensitive varieties than spring sowing (February–March) due to the relative lower temperature, and in high-temperature season (August), most varieties tend to grow smaller racemes than usual.

8.2.4 Drought Tolerance

Drought tolerance is one of the strengths of castor as a crop. Because castor oil is an only-industrial product, there is a possibility that the competition for land with food crops moves castor production into marginal soils. In that scenario, drought tolerance would be particularly important.

Castor plants are more sensitive to water stress in the early stages of growth. At the cellular level, water stress reduced callus initiation, nitrate reductase activity, and chlorophyll content (Manjula et al. 2003a, b). Drought stress increases cuticular wax load (Lakshmamma et al. 2009) and abscisic acid concentration in the phloem sap (Zhong et al. 1996).

Osmotic adjustment is an important mechanism for drought tolerance in castor. Osmotic adjustments in the leaves were demonstrated but with a wide variability in the intensity of the effect between genotypes (Babita et al. 2010). Among the osmotically active compounds, accumulated soluble sugars were by far the most important (61%), followed by free amino acids (17%), proline (12%), and K (2.8%). The seed yield of genotypes with higher osmotic adjustment was 53% greater compared to genotypes with low osmotic adjustment (Babita et al. 2010).

Under limited water supply, castor plants maintained efficient stomatal control while keeping a high level of net CO_2 fixation. Water loss by transpiration was minimized by an early stomatal closure (Sausen and Rosa 2010). Under drought stress, the photosynthetic apparatus of castor plants was preserved and that photosynthetic limitations were mostly due to diffusive resistance (Sausen and Rosa 2010). Castor plants were able to partially recover photosynthetic functions while experiencing stress due to severe drought. When the stress was removed, the plants completely recovered their normal photosynthetic function within 24 h. However, castor showed high levels of sensitivity to limited light (Funk and Zachary 2010).

8.2.5 Flooding and Submergence Tolerance

Castor is extremely sensitive to soil hypoxia. Within 2–6 h after subjecting castor plants to soil flooding, stomatal conductance, transpiration, CO_2 uptake, leaf elongation, root hydraulic conductance, and production of abscisic acid from flooded roots were reduced (Else et al. 2001). Castor plants subjected to continuous flooding were permanently damaged after 3 days and died after 4 days (Severino et al. 2005). In leaves of plants subjected to hypoxic condition, β-amylase activity, concentration of starch, protein and soluble sugars were increased, nitrate reductase activity was reduced (Beltrão et al. 2003, 2006) and seed yield was reduced (Baldwin and Cossar 2009).

8.2.6 Salinity Tolerance

For most crops, they could only survive in limited concentration of saline condition, while castor plant can grow in marginal lands with wide range of external salinities. Even at 160 mol m^{-3} NaCl, castor plant could also survive and produce viable seeds (Dieter Jeschke and Wolf 1988). Growth and production of castor were also inhibited by high salinity (Na) in either the irrigation water or in the soil (Silva et al. 2008). Plants were most sensitive in the early stages of development (Pinheiro et al. 2008). Increased salinity delayed and reduced total emergence of castor seed, but significant difference existed between genotypes (Silva et al. 2005).

Increasing levels of Na salinity apparently damaged the photosynthetic apparatus and induced proline accumulation in castor plants (Li et al. 2010). The threshold of Na salinity for castor emergence and growth is 7.1 dS m^{-1}, and in this high concentration salt environment, nutrient uptake and accumulation did not alter a lot. The emergence rate was delayed by 9 days and was 50% lower at a salinity of 13.6 dS m^{-1}. 60% of the seedlings did not survive when subjected to the same salinity level for 11 days (Zhou et al. 2010).

Saline stress negatively affected castor bean growth, regardless of cationic nature of water. Among the ions studied, castor plant was more sensitive to the presence of sodium in the irrigation water (de Lima et al. 2018), and calcium and magnesium did not alleviate the toxic effect of sodium on the emergence and initial growth of castor (Severino et al. 2014). Emergence and growth were more affected by the electrical conductivity than by the cationic composition of the irrigation water, and the order of the cations in the irrigation water, in terms of negative effects, was $Na^+ > Na^+ + Ca^+ > Ca^{2+} > Na^+ + Ca^{2+} + Mg^{2+} > K^+$. The cationic composition of the irrigation water influenced the time interval for inflorescence development and the opening of flower buds, and the most pronounced effects were observed in plants irrigated with calcic water (de Lima et al. 2016).

High salt concentration can alter basic physiological and biochemical processes (Parida and Das 2005), and reactive oxygen species hormones in plants

(Mizrahi et al. 1971). Gibberellic acid (GA_3) is one of the important hormones that plays a crucial role in the stress responses in plant (Shakirova et al. 2003), and presoaking castor seed with 25 μM GA_3 can promote the growth of castor seedlings in high salinity level.

8.2.7 Disease Resistance

The three major diseases affecting castor are: gray mold (*Botryotinia ricini* G.H. Godfrey or *Amphobotrys ricini* N.F. Buchw. in its anamorphic), vascular wilt (*Fusarium oxysporum* f.sp. *ricini* Nanda and Prasad), and charcoal rot (*Macrophomina phaseolina* [Tassi] Goid.). Several others diseases can sporadically cause severe outbreaks depending on the genotype and climatic conditions, such as the leaf spots caused by the fungi *Alternaria ricini* (Yoshii) Hansf. and *Cercospora ricinella* Saccardo and Berlese and the bacteria *Xanthomonas axonopodis* pv. *ricini* Hasse. Among these, gray mold deserves more attention because it is a seed-borne fungus that can also cause seedling blight and pod rot with seed yield losses reaching 70% (Holliday 1980).

Gray mold is probably the most serious castor disease worldwide. Breeding programs have failed to develop resistant genotypes, but genotypes with moderate levels of tolerance have been identified (Araújo et al. 2007; Anjani 2012). There has also been the development of diagrammatic scales to assess disease severity in the field (Sussel et al. 2009; Chagas et al. 2010) and a technique to screen germplasm resistance under controlled conditions (Soares et al. 2010). Although gray mold is a seed-borne fungus, the initial inoculum source of the disease is not likely to be the seed because there is a large time between planting and flowering (Soares 2012). Under tropical conditions, the initial inoculum source is probably the conidia produced on wild castor plants. The fungus infection on the first flowers produces abundant sporulation allowing multiple rounds of reinfection as this pathogen is spread by wind, rain, and probably insects. Additionally, gray mold has a wide host range within the family Euphorbiaceae, including both weeds and ornamentals (Soares 2012).

Fusarium wilt is regarded as the most important disease of castor in India (Desai and Dange 2003) and China. Fusarium wilt generally appears in patches and at all growth stages of the crop. The extent of yield loss depends on the stage at which plant wilts with the losses ranging from 77% at flowering to 63% at 90 days and 39% in later stages on secondary branches (Pushpawathi et al. 1998). The reduction due to wilt is 10–40% in yield, 8–14% in seed weight, and 1–2% in seed oil content (Lakshminarayana and Raoof 2006). Breeding for wilt resistance is hampered due to the limitation on number of genotypes that can be screened in a traditional 'sick plot (field) method'. A high-throughput screening method was established for large-scale phenotyping of castor genotypes for resistance to Fusarium wilt disease (Shaw et al. 2016). The use of varietal resistance, seed treatment, and crop rotation are the best practices to manage this disease. Several commercial hybrids and

breeding lines resistant to vascular wilt have been developed in India (Anjani et al. 2004; Anjani 2005a, c, 2012; Patel and Pathak 2011) and China.

Charcoal rot, also known as Macrophomina root rot, is a major disease in most countries where castor is cultivated (Araújo et al. 2007; Rajani and Parakhia 2009). Grezes-Besset et al. (1996) defined the criteria for developing castor resistance to charcoal rot, and some tolerant genotypes have been developed (Anjani et al. 2004; Anjani 2005b). Management of charcoal rot is primarily based on cultivar resistance, but crop rotation and organic matter amendments can reduce the severity of this disease (Rajani and Parakhia 2009).

8.2.8 Insect Resistance

In India, insect pests of economic importance are castor semilooper (*Achaea janata*), castor shoot borer (*Conogethes punctiferalis*), capsule borer (*Dichocrosis punctiferalis*), tobacco caterpillar (*Spodoptera litura*), red hairy caterpillar (*Amsacta* spp.), and leafminer (*Liriomyza trifolii*) (Basappa 2007; Anjani et al. 2010b). In Brazil, the major pests are stink bug (*Nezara viridula*); leafhopper (*Empoasca* spp.); defoliators including armyworm (*Spodoptera frugiperda*), *A. janata*, and black cutworm (*Agrotis ipsilon*); and the mites *Tetranychus urticae* and *Tetranychus ludeni* (Soares et al. 2001; Ribeiro and Costa 2008). In China, the major pests are black cutworm (*A. ipsilon*), leafhopper (*Empoasca* spp.), castor semilooper (*A. janata*), *Euproctis cryptosticta*, *Helibthis armigera* Hiibner, *Liriomyza sativae* Blanchard, and tobacco caterpillar (*S. litura*). In Colombia, cotton lace bug (*Corythucha gossypii*) was reported as a pest of castor plants (Varón et al. 2010).

Castor farmers in Andra Pradesh, India increased the seed yield by 28% when they used an Integrated Pest Management program with pesticides and crop rotation, insect traps, and neem extract (Basappa 2007). Purple-leafed cultivars of castor (with high levels of anthocyanins) were found to be tolerant to leafminer (Anjani 2005a). The presence of epicuticular wax (blooming) on castor leaves reduced infestation and defoliation caused by *A. janata* and *S. litura* (Sarma et al. 2006). In an experiment conducted at the Guangdong Ocean University, China, a whole farm was infested with the leafhopper pest during winter, many materials were susceptible but Heyuan 1 and Heyuan 2, wild castor plants collected from the Guangdong Province, remained resistant. In that experiment, they were observed as very resistant, and subsequent tests supported this finding.

8.3 Genetic Resources of Climate-Smart (CS) Genes

The taxonomy and geographic distribution of castor plant were thoroughly studied and documented earlier in the former USSR (Moshkin 1986), USA (Brigham RD), Brazil (Savy Filho 2005) and India (Kulkarni 1977; DOR 2003; Anjani 2010a).

Table 8.1 Major castor germplasm collections in the world as of 2011

Genebank	Number of accessions reported
National Bureau of Plant Genetic Resources (NBPGR), India	4307
Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences (ICGR-CAAS), China	2111
United States Department of Agriculture-Agricultural Research Service, USA	1390
Centro Naeional de Pesquisa de Algodao (CNPA), Brazil	1000
Vavilov Institute of Plant Industry (VIR), Russia	696
Institute of Biodiversity Conservation (IBC), Ethiopia	510
Others (52 institutes)	8699

Castor is reported to have a polyphyletic origin, both Africa and India were considered as the origin of castor based on its widespread cultivation, documents of its medicinal uses, and physical evidence. Due to its widespread survival and perennial nature, all possible transitions from an uncultivated plant to a weedy plant and from semi-cultivated to a field crop exist and there is no gap between uncultivated and cultivated castor. Castor was classified as Angiospermae, Eudicotyledone, Rosanae, and Malpighiales (Angiosperm Phylogeny Group III 2009).

The FAO's Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture (www.fao.org.docrep/013/i500e) states that 17,995 castor germplasm accessions are being held with various institutes in the world (Table 8.1) (Anjani 2012).

Germplasm banks are the basic providers of useful genes and genotypes needed to achieve the desirable genetic improvement in breeding programs; however, the resources available in castor germplasm worldwide have been barely tapped for castor genetic improvement and the majority of them have been poorly characterized (Bezerra et al. 2010). Nearly all the researches on castor germplasm focused on primary gene pool, but secondary and tertiary gene pools were seldom involved.

8.3.1 Sources of Resistance to Diseases

Resistance to Fusarium wilt was reported to be monogenic dominant (Hanumantharao et al. 2005; Singh et al. 2011), recessive (Sviridov 1988; Lavanya et al. 2011; Patel and Pathak 2011), incompletely dominant resistance (Lavanya et al. 2011), involvement of two complementary genes (Hanumantharao et al. 2005; Shankar et al. 2010), and polygenic control (Desai et al. 2001). Kammili and Raoof (2014) confirmed the digenic epistatic interactions on wilt resistance with F_1, F_2 and backcross generations of four crosses involving four resistant and three susceptible parents, including the 15:1, 9:7 and 13:3 ratios indicating duplicate dominant, duplicate recessive, and dominant and recessive epistatic interactions, respectively. It was reported that both

the parents should be wilt resistant for developing wilt-resistant hybrids (Patel and Pathak 2011) and that the mode of inheritance of offsprings of a cross is dependent on the inheritance mode of the parents (Anjani and Raoof 2014). Shaw et al. (2018) studied eight different populations and found the resistance to wilt is conferred by a single locus in one population, while the resistance was controlled by at least two complementary loci in other seven populations. In addition, different modes of inheritance were observed when the same resistant source was crossed with different susceptible parents, which led the wilt resistance to be a complex trait.

The first reported wilt-resistant genotypes, viz., Kitaiskii, Sangvineus, Gibridnyi, VNIIMK 18, VNIIMK 165 and Sizaya 7, were from VNIIMK (All-Russia Research Institute of Oil Crops, Russia) and VIR (N.I. Vavilov Institute of Plant Industry, Russia) (Podukuichenko 1977, 1991; Moshkin 1986). In India, several sources of stable resistance to Fusarium wilt were identified based on multiyear, multilocation screening in wilt sick plots under high disease pressure (Anjani et al. 2004, 2010a; Anjani and Raoof 2005). Anjani (2010b) showed wide genetic diversity based on agro-economic traits among wilt-resistant accessions. Over the years, global castor collections comprising 1779 Indian and 190 exotic accessions from 36 countries were screened against wilt, among which only 13 accessions (11 from India; 2 from the former USSR) consistently showed wilt resistance in both wilt sick plot and greenhouse conditions during multiple years (Anjani and Raoof 2014). These 13 resistant accessions (RG-43, RG-111, RG-109, RG-297, RG-1608, RG-1624, RG-2758, RG-2787, RG-2800, RG-2818, RG-2822, RG-3016 and RG-3105) would be of great value as donors of resistance.

In China, hybrid varieties Jinbi 2, Fenbi 10, and cultivar Yunbi 4 showed high resistance to wilt on natural inoculation conditions. Twenty-six wilt-resistant indigenous accessions, from wild or semi-wild castor plants collected from south China, were identified under natural and artificial infestation. Twenty-two wilt-resistant strains with high seed yield potential were identified from recombinant inbreed lines derived from a resistance-sensibility cross YC2 × YF1 (Xuegui Yin personal communication).

Grezes-Besset et al. (1996) characterized field resistance to root rot caused by *M. phaseolina* (Tassi) Gold in castor collected from Medagaskar. In India, nine accessions collected from Andaman and Nicobar Islands and Tamil Nadu State exhibited high resistance to root rot in root rot sick plot (Anjani et al. 2004; Anjani 2005d, 2006).

There are a very few studies on castor resistance to gray mold caused by *Botrytis ricini* (Godfrey). Batista et al. (1998) identified four resistant accessions under natural infestation. Milani et al. (2005) identified six gray mold resistant Brazilian castor genotypes (MPAI T63/6, Cinnamon Juriti, Sipeal 28, 2004 Sipeal, CNPA SM1, Ox Blood) based on screening under natural infestation. Anjani and Raoof (2010) identified five gray mold resistant indigenous accessions (RG 2787, RG 2836, RG 2980, RG 3126, RG 3139) based on screening under artificial epiphytotic conditions in glasshouse and field in 3 to 6 consecutive years.

Accessions possessing multiple resistance to Fusarium wilt and root rot (Anjani 2006), or wilt, root rot, reniform nematode (*Rotylenchulus reniformis*) and gray mold (Anjani and Raoof 2009), or wilt and leafminer (Anjani 2005a), or wilt, nematode and leafhopper are also present among Indian collections. The castor varieties Dawn, Hale, and Lynn developed by USDA and Texas Agricultural Experimental Station showed field resistance to Verticillium wilt, Alternaria leaf spot and tolerance to bacterial leaf spot (*Xanthomonas axonopodis* pv. *ricinicola*) (Brigham 1970a, b, c).

Anjani et al. (2018) identified trait-specific accessions in global castor germplasm core set. Among the 165 accessions comprising core set, eight accessions, viz., RG-43, RG-111, RG-224, RG-297, RG-558, RG-2430, RG-2818, and RG-2819 consistently exhibited resistance reaction with wilt incidence ranging from 0 to 18.8% in wilt sick plots and 0 to 15.4% in greenhouse; six accessions viz., RG-111, RG-2035, RG-2430, RG-2818, RG-2819, and RG-2821 exhibited resistance reaction against Macrophomina root rot in root rot sick plot (root rot incidence: 0–18.7%) and greenhouse (root rot incidence: 0–19.2%); Multiple resistance to both Fusarium wilt and Macrophomina root rot were observed in four accessions namely, RG-111, RG-2430, RG-2818, and RG-2819 in sick plots and greenhouse conditions. The accession, RG-1963 showed resistance reaction against gray mold with 4.3% disease severity and RG-3088 had moderate resistance reaction (33.6% disease severity).

8.3.2 Sources of Resistance to Insect Pests

Castor is a host to more than 100 species of insects and is used as an insect trap plant in several crops. Sources of resistance to some of the insect pests are available in castor gene pool. Jayaraj (1966, 1967) identified RC1098 Baker, RC1094, RC1092 Italy and RC1096 Cimmerron Coonoor as resistant sources and C3 Pakistan as a tolerant source against leafhopper (*Empoasca flavescens*). No-bloom and single-bloom types are reported to be less resistant to leafhopper than double and triple-bloom types in castor (Jayaraj 1968; Srinivas Rao et al. 2000; Vijaya Lakshmi et al. 2005). Seventeen Indian collections were identified as stable sources of resistance against leafhopper (Lakshminarayana 2003; Lakshminarayana and Anjani 2009). The Indian accession RG 43 is multiple resistant to leafhopper, wilt, and nematode. Five accessions, viz., RG-43, RG-631, RG-1621, RG-3037, and RG-3067 from global castor germplasm core set exhibited resistance reaction against leafhopper (0-1 hopper burn on 0-4 scale, the resistant check, DCH-519, the commercial castor hybrid, showed 0-1) at all the locations in all years of screening (Anjani et al. 2018). The Chinese accessions HY1 and HY2 showed strong resistance to leafhopper (Xuegui Yin personal communication).

Whitefly, *Trialeurodes ricini* (Homoptera: Aleyrodidae) is another serious sucking pest in castor. An exotic accession EC 103745 was reported to be resistant

to whitefly (Ramanathan 2004). Forty-three Indian accessions were identified as possible sources of resistance to whitefly (Lakshminarayana 2003; Anjani and Jain 2004).

Tobacco caterpillar (*Spodoptera litura* Fabr.) and semilooper (*Achoea janta* L.) are the most destructive defoliators in castor. Indian castor collections exhibited varying reaction against *Spodoptera* and semilooper when screened over years under natural infestation in hot spot areas in multilocation trials on castor. Thanki et al. (2001) observed moderate resistance to *Spodoptera* in an Indian castor cultivar CO-1, and five accessions (RG 5, RG 33, RG 221, RG 224 and RG 449) were reported to be tolerant to semilooper based on screening under natural infestation in hot spot areas.

Capsule borer, *Conogathes* (*Dichocrocis*) *punctifaralis* Guen. (Pyralidae: Lepidoptera), is another serious pest of castor. Yield loss to the tune of 53% in castor sole crop and 35–53% when castor was intercropped with green gram (*Vigna radiata*), sesame (*Sesamum indicum*), moth bean (*Vigna aconitifolia*) and cowpea (*Vigna unguiculata*) was reported (Patel and Patel 2009). Breeding for capsule borer resistance is an effective means to manage the pest. Indian collections were screened against capsule borer under caged conditions. Five accessions (RG 1934, RG 2546, RG 2770, RG 2543 and RG 2786) were identified as confirmed sources of resistance to capsule borer (Lakshminarayana 2003; Lakshminarayana and Anjani 2010).

Leafminer, *L. trifolii* (Burgess), Diptera Agromyzidae, causes severe damage to castor foliage right from cotyledenary stage to 150 days after planting. Four Indian accessions (RG 1930, RG 2008, RG 1766 and RG 1771) exhibited high resistance to leafminer. The former two are dark purple color morphotypes and the latter two are papaya leaf type morphotypes. High concentration of total phenols was observed in resistant genotypes (Prasad and Anjani 2000; Anjani et al. 2010b). Anjani et al. (2007) reported maternal inheritance of leafminer resistance when the leafminer resistant purple color accession RG 1930 was used as a female parent.

8.3.3 Sources of Resistance to Abiotic Stresses

Castor is susceptible to frost particularly in the early growth stages as germination is effected when temperature drops below 15 °C. The evaluation of castor germplasm in northeast USA at high latitudes identified accessions yielding 60–75% higher than the standard cultivar Hale under low soil temperature encountered in spring. Some geographically diverse castor accessions tested at Lubbock, TX, USA under dry condition showed drought tolerance. Tolerance to salinity was also observed in some of these accessions when tested at Pecos, TX, USA where salt concentration in irrigation water was approximately 3500 ppm. Some of these tolerant accessions yielded 30–60% higher than the standard cultivars Hale and Brigham under either salt or drought stress (Meeks et al. 2010).

In India, systematic screening of castor germplasm against moisture stress and temperature stress has been taken up using standard techniques such as temperature induction response (TIR) technique in the laboratory and moisture stress imposition during winter or summer season in the field. Root structures with controlled irrigation were used to study variation in root traits to determine water use efficiency of castor germplasm accessions. The accession series RG 122, RG 226, RG 232, RG 235, RG 242, RG 247, and RG 1096 were identified as thermal-tolerant sources (Lakshmi Prayaga and Lakshmamma 2006), which exhibited more than 80% recovery after induction temperature (35 °C for 2 h followed by 40 °C for 2 h and 45 °C for 1 h) and less than 20% death even at the lethal temperature (48 °C for 2 h). Lakshmamma et al. (2004) selected one hundred germplasm lines to study drought tolerance. Drought was imposed at 25 days after sowing (DAS) and released at 85 DAS, as a result, RG 72, RG 17, and RG 52 were identified for early maturity coupled drought tolerance and could be used for breeding. Parvathaneni et al. (2017) tested 15 germplasms under water stress for 2 years and found two varieties RG 1494 and RG 2139 that showed good performance in both years. Lakshmi Prayaga and Lakshmamma (2006) identified accessions RG 89, RG 122, RG 214, RG 232, RG 297, RG 298, RG 332, RG 707, RG 1117, RG 1449, and RG 1526 as sources of tolerance to drought. The accessions RG 17, RG 122, and RG 214 were developed from heterogeneous populations of introductions, viz., EC168752 from Hungary, EC103743 from an unknown country of origin and EC198496 (AMM 930) from UK (Anjani 2010a). Variability for root and shoot traits and drought tolerance was reported in castor germplasm (Lakshmamma et al. 2010; Lakshmamma and Lakshmi Prayaga 2010). The accessions RG 1450, RG 1611, RG 2122, RG 2149, RG 2714, RG 2797, and RG 2826, which are semi-wild and wild collections from India, exhibited longer root, higher root volume, and dry weight and lower ^{13}C value. The accessions RG 2149 and RG 2714 also recorded to possess high leaf area index, total dry matter, and SAP chlorophyll meter reading.

Falasca et al. (2012) developed an agroclimatic zoning model to determine potential production areas for castor bean in Argentina, the aptitude types were defined and mapped: optimal (>750 mm; temperature 24.0–27.0 °C; >−8 °C; >180 frost-free days); very suitable (>750 mm; temperature 21.0–23.9 °C; >−8; >180 frost-free days); suitable with humid regime (>750 mm; temperature 16.0–20.9 °C; >−8 °C; >180 frost-free days); suitable 1 with subhumid regime (450–750 mm; temperature 24.0–27.0 °C; >−8 °C; >180 frost-free days); suitable 2 with subhumid regime (450–750 mm; temperature 21.0–23.9 °C; >−8 °C; >180 frost-free days); suitable 3 with subhumid regime (450–750 mm; temperature 16.0–20.9 °C; >−8 °C; >180 frost-free days); marginal due to humidity (200–450 mm); marginal due to temperature (<16.0 °C); marginal due to frosts (<180 frost-free days or <−8°C) and not suitable areas (combination of 2 or more of the following variables: <200 mm; <16.0 °C; <−8 °C; <180 frost-free days). This agroclimatic zoning model can be applied to any part of the world, using the bioclimatic limits of the species presented in this work (Falasca et al. 2012).

8.3.4 Sources of High Yield and High Quality Traits

Among the 165 core set accessions evaluated at multilocations for ricinoleic acid level, and economic and phenological traits, four accessions, viz., RG-408, RG-2451, RG-2685, and RG-3233 stably expressed high levels of ricinoleic acid (90.4–91%) while the high-yielding hybrid, DCH-519 and variety, GC-3 had 87.5–88.7% ricinoleic acid content (Anjani et al. 2018). The high ricinoleic acid type accession RG-2685 yielded at par with the high-yielding check variety GC-3, possessed 48.7% mean oil content at par with GC-3 (48.4%) and was comparable to GC-3 in most of the agronomic and phenological traits. The accession RG-1647 possessed higher mean 100-seed weight (51.3 g) than the check varieties GC-3 (35.3 g) and DCS-107 (30.8 g). It gave 2607 kg/ha mean seed yield under irrigated conditions, which was comparable to seed yield of check GC-3 (2647 kg/ha), however its yield under rainfed conditions (1751 kg/ha) was far less than that of GC-3 (2346 kg/ha); RG-1647 recorded 50.4% mean oil content while the check GC-3 had 50% and DCS-107 had 49.2% oil content. A germplasm collection of 60 genotypes belonging to 12 castor accessions from six different provinces of Iran were evaluated for seven biochemical traits as important seed oil components and tested for significant association with the allelic profile of 16 ISSR primers, 37 marker-trait significant associations ($P \leq 0.05$) were identified and a maximum number of marker-trait associations (27.03%) were identified for stearic acid (Darvishzadeh 2016).

8.3.5 Effects of Nuclear Genome Duplication

The effects of nuclear genome duplication on the chlorophyll-protein content and photochemical activity of chloroplasts, and photosynthetic rates in leaf tissue, have been evaluated in haploid, diploid, and tetraploid individuals of castor plants. Analysis of this euploid series revealed that both photosystem II (2,6-dichlorophenolindophenol reduction) and photosystem I oxygen uptake (N, N, N', N-tetramethyl-pphenylenediamine to methyl viologen) decrease in plastids isolated from cells with increasingly larger nuclear complement sizes (Timko et al. 1980). Photosynthetic O_2-evolution and $^{14}CO_2$-fixation rates in leaf tissue from haploid, diploid, and tetraploid individuals were also found to decrease with the increase in size of the nuclear genome (Timko et al. 1981).

8.4 Glimpses on Classical Genetics and Traditional Breeding for CS Traits

8.4.1 Genetics of Important Traits

Many morphological and qualitative traits in castor are controlled by one or few genes. Stem color was reported to show epistatic interaction of two genes, '*M*' (mahogany) and '*G*' (green). The combination '*MG*' results in a rose coloring, '*Mg*' a mahogany, '*mG*' a green, and '*mg*', a tinged coloring on the stems (Harland 1928; Peat 1928). Tall plants are dominant over dwarf plants due to a monogenic factor. Characters like bloom, compactness of spike, the presence of spines on the capsule, and branching of the stalk appear to be controlled by partial dominance and simply inherited (Rao et al. 2009). Earlier studies indicated complete or partial dominance of presence of bloom (waxy stems) over its absence. This trait showed epistasis with either two (Zimmerman 1958) or four genes (Moshkin 1986). The intensity and distribution of bloom on different parts of the plant appear to be controlled by multiple genes (Lavanya and Gopinath 2008). The inheritance of sex expression is particularly important in the development of hybrids. There are three types of pistillate lines that could be used for hybrid production: N, S, and NES. In the N type, the occurrence of only female flowers is controlled by a recessive gene (*ff*). In the S type, the production of only female flowers is controlled by a polygenic complex with dominant and epistatic effects. In the S type, the plant starts as female, but a reversion to the production of dioecious flowers can occur at any time. In the NES type, the plant has the recessive gene (*ff*) that allows it to start as female, but when air temperatures exceed 31 °C, there is a sexual reversion (Zimmerman 1958; Shifriss 1960; Ankineedu and Rao 1973).

Characteristics of primary economic importance such as seed yield and seed oil content are usually inherited in a quantitative manner. Additive genetic effects were shown to be important in determining the number of nodes before flowering, number of racemes per plant, and seed oil content in studies with inbred lines evaluated in diallel crosses (Hooks et al. 1971; Swarnlata and Rana 1984). Traits such as the length of the primary raceme, the number of capsules per primary raceme, and the seed weight have also been shown to be additively inherited (Giriraj et al. 1974; Solanki and Joshi 2000; Solanki et al. 2003). A high heritability was reported for earliness, seed weight, and plant height (Solanki and Joshi 2000). An extensive report on the inheritance of the components of seed yield and oil content can be found in Moshkin (1986). Studies on the general combining ability and specific combining ability for seed yield, seed yield components, and other agronomic traits were made by Pathak and Dangaria (1987), Mehta (2000), and Nóbrega et al. (2010). Significant effects of general and specific combining ability on seed yield were found in all the studies, and in most cases, even genotypes with high specific combining ability had at least one parental line with high general combining ability. These results showed that selection in a conventional breeding could enhance these traits. Patel and Pathak (2011) studied the inheritance of the

resistance to wilt (*F. oxysporum* f.sp. *ricini* Nanda and Prasad) and found that both the parents should be wilt resistant for developing wilt-resistant hybrids. Several breeding programs around the world are currently focused on producing castor cultivars adapted to mechanical harvest. The perennial nature and indeterminate growth habit of castor have limited success when this crop is cultivated as an annual and mechanically harvested. Plants adapted to mechanized production would ideally be short (1.0–1.5 m), have uniform maturing racemes, and produce a minimum number of lateral branches. Baldanzi and Pugliesi (1998) were successful in increasing the frequency of non-branching plants after four cycles of selection, although successive rounds of self-pollination reduced plant vigor.

A natural mutant expressing high oleic acid and reduced ricinoleic acid in the seed was identified (Barros et al. 2004), and the high oleic acid trait appeared to be controlled by two independent genes (*ol*, *ML*) with epistatic interaction (Rojas-Barros et al. 2005).

8.4.2 Genetic Improvement of Castor Through Conventional Approaches

8.4.2.1 Variability

Castor, though being monotypic, has good variability for most of the morphological and agronomic traits, and this variability has also been exploited for its improvement. Great scope still exists for genetic improvement of castor through conventional breeding methods like interspecific hybridization and induced mutation. However, narrow information of genetics of little and unpredictable yields and susceptibility to diseases and insects are major challenges in successful breeding of castor.

The genetic improvement of any crop by conventional breeding approach depends on the available variability in the germplasm or different species of that crop. All varieties of castor plant have a chromosome number of $2n = 20$ (Perry 1943). Castor morphology varies from giant perennial plants to short internode and annual dwarf plants. Natural polyploidy is absent in castor, however, species with $2n = 20$ are believed to have originated from progenitors with $2n = 10$ and $x = 5$ (Singh 1976).

Good variability exists for number of nodes, days to maturity, length of primary spike, effective length of primary spikes, seed yield, and seed weight. Considerable variation has also been observed in length, thickness, rigidity, and presence or absence of capsule spines (Auld et al. 2009; Anjani 2012; Severino et al. 2012). Similarly, variation has also been reported for capsule wall thickness, which is associated with shattering of seeds as well as suitability to mechanical dehulling, seed size, and color (Severino et al. 2009). The main raceme usually produces more seeds with higher test weight. Variability observed in the bloom (a waxy coating on

leaves and stem of castor plant) has been reported to have correlation with insect resistance/susceptibility. No bloom trait is positively correlated to jassid susceptibility while triple bloom is positively correlated with susceptibility to white fly. Though several accessions have been reported to have varying degrees of susceptibility/resistance to *Fusarium*, very few have shown high levels of resistance to Fusarium wilt. The recent studies, however, indicate that even the accessions reported to have wilt resistance may be susceptible to several locally isolated races of the fungi. Substantial variation in the oil content (<39 to >60%) as well as the oil quality has been reported from the extensive germplasm surveys. So far, the castor improvement programs have relied mainly on the existing natural genetic variability and characters like yield, branching type, nonshattering capsules, and oil content in seeds have been successfully improved through conventional breeding. Traditional techniques like mass selection and pedigree methods have been successfully used for developing elite genotypes with desirable traits (Moshkin 1986; Weiss 2000; Hegde et al. 2003; Morris et al. 2011; Anjani 2012).

In general, castor, though being monotypic, has good variability for most of the morphological traits, except presence of toxins (Auld et al. 2001), and this variability has also been exploited for crop improvement (Morris et al. 2011; Anjani 2012). However, due to its intensive cultivation over the years, plant breeders have used most of the natural sources of tolerance/resistance to biotic and abiotic stresses and now there is a constant need of new variability in the crop for its genetic improvement. Today, successful breeding is severely hampered by limited functional genetic diversity for productivity traits and sources of resistance to pests and diseases (Severino et al. 2012).

8.4.2.2 Distant Hybridization

With the limiting variability available, plant breeders resort to distant hybridization for widening the genetic base and to incorporate various desirable traits into the agronomically desirable species. Distant hybridization, especially with *Jatropha* species though might have potential in genetic improvement (Sujatha and Prabakaran 2003), has so far remained unexploited, due to incompatibility in the castor-Jatropha combinations probably due to poor pollen germination and abnormal pollen tube development or incompatible interaction of pollen with stigma and style (Sathaiah and Reddy 1985; Reddy et al. 1987a, b; Sujatha 1996). Reddy et al. (1987a, b) reported that there was no success in interspecific hybridization of castor with several *Jatropha* species. Sujatha (1996) reported the development of some interspecific hybrid seeds of castor × *Jatropha integerrima* but the interspecific hybrid failed to survive. The closeness of *J. integerrima*, with castor, relative to other *Jatropha* species, in their seed protein profiles and reproductive characteristics, might have been responsible for the formation of interspecific hybrid embryos (Singh et al. 2015). The likelihood of using *J. integerrima*, which is an ornamental species, as a bridge species to transfer genes from agronomically suitable *Jatropha curcas*, was also indicated (Singh et al. 2015).

8.4.2.3 Mutation Breeding

The other techniques of crop improvement like induction of mutations, though random, have been attempted with some success in castor. Mutation breeding has played an important role in the development of the present-day high-yielding varieties/hybrids from the wild perennial progenitors (Weiss 2000; Hegde et al. 2003). Mutation breeding for specific traits has been attempted successfully, especially for dwarfness. However, mutation breeding failed to develop genotypes with increased resistance to biotic stress. Altered ricinoleic and oleic acid concentration in seed oil of castor was induced through mutagenesis. The inheritance pattern of this altered oil quality in natural mutant line (OLE-1) was also studied which indicated recessive nature of this mutation and presence of epistatic interaction for this trait (Rojas-Barros et al. 2005).

8.4.2.4 Development of Nontoxic Varieties/Hybrids

Ricin, a phytotoxin, is an extremely toxic ribosome inactivating protein present in castor. An oral dose of 1–20 mg/kg body weight of ricin may prove fatal to humans (Audi et al. 2005). Small particles in abrasions and eyes have also shown lethal effects. The other toxins/allergens present in castor are ricinine-an alkaloid, *R. communis* agglutin (RCA), 2S albumin, etc. Attempts to eliminate these toxic and allergen substances have been made through traditional breeding and advanced generation lines with 70–75% less ricin and RCA were developed (Auld et al. 2001, 2003). However, this partial reduction of toxins through conventional breeding does not eliminate the toxin associated problems in castor.

8.4.2.5 Development of Hybrids

Various studies involving analyses of yield and yield components indicate significant additive, dominance as well as the presence of epistatic interactions. However, the higher magnitude of dominance in most of the studies suggested exploitation of hybrid vigor through the development of hybrids as the most suited approach for yield improvement (Singh et al. 2015). Although castor is a monoecious plant, various pistillate conditions of castor provides opportunities for easy hybrid seed production. The proportion of female and male flowers being genetically governed by several genes is greatly influenced by environmental factors, viz., temperature, day length, soil conditions, etc. The first commercial hybrid, GCH-3 in India, was developed from pistillate line TSP-10R from Texas, USA and an indigenous selection J1-15. Subsequently, an indigenous pistillate line VP-1, based on TSP-10R, was developed and several high-yielding hybrids (GCH-4, GCH-2, and GAUCH-1) were released for cultivation in India (Hegde et al. 2003). Different types of pistillate conditions exist in castor and the S-type pistillate lines (VP1, Geeta, and LRES17) were developed by applying selection pressure for late

reversion (reversion of plants to monoecious state from pistillate after appearance of inflorescences in quaternary branches/fourth order) and have been very successfully used in hybrid seed production programs till date. In another type of pistillate condition, NES type, the plant produces male flowers (interspersed staminate flowers) only if the temperature is above 35 °C. JP65, the female parent of popular castor hybrid GCH6, is an example of this type of pistillate condition employed for hybrid seed production (Hegde et al. 2003).

Conventional breeding techniques have limitations in improving the resistance to biotic stresses as well as in developing ricin-free castor due to limited variability in the existing germplasm. The other limitations of the conventional breeding in castor are long time required and lack of precision in selecting plants with desirable traits, especially the polygenically governed traits. Therefore, molecular breeding appears to be time effective and alternative approach (Singh et al. 2015).

8.5 Brief on Diversity Analysis

8.5.1 Phenotype-Based Diversity Analysis

The main descriptions on variability of castor germplasm have been furnished in Sect. 4.2.1. Here are more latest examples.

Genetic diversity of 33 accessions in a wild form in isolation across India was assessed using 18 agro-morphological traits and 29 expressed sequence tag-simple sequence repeat (EST-SSR) markers (Kanti et al. 2016). High agro-morphological and molecular variability were observed among these accessions. However, EST-SSRs separated the accessions into more groups than did agro-morphological data, implying high efficiency and resolution of EST-SSR markers in the genetic analysis of castor germplasm.

Studies on genetic variability and relatedness were conducted on 86 castor accessions at three locations in Niger state, Nigeria (Salihu et al. 2017). This study aimed to provide information for the commencement of an improvement program of castor. There was a significant variation among the accessions for all the traits evaluated such as establishment counts (%), days to first spike flowering, days to first spike maturity, branches per plant, spikes per plant, plant height at maturity, seed yield (kg/ha), and seed physical properties. There was a high divergence in seed color, seed shape, seed mottle, seed caruncle, and seed sizes among the collections. Phenotypic coefficients of variation were high for all the studied traits except for days to maturity (5.33%). High phenotypic and high genotypic coefficients of variation were observed for 100-seed weight (71.87 and 52.43%), branches per plant (41.45 and 22.73%), and spike length (54.74 and 43.17%), respectively.

Rukhsar et al. (2017) conducted a study to evaluate the diversity of 27 diverse castor inbreds using 13 morphological characters and 14 SSR markers. The genetic

advance was between 8.06% for days to 50% flowering to 76.82% for some capsules on the main raceme. The phenotypic coefficient of variation was observed to be higher than the genotypic coefficient of variation for 100-seed weight, shelling out turn and oil content. The path coefficient analysis also revealed that most nodes at primary raceme had a significant direct effect on seed yield per plant. During phenotype-based cluster analysis, Manhattan distance generated five clusters at a cutoff value of 0.19.

Mullualem et al. (2017) also conducted a field experiment in Ethiopia to study the genetic variability and association of characters among yield and yield-related traits in castor accessions. Analysis of variance revealed that there was a highly significant difference among the accessions for most of the characters studied. For all traits, the phenotypic coefficient of variation was profoundly higher than the genotypic coefficient of variation; this indicates that there was an environmental influence on these traits. The range and mean of agronomic traits obtained in the study indicated that there is sufficient variability in castor germplasm. The range of oil content observed 42.4–53.53% with a mean of 42.53% is quite high as compared to the level in other oilseeds such as linseed and sunflower.

8.5.2 Genotype-Based Diversity Analysis

Genetic diversity of castor germplasm was assessed on 41 germplasm accessions representing 35 countries using SSR and amplified fragment length polymorphism (AFLP) markers, indicating low genetic diversity and no clear geographic structure (Allan et al. 2008). Subsequently, a small set of genomic SSR markers was developed and used to assess the genetic diversity of 38 accessions from the Brazil castor germplasm collection (Bajay et al. 2009, 2011). After the genome sequencing (Chan et al. 2010), single nucleotide polymorphism (SNP) markers were developed and became available. A set of 48 SNP markers were used for genotyping 488 castor germplasm samples from 45 countries. A low level of genetic diversity was revealed and the germplasm samples were classified into five distinct groups (Foster et al. 2010). From the EST database, 118 polymorphic EST-SSR markers were developed and used for genotyping 24 castor germplasm accessions. These accessions were classified into five distinct clusters showing the geographic pattern across genetic diversity centers of castor (Qiu et al. 2010), and displayed moderate gene diversity (He) with an average of 0.41. A set of 144 inbred lines derived from the Indian castor core collection through selfing and single seed descent were genotyped with 39 SSR markers and classified into four clusters (Senthilvel et al. 2016). Because 136 lines were from India and the remaining eight were from six other countries, this collection does not fully represent the worldwide distribution or country of origin of castor accessions.

There are six major centers for castor germplasm collection in the world holding from 11,300 to 18,700 accessions (Anjani 2012; Severino et al. 2012). Identifying redundant accessions in the collection is a major challenge for germplasm

management, especially for the establishment of a core collection. A castor core collection has not been established for the US castor germplasm collection to date. A core collection with large genetic variability and well representation can be established based on genotypic data, rather than phenotypic data (Hu et al. 2000). Since EST-SSR markers were developed from the transcribed gene regions, these markers may have high distinguishing power for discerning genetic diversity and identifying redundant accessions within the species (Wang et al. 2006, 2009). Compared with using SNP markers to assess genetic diversity, using EST-SSR markers may cost less and be easier to access for a laboratory that does not have high-throughput genotyping equipment.

The genetic diversity in 31 accessions of castor representing seven geographic areas in the world was studied using random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), and start codon targeted polymorphism (SCoT) primers (Kallamadi et al. 2015). The range of average polymorphic information content (PIC) values was from 0.20 (ISSR) to 0.24 (RAPD and SCoT). Combined data of the three marker systems classified the accessions into three major clusters, cluster I included four accessions each from USA and India; cluster II was large and included 10 accessions from Nigeria, three accessions from the USA, two accessions each from Kenya, India, and Egypt, one accession each from Brazil and the former USSR; cluster III included two accessions from India. Use of three dominant marker systems targeting different regions of the genome (random, repeat regions and functional regions of the gene) on castor germplasm from seven geographical regions indicated higher to modest level of genetic variability but led to the identification of accessions with several unique bands.

Twenty-eight primer pairs of EST-SSR were chosen to determine the genetic diversity among 27 castor accessions (Thatikunta et al. 2016). The accessions were grouped into two separate clusters by the dendrogram with a polymorphic information content value of 0.28–0.49 revealing a medium level of diversity in castor.

Based on the screening results of oil content, fatty acid composition, and country origins, 574 accessions were selected and genotyped with 22 polymorphic EST-SSR markers (Wang et al. 2017). The accessions were partitioned into four subpopulations, the clusters and subpopulations aligned with geographic origins. The US castor germplasm collection encompasses a moderately high level of genetic diversity (pairwise dissimilarity coefficient = 0.53).

A study was conducted with 27 diverse castor inbreds to evaluate diversity using 13 morphological characters and 14 SSR markers (Rukhsar et al. 2017). The genotypes showed the presence of ample variability for most of the traits with high heritability (>67%). A high phenotypic than genotypic coefficients of variation was observed for 100-seed weight, shelling out turn and oil content. Seed yield per plant was significantly correlated with 100-seed weight (0.62^{**}) and shelling outturn (0.56^{**}) at genotypic level. The PIC values ranged from 0.16 to 0.68 with a mean of 0.43. A weak correlation (0.31) was recorded between the morphological and molecular matrix. The results of the present study indicated that both morphotypes and molecular markers should be deployed simultaneously to capture the actual

genetic diversity of germplasm as well as to harvest greater heterosis through hybridization.

Target region amplification polymorphism (TRAP) markers developed by Simões et al. (2017a) for castor was never been applied in castor genetic diversity before. Therefore in a goal to evaluate the genetic divergence among 40 elite lineages of *R. communis*, which belong to the NBIO-UFRB Genetic Improvement Program in Brazil, Simões et al. (2017b) used TRAP markers involved in the biosynthesis of oil and ricin for their study. Thirty-five TRAP combinations had a PIC above 0.25, with higher discrimination power among the genotypes evaluated. The genetic distance, calculated by the amount of discordance between lineages (for the genetic dissimilarity of the 40 lineages), varied between 0.20 and 0.98, with an average of 0.66.

8.5.3 Relationship with Geographical Distribution

Foster et al. (2010) conducted a survey sequencing of the genomes of seven diverse castor cultivars and compared the data to a reference genome assembly of a widespread cultivar (Hale). The population genetic structure of 676 samples was determined using SNPs at 48 loci. From the best K value, the nuclear SNP genotypes of the worldwide collection of germplasm samples (n = 488) were described into five clusters (Fig. 8.1). However, the groupings were not consistent with the country of origin. The AMOVA results indicated that most of the molecular variance occurred within populations (74%) followed by 22% among populations, and 4% among continents, which were consistent with previous work (Allan et al. 2008). Despite limited genetic variation worldwide, few countries showed groupings where the majority of genotypes were considered part of the same cluster. For countries with greater than one sample, only Botswana, El Salvador, Iran, Syria, USA (Oregon only), and US Virgin Islands had similar groupings where all samples from the same country clustered together. Hence, 39 of 45 countries had samples with genotypes from more than one group. The admixture was also prevalent within each sample, with possible membership in >1 cluster for the majority of samples.

Castor plant populations worldwide clustered into five distinct groups that were not geographically structured even though there were often high levels of pairwise population differentiation based on country of origin. This suggests that plants within a particular region may have been derived from multiple sources or introductions, likely due to human-assisted migration via domestication. Plants from an accession or country did not fall into the same genetic-based cluster; possibly due to multiple sources or introductions to individual countries.

Rivarola et al. (2011) assembled the chloroplast and mitochondrion genomes extracting selected reads from the available whole genome shotgun reads. Using the chloroplast reference genome, the methylation filtration technique was used to obtain draft genome sequences of seven geographically and genetically diverse

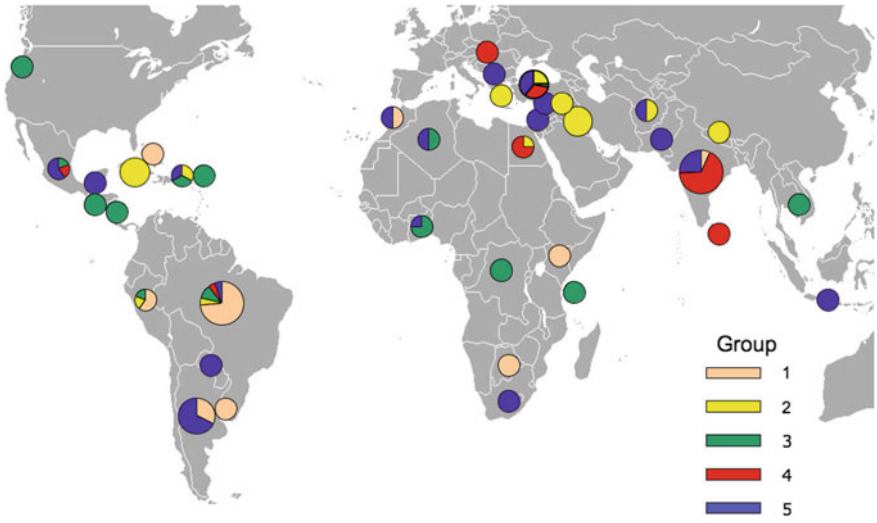

Fig. 8.1 Genotypes of *R. communis* from nuclear SNPs by Foster et al. (2010) described by five genetic clusters in a worldwide collection of 488 germplasm samples. Group colors correspond to images described in Bayesian cluster graph, and circle sizes represent a relative number of samples. Samples were only considered in a particular group if they meet a 60% threshold of group assignment. Thus, not all samples were assigned to a group because they shared an affiliation with several different groups

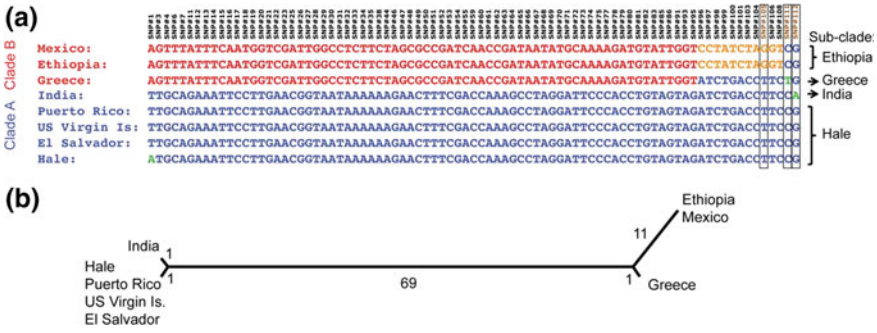

Fig. 8.2 SNP identification and phylogeny obtained from genomic sequencing of eight castor bean accessions. **a** 83 high-quality SNPs identified in the chloroplast genomes of the eight sequenced chloroplast accessions. The two major clades are shown in red and blue and SNPs that differentiate different subclades are shown in orange or green. **b** Phylogeny of the eight castor bean accessions based on the identified SNPs. Two major clades are shown separated by 69 SNPs. Members of each subclade are indicated in each branch of the phylogeny. (Rivarola et al. 2011) https://doi.org/10.1371/journal.pone.0021743.g004

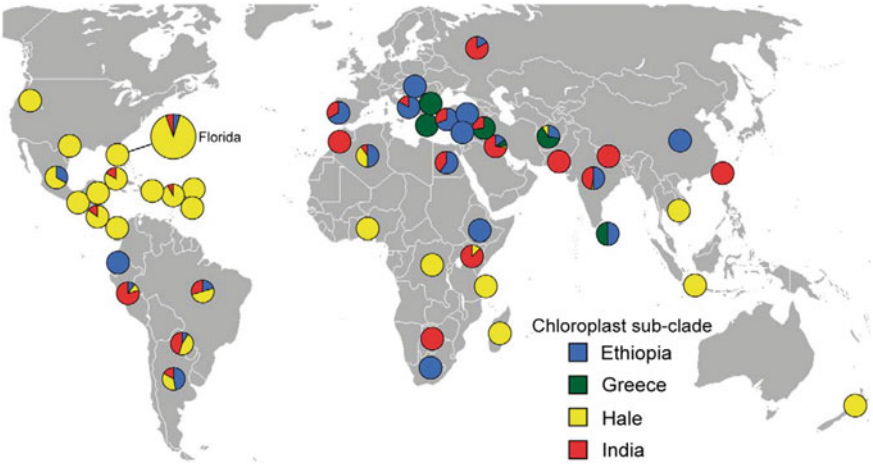

Fig. 8.3 Worldwide distribution of chloroplast genotypes of castor bean from a collection of 894 accessions. Distribution of chloroplast genotypes corresponding to the subclades shown in Fig. 8.2, based on the origin of each accession. The pie chart corresponding to Florida is expanded to reflect the larger number of samples (n = 272) that came from that state, relative to other parts of the world. (Rivarola et al. 2011) https://doi.org/10.1371/journal.pone.0021743.g005

castor accessions. SNP markers were identified using the sequence data. A phylogenetic analysis resulted in the identification of two major clades that were not discovered in previous population genetic studies using genetic markers derived from nuclear DNA (Fig. 8.2). Two distinct subclades could be defined within each major clade and large-scale genotyping of castor bean populations worldwide confirmed previously observed low levels of genetic diversity and showed a broad geographic distribution of each subclade (Fig. 8.3).

Wang et al. (2013) collected 39 accessions from North East China for their genetic diversity analysis. The maximum value of mean number of alleles (Na), the mean effective number of alleles (Ne), Nei's gene diversity (H), and Shannon's Information Index (I) all belonged to populations from the Inner Mongolia. Zhang (2010) assessed the genetic diversity 352 wild and semi-wild accessions collected from south China and revealed the highest diversity of populations from Guangdong province, followed by the Guangxi, and then Hainan province.

Wang et al. (2017) projected the results of the population structure and country of origin from the passport data in Germplasm Resources Information Network (GRIN). Over 55% of the accessions were obtained from the US, so the US accessions were classified into four different subpopulations with the genetic diversity of 0.49, 0.54, 0.39, and 0.39, respectively, showing obvious association with geographic distribution (Fig. 8.4).

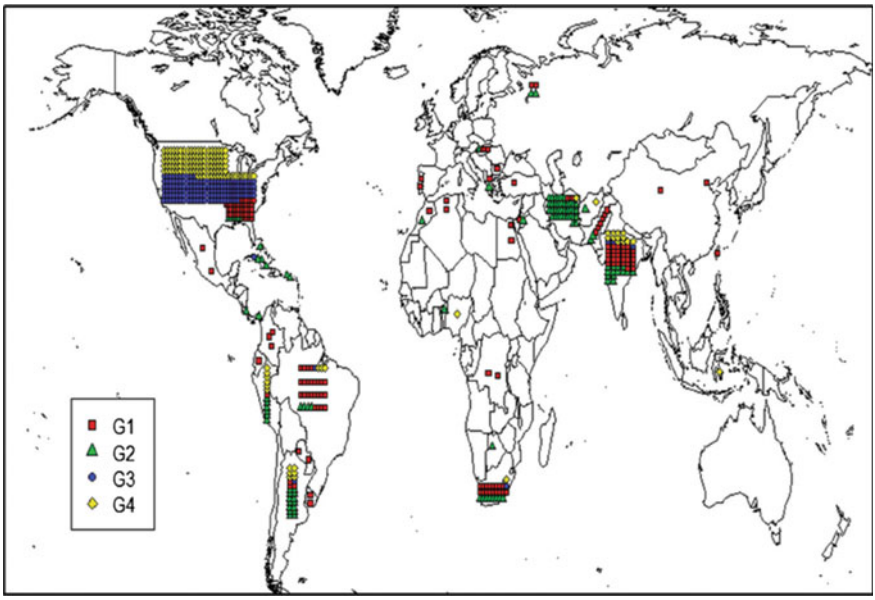

Fig. 8.4 Country origin record of castor accessions. The investigated accessions are projected to the world map. Each symbol represents one accession. The red squares, green triangles, filled blue dots, and yellow diamonds represent the accessions in subpopulations G1, G2, G3, and G4, respectively. Please note country origin records for 'The United States' indicate the location of the institution that donated the castor accessions, not necessarily the original collection site. (Wang et al. 2017)

8.6 A Brief Account of Molecular Mapping of CS Genes and QTLs

8.6.1 A Brief History of Mapping Efforts

Only two studies (Reddy et al. 2011; Singh et al. 2011) using molecular markers for Fusarium wilt control were reported before 2011. Liu et al. (2014) mapped a number of quantitative trait loci (QTLs) for plant height and related traits with SSR markers. Tomar et al. (2016) conducted another experiment on identifying molecular markers linked to Fusarium wilt resistance. Simões et al. (2017a) researched on developing TRAP primers, based on ESTs obtained from the NCBI (National Center for Biotechnology Information) databank. The forward primers were designed with the help of the Primer 3 software and, for the reverse, six arbitrary primers were used. A total of 336 combinations were obtained using the 56 fixed and six arbitrary primers. Tomar et al. (2017) researched with the aim to develop linkage map and to identify novel QTLs associated with charcoal rot resistance in castor. After screening 920 (520 RAPD, 100 ISSR, 300 SSR) primers

among the parental lines, 336 polymorphic markers were used for genotyping the mapping population to develop genetic linkage map.

8.6.2 Evolution of Marker Types

EST databases offer an opportunity for the rapid development of SSR markers. Sequence assembly and clustering of 57,895 ESTs of castor bean (Pranavi et al. 2011) resulted in the identification of 10,960 unigenes (6459 singletons and 4501 contigs) having 7429 SSRs. Averagely, the unigenes contained one SSR for every 1.23 kb of unigene sequence. These SSRs mostly consisted of dinucleotide (62.4%) and trinucleotide (33.5%) repeats. The AG class was the most common among the dinucleotide motifs (68.9%), whereas the AAG class (25.9%) was predominant among the trinucleotide motifs. A set of 611 primer pairs were designed for the SSRs, having repeat length more than or equal to 20 nucleotides, of which a set of 130 markers were tested, and 92 of these yielding robust amplicons were analyzed for their utility in genetic purity assessment of castor bean hybrids. Nine markers were able to detect polymorphism between the parental lines of nine commercial castor bean hybrids (DCH-32, DCH-177, DCH-519, GCH-2, GCH-4, GCH-5, GCH-6, GCH-7, and RHC-1), and their utility in genetic purity testing was demonstrated. These novelly developed EST-SSR markers are valuable addition to the growing molecular marker resources that could be used in genetic improvement programs of castor bean.

Three RAPD markers, viz., RKC231375, RKC211080, and OPBE18900 flanking the wilt resistance gene at a genetic distance of 5, 10.7, and 7.6 cM, respectively, were identified using linkage analysis carried out on 200 F_2 individuals obtained from a cross between the wilt-resistant cultivar 48-1 and the susceptible cultivar VP-1 (Singh et al. 2011). Another two RAPD markers, OPH-124973 and OPJ-154268 tightly linked to 'Haritha' and'48-1' at a distance of 5.0 and 7.0 cM, respectively, to wilt resistance were identified (Reddy et al. 2011). Tan et al. (2014) developed 1435 SSR primers by mining data from whole genome sequences. 2719 castor SSR markers were developed from the 5546 putative SSR loci mined from the sequenced and assembled castor genome, and an SSR genetic linkage map was constructed by Liu et al. (2016). Foster et al. (2010) reported 232 high-quality SNPs and used them to genotype a worldwide castor collection of 488 germplasm samples described in five genetic clusters.

Little experimental results were reported on gene/QTL mapping in castor. Until now, all the QTL identifications were conducted in temporary mapping populations, such as F_2 populations (Liu et al. 2014; Liu et al. 2016; Tomar et al. 2016) and $F_{2:3}$ population (Tomar et al. 2017). Software such as MapMaker/Exp3.0, QTLNetwork2.0, OneMap and R/QTL package of R software, WinQTL Cartographer 2.5, and JoinMap 4.0 were used to perform linkage analysis and linkage map construction.

8.6.3 Examples of Some Linkage Maps

Three preliminary linkage maps of castor have been published by now (Figs. 8.5, 8.6 and 8.7; Tables 8.2 and 8.3). Xuegui Yin et al. (unpublished) constructed a high-density genetic map in castor bean using specific length amplified fragment (SLAF) sequencing in 2017 (Fig. 8.8; Table 8.4).

8.6.4 Details on Traitwise QTLs

Singh et al. (2011) identified three RAPD markers (RKC231375, RKC211080, and OPBE18900) with a genetic distance of 5, 10.7 and 7.6 cM from the resistance gene responsible for Fusarium wilt resistance using 200 F_2 segregations derived from resistant parent 'cultivar48-1 ' and feeling sick parents 'cultivar VP-1'.

Reddy et al. (2011) identified two RAPD markers (OPH-124973 and OPJ-154268) with a genetic distance of 5 and 7 cM from the resistance gene responsible for Fusarium wilt with F_2 and BC_1F_1 populations derived from resistant parents Haritha and '48-1', and susceptible parent 'Kranthi'.

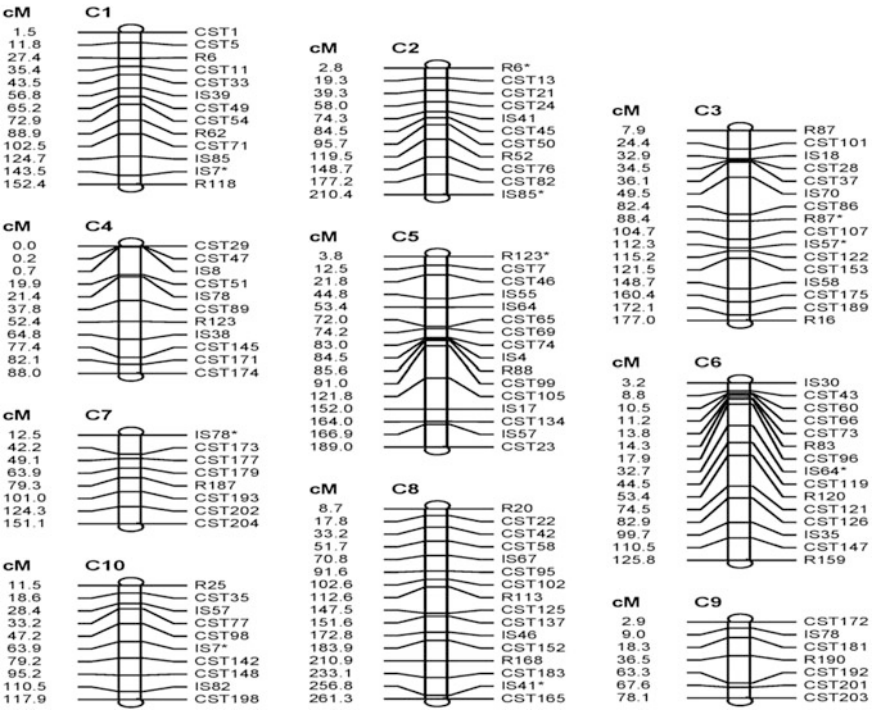

Fig. 8.5 A linkage map of castor by Tomar et al. (2016)

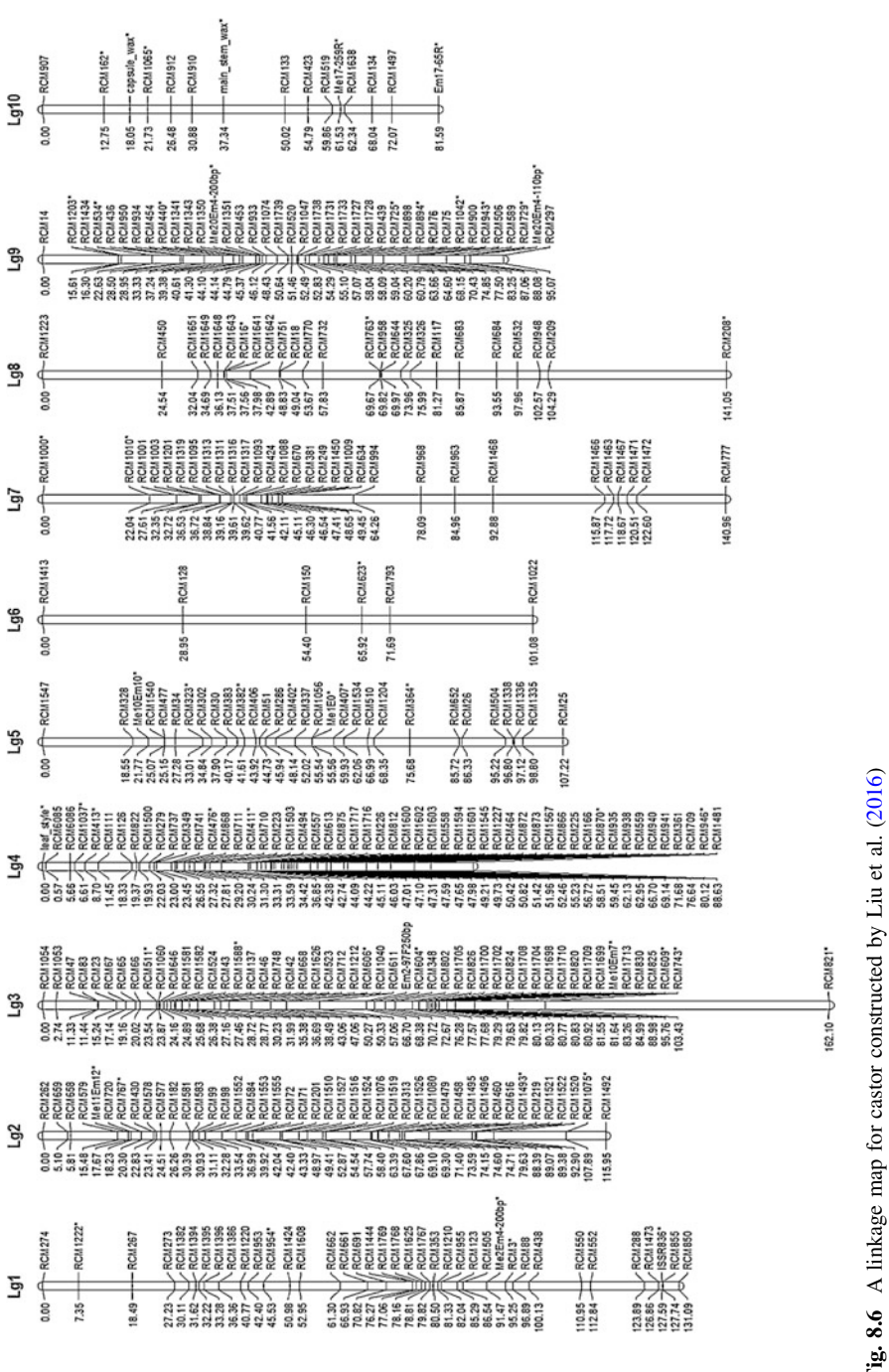

Fig. 8.6 A linkage map for castor constructed by Liu et al. (2016)

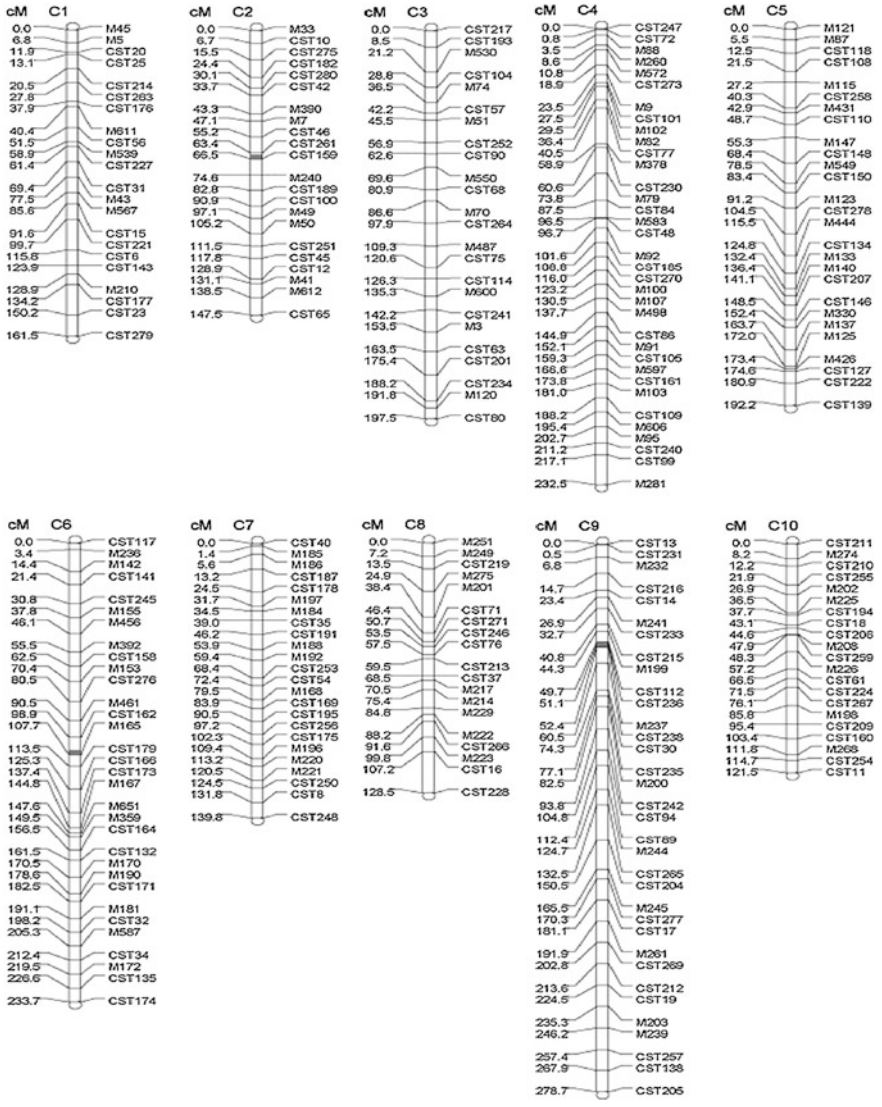

Fig. 8.7 A linkage map of castor developed by Tomar et al. (2017). Marker loci are on the right side, and cumulative recombination distance is on the left side. The presence of three QTLs is shown on the linkage group 2, 6 and 9

Liu et al. (2014) located the QTLs conferring the plant height related traits with YC2 × YF1 F_2 population (Tables 8.5 and 8.6) to reveal that that the additive effect was the main genetic components of plant height, main stem height, and internode length of main stem, while epistatic effect was the main genetic component of node number of main stem.

Table 8.2 Characteristics of the genetic linkage map of *R. communis* L. by Liu et al. (2016)

LG	LG size (cM)	Marker number	Average interval (cM)	Max gap	All marker percentage (%)	Segregation distortion marker number	Segregation distortion marker percentage (%)	Frequency of segregation distortion marker (%)
LG 1	131.09	38	3.54	11.14	11.48	5	9.43	13.16
LG 2	115.95	44	2.70	15.00	13.29	4	7.55	9.09
LG 3	162.11	51	3.24	58.68	15.41	8	15.09	15.69
LG 4	88.63	53	1.70	8.52	16.01	7	13.21	13.21
LG 5	107.22	30	3.70	18.55	9.06	7	13.21	23.33
LG 6	101.08	6	20.22	29.38	1.81	1	1.89	16.67
LG 7	140.96	30	4.86	22.99	9.06	2	3.77	6.67
LG 8	141.05	25	5.88	36.76	7.55	3	5.66	12.00
LG 9	95.07	39	2.50	15.61	11.78	10	18.87	25.64
LG 10	81.59	15	5.83	12.75	4.53	6	11.32	40.00
Total	1164.73	331	3.63	58.68	100.00	53	100.00	16.01

Table 8.3 Salient features of genetic map of castor by Tomar et al. (2017)

LG	No. of mapped markers				Density (marker/cM)	No. of interval (>1 cM)	No. Of gaps (>10 cM)	Average marker interval (cM)	Length (cM)
	PAPD	ISSR	SSR	Total					
LG1	4	3	15	22	0.13	21	5	7.34	161.5
LG2	3	5	14	22	0.15	21	1	6.70	147.5
LG3	5	4	15	24	0.12	23	8	8.23	197.5
LG4	11	8	16	35	0.15	34	3	6.64	232.5
LG5	12	2	13	27	0.14	26	7	7.11	192.2
LG6	16	0	16	32	0.14	31	5	7.30	233.7
LG7	7	3	14	24	0.17	23	1	5.82	139.8
LG8	8	1	10	19	0.15	18	3	6.76	128.5
LG9	5	5	24	34	0.12	33	16	8.19	278.7
LG10	5	2	14	21	0.17	20	0	5.78	121.5
Total	76	34	151	261	–	250	49	–	1833.4
Average	7.6	3.4	15.1	26.1	0.14	–	5.7	6.93	–

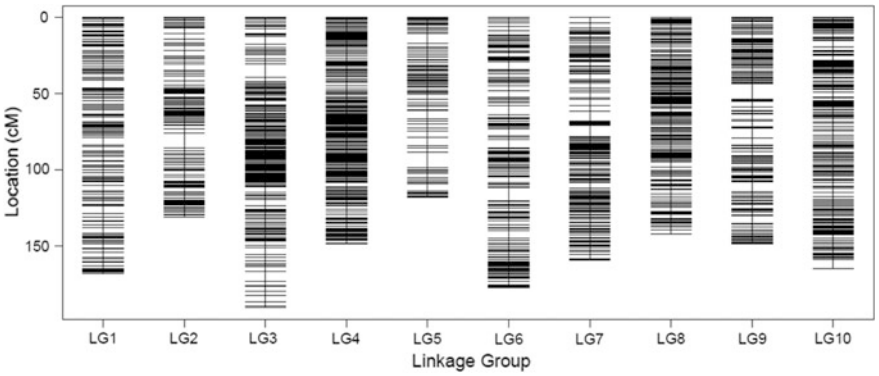

Fig. 8.8 High-density genetic map of castor bean based on SLAF markers by Xuegui Yin et al. (unpublished)

Table 8.4 Characteristics of the 10 LGs in the SLAF-based genetic map Xuegui Yin et al. (unpublished)

Linkage group ID	Total marker	No. of SSR marker	Total distance (cM)	Average distance (cM)	Max gap	SNP number	No. of anchored scaffolds	Scaffold length
LG1	272	11	167.79	0.62	5.35	344	55	22,278,757
LG2	179	13	131.02	0.74	9.74	230	32	13,871,998
LG3	707	17	190.47	0.27	8.86	1028	87	20,944,873
LG4	1064	16	148.5	0.14	2.54	1436	97	26,275,325
LG5	149	0	117.88	0.80	10.12	184	23	19,792,242
LG6	346	27	177.25	0.51	8.45	420	53	23,652,170
LG7	387	13	159.06	0.41	7.39	486	43	21,427,524
LG8	684	9	142.08	0.21	3.2	923	76	23,966,805
LG9	207	12	148.54	0.72	10.13	252	43	14,362,120
LG10	425	2	164.82	0.39	6.23	555	57	28,132,081
Total	4420	120	1547.41	0.35	10.13	5858	566	214,703,895

Tomar et al. (2016) detected two QTLs responsible for Fusarium wilt resistance in two linkage groups, one was present on linkage group 6 while the other was present on linkage group 8 at a threshold value of 90%. However, at a threshold value of 95%, only one QTL at linkage group 6 was identified with a LOD score of 13.5. Confidence interval at linkage group 6 indicated QTL location between 12 and 16 cM nearer to the marker CST 73 and R83. Of these identified markers, CST 73 is an SSR marker while R83 is a RAPD marker.

Table 8.7 shows the information of three novel QTLs for charcoal rot resistance identified by Tomar et al. (2017).

Table 8.5 QTLs plant height related traits detected by Liu et al. (2014)

Trait	QTL	Marker interval	Marker position (cM)	QTL position (cM)	Confidence interval (cM)
PH	*PH1-1*	RCM134–259R/Me17	26.0–43.8	41	33.0–47.8
	PH7-1	RCM219–RCM313	0–22.8	1	0–18.0
	PH8-1	RCM872–RCM226	33.0–55.7	46	31.0–48.0
	PH9-1	RCM579S–RCM182	242.7–263.1	242.7	237.5–250.7
	PH11-1	RCM51 h–RCM51	0–5.1	5	4.0–5.0
PRH	*PRH5-1*	RCM611–97F/Em2	330.5–347.6	334.5	326.3–341.5
	PRH7-1	RCM458–RCM1080	23.8–46.3	23.8	19.0–33.8
	PRH8-1	RCM872–RCM226	33.0–55.7	46	20.0–48.0
	PRH9-1	RCM579S–RCM182	242.7–263.1	242.7	238.5–253.7
MSNN	*MSNN6-1*	RCM88–Me12Em9	91.1–116.1	91.1	91.1–98.1
	MSNN6-2	Me12Em9–RCM855	116.1–137.6	116.1	115.1–121.1
	MSNN6-3	RCM855–ISSR836	137.6–137.6	137.6	132.1–141.6
	MSNN9-1	RCM948 h–RCM948c	174.0–184.1	178	167.2–182.0
	MSNN10-1	RCM520–RCM1047	43.8–48.2	43.8	38.8–45.8
	MSNN13-1	RCM933 h–RCM933	0–3.2	3	1.0–8.2
MSIL	*MSIL5-1*	65F/Em1–RCM611	315.3–330.5	320.3	315.3–327.3
	MSIL8-1	RCM126–RCM737	64.9–73.1	65.9	64.9–73.7
	MSIL9-1	RCM450–RCM732	74.8–93.6	81.8	74.8–91.8
MSD	*MMSD9-1*	RCM579B–RCM579S	236.5–242.7	241.5	235.5–242.5
	MMSD14-1	$Me2Em4_{-250bp}$– $Me2Em4_{-400bp}$	0–16.8	16	0–16.0

PH plant height; *PRH* bearing height of primary raceme; *MSNN* node number of main stem; *MSIL* length of main stem internode; *MSD* main stem diameter

8.7 Genomics-Aided Breeding for CS Traits

Through sequencing technology, draft genome of castor has been assembled, which helped researchers to understand the metabolic pathway such as the ricinoleic acid synthesis, the development of sex type, the formation of resistance, and tolerance, etc., and facilitated the development of castor varieties that can be grown on marginal lands (such as saline soil), heavy metal contaminated areas, and varying weather conditions (semiarid region) usually unsuitable for crops. In addition, castor could be used for phytoremediation.

Table 8.6 The effects of QTLs for plant height related traits detected by Liu et al. (2014)

Trait	QTL_i	QTL_j	a_i (h_a^2)	d_i (h_d^2)	a_j (h_a^2)	d_j (h_d^2)	aa_{ij} (h_{aa}^2)	ad_{ij} (h_{ad}^2)	da_{ij} (h_{da}^2)	dd_{ij} (h_{dd}^2)
PH	*PH9-1*		–10.34*** (6.04%)							
	PH7-1	*PH8-1*			33.59*** (23.7%)	20.71*** (2.6%)	17.8*** (0.7%)	26.5*** (3.23%)	–19.7*** (2.1%)	
	PH1-1	*PH11-1*							–40.7*** (1.6%)	107.0*** (5.9%)
PRH	*PRH7-1*	*PRH8-1*	8.01*** (8.0%)	14.88*** (7.4%)	13.0*** (11.6%)	9.28*** (3.0%)				
	PRH5-1	*PRH9-1*			–8.92*** (4.9%)				–17.5*** (5.5%)	–26.0*** (4.9%)
MSNN	*MSNN6-3*	*MSNN9-1*	–2.02*** (9.0%)		2.04*** (9.6%)	–1.6*** (0.8%)	–4.5*** (14.0%)	4.7*** (6.6%)	–3.8*** (3.76%)	4.9*** (3.07%)
	MSNN6-1	*MSN13-1*					2.3*** (4.5%)	–3.3*** (0.35%)	4.4*** (0.01%)	3.9*** (0.01%)
	MSNN6-2	*MSN13-1*					–8.07*** (0.32%)		–9.14*** (5.1%)	–5.85*** (1.81%)
	MSN10-1	*MSN13-1*						1.38*** (0.16%)	–2.8*** (7.0%)	
MSIL	*MSIL8-1*		1.33*** (36.28%)	1.07*** (10.4%)						
	MSIL5-1	*MSIL9-1*					–1.2*** (6.28%)			2.0*** (2.4%)
MSD	*MSD9-1*	*MSD14-1*						8.04*** (1.3%)	6.80*** (11.3%)	

*** Significance at 0.005 probability level. *PH* plant height; *PRH* bearing height of primary raceme; *MSNN* node number of main stem; *MSIL* length of main stem internode; *MSD* main stem diameter

Table 8.7 Quantitative trait loci confirming resistance to charcoal rot in castor

Linkage group	QTL peak (cM)	Marker locus		LOD	R^2 (%)	Additive effect
C2	65.6	CST261	CST159	6.5	71.2	0.86
C6	106.8	CST162	M165	3.4	12.5	0.85
C9	51	CST112	CST236	2.8	11.3	0.63

8.7.1 Details of Genome Sequencing

Castor is not a model plant, so its genome sequence level remains on scaffolds. Till now, there is only one draft genome published. Chan et al. (2010) developed draft genome of castor using a Biomek FX (Beckman Coulter) pipetting workstation in 2010. A total of 2,079,000 k high-quality sequences were generated and the average of read-length was 839 bp. Celera assembler was used to assemble genome with 'overlay-layout-consensus' strategy. The final assembly spans 350.6 Mb containing 25,828 scaffolds, and the size of the genome is consistent to the previous study. The details of draft genome are shown in Table 8.8. Over 50% of the draft genome sequence was considered as repetitive sequences (Table 8.9).

8.7.2 Gene Annotation

Multiple gene prediction strategies (including Homologous annotation, PASA tools, expressed sequence tags and Evidence Modeler program) were used to gain more accurate gene model. A total of 31,237 gene models (Table 8.10) were identified. Among them, 58.8% could be grouped in 3020 protein families, and ricin gene family, oil metabolism genes, and disease resistance genes were studied in detail.

Table 8.8 Genome assembly statistics for the draft sequence of the castor bean genome. (Chan et al. 2010)

	All scaffolds
Fold genome coverage	4.59
Number of scaffolds	25,828
Total span	350.6 Mb
N50 (scaffolds)	496.5 kb
Largest scaffold	4.7 Mb
Average scaffold length	14 kb
Number of contigs	54,000
Largest contig	190 kb
Average contig length	6 kb
N50 (contigs)	21.1 kb
GC content	32.50%

Table 8.9 Classification of repetitive sequences in the draft sequence of the castor bean genome (Chan et al. 2010)

	Length occupied (bp)	Total repeats (%)	Genome (%)
Retrotransposons	61,199,930	36.07	18.16
Gypsy	38,595,566	22.75	11.45
Copia	16,078,721	9.48	4.77
Line	465,220	0.27	0.14
Sine	1867	0.00	0.00
Other	6,058,556	3.57	1.80
Unclassified elements	105,387,872	62.12	31.26
DNA transposons	3,065,391	1.81	0.91
Total transposable elements	16,348,051	25.33	50.33
Low complexity sequences	6,348,051	0.95	1.88

8.7.3 *Impact on Germplasm Characterization and Gene Discovery*

The genome sequence (Chan et al. 2010) of castor could play a crucial role in accelerating genetic improvement of castor.

Ricin gene family is very important because it is related to biosecurity. This kind of gene is considered has three domains including one N-terminal RIP domain and two C-terminal lectin domains. Only 6–8 members were detected in ricin gene

Table 8.10 Genome annotation statistics for the draft sequence of the castor bean genome (Chan et al. 2010)

	All scaffolds
Gene models	31,237
Gene density	11,220 bp/gene
Mean gene length	2258.6 bp
Mean coding sequence length	1004.2 bp
Longest gene	15,849 bp
Mean number of exons per gene	4.2
Mean exon length	251 bp
Longest exon	6590 bp
GC content in exons	44.50%
Mean intron length	381 bp
Longest intron	33,291 bp
GC content in introns	31.80%
Mean intergenic region length	6846 bp
Longest intergenic region	691,597 bp
GC content in intergenic regions	30.70%

family (Halling et al. 1985; Tregear and Roberts 1992) before the draft genome. Through the sequencing of the genome, researchers found there were 28 putative genes of this family, which is about four times than previous studies. In addition, they found these genes' unique distribution through the whole genome-tend to be clustered in genome.

Ricinoleic acid is an important content in castor oil and were widely used in numerous bio-based products (Lung and Weselake 2006). Traditional techniques limited the mining of this kind of genes. For example, heterologous expression of castor bean genes in *Arabidopsis thaliana* increased only 17% hydroxyl-fatty acid content in seed oils, which is much lower than that in castor. While the published castor genome gave the possibility for researchers to study these genes in genome level (Cagliari et al. 2010). A total of 26 genes were discovered and classified in six groups, participating different steps in triacylglycerols (TAGs) biosynthesis.

Before the genome sequencing, it was difficult to develop SSR markers. Many of the development procedure is depending on microsatellite-enriched library, which is costly and inefficiently. As a result, there were less than 400 (Allan et al. 2008; Bajay et al. 2009; Qiu L et al. 2010; Bajay et al. 2011; Pranavi et al. 2011; Seo et al. 2011; Machado and Silva 2013) SSR markers available before 2014. Benefit from the release of genome database, using whole genome information to develop SSR markers become feasible. Tan et al. (2014) and Liu et al. (2016) developed 1435 and 2719 SSR primer pairs separately, which increased the available SSR markers greatly.

SNP is another popular marker, and more and more researchers were benefited from the genome sequencing. Through mapping sequencing to genome database, researchers can also find out methylation event (Xu et al. 2014) in castor's endosperm.

8.7.4 Application of Structural and Functional Genomics in Genomics-Aided Breeding

Biotechnological interventions play a vital role in the improvement of *R. communis* (Sujatha et al. 2008). Marker-assisted selection (MAS) is a useful process that an interested trait is selected based on marker linked to the trait and has achieved success in several crops. Through developing reliable markers relate to wilt-resistant genes, research accelerated the breeding program in evolving wilt-resistant cultivars (Kumar et al. 2015), drought tolerance, salinity tolerance, etc.

8.7.4.1 Disease Resistance: Wilt

Anjani (2005a) found that purple color castor, RG 2008 and RG 1930, had multiple resistances, including against wilt and leafminer. In addition, he found that the resistance was related to the color—when the female parent was purple color the hybrid showed purple, in contrast, if the male parent was purple color the F_1 showed intermediate color. Thus, purple-colored morphotypes can be used as genetic marker for selecting leafminer and wilt disease resistance materials in castor improvement.

Until now, several molecular markers linked to wilt resistance in castor had been reported and been used in breeding programs. Three RAPD markers, viz., RKC231375, RKC211080, and OPBE18900 flanking the wilt resistance gene at genetic distance of 5, 10.7, and 7.6 cM, respectively, were identified using linkage analysis carried out on 200 F_2 individuals obtained from a cross between the wilt-resistant cultivar 4-1 and the susceptible cultivar VP-1 (Singh et al. 2011). In another study, two RAPD markers, OPH-124973 and OPJ-154268 tightly linked to 'Haritha' and'48-1' at a distance of 5.0 and 7.0 cM, respectively, to wilt resistance were identified (Reddy et al. 2011). Tomar et al. (2016) identified a QTL responsible for wilt resistance on linkage group 6 nearer to marker CST 73 and R 83. Genome-wide association studies (GWAS) method has also been used to screen the wilt resistance in castor. Mir (2014) used 74 SSRs makers to associate the wilt resistance in 96 germplasm accessions (including cultivated and wild forms). General linear model (GLM) without Q matrix was used to identify the marker-trait association and only one marker, RCM9109, showed significant linkage to the wilt resistance. Tomar et al. (2017) reported three QTLs for charcoal rot resistance identified with the $F_{2:3}$ population. They are located on linkage group 2, 6, and 9, flanked by the two markers CST261 and CST159, CST162 and M165, and CST112 and CST236, respectively, and explained 71.2, 12.5, and 11.3% of phenotypic variation, respectively. Till now, researchers tried several methods to mine the candidate QTLs/genes related to disease resistance and have got initial progress.

Phyto-oxylipins are a group of biologically active molecules that play an important role in plant defense. A total of 12 candidate *LOX* genes were identified in the castor genome, and these *LOX* members could be clustered in two groups. Quantitative real-time (RT)-PCR implied *Rc-LOX5* plays an important role in wilt resistance in castor (Mhaske et al. 2013).

A strategy utilizing molecular markers to detect and exploit genetic variation in wilt resistance integrated with an understanding of the ultrastructural host–pathogen interaction could play a major role in understanding the genetic control of resistance to Fusarium wilt in castor (Kumar et al. 2015).

8.7.4.2 Insect Resistance

Castor plant is not toxic to most insects, even though contains toxic protein, ricin, and the alkaloid tricinine in vegetative parts of the plant (Weiss 1971). Thrips, corn earworms, armyworms, spider mites, leaf miners, lygus bugs, and green stink bugs have been observed in castor fields with minimal damage (Brigham 1993), while castor semilooper (*Achoea Janata* L.), *S. litura*, and capsule borer (*Dichocrocis Punctiferaus* Guen.) are voracious feeders causing extensive defoliation and yield losses (Kumar et al. 2011).

Anjani (2005a) found purple-colored castor to show multiple resistance including against serpentine leafminer. Patel and Patel (2009) presented an idea to manage shoot and capsule borer, *Conogathes punctifaralis* L., through intercropping. He found intercropping of castor crop with green gram or sesame to be profitable and could give higher seed yield and low infestation of capsule borer. In recent years, breeding for insect resistance is being approached by transgenic breeding. Transgenic plants containing *cry1AcF* were found to have insect tolerance ability (Malathi et al. 2006; Kumar et al. 2011). Anjani et al. (2010b) established significant correlation between high total phenol concentrations and leafminer resistance in castor and projected that selecting dark purple leaf phenotype in breeding programs is an effective visual and reliable approach for indirect selection of leafminer resistance.

8.7.4.3 Drought Tolerance

In recent years, researchers found that drought resistance is significantly correlated with osmotic adjustment (OA), root characters, and water use efficiency (WUE, efficient use of absorbed water). In addition, WUE showed significant genetic variability and also has heritability (Wright et al. 1988; Farquhar et al. 1989). OA is an active accumulation of solutes in response to drought, which gives plants drought adaptation in several crops. The hybrids, GCH4, DCH32, and DCH177, and their respective parents, VP-1, 48-1, LRES17, DCS5, DPC9, and DCS9, were studied for their OA, and found total soluble sugars (TSS) is the major contributor towards OA in castor leaves. In addition, OA showed positive relationship with seed yield under drought stress, and high OA (HOA) genotypes had higher total seed yield than the genotypes with low OA (LOA). For example, the hybrids GCH 4 and DCH 177 are HOA genotypes and have 53% higher total seed yield than the genotypes with LOA (Babita et al. 2010).

Nuclear factor-Y transcription factors, which function in responding to abiotic stresses, were identified and characterized in castor plant (Wang et al. 2018). The expression changes of *RcNF-Y* members in 2-week-old seedlings under drought, cold, hot, and salt stresses were investigated. The expression levels of 20 *RcNF-Y* members were found being changed and three *RcNF-Y* members might function in response to abiotic stresses.

Patrícia Favoretto Moraes et al. (2014) analyzed the differential expression of genes potentially related to tolerance to water deficit with three castor accessions (China Careca, IAC 2028 and PB 07) subjected to polyethylene glycol (PEG 6.000) for induction of drought. CAT, APX, SOD-Cu/Zn, Fe-SOD, and Mn-SOD genes had higher differential expression in China Careca, and the SOD-Cu/Zn gene was the most differentially expressed in China Careca when compared to control (3.57-times), indicating that China Careca can be considered of great importance for breeding programs by presenting the resistance to drought.

8.7.4.4 Heavy Metal Tolerance and Soil Remediation

Soil contaminated by heavy metal (Cadmium, Lead, Mercury and Arsenic) has become a global environmental problem and it is crucial to remediate this soil (Järup 2003). Phytoextraction is an environment-friendly technique used for the decontamination of soil polluted by heavy metal, and castor is a good choice (de Souza Costa et al. 2012).

Cadmium (Cd) is high toxic to plants, animals, and humans. Cd is easily accumulated in human body via the food chain (Cd-contaminated crop), endangering human health. The effects of Cd on tolerance and antioxidant activities of castor were widely studied (Niu et al. 2009; Huang et al. 2011; Zhang et al. 2014). High concentration of Cd (17.50 mg Cd kg^{-1} soil) was set for exploring the tolerance of castor and remediation. Cd at 51% was accumulated in roots and rest of the metal was transferred to the stem and leaves. The growth, biomass, and yield between control and Cd-treated plants did not show significant differences. The seed yield per plant was reduced only by 5% of Cd-contaminated plants than control (Bauddh and Singh 2012).

A hydroponic experiment was conducted to investigate the Cd accumulation and subcellular distribution characteristics in castor (Zibima 3) with different Cd doses in nutrient solution (0, 10, 25, 50, 100, 200, 400 μmol L^{-1}) (Chen et al 2014). The results showed no significant differences of MDA (malondialdehyde) contents between all Cd treatments, but chlorophyll contents are sensitive to Cd supply, so chlorophyll contents can be used as an important index of Cd tolerance in castor. The Cd contents and uptakes in roots, stems, and leaves increase with elevating Cd supply in nutrition solution. The Cd contents in the plant tissues are generally in the sequence root>stem>leaf. The roots demonstrate high accumulation and sequestration capacity of Cd. The Cd subcellular distribution is in the order of soluble fraction>cell wall>organelle in both roots and leaves. In addition, the Cd accumulation percentages in cell wall show increasing trend with elevating Cd supply. The low translocation ability of Cd from root to stem may be the limiting factor of accumulation ability in aerial parts.

Castor could grow normally in soils containing 800 mg/kg dry weight lead (Pb) for 30 days. Researchers reported that transcript levels of genes coding for ABC transporters were positively related to Pb concentration in soil and this gene family only expressed in roots (Pal et al. 2013).

The natural occurrence of arsenic (As) in ground and surface water hazards health of 200 million people all over the world (Naujokas et al. 2013). Extremely high concentration of Arsenic (5000 μg L^{-1}) did not result in severe toxicity symptoms in castor and did not affect the accumulation of nutrients as well (Melo et al. 2009). As a result, castor could be used for revegetation of As-contaminated areas. The transportation of As in phloem was also studied. As (V) was the main species in xylem exudate (55–83%) whereas As (III) predominated in phloem exudate (70–94%).

8.7.4.5 Transcriptomic Analysis on Pistillate Character

Transcriptomic analysis showed that identical dynamic changes of gene expression were indicated in monoecious and female apical bud during its development from vegetation to reproduction, with more genes expressed at the raceme formation and infant raceme stages compare to the early leaf bud stage. More than 3000 of differentially expressed genes (DEGs) were detected in *Ricinus* species at three developmental stages between two different sex types. A number of DEGs involved in hormone response and biosynthesis, such as auxin response and transport, transcription factors, signal transduction, histone demethylation/methylation, programmed cell death, and pollination, putatively associated with sex expression and reproduction were discovered, and the selected DEGs showed consistent expression between qRT-PCR validation and the DGE patterns. Most of those DEGs were suppressed at the early leaf stage in buds of the mutant, but then activated at the following transition stage (5-7-leaf stage) of buds in the mutant, and ultimately, the number of upregulated DEGs was equal to that of downregulation in the small raceme of the mutant (Tan et al. 2016).

8.8 Achievements of Transgenic Technology

Introduction of alien gene controlling specific agronomically important traits into castor through genetic transformation techniques, such as *Agrobacterium tumefaciens* mediated method, direct gene transfer to protoplast by chemical and electrical means and particle gun method, is of great importance in eliminating the problems associated with the integration of undesirable genes along with the useful traits from *R. communis* through sexual and parasexual hybridization. Castor is a monotypic genus, and the limited diversity of varieties restrained the breeding process. Besides, transgenic technology has another problem for castor because of its recalcitrance to tissue culture. Using meristem as the target tissue could be a successful trial of transgenic method in castor.

Auld et al. (2001) reported on 'knocking out' the genes responsible for ricin production as well as genes responsible for the production of ricinine and CB-1A. Random mutations were introduced into the castor genome through targeting

induced local lesions in genomes (TILLING) technique. McKeon and Chen (2003) transformed castor plant through *Agrobacterium*-mediated transformation using vacuum infiltration of wounded flower buds. The first successful attempt to develop a stable transformation system for castor using vegetative explants was reported by Sujatha and Sailaja (2005), and the frequency of recovery of putative transformants was only about 0.08%. Semilooper-resistant (Malathi et al. 2006) transgenic castor plant was produced by this method, and insect bioassays were also been studied that confirmed the toxicity of Cry1Ab protein expressed in the transgenic castor plants. This protocol has been extensively used for development of transgenic lines of castor resistant to major foliage feeders through the deployment of *Cry1Ec*, *Cry1Aa*, and *Cry1Ab* genes by Sujatha and Sailaja (2007). The first successful transformation protocol in castor bean based on particle delivery system through using embryonic axes as acceptois was reported by Sailaja et al. (2008). Sujatha et al. (2009) transformed castor (cv. DCS-9) through *Agrobacterium*-mediated and particle gun bombardment methods using appropriate vectors containing the *Bt* chimeric gene *cry1EC* to incorporate the resistance to *S. litura* and castor semilooper. Kumar et al. (2011) used the *Agrobacterium* strain EHA105/pBinBt8 harboring *cry1AcF* to infect 2-day-old seedlings and got the putative insect tolerance transformants. Another success of this method was the salt tolerant transgenetic plants (Patel et al. 2015). Through expressing the gene *SbNHX1*, which comes from extreme halophyte *Salicornia brachiata*, the castor lines enhanced their ability of salt endurance.

A relatively advanced genetic transformation system, the acupuncture-vacuum infiltration assisted *Agrobacterium* transformation in castor has been patented and created a batch of transgenic lines with a transformation rate of over 75% (Lu et al. 2016). Using this transformation system, the function of phenylalanine ammonialyase gene (*RcPAL*) and phosphoenolpyruvate carboxylase gene (*RcPEPC*) were studied. A batch of plants with oil content increased over 5% was obtained by introducing the antisense gene of *RcPEPC* 1 (YueguiYin, personal communication).

8.9 Genes Identified in Castor

The reference genome of castor (Chan et al. 2010) facilitated the identification and characterization of genes involved in biosynthesis of oil (Cagliari et al. 2010). Seed oil content can be increased by manipulation of the expression levels of key enzymes in the triacylglycerol biosynthetic pathway such as diacylglycerol acyltransferase (He et al. 2004), glycerol-3-phosphate dehydrogenase (Mi et al. 2011), and lysophosphatidyl-acyltransferases (Arroyo-Caro et al. 2013). The cloning and characterization of candidate genes involved in fatty acid metabolism are being attempted (Eastmond 2004; Kim et al. 2011). Gaining a better understanding of castor oil biosynthesis holds potential of even higher oil contents and modified fatty acids profiles (Auld et al. 2009). Phosphatidylcholine-hydrolyzing phospholipase D was also cloned in castor, which plays a significant role in the signal transduction

pathways (Wang et al. 1994). Application of genetic engineering to knock out the genes governing the production of toxins and allergens could make castor even more suitable for wide cultivation across various regions. The genes that produce toxic proteins are expressed at higher levels during seed development (Chen et al. 2004, 2005), however, the expression of these genes could be downregulated by 10,000 folds with the use of appropriate promoters and suitable antisense gene constructs for gene-silencing. The cloning and functional analysis of gene *RcPAL* (*R. communis* phenylalanine ammonialyase) were conducted to reveal its key role in lignin biosynthesis in castor bean (Jiannong Lu, Personal communication)

8.10 Role of Bioinformatics as a Tool

Bioinformatics is a very important research tool in genetics, genomics, and other omics. The gene anotation, gene function analysis, etc. need detailed information provided by bioinformatics, but the accumulated data in castor plant is too inadequate, relative to the main crops such as rice, wheat, maize, soybean, etc., to meet the requirements of corresponding research. Some databases with limited information are presented below.

8.10.1 Gene and Genome Databases

Till now, only one draft castor genome has been published. The genome sequence covered 4.6 folds of the whole genome and contained 31,237 genes (Chan et al. 2010). It can be downloaded from TIGR site or NCBI: http://castorbean.jcvi.org/downloads.php.

Organelle genome sequencing including of chloroplast and mitochondrion was assembled using the Celera Assembler, they contained 163,161 and 502,773 bp, respectively (Rivarola et al. 2011).

8.10.2 Comparative Genome Databases

Through comparison of different species in the same family of Euphorbiaceous, the information can help researchers better understand the structure and function of castor genes, and develop new strategies to improve the oil content and reduce the ricin in castor seeds. Three comparative genome studies of castor with different species including, cassava (Prochnik et al. 2012) and Jatropha (Sharma 2012; Zou et al. 2018) provided new insights into lineage-specific evolution in eudicots. Prochnik et al. (2012) made a global comparison of cassava and castor bean

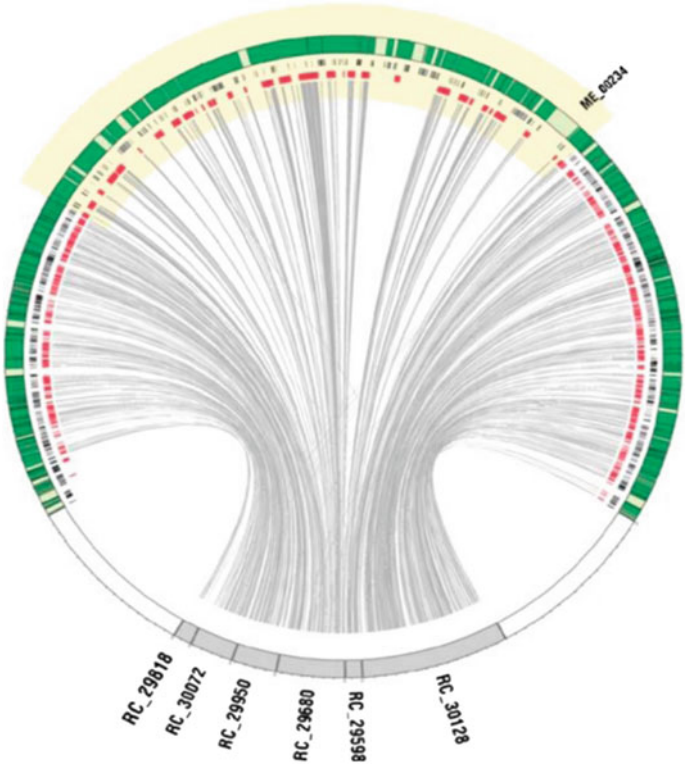

Fig. 8.9 Circos plot showing cassava-castor bean colinearity (Prochnik et al. 2012)

genomes, and the result indicated extensive colinearity between them. The Fig. 8.9 shows cassava-castor bean colinearity in a sample 2.7 Mb region.

Sharma and Chauhan (2011) studied the SSR markers in castor, and their transferability to Jatropha, 211 of 302 SSRs (approximate 70%) were successfully transferred to Jatropha from castor bean genome.

Fatty acid and TAG biosynthesis genes in different plant species were analyzed, perusing the correlation between sequences and contents or composition of fatty acids in seed oil. A total of 55 castor bean genes were selected to perform the structure comparison with Jatropha, which will be helpful to enhance the content of both saturated fatty acids and unsaturated fatty acids in oilseed plant species (Sharma 2012).

Papain-like cysteine proteases (PLCPs) play crucial roles in plant-pathogen/pest interactions (Zou et al. 2018). A total of 26 or 23 PLCP genes were identified from the genomes of castor bean and physic nut, respectively. A phylogenetic tree including 80 $PLCP_S$ from castor bean, physic nut, and Arabidopsis is shown below (Fig. 8.10), and these genes can be divided into nine subfamilies.

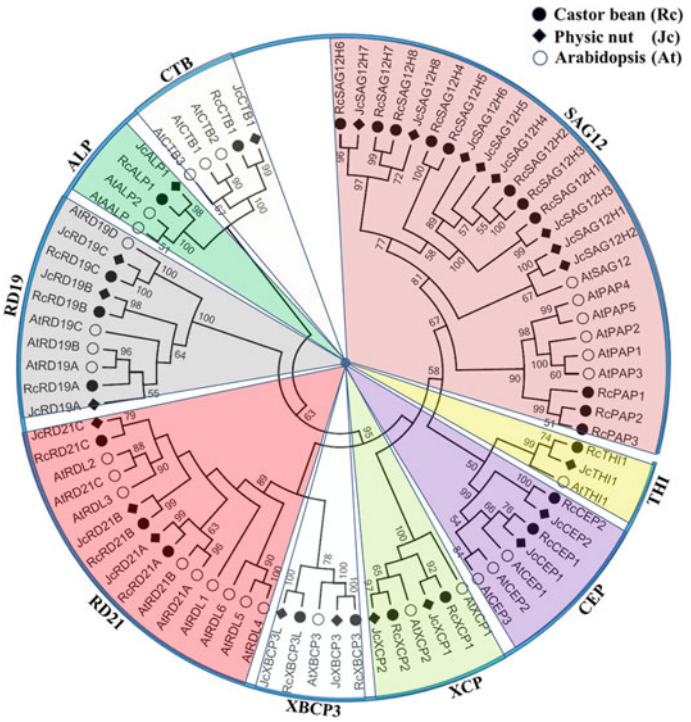

Fig. 8.10 Phylogenetic analysis of castor bean, physic nut, and Arabidopsis PLCPs

8.10.3 Gene Expression Databases

Compared with the main crops such as rice, maize, wheat, and model plant such as Arabidopsis, the researches on gene expression in castor has been inadequate. The existing databases were listed as below.

More than 62,105 ESTs from castor are currently available in Genebank https://www.ncbi.nlm.nih.gov/nucest. The transcriptome sequencing data on Triacylglycerol Lipid Biosynthetic Pathways in a variety of organizations including of developing seeds, germinating seeds, leaf and pollen-producing male flowers, was archived in Accession Number PRJEB2660, NCBI (https://www.ncbi.nlm.nih.gov/bioproject/204332). The data of responding to drought stress in various tissues were archived in NCBI (https://www.ncbi.nlm.nih.gov/bioproject/401329) with an accession Number PRJNA401329. Long noncoding RNA and SLAF (specific length of amplified fragment) data can be downloaded from NCBI (https://www.ncbi.nlm.nih.gov/sra/?term=SRP143459) and NCBI (https://www.ncbi.nlm.nih.gov/bioproject/414256) respectively.

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