Chittaranjan Kole *Editor*

Genomic Designing for Biotic Stress Resistant Pulse Crops

Springer

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Dedicated to

Prof. Roger D. Kornberg
Nobel Laureate in Chemistry 2006
Professor of structural biology at Stanford University School of Medicine

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

Preface

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

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

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

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

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

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

There are obviously a number of reviews and books on the individual aspects of plant molecular breeding, genetic engineering and genomics-aided breeding on crops or on agro-economic traits which includes the 100-plus books edited by me. However, there is no comprehensive reviews or books available that has coverage on crop commodity groups including cereals and millets, oilseeds, pulses, fruits and nuts, vegetables and technical or industrial crops, and modern strategies in single

volumes with precise focuses on biotic and abiotic stresses. The present volumes will fill this gap with deliberations on about 120 important crops or their groups.

This volume on "*Genomic Designing for Biotic Stress Resistant Pulse Crops*" includes nine chapters focused on Common Bean, Chickpea, Pea, Cowpea, Lentil, Pigeonpea, Faba Bean, Asiatic Beans and Grass Pea contributed by 61 scientists from 14 countries Argentina, Australia, Brazil, India, Italy, Lebanon, Morocco, Norway, Romania, South Africa, Spain, Switzerland, UK and USA. I remain immensely thankful for their highly useful contributions.

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

New Delhi, India Chittaranjan Kole

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Abbreviations

A	Andean
a.i	Active ingredient
AATF	African Agricultural Technology Foundation
AB	Ascochyta blight
ABA	Abscisic Acid
ABRII	Agricultural Biotechnology Research Institute of Iran
ADE	Adenine-based editing
ADP	Andean Diversity Panel
AFLP	Amplified fragment length polymorphism
AI	Artificial intelligence
ALS	Angular leaf spot
AM	Association mapping
AMF	Arbuscular mycorrhizal fungi
AMV	*Alfalfa mosaic virus*
ANOVA	Analysis of variance
ANT	Anthracnose
ARR	Aphanomyces root rot
ARS	Agricultural Research Service
ASC	Ascochyta blight disease score
ASM	Acibenzolar-S-methyl
ATP	Adenosine triphosphate
BAC	Bacterial artificial chromosome
BCMNV	*Bean common mosaic necrosis virus*
BCMV	*Bean common mosaic virus*
BGM	Botrytis graymold
BGMV	*Golden mosaic virus*
BHR	Brown hypersensitive resistant
BIC	Bean Improvement Cooperative
BIN	Binning of redundant markers
BLAST	Basic local alignment search tool

BLCMV	*Blackeye cowpea mosaic virus*
BLS	Bacterial leaf spot
BPL	Broad bean pure line
BPMV	*Bean pod mottle virus*
BSA	Bulked segregant analysis
Bt	*Bacillus thuringiensis*
BYMV	*Bean yellow mosaic virus*
CAAS	Chinese Academy for Agricultural Research
CABI	CAB International
CABMV	*Cowpea aphid-borne mosaic virus*
CAP	Common Agricultural Policy
CAPS	Cleaved amplified polymorphic sequences
Cas	CRISPR-associated protein
CBB	Common bacterial blight
CBD	Convention on Biological Diversity
CBDP	CAAT box derived polymorphism
CBE	Cytosine-based editing
CCMV	*Cowpea chlorotic mottle virus*
cDNA	Complementary DNA
CDPK	Calcium-dependent protein kinase
CDRK	California Dark Red Kidney
CG	Candidate gene
CGIAR	Consultative Group on International Agricultural Research
CGKB	Cowpea Genespace/Genomics Knowledge Base
CIAT	International Center for Tropical Agriculture
CIRAD	The French Agricultural Research Centre for International Development
CIYVV	Clover yellow vein virus
CLS	Cyclospora leaf spot
ClYVV	*Clover yellow vein virus*
cM	CentiMorgan
CMP	Consensus map construction from multiple linkage maps
CMV	*Cucumber mosaic virus*
CMV	*Cauliflower mosaic virus*
CMV/CPMV	*Cowpea mosaic virus*
CNR	Consejo Nacional de Investigation-Madrid
COPA-COGECA	European Farmers and European Agri-cooperatives
COVID	*Corona virus*
COYU	Combined Over Years Uniformity
CpCDV	*Chickpea chlorotic dwarf virus*
CpLV	*Chickpea luteovirus*
CPMMV	*Cowpea mild mottle virus*
CPMoV	*Cowpea mottle virus*
CPSMV	*Cowpea severe mosaic virus*
CpSMV	*Bean rugose mosaic virus*

CR	Collar rot
CRISPR	Clustered regularly interspaced short palindromic repeats
cry1Ab	Crystal protein 1Ab
cry1Ac	Crystal protein 1Ac
cry2Aa	Crystal protein 2Aa
CWR	Crop wild relative
CYMV	*Cowpea yellow mosaic virus*
DArT	Diversity array technology
DArT-Seq	Diversity arrays technology sequencing
DAS	Days after sowing
DDBJ	DNA databank of Japan
DDS	Direct disease resistance
DEG	Differentially expressed gene
DLO	Stichting Dienst Landbouwkundig Onderzoek
DPI	Department of Primary Industries Victoria
DRR	Dry root rot
ds	Double-stranded
EBI	European Bioinformatics Institute
EC	European Commission
ECPGR	European Cooperative Programme for Plant Genetic Resources
ELISA	Enzyme-linked immunosorbent assay
EMBL	European Molecular Biology Laboratory
EMBRAPA	Brazilian Agricultural Research Corporation
EMS	Ethyl methane sulphonate
ENU	Ethylnitroso urea
Ep	*Erysiphe pisi*
EST	Expressed sequence tag
ETI	Effector-triggered immunity
ETL	Economic threshold level
EU	European Union
EURISCO	European Genetic Resources Search Catalogue
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FBNYV	Faba bean necrotic yellow virus
FN	Fast neutron
Foc	*Fusarium oxysporum* f. sp. *ciciris*
FRR	Fusarium root rot
FW	Fusarium wilt
GAB	Genomics-assisted breeding
GBLUP	Genome-based linear unbiased prediction
GBS	Genotyping by sequencing
GCA	General combining ability
GEBV	Genomic estimated breeding value

GFP	Green fluorescent protein
GHG	Greenhouse house gas
GMO	Genetically modified organism
GP	Gene pool
GP	Glycoprotein
GRC	Genetic Resources Centre
GS	Genomic selection
GUS	Glucuronidase
GW	Genome-wide
GWA	Genome-wide association
GWAS	Genome-wide association study
H2020	Horizon 2020
HB	Halo blight
HD	Infinium iSelect high definition
HIGS	Host-induced gene silencing
HIK	Histidine kinase
HPR	Host plant resistance
HSP	Heat shock protein
HTS	Infinium iSelect high-throughput screening
IAR	Institute for Agricultural Research
IBPGR	International Board for Plant Genetic Resources
ICARDA	International Center for Agricultural Research in the Dry Areas
ICP	Insecticidal crystal protein
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IDM	Integrated disease management
IFAPA/Cordoba	Instituto de Investigación y Formación Agraria y Pesquera
IIPGR	Institute of Plant Introduction and Genetic Resources
IITA	International Institute for Tropical Agriculture
ILB	Inbred line broad bean
InDel	Insertion/deletion
INERA	Institut National de L'environnementet des RecherchesAgricoles
INRA	Institut National pour les Recherches Agrnomiques
INRB-IP	Instituto Nacional dos Recursos Biológicos
IOPG-PAS	Polish Academy of Sciences Institute of Plant Genetics
IP	Intellectual property
IPCC	Intergovernmental Panel on Climate Change
IPDM	Integrated pest and disease management
IPK	Institut Für Pflanzengenetik Und Kulturpflanzenforschung
IPM	Integrated pest management
IPR	Intellectual Property Rights
iProClass	Integrated Protein Classification
ISH	*In situ* hybridization

ISRA	Institut Senegalais de Recherches Agronomiques
ISSR	Inter-simple sequence repeat
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
iTRAQ	Isobaric Tag for Relative and Absolute Quantitation
JA	Jasmonic acid
JGI	Joint Genome Institute
KASP	Kompetitive allele-specific PCR
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LD	Linkage disequilibrium
LE	Larval equivalent
LEA	Late embryogenesis abundant
LG	Linkage group
LIS	Legume Information System
LOD	Logarithm of odds
Ma	Mesoamerican
MAB	Marker-assisted breeding
MABB	Marker-assisted backcross breeding
MABC	Marker-assisted backcross/backcrossing
MAGIC	Multi-parent advanced generation intercross
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
MAP	Mitogen activated protein
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MAT	Mating type
MAT	Mutually agreed terms
MCPA	2-Methyl-4-chlorophenoxyacetic acid
MDP	Mesoamerican diversity panel
miRNA	MicroRNAs
ML	Machine learning
MLO	*Mildew Resistance Locus O*
MNU	Methylnitroso urea
MP	Movement protein
mRNA	Messenger Ribonucleic acid
MUSCLE	MUltiple sequence comparison by log-expectation
MYA	Million years ago
MYMIV	*Mungbean yellow mosaic India virus*
MYMV	*Mungbean yellow mosaic virus*
NAM	Nested associated mapping
NARS	National Agricultural Research System
NBA	National Biodiversity Authority
NB-LRR	Nucleotide-binding leucine-rich repeat
NBPGR	National Bureau of Plant Genetic Resources
NBS	Nucleotide-binding site
NBS-LRR	Nucleotide-binding site-leucine-rich repeat

NCBI	National Center for Biotechnology Information
ncRNA	Non-coding RNA
NGS	Next-generation sequencing
NLR	Nucleotide-binding leucine-rich repeat
NM	Nanomaterials
NMR	Nuclear magnetic resonance
NP	Nucleocapsid protein
NPR -1	Non-expressor of pathogenesis-related gene 1
NPV	*Nuclear polyhedrosis virus*
NREF	Non-redundant reference
NSKE	Neem seed kernel extract
ODAP	Oxalyldiaminopropionic acid
OMRI	Organic Materials Review Institute
OP	Open pollinated
PAMP	Pathogen-associated molecular pattern
PB	Phytophthora blight
PBAI	Plant Breeding and Acclimatization Institute
PBR	Pod borer resistant
PCD	Pulse Crop Database
PCR	Polymerase chain reaction
PDS	Phytoene desaturase
PEBV	Pea early browning virus
PEG	Polyethylene glycol
PEMV	Pea enation mosaic virus
PFR	Plant and Food Research Limited
PGI	Pigeonpea Genomics Initiative
PGIP	Polygalacturonase-inhibiting protein
PGR	Plant genetic resources
PGRC	Plant Genetic Resources Center
PGRFA	Plant Genetic Resources for Food and Agriculture
PIC	Polymorphism information content
PIP	Plasma membrane intrinsic protein
PIR	Protein Information Resource
piRNA	Piwi-interacting RNA
PM	Powdery mildew
PPN	Plant parasitic nematodes
PPSMV	Pigeonpea sterility mosaic virus
PPVFR	Protection of Plant Varieties and Farmers' Right
PR	Pathogenesis related
PRGdb	Plant Resistance Genes database
PSbMV	*Pea seed-borne mosaic virus*
PSD	Protein Sequence Database
PSPDB	Plant Stress Protein Database
PvDREB	*Phaseolus vulgaris* dehydration responsive element binding
QRLs	Quantitative resistance loci

qRT-PCR	Quantitative real-time PCR
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RAXML	Randomized axelerated maximum likelihood
RdRp	RNA-dependent RNA polymerase
RDS	Root disease severity
RFLP	Restriction fragment length polymorphism
RGA	Rapid generation advancement
RGA	Resistance gene analog
R-gene	Resistance gene
RIL	Recombinant inbred line
RKHS	Reproducing Kernel Hilbert spaces regression
RKN	Root-knot nematode
RNAi	RNA interference
RS	Resistant starch
SA	Salicylic acid
SAGE	Serial analysis of gene expression
SAR	Systemic acquired resistance
SARI	Savanna Agricultural Research Institute
SB	Stemphylium blight
SBMV-CS	Southern bean mosaic virus cowpea strain
SCAR	Sequence characterized amplified region
SDL	Segregation distortion loci
SDN	Site-directed nuclease
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
siRNA	Small interfering RNA
SMD	Sterility mosaic disease
SMTA	Standard Material Transfer Agreement
SMV	Soybean mosaic virus
SNF	Symbiotic nitrogen fixation
SNP	Single nucleotide polymorphism
SRA	Sequence Read Archives
SRAP	Sequence-related amplified polymorphism
SSH	Suppression subtractive hybridization
SSR	Simple sequence repeat
ssRNA	Single stranded RNA
STMS	Sequence tagged microsatellite site
STS	Sequence tagged site
TALEN	Transcription activator-like effector nucleases
TBIA	Tissue blot immunoassay
TbyS	TILLING by sequencing
T-DNA	Transfer DNA
TF	Transcription factor
TILLING	Targeting-induced local lesions in genome

TIN	Temperature-insensitive necrosis
TIP	Tonoplast intrinsic proteins
TRIPS	Trade-related Aspects of Intellectual Property Rights
tRNA	Transfer RNA
TRV	*Tobacco rattle virus*
TSA	Transcriptome shotgun assembly
UN	United Nations
UNCBD	United Nation Conventions on Biological Diversity
UNDP	United Nations Development Programme
UNFCCC	United Nations Framework Convention on Climate Change
UniProt	Universal Protein Resource
UPGMA	Unweighted pair group method with arithmetic mean
UPOV	Union for the Protection of New Varieties of Plants
URGI	Nité de Recherche Génomique Info
USDA	United State Department of Agriculture
USEPA	United States Environmental Protection Agency
UTR	Untranslated region
VIGS	Virus-induced gene silencing
VIR	Vavilov Institute of Plant Genetic Resources
VIR/St Petersburg	The St. Petersburg Institute of Plant Industry
WB	Web blight
WClMV	*Pea white clover mosaic virus*
WGS	Whole genome shotgun
WIPO	World Intellectual Property Organization
WM	White mold
WMV	*Watermelon mosaic virus*
WS	Water soluble
WTO	World Trade Organization
Xav	*Xanthomonas axonopodis* pv. *vignicola*
YMD	*Yellow mosaic disease*
ZFN	Zinc finger nuclease

Chapter 1
Common Bean Genetics, Breeding, and Genomics for Adaptation to Biotic Stress Conditions

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Abstract Sustainable Development Goal 2 from the United Nations (Zero Hunger) states that there is a pressing need for increasing food production and quality through sustainable agricultural practices to feed the ever-growing human population. One of the key aspects to achieve a sustainable food production is to control plant pests, diseases and weeds through integrated crop management which mainly aims at

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reducing the widespread use of phytochemicals due to their persistence in the air, soil, water and food, as well as the development of biotic stress such as parasite resistance. Legume crops plants are, after cereals, the main source of food for the world population. These plants provide proteins, carbohydrates, minerals, vitamins, oils, fiber and other compounds of high nutraceutical value and beneficial properties for human health. The common bean (*Phaseolus vulgaris* L.) is the most widely used food legume for direct human consumption, and is present in regional, national and international marketson all continents by small farmers and large producers, with both green pods and dried seeds being marketed. Like other crops, beans need to adapt to changing conditions, in the current conditions of climate change. These conditions are producing new situations of abiotic and biotic stresses (mainly

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pests and diseases).Genetic improvement of the common bean includes the knowledge of its genetic diversity and the genome and gene function in response to the current changing environmental conditions. An important long-term challenge is the knowledge of the gene(s) that control relevant traits such as pest and disease tolerance/resistance that affects the crop yield and food security. New technologies built around the recently released common bean genome sequence that facilitates the arise of genomic resources, but they need the support of phenotypic data. Generating new bean cultivars or genotypes with enhanced resistance to different parasitesand new knowledge on possible innovative control methods are relevant for the improvement of a sustainable productivity of bean and its quality in different agrosystems.

Keywords Breeding · Common bean · Diseases · Genetics · Legumes · Pests · *Phaseolus vulgaris*

1.1 Introduction

1.1.1 Domestication and Distribution

Understanding the effects of domestication on genetic diversity of common bean (*Phaseolus vulgaris* L.) is of great importance, not only for crop evolution but also for possible applications, such as the implementation of appropriate biodiversity conservation strategies, and the use of genetic variability in breeding programs under the effects of the climatic changes. An important and widespread characteristics of plant domestication is the reduction in genetic diversity, during the initial domestication process and also during the adaptive radiation from the domestication centers to other areas. This reduction in biodiversity is usually more intense in self-pollinating species such as the common bean compared to cross-pollinated species (Jarvis and Hodgkin 1999). This reduction is caused by stochastic events (that is, a bottleneck and genetic drift due to a reduction in population size) and by natural selection adaptive processes and by artificial selection (Vigouroux et al. 2002).

Bitocchi et al. (2012, 2013) defended a Mesoamerican origin of the common bean, based on the analysis of the diversity and the population structure within the Mesoamerican gene. Furthermore, these authors suggested that wild beans from northern Peru and Ecuador represent an ancient germplasm that includes a part of the genetic diversity of ancestral populations of common bean. The resequencing of the common bean genome confirmed this hypothesis.

Domestication took place after the formation of the Mesoamerican and Andean genetic pools pools, thereforetheir population structure isclearly manifested in the wild populations and the domesticated varieties (Papa and Gepts 2003; Papa et al. 2005, 2007; Rossi et al. 2009).This subdivision of common bean germplasm has been defined by several authors (Papa et al. 2007; Angioi et al. 2009; Bitocchi et al. 2012, 2013) although the domestication events into each genetic pool group is under discussion. Bitocchi et al. (2013) proposed a single domestication event in each

genetic pool and suggested the Oaxaca Valley in Mesoamerica and southern Bolivia and northern Argentina in the Andean regios as tentative areas of domestication of the common bean.

Each of the two domesticated gene pools of the common bean isadditionally subdivided into several ecogeographic races, with a long history of adaptation to specific environmental conditions: Durango, Jalisco, Mesoamerica, and Guatemala in the Mesoamerican gene pool; and Chile, Nueva Granada, and Peru in the Andean gene pool (Singh et al. 1991; Beebe et al. 2001).

The introduction of some exotic species in new agricultural agrosystems raises relevant questions about adaptation, taking into account the requirements of tolerance to several stresses, as well as competence with other native crops (De Ron et al. 2016). Zeven (1997) reported that no records of common bean earlier than 1543 have been found in European herbariums. The dispersion of the common bean to Europe started from the Iberian Peninsula (Spain and Portugal), where this species was introduced mainly from Central America around 1506 and from the southern Andes after 1532, through sailors and traders (Brücher and Brücher 1976; Debouck and Smartt 1995). The pathways of dissemination of the crop across Europe included introductions from America combined with direct seed exchanges between European and other Mediterranean countries (Papa et al. 2007). The phaseolin protein was used as a marker to explain the worldwide dissemination of common bean (Gepts 1988). A high frequency of phaseolin Andean types (T, C, H, and A) was identified compared to the Mesoamerican ones (S, B, M) (Lioi 1989a, 1989b; Santalla et al. 2002; Logozzo et al. 2007).

As mentioned before, the common bean was originated and domesticated in tropical highlands. This means that abiotic and biotic conditions had an influence on the development of European varieties (Rodiño et al. 2006, 2007). In some cases, bean breeders have had to incorporate tolerances to abiotic stresses from sources outside the primary gene pool of common bean. For example, tepary bean could also provide tolerance to heat or drought, and runner bean, tolerance to low soil fertility (Miklas et al. 2006). In the case of rhizobia symbiotic system, it is possible that migration of the species had not been parallel, so additional efforts are underway to achieve efficient symbiotic genotypes of common bean and rhizobia (Rodiño et al. 2011). As a result of plant-rhizobia coevolution, a spectrum of compatible specific rhizobia is recognized for one or more legume species.

1.1.2 Economic Importance of Common Bean as a Food Resource

With more than 19,500 species and 770 genera, legumes (family Fabaceae or Leguminosae) constitute, after the families Asteraceae and Orchidaceae, the third most abundant angiosperm plants in number of species. Legumes played an important role in the early development of agriculture, were domesticated along with grasses, and

today occupy diverse aquatic and terrestrial environments in nearly every biome on Earth, even the most extreme habitats.

Grain legumes are relevant sources of food for a large part of the world population, providing protein, carbohydrates, minerals, vitamins, oil, fiber and other compounds with nutraceutical value and health-promoting properties (Champ 2002). From a nutritional point of view, the amino acid profile of legume storage proteins reveals low amounts of the essential sulfur-containing amino acids (i.e., methionine and cysteine) and tryptophan, while lysine, another essential amino acid, is quite abundant. Legume proteins complement very well those of cereals, which are normally rich in sulfur amino acids and poor in lysine and threonine. Besides the composition in essential amino acids, the nutritional quality of seed proteins is also largely determined by their digestibility; in fact, amino acids composition only represents the potential nutritional quality of a protein, being their bioavailability critical for the supply of amino acids in the diet (Sparvoli et al. 2015).

Beans are produced and consumed mainly as a dry food legume, due to the high protein content of the grain, but the use of the fresh pod as vegetable (snap bean) is common in many areas. Common bean is highly consumed Africa and Latin America (as the most important source of plant protein), as is relevant also in traditional diets of the Middle East, Europe (Broughton et al. 2003; Casquero et al. 2006) and the USA (Blair and Izquierdo 2012).

The role of bean in human diet is on its protein content and alsoon the functional properties. The consumption of common bean could reduce the risk of some diseases such as obesity, diabetes, cardiovascular diseases and colon, prostate, and breast cancer (Hangen and Bennik 2003; Thompson et al. 2009; Sparvoli et al. 2015).

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

1.1.3.1 Brief Account on Behavior of Beans Under Thermohydric Stress

The bean crop can grow at different latitudes where mean air temperature varies from 14 to 35 °C. Being originated in the medium to high altitude regions, it is sensitive to heat, whereas day and night temperatures above 30 or 20 °C, respectively, result in significant yield reduction (Beebe et al. 2011). According to Araújo et al. (2015), common bean fromthe Andean area adapts better to cooler climate and high altitude (1400–2800 masl) regions, whereas genotypes of Mesoamerican origin adapt to higher temperatures in low to medium altitude (400–2000 masl) regions.

Extensive areas are almost permanently subjected to the action of thermohydric stress conditions. This is highlighted by the aridity index, calculated according to De Martonne's formula, which frequently varies for temperate areas between 22 and 24 °C (Păltineanu et al. 2005). Stress conditions may increase in the future

due to climate change that is affectingmany countries in the world. These changes, caused by the accumulation of greenhouse gases, mainly lead to higher temperatures, increased water stress and increased frequency of storms, factors that limit the level of agricultural production and its quality.

According to Easterling et al. (2007) increasing the average of annual temperature by less than 2 °C has a positive effect on crops in temperate zones but increasing above this limit can have negative effects on plant metabolism and water regime.

Dawson and Spannagle (2009) estimate that in subtropical areas and mid-latitudes in the northern hemisphere, the climate will become drier. If the temperature increases 2 °C by 2050, the precipitation in these areas will be lower by about 30%. But if the temperature increases by 3 °C during this period, the precipitation will decrease by up to 50%. The precipitations will increase in the northern regions of Europe, Asia and America. In areas with temperate climates, precipitation will be reduced in the spring and summer seasons.

Recent projections reported by CGIAR showed that the area suited for bean in eastern and central Africa could shrink up to 50% by 2050. Affecting mainly lowland areas, heat stress will pose a particularly serious problem for bean crops in Malawi and the Democratic Republic of the Congo (DR Congo), followed by Tanzania, Uganda, and Kenya. Across Latin America, the situation is also dire. Bean production in Nicaragua, Haiti, Brazil, and Honduras, as well as Guatemala and Mexico, would be most impacted (CGIAR 2015).

An experiment conducted by Alves da Silva et al. (2020) showed that the crop season factor significantly influenced the performance of genotypes and the high temperatures observed in the summer crop season drastically reduced the grain yield of the cultivars. Due to the high interaction of genotype versus location and season versus location for grain yield, it was observed that investigated genotypes do not exhibit wide adaptability for high temperature, being necessary to carry out the evaluations and selections in unfavorable environments.

1.1.3.2 Limits of Thermohydric Stress at Bean Plants

In the case of worsening thermohydric stress conditions, it is necessary to know the limits of its negative effects, how plants recognize stressors and what is the answer to acclimatization that allows them to survive a shorter period in these conditions. Soil temperature and humidity, within optimal limits, are the main factors that determine the growth and development of bean plants. Outside the optimal limits, temperature and humidity are stressors, effect of which is accentuated as the differences from the optimal limits increase and the duration of action is longer.

The optimum temperature varies mainly depending on the process, organ and phenophase. Thus, the optimum temperature for bean seed germination varies between 8 and 25 °C (Lin and Markhart 1996); for flowering, between 20 and 25 °C (Angelini 1965); for pod setting between 22 and 25 °C and for photosynthesis the optimum temperature is 25 °C (Fraser and Bidwell 1974).

Temperatures outside these limits have a stress effect. Thus, the temperature of 32 °C determines the reduction of the leaf surface, the length of the roots, the rhythm of the net assimilation and the accumulation of the dry matter (Lin and Markhart 1996). The growth rate of bean plants at this temperature is slower compared to that determined at 25 °C. Similar results were obtained by Udomprasert et al. (1996), who found that exposing the roots and stems to high temperature of 45 °C for 5 h reduced the intensity of the photosynthesis process and the growth process.

Critical temperatures, below 8 °C, have a negative effect on the metabolism of bean plants. Thus, Pardossi et al. (1992), stated that seedlings exposed to a temperature of 3 °C have a slow process of abscisic acid biosynthesis and wither quickly because the stomates remain open for the first 24 h. The turgidity of the leaf cells returns to normal after 30–40 h, with the change in the endogenous concentration of abscisic acid. Also, at critical temperatures, lipids in the mitochondrial membranes of bean plants, which have a higher content of saturated fatty acids, passes to the gel phase, inhibiting the transport of pyruvic acid (Lyons and Raison 1973), which accumulates in the cytoplasm, where it is biodegraded anaerobically to acetic aldehyde and ethyl alcohol. The accumulation of these substances causes the characteristic symptoms of the physiological disorder known as low temperature breakdown.

Chasompongpan et al. (1990) found that exposure of bean plants for 5 min at 42 °C reduced the amount of oxygen produced in photosynthesis by 50–95%, and at 45 °C oxygen production is completely canceled. According to Angelini (1965) the minimum temperature for flowering of bean plants is 15 °C. Increasing the temperature during the day to 32 °C has small effect on the abscission of flower buds and flowers but increasing the temperature during the night to 27 °C has reduced the production of pods and seeds due to the abscission of flower buds, flowers, and small pods. Thermal stress (2 days at 35 °C, 10 h per day), affected the pollen more, compared to the pistil. The critical period for thermal stress is between 6 days before flowering, when it can cause abscission of 82% of pods less than 2 cm long (Monterroso and Wien 1990).

1.1.3.3 Effects of Thermohydric Stress on Bean Plants

During the vegetation period, the plants are subjected to longer or shorter periods with thermo-hydric stress. Boyer (1982) considers that water stress is widespread and is the most important abiotic limiting factor for most plants. The sensitivity of plants to the action of thermohydric stress differs, depending on the species and variety, the level of stress, the rate of change and the phenophase in which it manifests itself. Hsiao (1973) considers that water stress is moderate, if the foliar water potential varies between −1.2 and −1.5 MPa and is severe, when the water potential falls below −1.5 MPa. It causes the appearance in bean plants of numerous morphological, physiological, and biochemical changes, which ultimately lead to a decrease in its yield and quality.

From the synthesis of the research results carried out by Trewavas (2003), it resulted that water stress causes changes in the synthesis process of cell walls, cuticle

thickness, stomatal conductivity, leaf size, stomatal density and phenophase development. Thermohydric stress reduces the leaf area of bean plants, both by reducing the number of pods and by reducing their growth rate.

The water requirements of bean plants are higher during flowering and fertilization. Lack of water during this period can cause abscission of flower buds and flowers. Drought resistance of plants is a genetic characteristic that is determined by many factors. Drought tolerance is a complex quantitative trait controlled by many genes and is one of the most difficult traits to study and characterize (Sayadi Maazou et al. 2016). This is highlighted by the drought index which represents the ratio between the yields obtained on non-irrigated plots and on irrigated ones. The value of this indicator varies depending on the genotype between 0.22 and 0.90.

1.1.3.4 Thermohydric Stress Reception

Both thermal and water stress have a common effect, reducing the water content of the soil, generating conditions of osmotic stress. For this reason, the reception of signals induced by thermohydric stress can be done by protein receptors, mainly located in the root cell plasmalemma (Trewavas and Malho 1997). The change and intracellular pressure are received by proteins that act as osmosensors and have been identified in the bacterium *Synechocystis* spp. and called histidine kinases 33 (Mikami et al. 2002). A similar receptor was later identified in *Arabidopsis thaliana*, that was named ATHK1, which is also a histidine kinase (Scheel and Wasternack 2004). The decrease in intracellular pressure changes the configuration of the osmosensor and activates its cytoplasmic component, which acts as a kinase.

The transmission of signals induced by osmotic stress is performed after Lata et al. (2015) with the participation of MAP-kinases (mitogen activated protein kinase), which is the main way of transmitting signals induced by osmotic stress. The transmission of signals can also be achieved with the help of a family of protein kinases (CDPKs) are serine threonine protein kinase Ca^{2+}-dependent protein kinases that have a molecule of calmodulin to the terminal carbon, to which calcium binds. By binding calcium ions to calmodulin, a conformational change occurs that activates the kinase by phosphorylation. The transmission of signals through the protein kinase chain is achieved by successive phosphorylation and dephosphorylation of protein kinases, which finally activate, by phosphorylation, specific transcription factors.

Transcription factors are proteins that activate in the cytoplasm or nucleus and have three structural domains: a binding domain to the gene encoding the response, a transcriptional activating domain, and a ligand binding domain. Transcription factors bind to the cis-regulatory sequence of DNA and activate the transcription process that results in a specific mRNA, which encodes the synthesis of proteins involved in acclimatization reactions to thermohydric stress. Seki et al. (2003) monitored the expression of 7,000 genes induced by drought, salinity and low temperatures and specified that in the case of drought stress occurs the expression of 277 genes and the repression of another 79 genes.

According to Konzen et al. (2019) PvDREB genes are involved in tolerance to abiotic stress, and Soltani et al. (2019) mention that the HSP21, ABA4 and KHCB4.3 genes provide protection of photosystem II to the action of water stress.

The acclimatization process determines the increase tolerance of the plants to the subsequent exposure to more severe thermohydric stress conditions. Key et al. (1981) and Jenks and Hasegawa (2005) found that acclimatization reactions to heat stress are triggered when the ambient temperature is 5–10 °C higher than the optimal value for plant growth. Lin et al. (1984) obtained similar results and found that acclimatization to thermal stress can be achieved by exposing plants to low thermal shock. This led to changes in gene expression and synthesis of heat shock proteins, which prevented the denaturation of cellular proteins under the action of temperatures of 45 °C. The reactions of plants to the action of these stressors are particularly complex. On one hand, stressors stimulate some processes (as free radical synthesis) and on the other, inhibit other processes (as photosynthesis). At the same time, it determines the performance of passive protective reactions, such as the passive closing of the stomata, the change of the position of the leaves in relation to the solar radiation, the withering, etc.

Exposure, a short period of time of bean plants to temperatures and humidity with stress effect, determines their acclimatization, which consists in achieving active changes, genetically coordinated, through which plants exhibit tolerance to stressors, changes that are not transmitted to offspring. The specific receptors, the signal transmission chain, the transcription factors, and the specific genes involved in carrying out these reactions participate in the acclimatization reactions.

1.1.3.5 Synthesis of Abscisic Acid

Thermohydric stress is a signal for specific receptors involved in the synthesis of abscisic acid: histidine kinases HIK33 or AtHK1 that function as osmosensors. Their activation under conditions of osmotic stress, by phosphorylation, the transmission of stress signals through the cascade of phosphorylations and successive dephosphorylation of MAP-kinases and the activation of transcription factors such as ABF1 and AREB2/ABF4 activate genes encoding the enzymes involved in the synthesis of abscisic acid.

Abscisic acid is transported quickly to the leaves, but can also be synthesized at their level, where it causes the opening of calcium channels in the guard cell plasmalemma (MacRobbie 1998). It causes inhibition of the activity of proton pumps in plasmalemma, depolarization of plasma membranes and opening of channels for potassium and anions (Ishkawa et al. 1983). After depolarization of the membranes, potassium is no longer retained by the negative bioelectric potential in the cells and passes through diffusion into the adjoining cells, followed by water exosmosis, loss of guard cell turgidity and hydro active closure of the stomata.

The presence of abscisic acid in the root cells is received by soluble sensors, made up of three proteins, which have received the name: PIR/PYL/RCAR. Activation of these receptors can activate the Ca^{2+}-dependent protein kinase chain and transcription

factors and determines the expression of genes involved in the response to water stress only in the presence of endogenous abscisic acid, while other genes respond both to the action of water stress and in the absence of this hormone (Shinozaki and Yamaguchi-Shinozaki 1999). Shinozaki and Yamaguchi-Shinozachi (1999) note that thermohydric stress causes the induction of genes encoding enzymes involved in the synthesis of proteins with a protective role hormones (abscisic acid), (thermal stress proteins HSP, LEA-proteins, osmotin), osmoprotective substances (soluble carbohydrates, proline, glycine, polyols, etc.), aquaporins involved in the transport of water through plasma membranes, enzymes involved in cell detoxification (catalase, superoxide dismutase, glutathione-S-transferase) and antioxidants.

1.1.3.6 Heat Shock Proteins

Moderate heat shock allows plants to acclimatize and survive in conditions of more severe heat stress through the synthesis of heat shock proteins. Vierling (1991) estimates that 1–2 h after the action of thermal stress on plants, the expression of heat shock protein (*HSP)* genes takes place, which determines the rapid synthesis of a new messenger RNA encoding new proteins (HSPs), thermal shock proteins. The high soil temperature (35–40 °C) stimulated the synthesis of 14 heat shock proteinsin resistant varieties of beans and only six proteins in the sensitive ones (Michiels 1994). The presence of these proteins has been identified in mitochondria, chloroplasts, and the endoplasmic reticulum. They have a molecular weight between 10 and 114 kDa and were classified according to molecular weight in five families, depending on molecular weight.

The role of HSP is diverse. Thus, heat shock proteins in mitochondria and chloroplasts protect the electron transport chain, some prevent the aggregation of proteins in cells, others promote their replication, help stabilize partially unfolded proteins, participate in achieving specific conformation of proteins. HSP bind to proteins that due to stress do not have the natural conformation, modify this conformation in the presence of ATP, release the protein, which in the presence of another HSP returns to normal structure (Mahmood et al. 2010). Thermal shock proteins have the role of molecular chaperones that prevent the aggregation of proteins, recognize, and bind denatured proteins in the inactive stage and promote their replication.

Neumann et al. (1995) specified that HSP form granules in the cytoplasm that stabilize the proteins and prevent their irreversible aggregation. Harndahl (1999) found that plants exposed to high temperatures synthesize thermal shock proteins with a molecular mass of 21 kDa, which prevents the aggregation of proteins, and after Lee et al. (1997) they keep them in non-negative form, the state in which they can be folded again. These stabilized proteins can return to the native form, through a folding mechanism, in which the HSP-70 protein is involved (Lee and Vierling 2000). During severe stress, insoluble complexes form. The role of HSP-100 proteins is to resolubilize these aggregates and transfer proteins released from insoluble complexes to the HSP-70/HSP-40 folding mechanism (Schirmer et al. 1996). Gurley (2000) notes that Hsp-100 is not used by all organisms to solubilize

protein aggregates and in some species, the role of HSP-100 proteins is taken over by lower molecular weight HSP proteins.

HSP have been identified in many horticultural plants, which have been exposed to moderate heat stress. Sanchez et al. (1992) consider that severe thermal stress, which is usually lethal to plants, can be tolerated for short periods of time if they were initially exposed to pre-adaptation. This consists of prior exposure to conditions of moderate stress, which determines the synthesis of the HSP-101 protein, and optimizes thermotolerance.

Souza et al. (2011), concluded that the action of an increase in temperature above the critical value for a specific period can cause irreversible damage. In this way it was reconfirmed the fact the tolerance limit of the plant under temperature stress may vary according to different factors as species, genotype, the phenological phases of the same species and genotype.

1.1.3.7 Late Embryogenesis Abundant (LEA) Proteins

Drought-induced osmotic stress causes the synthesis of LEA proteins that are synthesized and accumulated in seed embryos, during their maturation period (seed dehydration), as well as in various plant tissues exposed to water stress. These proteins have a low molecular weight (10–30 kDa). In plants exposed to water stress, saline stress, stress caused by low temperatures and in response to the action of abscisic acid they accumulate in greater quantities in the nucleus (Goday et al. 1994), in the endoplasmic reticulum (Lee et al. 2000), in plastids, in the cytoplasm (Rorat 2006), but also in plasma membranes. The accumulation of these substances contributes to the achievement of tolerance to dehydration. They are considered as intrinsic, hydrophilic, unstructured proteins and have no secondary or tertiary structure. They have a high degree of hydrophilicity and can bind water, reducing its loss under stress.

Dehydrins are *LEA* proteins, synthesized by the *Dhn* gene family and have the role of retaining water in cells, protect the structure of membranes and prevent clotting of cellular proteins under conditions of water stress and maintain the structural integrity of cells (Campbell and Close 1997). The presence of LEA18 proteins, from group 4, with a molecular weight between 8.4 and 18.8 kDa, was identified in bean plants. These proteins can bind to membranes, maintaining their structural integrity, and can bind ions, protecting the cytoplasm from the negative effect of their excess. The role of *LEA* proteins has not yet been well defined. It is estimated that the disordered structure of these proteins gives them a high reaction rate, form reversible bonds and may play a role in transmitting information at the cellular level (Kovács et al. 2008). These proteins have the role of protecting cellular structures, or restoring them, after the action of water stress. According to Ingram and Bartles (1996), severe water loss from cells causes changes in the structure of cytoplasmic proteins, and *LEA* proteins can maintain the structure under conditions of water stress.

1.1.3.8 Synthesis of Osmotically Active Substances

The absorption of water by plants from the soil solution is achieved through a process of endosmosis. In drought conditions, the concentration of soil solution increases, which prevents the absorption of water by plants and causes a process of exosmosis. Acclimatization of plants to drought conditions can be achieved by increasing the osmotic potential of root cells, by synthesizing and accumulating osmotically active substances, such as: carbohydrates (glucose, fructose, sucrose), amino acids (proline, serine, asparagine), organic acids (oxalate, malate) and small amounts of mineral ions. The accumulation of these substances causes the concentration of cellular juice, the decrease of osmotic potential, which ensures the plants the ability to absorb small amounts of water from dry soils. It follows that the osmotically active substances are represented by organic compounds and to a lesser extent by inorganic compounds. Of these, high concentrations of ions can cause adverse reactions in plants, which affect metabolic processes. For this reason, organic compounds are the most important osmoregulatory compounds in the plant world. Osmoregulatory substances accumulate in the cytoplasm or vacuole and facilitate osmotic adjustment and maintenance of cell turgor.

1.1.3.9 Aquaporins Synthesis

Water transport in plant root cells takes place among the phospholipid molecules that make up plasma membranes, but can take place at a higher rate through specialized water channels: aquaporins. Aquaporins are found in both plasmalemma (PIP) and tonoplast (TIP) and are protein tetramers that delimit a pore. Their synthesis is genetically coded and under conditions of water stress, the number of aquaporins increases, favoring the absorption and transport of water through the plant (Fray et al. 1994). Aquaporins also participate in the rapid hydration of cells and in the restoration of cell turgor, in the cessation of water stress. Increasing the number of aquaporins in the plant root cell plasmalemma, under conditions of water stress, is considered as an adaptation to faster water absorption.

1.1.4 Perspectives

According to different studies heat stress was estimated to be the most constraint abiotic stress, responsible to severe limitation of yields at global level due to the climatic changes.

CGIAR reported in 2015 that in Africa and Latin America, the production of beans is highly vulnerable to climate change impacts, which include higher temperatures and more frequent drought. Within the last 15 years, CGIAR researchers have registered key advance—particularly the development of drought-tolerant and

disease-resistant varieties—that will help make production more resilient in the face of future threats.

Current approaches aimed to develop tolerant and resistant genotypes involve obtaining transgenic plants featured by different tolerance traits. The benefits are related to shorter time as compared to classical breeding programs. For this goal, environmentally-controlled experiments need to be validated in long-term field experiments and this approach decrease severely the real advantage between the genetic approaches over the classical breeding. Moreover, legal limitations exist related to cultivation of transgenic plants in field, it remains arguable whether transgenic plants produced under controlled conditions to enhance tolerance really perform in field experiments in which other confounding variables may occur (Kapoor et al. 2020).

Population growth results in an increase in the absolute number of the population and an increase in the standard of living. These two determinants are associated with extraction and consumption of natural resources. The emission of greenhouse gases (GHGs) is a function of total population because every mouth must be fed. The growing population is putting stress on agricultural production systems that aim to secure food production (Vetter et al. 2017a, 2017b). On the contrary, food production contributes a substantial amount of GHGs, including carbon dioxide (CO_2), methane (CH_4) and nitrous oxide to the atmosphere (Steinfeld et al. 2006; Pitesky et al. 2009; Cohen 2010; Wolf et al. 2010a, 2010b; Vetter et al. 2017a, 2017b). Agriculture has a noteworthy contribution to ensure national food security, especially for developing countries. Methane generated from agricultural practices is the second major source of GHGs emission in the world (United States Environmental Protection Agency [USEPA] 2018). Furthermore, industrialization and development interventions contribute enormous GHGs emissions (He 2014). GHGs are the most important driver of observed climate change on Earth since the mid-twentieth century (USEPA 2018). In sober fact, the more population on Earth indicates more consumption and more emissions, which intensifies climate change.

Climate change is the single most pressing environmental issue for the Earth's biotic environment with adverse implications for food security, freshwater supply and human health (United Nations Framework Convention on Climate Change [UNFCCC] 2007). Climate change is also the biggest challenge for tropical and subtropical countries of the world, especially for coastal areas and islands. The impact would be particularly severe in the tropical areas, which mainly consist of developing countries (Sathaye et al. 2006).

Population includes the number of people; their demographic characteristics like age, sex, health, education and familial status; their demographic processes like birth, death, migration, the formation of unions and families and their dissolution; and the spatial distribution of people by geographic regions and size of settlements, from rural to urban (Cohen 2010). Therefore, population growth has diversified effects on development. On the contrary, the relationship between climate change and development is reciprocal. Social and economic development may be influenced by climate change, while society's precedence on sustainable development influences the level of emissions of GHGs that are causing climate change (IPCC 2007).

1.2 Description on Different Biotic Stresses

Dry beans are susceptible to biotic and abiotic stresses and depending on the severity of the stress and the plant's ability to tolerate them, yield can be severely affected. Biotic constraints such as fungal, bacterial, viral diseases and other diseases as well as insect pests, can cause serious yield losses especially when the climate is conducive to their development. Depending on the occurrence and severity of individual and collective diseases occurring in the same field, yield losses can range from 20 to 100% (Singh and Schwartz 2010). Variables such as production systems, management practices, cultivar choice and crop stage will all play a role in not only yield loss, but also quality of harvested seed, germinability, and its market value.

1.2.1 Antrachnose

It is caused by *Colletotrichum lindemuthianum* (Sacc. et Magn.) Briosi and Cavara. and is one of the most important diseases that affect common bean cultivars, especially in regions with moderate to cold temperatures (17–24 °C and relatively high humidity of more than 92% (Pastor-Corrales and Tu 1989; Thomazella et al. 2002). This disease can cause losses of up to 100% under favorable environmental conditions (Singh and Schwartz 2010). All aboveground parts of the plant can be affected. The first sign of the disease can be noticed as a brick-red discoloration along the veins on the lower surfaces of the leaves. Discoloration can be seen at a later stage on the upper surface of the leaves and petioles can also be affected. Symptoms on the pods begin as small brown spots that later enlarge to brown sunken lesions with a reddish-brown border. Symptomless seed infections will infect the hypocotyl. Survival of *C. lindemuthianum* in the debris of infected dry bean crops has been reported by Dillard and Cobb (1993) and Ntahimpera et al. (1997). Therefore, crop rotations of 2–3 years with non-host species is generally recommended as an important component in the integrated control of anthracnose (Dillard and Cobb 1993; Schwartz et al. 2005). Seed-borne transmission of anthracnose fungus is an important factor in the spread of the pathogen to new bean producing regions of the world, as well as between fields in a growing region and can result in the introduction of new races into a region (Tu 1992; Conner et al. 2009).

Genetic resistance can minimize production costs and reduce damage to the environment (Falleiros et al. 2018). However, the large virulence diversity of *C. lindemuthianum* with hundreds of races (Pastor-Corrales et al. 1995) limits disease control and development of new cultivars with durable resistance (Pinto et al. 2012; Gilio et al. 2020). The races of the anthracnose pathogen comprised two separate groups based on their virulence; one group called Andean, causes disease only on cultivars from the Andean gene pool of common bean. The second group, designated Mesoamerican, causes disease on both Andean and Middle American cultivars; however, it is more virulent on cultivars of The Middle American gene pool (Pastor-Corrales et al.

1995; Pastor-Corrales 1996). Anthracnose resistance in common bean is conferred by multiple single, independent and mapped genes. Most of these genes have been assigned the *Co*-symbols, as follows: *Co-1* (with four alleles), *Co-2*, *Co-3* (with four alleles), *Co-4* (with two alleles), *Co-5* (with one allele), *Co-6*, *Co-11*, *Co-12*, *Co-13*, *Co-14*, *Co-15*, *Co-16*, and *Co-17*. With the exception of the recessive *Co-8* gene, all other genes are dominant genes (Kelly and Vallejo 2004; Gonçalves-Vidigal et al. 2011; de Lima et al. 2017; Valentini et al. 2017, Gilio et al. 2020). The nine resistance genes Co-2 to *Co-6*, *co-8*, Co-11, *Co-16* and *Co-17* are Middle American in origin and *Co-1, Co-12* to *Co-15*, and *Co-AC* (Gilio et al. 2020; Valentini et al. 2020) are from the Andean gene pool. An order of dominance exists among the four alleles at the *Co-1* locus.

1.2.2 Angular Leaf Spot (ALS)

It is caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous et al. (2006). Several articles reviewing the most important aspects of the ALS disease and genetic studies to find resistance loci in common bean have been published (Correa-Victoria et al. 1989; Liebenberg and Pretorius 1999; Nay et al. 2019). The ALS disease has been reported occurring in all continents but Antarctica (Zaumeyer and Thomas 1957; Liebenberg and Pretorius 1997; Correa-Victoria et al. 1999; Stenglein and Balatti 2006; Aggarwal et al. 2003). However, ALS is a particularly recurrent, severe and widely distributed disease in tropical and subtropical areas, especially in South and Central America, Mexico, the Caribbean, and in Eastern and Southern Africa (Correa-Victoria et al. 1989; Liebenberg and Pretorius 1997; Aggarwal et al. 2004; Nay et al. 2019). ALS also occurs on dry beans produced in temperate regions (Correa and Saettler 1987; Melzer and Boland 2001; Landeras et al. 2017).

While ALS occurs predominantly on dry beans, it has also been reported occurring on French (snap) beans in Africa (Kimno et al. 2016). The ALS disease affects aerial parts of the common bean plant, particularly foliage and pods, during the growing season. Temperatures between 17 and 24 °C, with an optimum of 24 °C, and high humidity favor the development of the ALS disease. The characteristic symptoms on leaves initially are small brown and gray lesions between the leaf veins that become necrotic and that later assume an angular shape, which is the typical symptom of the ALS disease on the foliage. Stems often are covered with necrotic spots. The symptoms on the pods appear as dark reddish brown and often round, roughly circular lesions, frequently covered with sporulation of the ALS pathogen. Sporulation is also common on the lower side of the leaves. In general, ALS tends to be most destructive during and after flowering, causing premature defoliation, reduced seed size and quality that can result in severe yield losses reaching 80% (Schwartz et al. 1981; Rava Seijas et al. 1985; de Jesus Junior et al. 2010). Volunteer plants, off-season crops and ALS infected plant debris have been reported as the principal sources of inoculum (Correa-Victoria et al. 1989). Infested seed can also cause infections but is generally not regarded as an important source of inoculum (Liebenberg and

Pretorius 1997). Planting pathogen-free seeds treated with effective fungicides, crop rotations, and use of foliar fungicides have been reported as options to control the ALS disease; however, fungicides are often expensive or not readily available to smallholder farmers, the predominant producers of common bean in the tropics. Hence, planting cultivars with resistance to *P. griseola* present a cost-effective, easy to use, and environmentally friendly strategy to manage the ALS disease (Pastor-Corrales et al. 1998; Aggarwal et al. 2004; Gonçalves-Vidigal et al. 2011, 2013). Nevertheless, resistant varieties may become susceptible due to the appearance of new virulent strains, known as races, of *P. griseola.* Due to the appearance of new races, varieties that previously were resistant in a given year or location can suddenly become susceptible.

The ALS pathogen is known for its extensive virulence diversity that comprises hundreds of different virulent races (Pastor-Corrales et al. 1998; Busogoro et al. 1999; Mahuku et al. 2002; Sartorato 2004; Aggarwal et al. 2004; Stenglein and Balatti 2006; Nay et al. 2018). These races are identified by inoculating each isolate on an internationally accepted set of 12 differential cultivars, six Andean and six Mesoamerican cultivars, developed by Pastor-Corrales (1996). The large number of races of *P. griseola* separate into two distinct virulence groups; Andean races infecting only Andean differential cultivars and Mesoamerican races, infecting Mesoamerican and Andean differential cultivars (Guzman et al. 1995; Pastor-Corrales 1996; Mahuku et al. 2002; Aggarwal et al. 2004). Resistance to the ALS pathogen is conferred by several single dominant resistance loci and quantitative resistance loci (QTLs) as reviewed by Nay et al. (2019). Currently five ALS resistance genes have been given official names (Souza et al. 2016). These include three dominant and independent *Phg* loci named *Phg-1* [present in Andean (A) common bean AND 277], *Phg-2* [present in Mesoamerican (MA) common bean Mexico 54] and its allele Phg-2^2 (present in MA common bean BAT 332), *Phg-3* (present in MA common bean Ouro Negro) and two major quatitative trait loci (QTLs) named *Phg-4* (present in A common bean G5686) and *Phg-5* (present in A common beans CAL 143 and G5860 (Gonçalves-Vidigal et al. 2011, 2013; Oblessuc et al. 2012, 2013; Keller et al. 2015; Nay et al. 2019). Several molecular markers linked to these resistance loci are available and can be used to efficiently incorporate the resistance loci in new bean varieties (Nay et al. 2019).

1.2.3 Rust

The common bean rust disease is caused by the basidiomycete fungus *Uromyces appendiculatus* (Pers.: Pers.) Unger. This disease has worldwide distribution and occurs in most dry and snap bean growing areas of the world and particularly in locations with cool temperature (17–22 °C) and high humid conditions (>95%) maintained for 8–10 h and long dew periods. Rust is rare in arid climates except under irrigation. Bean rust has been reported occurring throughout Latin America where it is an important disease in Brazil, Central America, Mexico, and the Caribbean. It also

has been reported in multiple countries of Eastern and Southern Africa. In addition to infecting dry beans, the rust pathogen also infect snap bean where it is often a recurrent and severe disease of snap beans grown in East Africa, Latin America and Asia (Zaumeyer and Thomas 1957; Stavely and Pastor-Corrales 1989). Yield losses depend on the climatic conditions favoring rust development, and the earliness and severity of the infection. Infections occurring during the pre-flowering and flowessring stages usually result in high to extremely high yield losses approaching 100%. High losses have been reported in many countries of the Americas, Africa and other geographic areas (Stavely and Pastor-Corrales 1989).

The bean rust pathogen is an obligate parasite of common bean and it cannot live independently of its host. This fungus cannot be cultured on artificial media; thus, it depends on wild and cultivated common beans for its survival. This pathogen has a complex life cycle that includes five different spore stages and three nuclear conditions (Groth and Mogen, 1978; McMillan et al. 2003), which are suggestive of its capacity for genetic recombination. The entire life cycle is completed on common bean. The rusty cinnamon brown pustules present on the foliage of common beans during the planting season, gives the disease its "rust" name. The pustule or uredinia which occur on leaves, stems and pods, contain thousands of spiny cinnamon brown spores named urediniospores. Repeated generations of urediniospores happen during the growing season. Toward the end of the growing season and under appropriate conditions, the next spore stage is named telia that develops within the aged uredia and produces dark brown, nearly black, ovoid teliospores. The other three spore stages occur later but are not easily seen. Many publications have revealed the extensive virulence diversity of this pathogen. Hundreds of different virulent races of *U. appendiculatus* have been reported around the world (Stavely 1984; Stavely and Pastor-Corrales 1989; Stavely et al. 1989; Araya et al. 2004; Acevedo et al. 2012; Arunga et al. 2012). Different races produce dissimilar virulent phenotypes when they are inoculated on a set of differential cultivars. A new set of 12 differential cultivars, created by Pastor-Corrales, containing six Andean and six Middle American cultivars was approved for international use during the 2002 International Bean Rust Workshop that took place in South Africa (Steadman et al. 2002). This new set replaced the previous set containing 19 differential cultivars that was adopted during the 1983 Bean Rust Workshop held at Mayaguez, Puerto Rico (Stavely et al. 1983). In addition to adopting a new set of 12 differential cultivars, it was also agreed to name the new races of *U. appendiculatus* using a "Binary System" in which each of the six Andean and six Middle American cultivars were assigned a numeric value. The name of each race included two digits separated by a hyphen.

These two numbers specify which rust resistance genes present on the differential cultivars were susceptible. Using this new set of differential cultivars and molecular markers, the races of the bean rust pathogen have been segregated into two different groups, one Andean and another Mesoamerican that correspond to the Andean and Middle American gene pools of the common bean, respectively (Pastor-Corrales and Aime 2004). Genetic resistance is the most cost-effective strategy to manage the bean rust pathogen. Rust resistance in common bean is conferred by single and dominant genes identified by the Ur- symbol (Kelly et al. 1996). Currently, 10 rust

resistance genes have been named, mapped and tagged mostly with random amplified polymorphic DNA (RAPD) and sequence characterized amplified region (SCAR) molecular markers (Miklas et al. 2002; Hurtado-Gonzales et al. 2017). Six genes (*Ur-3, Ur-5*, *Ur-7, Ur-11, Ur-13*, and *Ur-14*) are present in common beans that belong in the Middle American gene pool, while the other four genes (*Ur-4, Ur-6, Ur-9*, and *Ur-12*) are in common beans belonging in the Andean gene pool. The Andean rust resistance genes are preponderantly susceptible to Andean races of *U. appendiculatus*; however, these genes often confer resistance to highly virulent Mesoamerican races. Conversely, the Middle American rust resistance genes are usually broader in their resistance spectrum than the Andean resistance genes and are very effective against most Andean races of *U. appendiculatus.* All rust resistance genes differ in their spectrum of resistance to the known races of the rust pathogen. None of these genes are either susceptible or resistant to all known races. The *Ur-11* gene present in the PI 181,996 accession has the broadest spectrum of resistance of all named rust resistance genes (Hurtado-Gonzales et al. 2017). Similarly, the *Ur-14* gene present in the Ouro Negro landrace is also broadly resistant (Souza et al. 2011.) Combining rust resistance genes from the Andean and Middle American gene pools results in broad spectrum resistance to all known races of *U. appendiculatus.*

The pinto bean germplasm line BelDakMi-RMR 18 and six great northern bean germplasm lines (BelMiNeb-RMR-8 to BelMiNeb-RMR-13), developed at the ARS-USDA Beltsville Agricultural Research Center, Beltsville, Maryland, USA, combine the Andean *Ur-4* and *Ur-6* and Middle American *Ur-3* and *Ur-11* rust resistance genes. All these seven lines have been evaluated as resistant under greenhouse conditions to more than 70 Andean and Mesoamerican races of the rust pathogen (Pastor-Corrales et al. 2007). These lines have also been evaluated as resistant to rust in small plots planted under field conditions in various dry bean producing states in the United States and in other locations including Puerto Rico, Honduras, Brazil South Africa, and other sites. These results support the proposition that combining rust resistance genes of Andean and Middle American origin can result in common bean cultivars with broad resistance to the highly virulence variable rust pathogen of common bean.

1.2.4 Rhizoctonia Solani *Kuhn. Teleomorph:* Thanatephorus cucumeris *(A. B. Frank) Donk*

It is a heterogenous multinucleate species complex that includes 15 anastomosis groups (Carling et al. 2002; Godoy-Lutz et al. 2008; Bolton et al. 2010) based on hyphal fusion, cultural morphology, pathogenicity, or virulence and DNA homology (Godoy-Lutz et al. 2003; Harikrishnan and Yang 2004). The diversity of this soil-borne pathogen is a major reason for the difficulty in managing *R. solani* root rot. *R. solani* can occur during any stage of the common bean growth stage (Valentín Torres et al. 2016). It can cause severe plant diseases which can differ in symptomology like collar rot, root rot, damping off and wire stem (Ogoshi 1996) as well as complete

defoliation, leading to complete crop failure (Singh 2001). Because of its facultative parasitic ability, it can survive as a saprotroph (Zhao et al. 2005) in the form of sclerotia on infected plant debris. *R. solani* then act as an inoculum for susceptible plants such as sugar beet (*Beta vulgaris* subsp. vulgaris) (Plyler-Harveson et al. 2011), dry beans (*Phaseolus vulgaris*) (Das et al. 2020), potato (*Solanum tuberosum*) (Wendels et al. 2009), and soybean (*Glycine max*) (Liu and Klein 2012). *R. solani* can also spread by airborne basidiospores (produced by the teleomporph *T. cucumeris*) as well as mycelial bridges between plants and infected seed (Godoy-Lutz et al. 2003). Hagedorn (1994) and Singh and Schwartz (2010) reported that *R. solani* severely impacts seed yield of common bean, resulting in upwards to 100% seed yield loss.

Although genetic resistance is considered the most cost effective and sustainable management of root rots in common bean (Abawi and Pastor-Corrales 1990; Park and Rupert 2000; Abawi et al. 2006), sources with resistance is limited. Oladzad et al. (2019) reported evidence for major as well as minor genes involved in resistance to *R. solani* in common bean. According to Harman et al. (2004) and Siameto et al. (2011), *T. harzianum* inhibit fungal growth through competition for space and nutrients, mycoparasitism and production of antibiotic compounds. Matloob and Juber (Matloob and Juber 2013) reported that *A. chroococcum*, *G. intraradices* and *T. harzianum* decreased *R. solani* root rot disease incidence (field trials) and increased plant resistance against infection with *R. solani* and improve plant growth and yield.

1.2.5 Pythium

It is a complex genus containing over 200 described species with a broad host range and occupying a variety of terrestrial and aquatic ecological habitats (Dick 2001). *Pythium* spp. that cause root rot of common bean can be found worldwide (Paul 2004). An increase in root rot producing *Pythium* spp. have been reported over the last 20 years in countries such as Eastern and Central Africa, Burundi, the Democratic Republic of Congo, Kenya and Uganda (Otsyula et al. 2003). For example, in Western Kenya and in Rwanda, many farmers stopped growing beans between 1991 and 1993 due to a severe outbreak of root rots, which caused serious food shortages and price increases beyond the reach of many resource-poor households (Nekesa et al. 1998).

Depending on the *Pythium* spp. involved, symptoms can include general root rot symptoms, any combination of various traits such as poor seedling establishment, damping-off, uneven growth, leaf chlorosis, premature defoliation, death of severely infected plants and lower yield (Abawi et al. 2006; Schwartz et al. 2007). *Pythium* spp. can reproduce both asexually and sexually. Asexual reproduction takes place through the zoosporangia and zoospores (Nzungize et al. 2012). Structures such as oospores, zoospores and sporangia enable this species to survive in soil for long periods (Onokpise et al. 1999). There are many specific pesticides such as benomyl, captafol, captan, carboxin, metalaxyl, propamocarb hydrochloride and etridiazole, which have already proven to be efficient in controlling Pythium root rot diseases

on beans. However, some pesticides, such as benomyl, are only active on growing mycelium, but not during the resting stage of the mycelium (Nzungize 2012).

The coating of bean seeds usually results in effective protection of seeds and young seedlings for about 2–3 weeks after sowing (Abawi et al. 2006; Schwartz et al. 2007). Beneficial microorganisms of interest for biological control of plant pathogenic *Pythium* spp. have been identified among fungi and bacteria. Isolates of *Trichoderma* spp. and *Gliocladium* spp. are antagonists of Pythium induced soil-borne diseases and several strains are already commercially available for the biological control of Pythium root rots (Howell et al. 1993; Fravel 2005). Although the use of resistant common bean cultivars can be the most efficient management strategy against root rot diseases, these cultivars should have resistance to all the major root rot pathogens that prevail in a given bean growing region (Abawi et al. 2006). Cultural practices such as deep plowing and the use of raised ridges to grow beans has been found to reduce root rots favored by high moisture (Rhizoctonia, Fusarium and Pythium root rots) (CIAT 1992).

1.2.6 Fusarium Root Rot

It is caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* W.C. Snyder and H.N. Hansen, has been considered as one of the major yield-limiting diseases of dry bean worldwide (Kraft et al. 1981; Bilgi et al. 2008; Mwang'ombe et al. 2008). *F. solani* is commonly found as part of a complex with *Rhizoctonia solani* and *Pythium* spp. Fusarium root rot can cause severe yield losses, especially when adverse environmental conditions (such as soil moisture and soil compaction) persist after planting and at flowering stage (Román-Avilés et al. 2003). Unlike other root-rotting diseases, *F. solani* does not cause seed rot, or damping off of seedlings. Symptoms of Fusarium root rot on common bean are narrow, dark brown to rust colored lesions on the stems where lengthwise cracks can develop. Lesions extend down the main taproot (Román-Avilés et al. 2003) and can cause shriveling decay and death of the taproot. Lateral roots or adventitious roots commonly develop above the shriveled taproot and under ideal growth conditions, they can limit above ground symptoms. When lesions on the lower hypocotyl coalesce as the disease progress, it can result in complete rot of the root system (Abawi 1989). When left unmitigated, Fusarium root rot can cause up to 84% yield loss (Schneider et al. 2001).

Managing Fusarium root rot can be difficult due to the durability and extended viability of chlamydospores in soil and plant debris (Katan 2017). Current management strategies include the use of seed treatment chemicals, avoiding infested fields, croprotation, and planting certified seeds. However, the most sustainable and durable approaches for controlling the disease is genetic resistance (Rubiales et al. 2015). While foliar disease resistance is a target for crop improvement, less emphasis has been given to breeding for root rot resistance in common bean and there were fewer sources of root rot resistance available.

Because of this paucity and a shift in research focus, authors from multiple studies have characterized and identified sources of resistance within common bean germplasm collections (Román-Avilés and Kelly 2005; Bilgi et al. 2008; Nicoli et al. 2012; Hagerty et al. 2015; Nakedde et al. 2016; Vasquez-Guzman 2016).

The high variability in cultural characteristics exhibited by *F. solani* f. sp. *phaseoli* isolates (Nelson et al. 1983; Nirenberg 1989) poses a challenge to efforts aimed at breeding for resistance to bean root rot disease. Moreover, host specificity (Li et al. 2000) as well as a ribosomal DNA nucleotide sequence (Suga et al. 2000) has shown that *phaseoli* is a very diverse form, almost indiscernible from other related forms such as *glycines*. In general, cultivars developed from the large-seeded Andean gene pool such as red kidney bean tend to be more susceptible to Fusarium root rot than those developed from the small-seeded Mesoamerican gene pool, such as black bean (Beebe et al. 1981).

1.2.7 White Mould

It is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary and is a major disease concern for bean growers in cool subtropical and temperate climates where moist conditions prevail due to irrigation or rainfall (Miklas et al. 2006). It is a highly destructive disease, affecting both dry bean yield and quality (Pynenburg et al. 2011). White mold symptoms can be observed on all aerial plant parts (Schwartz and Singh 2013). Infected flowers may develop a white, cottony appearance as mycelium grows on the surface. Lesions on pods, leaves, branches, and stems are initially small, circular, dark green, and water soaked but rapidly increase in size, may become slimy, and may eventually encompass and kill the entire organ. Under moist conditions, these lesions may also develop a white, cottony growth of external mycelium. Affected tissues dry out and bleach to a pale brown or white coloration that contrasts with the normal light tan color of senescent tissue (Schwartz and Singh 2013). The epidermis easily sloughs off when the stem or pod is rubbed. Entire branches or plants may be killed (Steadman and Boland 2005).Colonies of white mycelium (immature sclerotia) develop into hard, black sclerotia in and on infected tissue. Sclerotia (approximately 5–10 mm long) allow the fungus to survive in a dormant state for periods of months to years (Koike et al. 2007), providing primary inoculum for successive susceptible crops.

In bean, the dominant mechanism of sclerotial germination is carpogenic, during which stipes push to the soil surface and form small, tan, cup-shaped apothecia that produce copious numbers of wind-borne ascospores (Willetts and Wong 1980). Primary infections in bean, are most commonly by ascospores infecting senescent flower tissue, which is subsequently colonized by the fungus. The senescent tissue provides the fungus with an energy source for later infection of healthy tissues (Abawi and Grogan 1979). Secondary spread within the canopy may occur when infected petals fall and make contact with other plant parts, including pods, leaves or stems (Abawi and Grogan 1979). White mould is difficult to control due to a wide host

range and the ability of the fungus to survive for long periods as sclerotia. There is a lack of commercially suitable bean cultivars with resistance (Jones et al. 2011). The combination of genetic resistance with avoidance mechanisms, including upright and open plant structure, less dense canopies and branching patterns, elevated pod set, and reduced lodging (Schwartz et al. 1987), is the current breeding strategy for reducing white mold damage in dry bean (Kolkman and Kelly 2002).

1.2.8 Bacterial Diseases

They include common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin et al. and *Xanthomonas fuscans* subsp. *fuscans*, (recently reclassified by Constantin etal. (2016) as *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*, respectively), halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) (Burkholder) Gardan et al., bacterial brown spot (*Pseudomonas syringae* pv. *syringae*), van Hall, and bacterial wilt *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges, Collins and Jones). Pathogenic variation exist within the halo blight pathogen, with nine races reported worldwide (Taylor et al. 1996). Despite several reports on pathogenic variation within the common blight pathogen, no evidence for the existence of races have been reported using common bean differential lines (Mutlu et al. 2008). Bean bacterial diseases are seed-borne and affect foliage, stems, pods and seeds of beans (Yoshii 1980) with losses of up to 45% reported (Singh and Schwartz 2010). Effective and economical control of bacterial diseases can only be achieved using an integrated approach, including cultural practices, chemical sprays and genetic resistance. Planting of pathogen-free seed is the most important primary control method (Gilbertson et al. 1990), however, it does not guarantee disease control (Allen et al. 1998). Additional cultural practices such as removing, destroying or deep ploughing of debris, effective weed control, crop rotation and minimized movement in fields, especially when foliage is wet, may be effective (Allen et al. 1998; Schwartz and Otto 2000). Copper-based bactericides protect foliage against bacterial diseases and secondary pathogen spread. Efficacy of chemical control, however, is limited (Allen et al. 1998) and resultant yield increases are minimal (Saettler 1989). The most important factor of an integrated approach is use of resistant cultivars. Singh and Schwartz (2010) recently reviewed the status of breeding for resistance to bacterial diseases and although significant progress has been made in identifying resistance genes, common bean cultivars with adequate levels of resistance are still lacking.

1.2.9 Diseases Caused by Viruses

Bean Common Mosaic Virus (BCMV) and Bean Common Mosaic Necrosis Virus (BCMNV)

They belong to the genus Potyvirus; Potyviridae are closely related. They induce similar symptoms in bean, and exist as a complex of strains with multiple isolates which differ in their virulence on common bean cultivars. BCMV and BCMNV can be disseminated by seed and vectors such as aphids and leaf beetles. Seed transmission of BCMV can range from 18 to 80% (Hall 1991; Klein et al. 1994; Bashir et al. 2000). Wild plants and weeds can act as virus reservoirs for transmission by vectors, as demonstrated by infection of wild legume species with BCMV (Flores-EsteÂvez et al. 2003; Melgarejo et al. 2007; Nordenstedt et al. 2017). Even with low seedborne transmissions, severe disease epidemics can be expected when combined with the efficient spread by vectors to susceptible cultivars (Johansen et al. 1994). BCMV and BCMNV are the most common and destructive viruses and the interaction between bean variety, virus strain and time of infection, will determine yield losses. Hall (1991) and Bashir et al. (2000) reported yield losses of up to 15% in plants of cv. Red Mexican U.I.34 that were either moderately or severely infected. Pod yields were reduced by 50 and 64% and seed yields were reduced by 53–68% respectively.

BCMV and BCMNV isolates are classified into seven pathotypes according to their reactions on 12 to 14 bean differentials with known combinations of resistance genes (Drifjhout 1978). Necrotic strains evolved more recently in the African continent (Spence and Walkey 1995) as recombination between strains of BCMV and BCMNV has been reported (Larsen et al. 2005). Five resistance genes govern interactions of BCMV and BCMNV isolates with common bean—one strain-nonspecific dominant I gene, and four strain-specific recessive genes: *bc-u*, *bc-1*, *bc-2*, and *bc-3* (Drifjhout 1978). If a BCMNV isolate is inoculated into an I-gene-carrying cultivar, a necrotic reaction occurs, regardless of the temperature, varying from limited vein necrosis on the inoculated leaf to a severe, whole-plant necrosis, called "black root syndrome". This reaction is called temperature-insensitive necrosis (TIN). When such necrotic reaction occurs, no virus replication is detected in leaf tissues surrounding necrotic tissue and no virus transmission through seed can be detected, resulting in a resistance reaction at the plant level (Feng et al. 2014).

1.2.10 The Bean Fly (Ophiomyia *Spp.)*

It is also known as the bean stem maggot and is considered as the most important insect pest of common bean. Though, it has been reported in Africa, Asia and Austria, it is widely distributed in Africa (Nkhata et al. 2019). The bean fly is a tiny insect of Agromyzidae family. The family consists of the following species: *Ophiomyia phaseoli* Tyron (*O. phaseoli*), *O. spencerella* Greathead (*O. spencerella*), *O. centrosematis* de Meij (*O. centrosematis*), *Melanagromyz sojae* Zehntner (*M. sojae*), *M.*

phaseolivora Spencer (*M. phaseolivora*) (Allen and Smithson 1986; Nkhata et al. 2019). The first three are considered as the most destructive, whereas the last two are either minor or occasional pest of common bean. Both the adult and larvae of the bean fly cause significant crop damage. However, the larvae causes the most significant damage (Kayitare 1993; Davies 1998). After oviposition under the surface of young bean leaves, larvae burrow under the thin layer of the leaf (epidermis), and tunnel along the veins down to the stem, and lodges where the stem touches the soil. Pupation takes place inside the bean stem, resulting in swelling and cracking of the stem at the point where the pupae are lodged, which destroys the transport system of the plant nutrients from the roots as well as products of photosynthesis from the leaves, leading to stunted growth, and yellowing of leaves at an early plant stage. Heavy infested crop stands are characterised by premature leaf drop and plant death (Davies 1998; Ojwang' et al. 2011). The occurrence and epidemic levels of the bean fly is dependent on suitable environmental conditions and availability of host plant species. High temperature, relative humidity and drought are reported to be favourable condition for bean fly. The pest causes up to 100% yield losses (Nkhata et al. 2021b).

Control strategies for bean fly includes, chemical pesticides, cultural practices, use of biological agents and host plant resistance. Chemical control method using seed dressing with sulphate based insecticides has been reported to be effective (Mutune et al. 2016). In addition, pesticides such as Gaucho 600 (active ingredient imidacloprid) and Pesthrin 6% EC (active ingredient pyrethrins) are used to control the bean fly at seedling or adult plant stages (Ambachew et al. 2015; Muthomi et al. 2018). Chemical insecticides are effective in controlling bean fly though they pose potential hazards to the ecosystem (Alavanja 2009). Chemical control expensive for majority of smallholder farmers (Laizer et al. 2019). Additionally, pesticide resistance can occur due to excessive use (Damalas and Eleftherohorinos 2011). Cultural practices such as early sowing, crop rotation, intercropping with maize, earthing up soil around the seedlings and fertilizer application are reported to control bean fly (Kapeya et al. 2005; Nkhata et al. 2021a). Early sowing allows the bean crop to avoid the insect pest in the field, while crop rotation and intercropping suppresses bean fly population in the field (Nkhata et al. 2021a). Earthing up promotes the development of new roots above the swelling caused by bean fly larva damage. The newly developed roots help to sustain the crop, while overcoming the impact of the damage whereas, fertilizer application ensures availability of nutrients for plant growth and maintenance of vigor (Nkhata et al. 2021a). Biological control involving the use of bean fly parasitoids such as *Opius phaseoli* Fisher and *O. importatus* suppress bean fly population but they are not very effective (Davies 1998). Incorporating host resistance is an effective, reliable and environment friendly method to control bean fly (Nkhata et al. 2021b).

Although host resistance is considered as the most effective control of bean fly. Resistance to bean fly in bean is still scarce despite decades of screening for resistance (Miklas et al. 2006; Nkhata et al. 2021b). Lack of a systematic screening procedures that exert uniform infestation of the genotypes has been the main attribute of scarcity of resistance (Hillocks et al. 2006). Bean fly resistance is quantitative and having

significant interaction with the growing environment (Mushi and Slumpa 1996; Wang and Gai 2001; Ojwang et al. 2011). Due to the high genotype-by-environment interaction, evaluation and selection of germplasm for bean fly resistance should be conducted under the target production environment (Nkhata et al. 2021b).

The resistance has been linked with morphological markers such as phenolic compounds, internode length, leaf hairiness, stem diameter and stem color in common bean and other related species (Rogers 1980; Abate 1990; Ambachew et al. 2015). Phenolic compounds serve as toxicants that inhibit the growth of bean fly (Chiang and Norris 1983). Narrow stem and short internode in common bean result into highly lignified stem, making it more difficult for the bean fly larvae to burrow into the stem (Abate 1990; Kayitare 1993; Ambachew et al. 2015). These morphological markers are useful under conventional breeding. Application of genomic tools have not be fully exploited in bean fly resistance such that there are few genomic studies on bean fly resistance compared to similar important traits in common bean (Miklas et al. 2006; Ojwang et al. 2019). The few genomic studies have mapped genes linked to bean fly on *B1, B2, B6, B8* and **B10** (Ojwang et al. 2019; Wilson Nkhata, unpublished data). The identified genomic regions offers prospects of genomic selection of bean fly resistance in common bean.

1.3 Genetic Resources of Resistance Genes

Nikolai Ivanovich Vavilov was a pioneer in recognizing the high potential value of plant genetic resources (PGR) for humankind. He highlighted the importance and potential value of collecting, conserving and exploiting the wide genetic diversity of crops and its wild relatives (CWRs) (Vavilov 1920, 1922). Harlan and de Wet (1971) formalized this particular issue by the introduction of the "gene pool concept", where crops and its related species can be divided into primary, secondary and tertiary gene pools according to how easy it is to use crop relatives in breeding (Maxted et al. 2006).

Diversity of germplasm stored in gene banks is a vital source for discovering useful genes that serve as a resource for common bean breeding programs. There are currently more than 1700 genebanks (FAO 2010), and more than 150,000 conserved *Phaseolus* accessions around the world (FAO Wiews 2019; Genesys 2020). The International Center for Tropical Agriculture (CIAT) in Columbia holds the largest *P. vulgaris* collection with 37,938 accessions, followed by the Western Regional Plant Introduction Station, United States Department of Agriculture (USDA-ARS) with 17,672 accessions, and the Brazilian gene banks Embrapa Arroz e Feijão, with 16,647 accessions, and Embrapa Recursos Genéticos e Biotecnologia, with 12,618 accessions. A great number of *Phaseouls* accessions are also held by theGerman gene bankLeibniz Institute of Plant Genetics and Crop Plant Research (IPK) with 9,004 accessions. Furthermore, the Russian collection of *Phaseouls*at the N. I. Vavilov Institute of Plant Genetic Resources (VIR) is one of the oldest in the world with 6,543 accessions.

Among the accessions, the following species are conserved: 135,582 *Phaseolus vulgaris*, 7,996 *Phaseolus lunatus*, 5,000 *Phaseolus coccineus*, 1,443 *Phaseolus acutifolius*, 629 *Phaseolus dumosus*, 224 *Phaseolus leptostachyus*, 127 *Phaseolus multiflorus*, 120 *Phaseolus* x *multigaris*, and some 2,000 accessions are unspecified species (*Phaseolus* sp.). The majority of the accessions are classified as traditional cultivar/landrace (82,742 accessions), followed by advanced/improved cultivar (20,720), wild (3,966), breeding/research material (1,667), breeders line (847), weedy (791), semi-natural/wild (150), natural (87), inbred line (76), and some 43,000 are not specified or specified as others (Genesys 2020). The top six countries of origin of the material are Mexico (10,650 accessions), Colombia (6,942 accessions), Brazil (6,737 accessions), Turkey (5,183 accessions), United States of America (4,986 accessions), and Peru (4,717 accessions).

At European level, the European Phaseolus Database (2020) was established in 1995 on the initiative of the European Cooperative Programme for Plant Genetic Resources (ECPGR) and holds in total 46,128 accessions from around 50 collection holders. Although the high number of total accessions, one-third of the global accessions (43,809 *Phaseolus* accessions) are not available for distribution, which highlight the challenges that many genebank have with backlogs and make accessions and information available. Morphological traits are often well described (for example, Fig. 1.1 shows variation in seed color) but to access resistance and tolerance traits, one often need to contact the collection holders or review published literature but collection holders try to make relevant information more accessible. For example, at CIAT (2020) there is a searchable facility for reactions to biotic and abiotic stresses with information on resistance to BCMV (Bean Common Mosaic Virus), and where 3,848 accessions (or around 10%) show up as resistant. So far, information on other reactions to biotic and abiotic stresses are not available online. This accounts from most genebanks although evaluations have been carried out. For example, the USDA genebank has data on resistance to Mexican bean beetle, BCMV, white mold (*Sclerotinia sclerotiorum*), Fusarium wilt (*Fusarium oxysporum* f. sp. *phaseoli*), rust *Uromyces phaseoli*, halo blight(*Pseudomonas syringe*),and bacterial wilt (*Curtobacterium flaccumfaciens*), but the data are not easily available.

1.3.1 Primary Gene Pool

The primary gene pool of common bean consists of *P. vulgaris* itself and its subspecies that are easy to interbreed, mainly *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet.

Domestication of common bean took place at two places with the formation of the Mesoamerican and Andean types (Papa and Gepts 2003). Regarding abiotic stress, Beebe et al. (2012) reported that Durango lines originatedat higher altitude in semiarid zones of Mesoamerica had the highest drought tolerance. This type is therefore useful in breeding more drought tolerant cultivars (Terán and Singh 2002; Frahm et al. 2004). Here, the growth habit seems to influence the result and plants with indeterminate, prostrate habits tend perform relatively well under dry

Fig. 1.1 Illustration of the variation in seed coat color of Swedish accessions (Photo S.Ø. Solberg)

conditions (Beebe et al. 2013a). Furthermore, deep rooting is another advantage as well as small seed size, where accessions with a short seed-filling period are less exposed to stress than large-seeded accessions with a longer seed-filling period (Beebe et al. 2001). Regarding heat tolerance there is some of the same patterns. Small-seeded Mesoamerican types are often more tolerant than large-seeded Andean types (Beebe et al. 2013). A few exceptions exist: 'G122' and 'Indeterminate Jamaica Red', landraces from in India, and 'Sacramento' and 'Celrk', lines developed in California, show relatively high heat tolerance (Román-Aviles and Beaver 2003). In Lima bean, a similar relationship is found with Mesoamerican types having higher tolerance to abiotic stresses as heat and drought compared to large-seeded Andean types (Long et al. 2014).

1.3.2 Secondary Gene Pool

The secondary gene pool consists of taxa more remotely related to the crop but still possible to cross and give rise to some fertile progenies. According to Vincent et al. (2013), 36 crop wild relatives of *Phaseolus* are documented and have potential value as genetic resources for crop improvement. In the secondary gene pool, we find *P. albescens* McVaugh ex Ramirez-Delgadillo and A. Delgado, *P. coccineus* L., *P. costaricensis* Freytag & Debouck, *P. dumosus* Macfad, and *P. persistentus* Freytag and Debouck. Secondary gene pool has been used extensively as a source of disease resistance (reviewed in Porch et al. 2013) and to introgress tolerance to aluminum toxicity into common bean (Butare et al. 2011). Table 1.1 gives an overview on crop wild relatives with confirmed or potential interesting stress tolerance traits.

1.3.3 Tertiary Gene Pool

The tertiary gene pool consists of taxa remotely related to the crop and naturally incapable of interbreeding with the crop, but that can be carried out with specific techniques such as protoplast fusion, embryo rescue or genetic engineering. Such genetic resources are only used if there are major limits for the genetic improvement within the primary and secondary gene pools, for example by introgressing genes for tolerance to abiotic and biotic stress. According to Vincent et al. (2013), here are *P. acutifolius* A. Gray, *P. angustissimus* A. Gray, *P. carteri* Freytag & Debouck, *P.filiformis* Benth., *P. maculatus* Scheele, and *P. parvifolius* Freytag.

The tepary bean *P. acutifolius,* is recognized as having greater heat and drought tolerance than common beans (Federici et al. 1990; Teran and Singh 2002; Acosta-Gallegos et al. 2007) hence, it could be used as a model to increase abiotic stress tolerance in common bead (Rao et al. 2013). Interspecific crosses between common bean and tepary bean have already been used to transfer heat resistance genes (CIAT 2015) and common bacterial blight resistance genes (Thomas and Waines 1984; Parker and Michaels 1986; Singh and Muñoz 1999).Only a small portion of genetic variability in tepary bean has been used for common bean improvement, hence there is still potential for large gains to be made through interspecific gene transfer (Singh 2001).

1.3.4 Artificially Induced/Incorporated Traits/Genes

Genetic engineering is a powerful tool to incorporate genes from sources that are inaccessible through traditional crosses (Svetleva et al. 2003). Several attempts have been made to develop reliable transformation methods for engineering common beans with various traits (Rech al. 2008). Genetic manipulations have been conducted

Table 1.1 Confirmed and potential* use of different species in common bean breeding for abiotic and biotic stress resistance

Trait	Species	References
Drought tolerance	*P. vulgaris* var. *aborigineus* *P. acutifolius*	Blair et al. (2016) Mejía-Jiménez et al. (1994)*
Heat tolerance	*P. acutifolius*	Munoz et al. (2004)*
Cold tolerance	*P. coccineus,* *P. costaricensis* *P. dumosus*	Singh (2001)*
Frost tolerance	*P. angustissimus*	Balasubramanian et al. (2004)*
Soil salinity tolerance	*P. acutifolius*	Munoz et al. (2004)*
Aluminum tolerance	*P. coccineus* *P. coccineus*	De Ron et al (2015) Porch et al. (2013)*
Angular leaf spot resistance	*P. coccineus,* *P. dumosus*	Singh (2001) Mahuku et al. (2003)
Anthracnose resistance	*P. coccineus* *P. dumosus*	Mahuku et al. (2002)
Ascochyta blight resistance	*P. dumosus*	De Ron et al. (2015)
Bean stem maggot resistance	*P. coccineus*	De Ron et al. (2015)
Bean yellow mosaic virus resistance	*P. coccineus*	De Ron et al. (2015)
Bruchid resistance	*P. vulgaris* var. *aborigineus*	Osborn et al. (2003)
Common bacterial blight resistance	*P. acutifolius* *P. coccineus* *P. vulgaris* var. *aborigineus*	Singh (2001) Freytag et al. (1982) Beaver et al. (2012)
Fusarium root rot resistance	*P. coccineus*	Singh (2001)
Fusarium wilt resistance	*P. acutifolius*	Porch et al. (2013)
Web blight resistance	*P. vulgaris* var. *aborigineus*	Beaver et al. (2012)
White mold resistance	*P. coccineus* *P. costaricensis* *P.vulgaris* var. *aborigineus*	Schwartz and Singh (2013) Mkwaila et al. (2011)

with biolistic-mediated method but at low efficiencies (0.03–0.9%) (Russell et al. 1993; Aragao et al. 1996; Vianna et al. 2004). Despite the regulatory approval of the transgenic "Embrapa 5.1" common bean with resistance to the Golden Mosaic Virus (BGMV) in Brazil in 2011 (Aragao et al. Aragao 2014; Balsamo et al. 2015; Souza et al. 2018), no genetically modified common bean has been commercialized to date. There is still a lack of an appropriate and reproducible transformation method for generating stable transgenic common bean plants. The key drawback has been the recalcitrance of common bean genotypes from non-meristem-containing tissues to in vitro regeneration (Veltcheva et al. 2005; Mukeshimana et al. 2013; Solis-Ramose et al. 2019).

1.4 Glimpses on Classical Genetics of and Traditional Breeding for Biotic Stress Resistance

Farmers and breeders had only phenotypic traits before the development of molecularmarkers to choose suitable individuals to interbreed. To assess and select useful genotypes, relatively longperiods of time, many generations and significant economic resources were required. This changed when the use of DNA-based molecularmarkers in breeding programs started in the 1980s (Kole and Gupta 2004). More recently, the advancement of large sequencing technologies has resulted inthe systematic use of thousands of molecular markers. Breeders can now use these high-performance sequencing methods to sequence large populations, research the genetic makeup of crop varieties, understand evolutionary relationships between cultivarsand wild relatives, and possibly provide the basis for modeling complex relationships between genotype and phenotype at the whole-genome level (Cobb et al. 2013; Varshney et al. 2014).

In recent years, new genetic tools, including intraspecific and interspecific mapping populations, molecular and associationmaps, quantitative trait loci (QTL), marker-assisted selection (MAS), and genomic selection (GS), have been created and accumulated. The genome of common beanwill enable a deeper, faster and clearer understanding of its genomic architectureand deliver climate resilient and high nutrition varieties for a sustainable agriculture both from ecological and an economic point of view.

Common beans are grown all over the world under much contrasted conditions, from the humid tropics in Latin America and Africa to the semi-arid highlands of Central America and Mediterranean basin and the High Plains of the US and Canada. In each area there are many production methodsand a unique set of biotic and abiotic constraints. Therefore, the goals of breeding program in common bean must be tailoredto meet the needs of farmers who use the cultivars (Kelly 2001; Santalla et al. 2001; Singh 2001).

Improvement in yields remain themost significant target trait for most common bean breeding programs. Improving yield includes addressing several biotic and

abiotic stresses, using a wide set of techniques, including different germplasms as parents, making several crosses, choosing major gene traits under conditions conducive to selection, and testing a large number of breeding lines. For each growing area and/orgrowing type, these stresses are unique. In most cases, however, fungal diseases are the major biotic stress, along with viruses and insects, while the key abiotic stresses are drought and heat stress at flowering, along with cold, low phosphorus, aluminum toxicity, manganese toxicity, and salinity (Singh 1992; Beebe 2012).

Over the past 40 years, convencional breeding has yielded significant achievementsin common bean. Moderate progress has been made in the production and release ofdry bean cultivars with greater seed yield using traditional plant breeding techniques (Singh 1991; Kellyet al. 1998). Improvement in pods/plant, seed/plant, and seed weight has contributed to the majority of efforts to increase seed yield in favorable environments (Bezaweletaw et al. 2006; Ribeiro et al. 2008). Several studies founded that hybridization of interracial bean varieties increased yield, particularly in crosses between Mesoamerican and Durango or Jalisco races (Beebe 2012). Increasing yield potential has also been achieved through breeding for abiotic stress tolerance. Beebe et al. (2008) stated that through photosynthate remobilization and biomass translocation, yield may increaseunder drought conditions, suggesting that yield improvements can be made under abiotic stress conditions.

Important progress has been made in the production ofresistant cultivars for different diseases using traditional breeding methods.For several disease resistance genes, molecular markers were developed and successfully used to improve common bean cultivars and germplasm (see Sect. 1.6 in this chapter and Table 1.2 for a summary of several important resistance-mapping research).

In response to climate change and increased use of marginal environments for bean production, selection for greater tolerance to abiotic stress such as drought, heat and low soil fertility is expectedto rise significantly. Improved common bean cultivars and breeding lines have been developed with enhanced tolerance to many important abiotic stresses such as drought (Frahm et al. 2004; Muñoz-Perea et al. 2006; Brick et al. 2008), low soil P (Lynch 2007; Beebe et al. 2008), aluminum (Yang et al. 2013), high temperature (Rosas et al. 2000; Beaver et al. 2008), and with improved symbioticnitrogen fixation (SNF) ability (Faridet al. 2017).

In recent years, nutrition and quality traits have become prioritiesfor severalbreedingprograms. Diversity in common bean seed micronutrient concentration (Beebe et al. 2000) and an approach to improving iron and zinc bioavailability for humans have been described. Cooking time (Elia et al. 1997; Cichy et al. 2019) and quality of canner (Hosfield and Uebersax 1980) are two major factors associated with consumer preference of dry beans. The quality of canned bean products can be evaluated on seed coat splitting, seed clumping, broth viscosity, extruded starch, or undesirable seed shape, color or size. Quality can be variable and is impacted by seed quality, canning protocol and genotype (Ghasemlou et al. 2013).

Conventional breeding approaches have allowed the development of many important common beancultivars over the past decades. Recent advances in common bean genomics have enabled greater access to key genomicregions that affect different

Table 1.2 Molecular linkage maps in common bean

Parents	Map size	Markers/traits mapped[a]	References
XR235-1–1/Calima (BC_1)	960	224 RFLPs, 9 seed proteins, 9 isozymes, *P*	Vallejos et al. (1992)
BAT 93/Jalo EEP558 (F_2)	1226	194 RFLP, 24 RAPDs, 15 SSR/ALS, ANT, CBB, *V, C,* Rhizobium	Nodari et al. (1993), Gepts (1999), Yu et al. (2000a)
Corel/Ms8EO2 (BC_1)	567.5	51 RFLP, 100 RAPD, 2 SCAR/ANT	Adam-Blondon et al. (1994)
Midas/G 12,873 (RIL)	1,111	77 RFLPs, 5 isozymes /domestication traits	Koinange et al. (1996)
DOR364/XAN176 (RIL)	930	147 RAPDs, 2 SCARs, 1 ISSR/ASB, BGYMV, CBB, *R*, *V*, *Asp*, rust	Miklas et al. (1998, 20001996)
BAC6/HT7719 (RIL)	545	75 RAPDs/CBB, WB, rust	Jung et al. (1996)
PC50/XAN159 (RIL)	426	168 RAPDs/CBB, *C*, *V*, rust, WM	Jung et al. (1997), Park et al. (2001)
BAT 93/Jalo EEP558 (RIL)	1226	120 RFLP, 430 RAPD, 5 isozymes/BCMV	Freyre et al. (1998)
BelNeb-RR-1 /A55 (RIL)	755	172 RAPDs, 2 SCARs/BBS, HB, BCMV	Ariyarathne et al. (1999), Fourie et al. (2004)
Eagle/Puebla152 (RIL)	825	361 RAPDs/RR	Vallejos et al. (2001)
Jamapa/Calima (RIL)	950	155 RAPDs, 88 RFLPs/RGA	Vallejos et al. (2001)
OACSeaforth/OAC 95–4 (RIL)	1,717	49 AFLPs, 43 RFLPs, 11 SSRs, 9 RAPDs, 1 SCAR/CBB, agronomic traits	Tar'an et al. (2001, 2002)
CDRK/Yolano (RIL)	862	196 AFLPs, 8 RFLP/SY, *C*	Johnson and Gepts (2002)
DOR364/ G19833 (RIL)	1,720	78 SSR, 48 RFLPs, 102 RAPDs, 18 AFLPs	Blair et al. (2003)
ICACer/G24404 (RIL)	869,5	80 SSRs, 1 SCAR/ *C*, *fin*, *st*, agron traits	Blair et al. (2006b)
G14519/G4825 (RIL)	915.4	46 RAPDs, 68 SSRs/seed Fe and Zn concentrations and contents	Blair et al. (2010)
BAT 93/Jalo EEP558 (RIL)	1,545	199 gene-based, 59 core and 17 other markers	Hanai et al. (2010), McConnell et al. (2010)

(continued)

Table 1.2 (continued)

Parents	Map size	Markers/traits mapped[a]	References
DOR364/BAT477 (RIL)	2,041	1,060 (SSR, EST-SSR, BES-SSR, gene-based markers)/SW, Y, DF, DM	Blair et al. (2012), Galeano et al. (2011, 2012)
IAC-UNA/CAL143 (RIL)	1,865.9	198 SSRs, 8 STS-DArT, 3 SCAR/ALS	Oblessuc et al. (2012, 2013)
SEA5/CAL96 (RIL)	1,351	2,122 SNPs/SW, Y	Mukeshimana et al. (2014)
Stampede/Red Hawk (RIL)		7,276 SSRs and SNPs	Schmutz et al. (2014)
Iapar 81/ LP97-28 (RIL) CDRK / Yolano (RIL)	815.9 936	773 SNPs/$SY9^{IL}$ 5,398 SNPs	Elias (2018) Valentini et al. (2018)

[a]ALS: Angular Leaf Spot, BCMV: Bean Common Mosaic Virus, CBB: Common Bacterial Blight, HB: Halo Blight, RR: Root Rot, WM: White Mold, SW: Seed Weight, SY: Seed Yield, DF: Days to Flowering, DM: Days to Maturity, Y: Yield, *fin*: Determinacy, *Ppd*: Gene for Photoperiod Sensitivity, *V*: Flower Color, *C*: Seed Color

biotic and abiotic stress tolerance andgrain yield. Availability of common bean reference genome sequence will be of great importance for addressing the domestication and evolution-related queries and functional dissection of traits of breeding relevance.

Future targets in common bean breeding include (i) increased and equitable access to improved dry bean varieties resistant to multiple environmental and climate change-related stresses; (ii) increased access to micro nutrient rich bean varieties and the adaptation of seed composition to novel end-use application possibilities, and (iii) increased access to high value bean products (varieties) targeted to niche markets.

1.5 Brief on Diversity Analysis

1.5.1 Phenotype-Based Diversity Analysis

Wild forms, landraces, and commercial cultivars from all over the world have been extensively collected and characterized using standardized sets of descriptors (CIAT 1980; IBPGR 1982), which are used to describe accessions and divide them into subgroups due to phenotypic variation (Leakey 1988; see Sect. 1.8 in this chapter). This simplistic phenotypic method has been considered useful in order to understand the extent of genetic variation between accessions.

Major classifications of common beans are based on market classes and agro-morphologic traits (Voysest and Dessert 1991; Santalla et al.2002). Market classes in common bean are mostly characterized by distinctive in pod color, shape, and size as well as seed shape (round, oval, cuboid, kidney, and elongate), seed size (varies from small-medium to large size), seed color (white, cream, yellow, brown, pink,

red, purple, black, and other like gray/green/etc.), seed pattern or striation (striped, mottled, and bi-color) (Singh 2001). Seed also varies in terms of surface texture from shiny (brilliant) to opaque to intermediate. Common bean genotypes can also be grouped according to growth habit into five groups: Type I (determinate bush), Type II (indeterminate bush), Type III (indeterminate semi climber), and Type IV (indeterminate climber), Type V (determinate climber) (Singh 1991). In addition to growth habit, beans are often categorized by origin, primarily by the two Andean and Mesoamerican gene pools and by races within the two gene pools (Singh et al. 1991; Beebe et al. 2013). Compared to the Andean gene pool, the Mesoamerican gene pool is characterized by either small (<25 g 100 seed weight^{-1}) or medium (25–40 g 100 seed weight^{-1}) seeds. In the Andean gene pool, race Nueva Granada includes large-seeded light and dark red kidney, white kidney, bush cranberry, most green beans, and yellow beans, while race Chile includes the vine cranberry bean (Gioia et al. 2019). Within the Mesoamerican gene pool, race Mesoamerica includes the small-seeded black, white and navy beans; while race Durango includes the medium-seeded pinto, great northern, small red, and pink beans (Gioia et al. 2019).

Exploitation of genetic resources in common bean breeding is still limited in comparison to availability of materials, and the potential impact of their use is far from optimal. Hundreds of accessions are conserved and maintained in gene banks with very little information available (i.e., lack of comprehensive information regarding passport data and descriptors useful for users, accession heterogeneity, non-harmonized data, e.g.), making their selection and use for specific purposes by researchers and breeders difficult.

1.5.2 Genotype-Based Diversity Analysis

Various marker systems have been applied to analyze diversity or polymorphisms in common bean but more recently single nucleotide polymorphism (SNP) markers are of interest (Hyten et al. 2010; Felicetti et al. 2012; Blair et al. 2013; Goretti et al. 2014; Zou et al. 2014, see also Sect. 1.6 in this chapter). Expressed sequence tags (ESTs) have been used at the transcriptional level to discover and classify genes differentially expressed under different conditions. Whole genome transcriptome analysis is also an efficient way to exploit key factors involved in transcriptional and metabolic activities for common bean responses to biotic and abiotic stress (Schmutz et al. 2014; Vlasova et al. 2016).The genomics era has resulted in a rapid increase in available sequence data, which can provide a more accurate picture of the genetic diversity and structure of germplasms of crops, along with the identification of genetic variants on the basis of important heritable target traits (Luikart et al. 2018).The current availability of high-throughput sequencing platforms has enabled the release of the high-quality reference genomes of the Andean genotype G19833 (Schmutz et al. 2014) and the Mesoamerican genotype BAT93 (Vlasova et al. 2016). A further high-quality common bean reference genome of race Durango pinto UI111 genotype was recently released (*Phaseolus vulgaris* UI111 v1.1, DOE-JGI and USDA-NIFA, http://

phytozome.jgi.doe.gov/). The assembly of the *P. vulgaris* genome is allowing a better and deeper understanding of its genomic architecture and will serve as an invaluable genomic guide to further develop our molecular-level knowledge of common beans and can be extended to molecular breeding for plants with improved biotic and abiotic tolerance.

1.5.3 Relationship with Other Cultivated Species and Wild Relatives

P. vulgaris exists as wild in South America with further five closely related taxa: *P. coccineus, P. dumosus, P. costaricensis, P. albescens,* and *P. persistentus*, which again relate to a number of other *Phaseolus* species. Wild *P. coccineus*grows wild from Mexico to Guatemala (Nabhan 1985; Debouck et al. 1995), while *P. dumosus* grows wild in western Guatemala and the Northern Andes, often as a weed (Schmit and Debouck 1991). *P. costaricensis* grows in the mountains of Costa Rica and Panama (Freytag et al. 1996; Araya-Villalobos et al. 2001), while *P. albescens* grows in the forests of Mexico (Ramírez-Delgadillo and Delgado-Salinas 1999).

Introgression, especially between cultivated and wild *P. vulgaris* and with *P. coccineus*, occurred in these centers of diversity (Wall 1970).

1.5.4 Relationship with Geographical Distribution

After domestication, crop species have extended their geographical distribution to large areas exploring highly diverse habitats from their relatively small canters of origin located in particular ecological niches. Through the selection of local varieties (i.e. landraces) correlated with adaptation to new and sometimes intense conditions, this process led to crop diversification. Bellucci et al. (2014) found that in common bean a small fraction (2.8%) of the genes detected as domestication outliers resulted in the wild forms fixed (monomorphic), whereas in the domesticated were highly polymorphic. Adaptive processes are expected to be connected to this new functional diversity. Bitocchi et al. (2017), which examined nucleotide sequences at 49 gene fragments on a collection of 45 *P. vulgaris* accessions, mostly wild and domesticated from Mesoamerica, also reported similar findings. Moreover, Bitocchi et al. (2017), in five genes of domesticated forms, detected an increase in functional diversity, and the function of these genes, expressed as plant reaction to biotic and abiotic stresses, suggests that they are involved in adaptation.

The Colombian Exchange that started after the voyage of Columbus 1492 was a major event that facilitated the dissemination of common bean and several other crop species worldwide. This process is very recent in its evolutionary scale (i.e. 400–500 generations for annual crops) and is an important experimental model for

understanding the rapid adaptation of crop plants to evolving environments and dissecting the genomic basis for adaptation to the environment.

Common bean represents an ideal model for these studies as it was rapidly disseminated out of the New World but also due to its two highly differentiated gene pools (Andean and Mesoamerican) that were introduced in different proportion in different continents. In Europe, a higher proportion of Andean genotypes are found (Gepts and Bliss 1988; Lioi 1989a, 1989b; Logozzo et al. 2007; Angioi et al. 2010; Gioia et al. 2013), while Mesoamerican forms are largely present in Argentina (Burle et al. 2010) and China (Zhang et al. 2008) and a balanced proportion of Mesoamerican and Andean types is found in Africa (Gepts and Bliss 1988; Asfaw et al. 2009; Blair et al. 2010). The breakdown of the spatial geographical barriers between the Mesoamerican and Andean types is especially interesting in terms of genetic variability and adaptation. This favored hybridization and recombination between the two gene pools and lead to the occurrence of novel genetic combinations and, consequently, novel genotypic and phenotypic variation (Angioi et al. 2010; Gioia et al. 2013), which again has been a key tool for breeding programs aimed to develop novel varieties. Evidence of this phenomenon has been detected using phaseolins, allozymes, and morphological data (Santalla et al. 2002; Rodiño et al. 2006), as well as inter-simple sequence repeats (ISSRs) and simple sequence repeats (SSRs) data from both the chloroplast and nuclear genomes (Sicard et al. 2005; Angioi et al. 2010; Gioia et al. 2013). Gene flow between both gene pools appears to be relatively common in the Andean (Debouck et al. 1989; Beebe et al. 1997; Chacón et al. 2005) and European zones (Santalla et al. 2002; Sicard et al. 2005; Angioi et al. 2010; Gioia et al. 2013).

1.5.5 Extent of Genetic Diversity

Modern varieties of common bean are inbred but wild plants, and to some extent landraces, have proportion of outcrossing. Landraces are therefore usually more diverse than modern varieties, and landraces often comprise of a mixture of more or less homozygous lines (Fig. 1.2) (Pierson 2012). Therefore, such varieties are population varieties, often with a high within-population diversity. Number of plants used in diversity studies is therefore of importance, and for genebanks, regeneration

Fig. 1.2 Photo of the landrace 'Götlandsböna' (NGB11554), collected on the Swedish island Gotland and that include at least two distinct lines (Photo S.Ø. Solberg)

a high number of plants applies for maintaining this diversity (FAO 2014). The extent of genetic diversity is developed further in Sects. 1.7 and 1.8.

1.6 Molecular Mapping of Resistance Genes and QTLs

1.6.1 Brief History of Mapping Efforts

Genetic linkage maps are useful tools for genetic analysis and have played a major role in genetic common bean improvement. They have been extensively used for many genetic applications such as tagging genes of interest to facilitate marker assisted selection (MAS) programs, cloning of agronomically important genes, comparative mapping, and analysis of germplasm diversity (Gepts 1999; González et al. 2017). In the literature, several common bean linkage maps have been reported (Table 1.2), and they include different features such as the types of parents and segregating population used, the type of markers and traits segregating in each population, the total map length, and the degree of saturation of the genome. The first common bean linkage maps were developed using markers with low-throughput markers, which resulted in low density maps. In this context, an international consortium called 'Phaseomics' and the BeanCAP project (USDA Common Bean Coordinated Agricultural Project) were developed to establish the necessary framework of knowledge and materials for the advancement of bean genomics, transcriptomics, and proteomics (Gepts et al. 2008; Fonsêca et al. 2010; Hyten et al. 2010). As a result, genotyping technologies and high-throughput molecular marker technology have contributed to produce high density maps enabling high precision QTL mapping (or high-density functional maps) and will accelerate MAS and genomic-assisted breeding (GAB) in common bean.

1.6.2 Marker Types

The first genetic common bean linkage map was based on morphological markers and showed a reduced genomic coverage (Lamprecht 1961). Later, it was further extended with seed proteins and isozymes (Bassett 1988; Koenig et al. 1990; Vallejos and Chase 1991) providing the baseline for the development of the DNA-based linkage maps.

There was a parallel evolution of common bean genetic maps and the development of molecular marker technologies. Moreover, molecular markers allowed to increase to a great extent the number of polymorphic loci in the mapping populations. Initially, linkage maps were developed based on DNA hybridization markers like restriction fragment length polymorphism (RFLP) which allowed the development of the first DNA-based genetic maps in common bean due to their great robustness and repeatability (Vallejos et al. 1992; Nodari et al. 1993). These maps were subsequently used to

compare and integrate different genetic maps (Adam-Blondon et al. 1994; Koinange et al. 1996; Freyre et al. 1998; Gepts 1999; Yu et al. 2000b). With the development of the PCR, new molecular markers were used for genetic mapping, among which random amplified polymorphic DNA (RAPD) (Williams et al. 1990), amplified fragments length polymorphism (AFLP) (Vos et al. 1995), simple sequence repeats (SSR) (Tautz 1989), and inter-simple sequence repeat (ISSR) (Zietkiewicz et al. 1994) have been the most applied. PCR-based molecular markers were used for saturating the RFLP maps and to create new ones based on additional mapping populations (Freyre et al. 1998; Ariyarathne et al. 1999; Yu et al. 2000b; Blair et al. 2003, 2010; Fourie et al. 2004).

The first RFLP-based genetic map, constructed using 224 RFLP marker loci, nine seed proteins, nine isozyme markers and the seed and flower color marker *P*, was developed by Vallejos et al. (1992). These markers were distributed into 11 linkage groups (LGs) covering 960 cM of the common bean genome. A second RFLP-based genetic map included 108 RFLPs, seven RAPDs, seven isozymes and 18 loci corresponding to 15 known genes, the *I* gene for bean common mosaic virus (BCMV) resistance, a seed color pattern gene, and a flower color gene (Nodari et al. 1993). All these markers were grouped into 15 LGs, with an average interval of 6.5 cM between markers, covering 827 cM of the bean genome. This map was later improved by Gepts et al. (1993) which included 204 markers grouped into 13 LGs covering 1060 cM. Adam-Blondon et al. (1994) constructed a third map including 157 markers: 51 RFLPs, 2 SCARs (sequence characterized amplified regions), 100 RAPDs and four morphological markers, spanning 567.5 cM of the bean genome. A preliminary correspondence with the map developed by Vallejos et al. (1992) was established by Adam-Blondon et al. (1994) since 19 RFLP markers were shared between both maps. The first core linkage map of common bean was constructed by Freyre et al. (1998) on the base of the shared RFLP markers among these three previous maps (Vallejos et al. 1992; Nodari et al. 1993; Adam-Blondon et al. 1994). This map included 563 markers: 120 RFLPs and 430 RAPDs, in addition to a few isozyme and phenotypic marker loci, which were sorted into 11 LGs covering 1226 cM.

In successive years, SSR markers, which are highly polymorphic PCR-based markers, replaced RFLP markers to anchor different genetic maps. The first successful assignment of 15 SSRs to a framework map based on RAPD and RFLP markers was published by Yu et al. (2000b). With the availability of EST (expressed sequence tag) sequencing programs several functional markers have been developed from coding genomic regions. Blair and collaborators in 2003 were the first to incorporate SSR markers developed from EST databases in a linkage map, which comprised a total of 246 loci (78 SSR, 48 RFLP, 102 RAPD and 18 AFLP markers) covering 1720 cM. In successive years, EST libraries become an important source of gene-based markers, like EST-SSR, single nucleotide polymorphism (SNP) and insertion/deletion (InDel), which are valuable markers representing transcribed sequences that can be associated with phenotypic characteristics (Hanai et al. 2010; Galeano et al. 2012; Oblessuc et al. 2012). Since then, functional markers have been progressively incorporated in the common bean linkage maps. Furthermore, functional maps allowed synteny comparisons between the common bean and other

genomes since EST based markers are highly conserved between species (McConnell et al. 2010).

With the advent of the next-generation sequencing (NGS) technology, high-throughput genotyping approaches were developed allowing the rapid and cost-effective generation of high-density functional maps. In this way, a SNP resource developed by the USDA BeanCAP project, the Illumina Infinium assay BARCBean6K_3 BeadChip resulted a valuable tool for high-throughput genotyping leading to the direct gene tagging for QTL mapping of different common bean resistance loci by using standard bi-parental populations or association panels (Brisco et al. 2014; Miklas et al. 2014; Hagerty et al. 2015;Viteri et al. 2015;Nakedde et al. 2016; Zuiderveen et al. 2016).

Moreover, with NGS technology sequencing of complete plant genomes has become increasingly more accessible and routine. Nowadays, the whole genome of common bean has been sequenced and the complete genomes of the Mesoamerican and Andean beans BAT93 and G19833 are available (Schmutz et al. 2014; Vlasova et al. 2016). Moreover, the PhaseolusGenes database, (http://www.beancap.org/; http://phaseolusgenes.bioinformatics.ucdavis.edu/), developed as part of the BeanCAP project resulted a useful tool to place markers on assembled common bean and soybean genomes. The whole genome sequence accelerates the development of markers for high throughput genotyping to be used in plant breeding and genetic studies promoting the identification of makers tightly linked to agronomical important traits (Moghaddam et al. 2014; Meziadi et al. 2016; Valentini et al. 2017).

1.6.3 Mapping Populations Used

In common bean genetic mapping, several segregating populations have been used (Table 1.2). As common bean breeding programs have different economic traits of interest, widely divergent parents were chosen to maximize the genetic polymorphism at the nucleotide level, the phenotypic variation and variability for disease resistance and other traits. Also, in many cases, the parents were chosen to belong to different gene pools because in this way the polymorphism among genotypes was markedly increased (Nodari et al. 1992; Haley et al. 1994). For instance, to develop the first linkage map, Vallejos et al. (1992) used a mapping population which consisted of a backcross progeny (BC_1) between the Mesoamerican line XR-235–1-1 with the Andean cultivar Calima (XC). In another study, Adam-Blondon et al. (1994) used a BC_1 population derived from an inter-gene pool cross between two European bean genotypes: Ms8EO2 and Corel (MsCo), whereas Nodari et al. (1993) used a F_2 population derived from the cross between the Mesoamerican line BAT 93 with the Andean cultivar Jalo EEP558 (BJ).

Recombinant inbred line populations (RIL; F_2-derived lines) have been extensively used in common bean mapping (Table 1.2). For example, the BJ F_2 mapping population was advanced to a RIL to develop the first core linkage map of common bean (Freyre et al. 1998), and then was later improved by McConnell et al. (2010)

and Hanai et al. (2010). Furthermore, the base map developed by Blair et al (2003) using SSR markers was performed using a RIL derived from the cross between the Mesoamerican variety DOR364 and the Andean landrace G19833 (DG). Likewise, numerous RIL populations were developed and used for bean genetic mapping studies and QTL identification in the last years (Blair et al. 2006b, 2010; Hanai et al. 2010; Oblessuc et al. 2012). For genetic mapping studies, the RIL populations derived from the BJ and DG inter-gene pool crosses have been broadly used since they are considered core mapping populations (Freyre et al. 1998; Blair et al. 2003, 2006a; Galeano et al. 2009, 2011, 2012; McConnell et al. 2010; Hanai et al. 2010).

1.6.4 Mapping Software Used

A genetic map is a list of genetic elements ordered according to their co-segregation patterns. Genetic maps of plants species whose genomes are yet to be sequenced provide an essential resource to understand the order and spacing of markers, and to leverage additional genetic information through comparative mapping with genetic maps and genome sequences of other plant species. Further, genetic maps allow studies of plant genes implicated in key plant traits (Cheema and Dicks 2009). In species whose genomes have been sequenced, genetic maps provide a scaffold for genome sequence assembly and validation, and they enable the suggestion of candidate genes conditioning any specific trait. Additionally, genetic maps can be used for marker-assisted selection in breeding programs (Cheema and Dicks 2009). Mapmaker is used to construct linkage maps, developed by Lander et al. (1987), using an algorithm for the simultaneous multipoint analysis of various loci. Polymorphic marker loci are essential to obtaining genetic linkage maps, and the advent of restriction fragment length polymorphism (RFLP) first allowed such genetic studies. Linkage analysis uses the maximum likelihood to construct genetic linkage maps from F_2 intercrosses, or from two- and three-generation nuclear families in natural populations (Lander et al. 1987). With the emergence of advanced genomic sequencing technologies, genotyping becomes easier and faster, frequently using SSR, SNP, and KASP markers to construct linkage maps.

JoinMap was developed by Stam in 1993 and comes with the differential of construct genetic maps with linkage data collected in different experiments. It performs a sequential build-up of the map and, at each step, a numerical search for the best fitting order of the markers, wherein the distance is estimated by weighted least square. Building an integrated map is necessary to determine marker positions segregating only one parent relative to another, and the linkage analysis of experiments based on inbred line crosses is less complicated than for other crosses (Van Ooijen 2011). Most plant model species and many important crop species are autogamous, which has propitiated linkage analysis for species inbreeding. Molecular markers, such as Single-Nucleotide Polymorphisms (SNPs), are now widely used for constructing linkage maps in all major crops.

A genetic linkage map of the common bean based entirely on SNPs is useful for identifying genes/QTL-controlling traits and marker-assisted selection. High-density common bean linkage maps containing thousands of SNP markers were constructed by Song et al. (2015). These SNPs were identified by aligning millions of reads to the Andean reference sequence (G19833) of the common bean (Schmutz et al. 2014). For this purpose, a total of 110 RILs from the mapping population California Dark Red Kidney (Andean) × Yolano (Mesoamerican) were used in this study. The development of the CY population was described by Johnson and Gepts (1999, 2002).

Seeds of each line were multiplied at the greenhouse of the Universidade Estadual de Maringá, Paraná, Brazil. Leaf tissue harvested from single plantlets and high-quality genomic DNA for SNP genotyping was isolated with the PureLink® Genomic DNA Mini Kit, following the manufacturer's instructions. The 110 CY RILs and parents were screened with 5,398 SNP markers of the Illumina BeadChip BARCBEAN6K_3 (Song et al. 2015), following the Infinium HD Assay Ultra protocol (Illumina, Inc., San Diego, CA). The fluorescence intensity obtained by the BeadChip was visualized using Illumina BeadArray Reader. SNP alleles were automatically called using Illumina GenomeStudio V2011.1 (Illumina, Inc., San Diego, CA). Allele calls were visually inspected and errors in allele calling were manually corrected. Molecular analysis was performed at the USDA-ARS Soybean Genomic and Improvement Laboratory in Beltsville, MD. For linkage map construction, a pre-selection of SNPs was carried out in Microsoft Excel. Distances between markers were calculated using Kosambi's mapping function with default parameters in JoinMap 4.1.

1.6.5 Details of Genetic Linkage Maps

Song and collaborators in 2015 studied the mapping population of 267 F_2 plants derived from the Stampede × Red Hawk common bean cross developed by Dr. Phil McClean at North Dakota State University. Linkage maps were constructed using JoinMap 4.0 (Van Ooijen 2006). As a result, the 267 F_2 plants of the Stampede × Red Hawk population were genotyped with the BARCBean6K_1 and BARCBean6K_2 BeadChips. After elimination of SNPs with missing data >10%, or loci with significant segregation distortion from a 1:2:1 ratio as measured by $\chi 2$ at the 1% significant level, 6,531 SNPs were retained for linkage analysis. Analysis of 7,040 markers, including 25 framework markers and 484 previously mapped SNPs, produced a genetic map consisting of 11 consensus linkage groups that spanned 1042.2 cM of Kosambi map distance. The average number of markers mapped per linkage group was 640, ranging from 225 to 979.

Previous studies performed the co-segregation analysis of resistance to *C. lindemuthianum* races 7 and 73 and *P. griseola* race 63–39 in a Ouro Negro cultivar using an F_2 population from the Rudá × Ouro Negro cross and $F_{2:3}$ families from the AND 277 × Ouro Negro cross. Results from Gonçalves-Vidigal et al. (2013) revealed that the ANT resistance gene *Co-3*^{4} and the ALS resistance gene *Phg-3* co-segregated

and were tightly linked to marker g2303 at a distance of 0.0 cM on Pv04 (Fig. 1.3). The close linkage between the *Co-3*^{4} and *Phg-3* genes and prior evidence is consistent with the existence of a resistance gene cluster at the end of chromosome Pv04, which contains the ANT resistance QTL *ANT4.1*^{UC} in addition to *Co-3*^{4} and *Phg-3* (Oblessuc et al. 2014).

Studies conducted by Valentini and collaborators in 2018 resulted in a common bean high-density SNP map using a California Dark Red Kidney (CDRK) × Yolano (CY population) RIL population. A total of 110 CY lines and parents CDRK and Yolano were screened with 5,398 SNP markers of the Illumina BeadChip BARCBEAN6K_3 following the Infinium HD Assay Ultra protocol (Illumina, Inc., San Diego, CA). After elimination of SNPs with a high frequency of missing data, or loci with a minor allele frequency of 30%, 3,277 SNP markers were selected to participate in linkage mapping analysis. Distances between markers were calculated using Kosambi's mapping function with default parameters in JoinMap 4.1.

The final linkage map for the population CDRK × Yolano, comprising 11 consensus linkage groups, spanned 936 cM with an average interval of 0.3 cM between markers (Table 1.3). The average number of markers mapped per linkage group was 290, ranging from 160 to 406. This map covered 512.15 Mbp of the genome, based on the physical distance (bp) between the first and last SNPs mapped to each chromosome. The average recombination rate (Kb/cM), measured by the physical (Kb) and genetic (cM) position of the last marker mapped in each chromosome, was 565.7 Kb, like an earlier observation around the Phaseolin locus. The

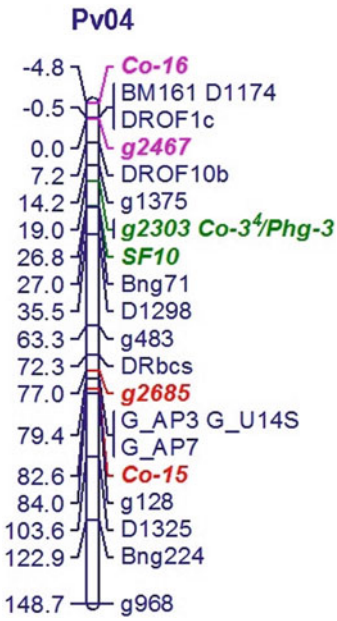


Fig. 1.3 Genetic distances and locations of the *Co-3*^{4} gene for resistance to common bean ANT, the *Phg-3* gene for resistance to ALS, and the molecular markers g2303 on linkage group Pv04 of *Phaseolus vulgaris* L. Map drawn with MapChart (Voorrips 2002; Gonçalves-Vidigal et al. 2013)

Table 1.3 Linkage group, number of SNPs, genetic and physical length, and recombination rate (Kb/cM) per chromosome for the RIL population CDRK × Yolano. Physical length (Kb) is based on the physical distance (bp) between the first and last SNPs mapped to each chromosome

Linkage group	Number of SNPs	Genetic length (cM)	Physical length (Kb)	Kb/cM
Pv01	328	90.17	51,870.7	575.3
Pv02	365	111.38	49,027.0	440.2
Pv03	264	116.82	52,103.9	446.0
Pv04	290	83.73	45,759.9	546.5
Pv05	406	90.22	40,452.9	448.4
Pv06	208	56.49	31,957.2	565.7
Pv07	332	90.47	51,531.0	569.6
Pv08	369	85.43	59,494.1	696.4
Pv09	285	90.61	37,039.7	408.8
Pv10	270	55.83	42,724.4	765.3
Pv11	160	65.23	50,187.1	769.4
Total	3,277	936.38	512,147.9	–
Mean	290	90.17	49,027.0	565.7

genetic position of most SNPs in the linkage maps was consistent with the physical positions along each chromosome of the *Phaseolus vulgaris* genome assembly V1.0 (Fig. 1.4). Additionally, the genetic and physical distances for the 3,277 SNPs mapped using the CY RIL population were correlated with the observed distances reported by Song et al. (2015).

To determine the genetic basis of disease resistance in the genotype CDRK, 110 RILs derived from the California Dark Red Kidney × Yolano (CY) RIL population described by Johnson and Gepts (1999) were used. SNP markers that were polymorphic between the parents CDRK and Yolano segregated at a 1:1 ratio in the RIL population, as measured by the $\chi 2$ test at $p = 0.01$, were used to create a linkage map using the default settings of JoinMap 4.1 (Van Ooijen 2006). Briefly, the regression-mapping algorithm based on the Kosambi map function was used to define the linkage order and genetic distances in centiMorgans (cM). A minimum likelihood of odds (LOD) ≥ 3.0 and a maximum distance of $\leq$50 cM were used to test linkages among markers. A genetic linkage map was created using MapChart (Voorrips 2002). SNP markers flanking the genomic locations associated with ANT and ALS disease reactions were used to define the physical region of these loci based on the bean reference genome v.1.0 (Schmutz et al. 2014), available in NCBI v.1.0 (http://phytozome.jgi.doe.gov).

Genetic linkage analysis conducted between the $CoPv01^{CDRK}$/$PhgPv01^{CDRK}$ loci and SNPs showing the expected segregation of 1:1 in the RIL population revealed that loci are flanked by the SNP markers ss715645251 and ss715645248 (Fig. 1.5) in a genomic region on chromosome Pv01 (Gonçalves-Vidigal et al. 2020). Based on the bean reference genome v1.0 (https://www.ncbi.nlm.nih.gov), these markers

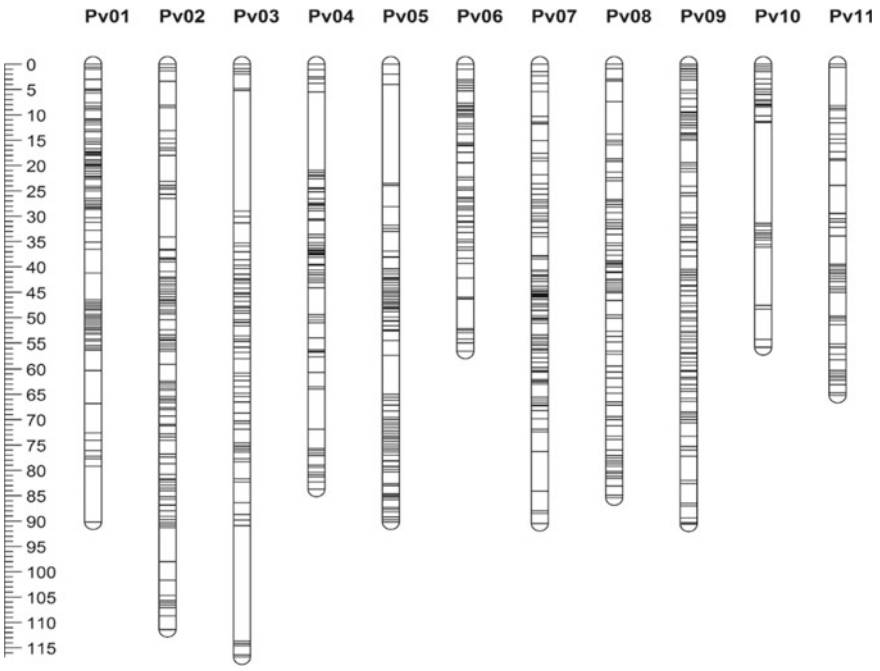


Fig. 1.4 Genetic mapping of the RIL population CDRK × Yolano using 3,277 SNP markers assigned to the 11 linkage groups of common bean. Scale in centiMorgan (cM) distance indicated on the left side. Distances between markers were calculated using Kosambi's mapping function with default parameters in JoinMap 4.0 (Van Ooijen 2006). Genetic linkage maps were designed using MapChart (Voorrips 2002; Valentini et al. 2018)

are located at positions of 50,301,532 bp and 50,546,985 bp, respectively, which correspond to a distance of 245.6 Kb.

A fine linkage map was developed with 17 SNPs, two additional SSRs (BARCPVSSR01358 and BARCPVSSR01361), and the STS CV542014 markers (http://phaseolusgenes.bioinformatics.ucdavis.edu/markers/1009). To reduce the distance between SNP markers ss715645251 and ss715645248 markers in the genomic region containing the $CoPv01^{CDRK}$/$PhgPv01^{CDRK}$ loci, Gonçalves-Vidigal et al. (2020) performed a fine-mapping analysis by genotyping 19 RILs that showed recombination events in the 245.6 Kb region. Recombination events were identified based on the genotypic data of all 110 RILs obtained with the BARCBEAN6K_3 BeadChip.

Upon genotyping these 19 RILs with 12 SNPs, two SSRs, and one STS marker, we observed that the susceptible CY5 RIL and the resistant CY48 RIL contained recombination events (Table 1.4) that allowed us to delimit the $CoPv01^{CDRK}$/$PhgPv01^{CDRK}$ loci region to the area between the CV542014 and ss715645248 markers. Based on the bean reference genome (Schmutz et al. 2014) these new $CoPv01^{CDRK}$/$PhgPv01^{CDRK}$ loci flanking markers are located at positions

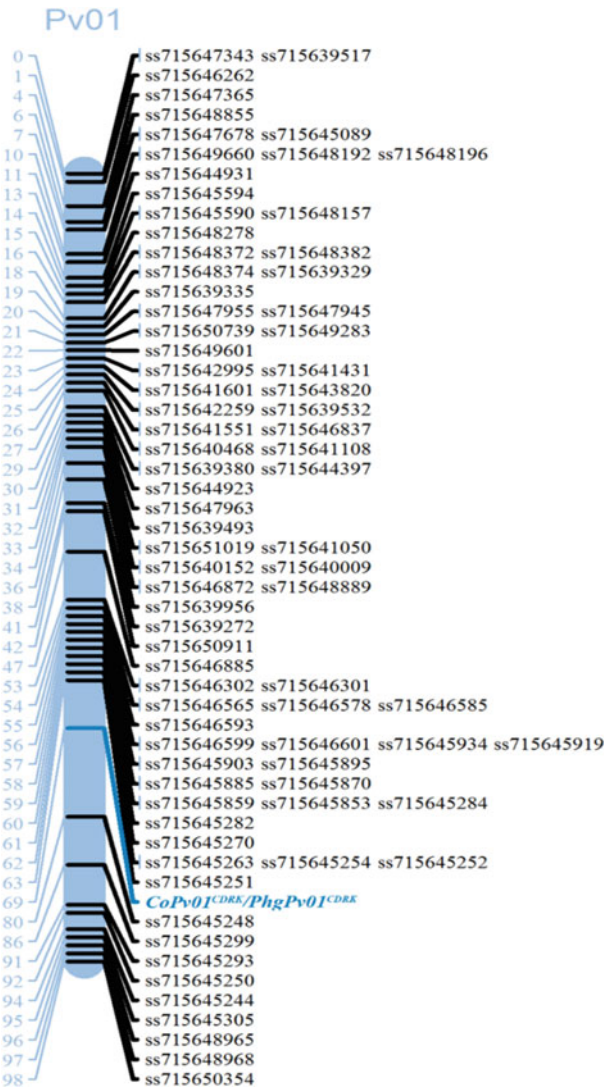


Fig. 1.5 Genetic map of common bean linkage group Pv01 containing the anthracnose and angular leaf spot resistance loci and linked single nucleotide polymorphism (SNPs) markers used to genotype the F_{10} population California Dark Red Kidney × Yolano. Recombination distances are indicated on the left side of the linkage group in centiMorgans (cM), and the marker names are shown on the right side. The *CoPv01*^{CDRK}/*PhgPv01*^{CDRK} resistance loci were flanked by SNP markers ss715645251 and ss715645248 in F_{10} mapping population. The map was drawn with MapChart (Voorrips 2002; Gonçalves-Vidigal et al. 2020)

Table 1.4 Genotype and phenotype of 19 F_{10} recombinant events in the region of Pv01 used for fine mapping of the ANT and ALS resistance loci in CDRK. Genotyping was achieved using flanking markers—12 SNP, two SSR, and one STS—that positioned *CoPv01*^{CDRK}/*PhgPv01*^{CDRK} *l*oci in a 33 kb genomic region flanked by markers CV542014 and ss715645248

Marker	SNP position	Recombinant lines from CDRK × Yolano																		
		5	12	19	20	33	38	43	47	48	62	70	73	79	87	88	91	115	96	146
ss715645260	50115685	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	AA	BB	BB	AA	BB	AB	AB
ss715645259	50130201	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	AA	BB	BB	AA	BB	AB	AB
ss715645258	50155987	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	AA	BB	BB	AA	BB	AB	AB
ss715645257	50161526	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	AA	BB	BB	AA	BB	AB	AB
ss715645256	50182775	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	AA	BB	BB	AA	BB	AB	AB
ss715645254	50203547	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	AA	BB	BB	AA	BB	AB	AB
ss715645252	50222584	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	AA	BB	BB	AA	BB	AB	AB
ss715645251	50301592	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	BB	BB	BB	AA	BB	AB	AB
BARCPVSSR01358	50350345	AA	AA	BB	BB	AA	BB	-	AA	AA	BB	AA	AA	BB	BB	BB	AA	BB	BB	AA
BARCPVSSR01361	50388017	AA	AA	BB	BB	AA	BB	-	AA	AA	BB	AA	AA	BB	BB	BB	AA	BB	BB	AA
CV542014	**50513853**	AA	AA	BB	BB	AA	BB	AA	AA	AA	BB	AA	AA	BB	BB	BB	AA	BB	AB	AB
CoPv01^{CDRK}/*PhgPv01*^{CDRK}		**BB**	**AA**	**BB**	**BB**	**AA**	**BB**	**AA**	**AA**	**AA**	**BB**	**AA**	**AA**	**BB**	**BB**	**BB**	**AA**	**BB**	**BB**	**AA**
ss715645248	**50546985**	BB	AA	BB	BB	AA	BB	AA	AA	BB	BB	AA	AA	BB	BB	BB	AA	BB	AB	AB
ss715645299	51353193	BB	BB	AA	BB	AA	AA	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	AB	AA
ss715645293	51617802	BB	BB	AA	AA	BB	AA	BB	BB	BB	AA	AA	BB	BB	AA	AA	BB	AA	AA	AA
ss715645250	51726047	BB	BB	AA	AA	BB	AA	BB	BB	BB	AA	AA	BB	BB	AA	AA	BB	AA	BB	AA
ss715645244	51764167	AA	BB	AA	AA	BB	AA	BB	BB	BB	AA	AA	BB	BB	AA	AA	BB	AA	BB	AA
ss715645305	51786948	AA	BB	AA	AA	BB	AA	BB	BB	BB	AA	AA	BB	BB	AA	AA	BB	AA	BB	AA
ss715645301	51819821	AA	BB	AA	AA	BB	AA	BB	BB	BB	AA	AA	BB	BB	AA	AA	BB	AA	BB	AA
ss715648967	51883712	AA	BB	AA	AA	BB	AA	BB	BB	BB	AA	AA	BB	BB	AA	AA	BB	AA	BB	AA
ss715648965	51896315	AA	BB	AA	AA	BB	AA	BB	BB	BB	AA	AA	BB	BB	AA	AA	BB	AA	BB	AA

AA = Resistant; BB = Susceptible; AB = Heterozygous; - = not available.

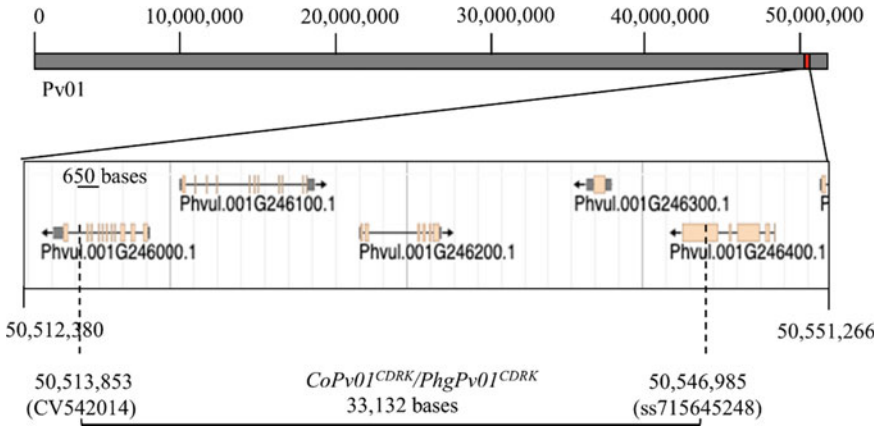


Fig. 1.6 Fine mapped region for the CDRK resistance loci*CoPv01*^{CDRK}/*PhgPv01*^{CDRK}. The upper bar represents the entire chromosome Pv01, with the *CoPv01*^{CDRK}/*PhgPv01*^{CDRK} region highlighted in red. The five predicted genes in this region are shown, with the *CoPv01*^{CDRK}/*PhgPv01*^{CDRK} flanking markers CV542014 and ss715645248 indicated by dashed lines, within the predicted genes Phvul.001G246000 and Phvul.001G246400, respectively. The genomic region between these markers is indicated by the lower bar and covers around 33 Kbp of the genome (Gonçalves-Vidigal et al. 2020)

50,513,853 bp (CV542014) and 50,546,985 bp (ss715645248) of chromosome Pv01, spanning 33 Kb (Fig. 1.6).

1.6.6 Enumeration of Mapping of Resistance Genes and QTLs

1.6.6.1 Disease Resistance

Fungal Diseases

Resistance to anthracnose (ANT), caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara, is conferred by independently segregating individual loci in a series named *Co* and mapped to date (Table 5). Several of these anthracnose-resistance genes are in clusters, where they are tightly linked to other resistance genes (for angular leaf spot, rust, etc.), and these clusters are often positioned at the ends of chromosomes (Vaz Bisneta and Gonçalves-Vidigal 2020). Currently, the known *Co* genes are *Co-1* and its alleles, *Co-Pa*, *Co-x*, *Co-w*, *CoPv01*^{CDRK}, and *Co-AC* on chromosome Pv01 (Geffroy et al. 2008; Mahiya-Farooq et al. 2019; Gonçalves-Vidigal et al. 2011; Richard et al. 2014; Chen et al. 2017; Lima Castro et al. 2017; Gonçalves-Vidigal et al. 2020; Gilio et al. 2020); *Co-u* and *CoPv02* on chromosome Pv02 (Campa et al. 2014; Geffroy et al. 2008); *Co-13* and *Co-17* on chromosome

Pv03 (Lacanallo and Gonçalves-Vidigal 2015; Trabanco et al. 2015); *Co-3*, *Co-3*^{2}, *Co-3*^{3}, *Co-3*^{4}*/Phg-3*, *Co-y*, *Co-z*, and *Co-RVI* on chromosome Pv04 (David et al. 2008; Gonçalves-Vidigal et al. 2013; Murube et al. 2019); *Co-5*, *Co-6* and *Co-v* on chromosome Pv07 (Young et al. 1998; Geffroy et al. 2008; Sousa et al. 2014); and *Co-2* on chromosome Pv11 (Kelly and Young 1996; Meziadi et al. 2016). Additionally, recent studies conducted by Azevedo et al. (2018) have revealed that COK-4, a putative kinase encoded in the ANT resistance locus *Co-4* that is transcriptionally regulated during the immune response, is highly like the kinase domain of FERONIA (FER) in *Arabidopsis thaliana,* a factor that has a role in balancing distinct signals to regulate growth and defense.

Several sources of resistance to angular leaf spot (ALS), which is caused by the fungus *Pseudocercospora griseola,* (Sacc.) Crous and Braun, have been identified in common bean. Furthermore, single, dominant resistance loci, as well as QTLs conferring resistance to ALS, have been reported (Miklas et al. 2006; Mahuku et al. 2009; 2011; Gonçalves-Vidigal et al. 2011; 2013; Oblessuc et al. 2013; Keller et al. 2015). The genes conferring resistance to ALS formally accepted by the Bean Improvement Cooperative (BIC) Genetic Committee are presented in Table 5. The *Phg-1* on chromosome Pv01 is tightly linked (0.0 cM) to the ANT locus *Co-1*^{4} in cultivar AND 277, which led to the designation of the locus as *Co-1*^{4}*/Phg-1* (Gonçalves-Vidigal et al. 2011). The *Phg-1* locus was discovered using F_2 plants from crosses of AND 277 × Rudá and AND 277 × Ouro Negro inoculated with *P. griseola* race 63–23.

A previous study conducted by de Carvalho et al. (1998) used the name *Phg-1* before describing a resistance locus in AND 277 crossed with Rudá. The molecular marker CV542014^{450} have been found to be linked with the *Co-1*^{4}*/Phg-1* loci at 0.7 cM (Gonçalves-Vidigal et al. 2011). The ALS resistance gene *Phg-2* in Mesoamerican cultivar Mexico 54 was discovered using a cross between Mexico 54 × Rudá and *P. griseola* race 63–19. The authors identified RAPD markers OPN02^{890}, OPAC14^{2400}, and OPE04^{650} as being linked to *Phg-2* at 5.9, 6.6, and 11.8 cM, respectively, on chromosome Pv08. Similarly, the RAPD marker OPE04 was found in all resistant individuals but was absent in those scored as susceptible based on virulence data (Namayanja et al. 2006). Additionally, an allelism test between Mexico 54 and BAT 332 inoculated with *P. griseola* race 63–39 showed that a single, dominant gene controls ALS resistance, suggesting that the resistance to ALS in Mexico 54 and BAT 332 is conditioned by the same resistance locus (Namayanja et al. 2006).

The *Phg*-2^2 allele of BAT 332 is the only allele officially accepted by the BIC Genetics Committee. *Phg-3* has initially been published as *Phg-ON* in cultivar Ouro Negro. This cultivar is an essential of resistance for ALS and other diseases in common bean, such as ANT and rust. Inheritance studies in an F_2 population derived from the Ouro Negro × US Pinto 111 cross revealed one dominant resistance gene conferring resistance to race. To investigate associations between *Co-3*^{4} and the *Phg-3* genes, Gonçalves-Vidigal et al. (2013) conducted a co-segregation analysis of resistance to *C. lindemuthianum* races 7 and 73 and *P. griseola* race 63–39 in Ouro Negro using an F_2 population from the Rudá × Ouro Negro cross and $F_{2:3}$ families from the AND 277 × Ouro Negro cross. This co-segregation analysis showed that *Co-3*^{4} and *Phg-3* are inherited together. Additionally, the genes *Phg-3* and *Co-3*^{4} were

found to be tightly linked to marker g2303 at a distance of 0.0 cM (Gonçalves-Vidigal et al. 2013) on chromosome Pv04.

Furthermore, seven QTLs on five LGs have been reported by Oblessuc et al. (2012). Among these, the major QTL *ALS4.1*^{GS,UD} on Pv04 and *ALS10.1*^{DG,UC} and *ALS10.1*$^{DG,UC,\ GS}$ on Pv10, identified in G5686 and CAL143 (Mahuku et al. 2009; Oblessuc et al. 2012; Keller et al. 2015), have been recently named as *Phg-4* and *Phg-5* (Souza et al. 2016). The *Phg-4* locus was first discovered by evaluating the G5686 × Sprite F_2 population with race 31–0 and was published as *Phg*$_{G5686A}$ (Mahuku et al. 2009). This QTL was later fine mapped to a 418-kb region on chromosome Pv04 and named *ALS4.1*^{GS,UC} (Keller et al. 2015). As this major locus had consistent and significant effects across different environments and populations (Mahuku et al. 2009; Oblessuc et al. 2012, 2013; Keller et al. 2015), the BIC genetics committee accepted the name QTL *ALS4.1*^{GS,UC} for *Phg-4* in G5686 (Souza et al. 2016). The resistance *Phg-5* locus on chromosome Pv10 was discovered using the CAL 143 × IAC-UNA RIL population. The RILs were evaluated under natural infection in the field and in the greenhouse inoculated with race 0–39, whereby QTL *ALS10.1* exhibited a strong effect in all environments (Oblessuc et al. 2012). Keller et al. (2015) confirmed the QTL *ALS10.1* in G5686. Because of its strong effect on resistance to ALS in different environments, the BIC Genetics Committee proposed officially named *Phg-5 ALS10.1* in both G5686 and CAL143 (Souza et al. 2016).

Correspondingly, several genes conferring race-specific resistance to the rust pathogen *Uromyces appendiculatus* (Pers.) Unger has been identified, named, and mapped in different LGs in the common bean genome (Table 1.6). Indeed, three large clusters harboring many resistance genes located at the ends of chromosomes have been identified on Pv04, Pv10, and Pv11 of the *Phaseolus vulgaris* genome (Schmutz et al. 2014). Among these, one of the most complex disease-resistance clusters containing many genes that confer resistance to various common bean pathogens has been identified at the end of the short arm of chromosome Pv04 (Geffroy et al. 2009; Richard et al. 2014). Moreover, 10 major rust resistance genes have been named and mapped in six different LGs of the common bean genome Pv01, Pv04, Pv06, Pv07, Pv08, and Pv11 (Kelly et al. 1996, Miklas et al. 2002; Miklas et al. 2006a; Hurtado-Gonzales et al. 2017). Mesoamerican rust resistance genes include *Ur-3*, *Ur-5*, *Ur- 7*, *Ur-11* and *Ur-14* (Stavely 1984, 1990; Souza et al. 2011). Andean rust resistance genes include *Ur-4*, *Ur-6*, *Ur-9*, *Ur-12,* and *Ur-13*. Besides, several genes conferring resistance to various common bean pathogens are arranged in clusters of tightly linked genes, often located at the end of the chromosomes. For example, *Ur-9* (Pv01), *Ur-5* (Pv04), and *Ur-3* (Pv11) co-localize with ANT resistance genes *Co-1* (Pv01), *Co3* (Pv04) and *Co-2* (Pv11), respectively (Kelly et al. 2003; Geffroy et al. 1999, 2000). Similarly, *Ur-13* maps close to the *Phg-2* gene for ALS resistance on Pv08 (Garzon and Blair 2014).

Table 1.6 Enumeration of mapping of simply-inherited CS traits and CS QTLs associated with resistance in common bean

Disease	Gene symbol	LG	Resistant parent	References
Angular Leaf spot (ALS)	*Phg-1*	1	AND277	Gonçalves-Vidigal et al. (2011)
	Phg-2, *Phg-2*^{2}	8	Mexico 54 BAT332	Namayanja et al. (2006), Mahuku et al. (2011),
	Phg-3	4	Ouro Negro	Gonçalves-Vidigal et al. (2013)
	Phg-4, Phg-5	4, 10	CAL143 G5686	Mahuku et al. (2009), Oblessuc et al. (2012), Keller et al. (2015)
Anthracnose (ANT)	*Co-1* *Co-1*^{2} *Co-1*^{3} *Co-1*^{4} *Co-1*^{5} *Co-1*^{HY} *Co-14* *Co-Pa* *Co-AC* *CoPv01*^{CDRK}	1	Michigan Dark Red Kidney Kaboon Perry Marrow AND277 Widusa Hongyundou Pitanga Paloma Amendoim Cavalo California Dark Red Kidney	McRostie (1919), Melotto and Kelly (2000), Melotto and Kelly (2000), Gonçalves-Vidigal et al. (2011), Gonçalves-Vidigal and Kelly (2006), Chen et al. (2017) Gonçalves-Vidigal et al. (2012) Lima Castro et al. (2017), Gilio et al. (2020) Gonçalves-Vidigal et al. (2020)
	Co-2	11	Cornell 49–242	Adam-Blondon et al. (1994)
	Co-3 *Co-15* *Co-16*	4	Mexico 222 Corinthiano Crioulo 159	Geffroy et al. (1999), Méndez-Vigo et al. (2005) Coimbra-Gonçalves et al. (2016)
	Co-4^3*/Co-3*^{3}	8, 4	PI207262	Alzate-Marin et al. (2007)

(continued)

Table 1.6 (continued)

Disease	Gene symbol	LG	Resistant parent	References
	Co-4 *Co-4²*	8	TO SEL1308	Fouilloux (1979) Young et al. (1998) de Arruda et al. (2000) Awale and Kelly (2001)
	Co-5 *Co-5²* *Co-6*	7	TU MSU 7–1 AB136	Young and Kelly (1996), Young et al. (1998), Sousa et al. (2014) Kelly and Young (1996), Gonçalves-Vidigal (1994)
	Co-4²/Co-5²/Co-3⁵	8, 7, 4	G2333	Young et al. (1998)
	Co-11	-	Michelite	Gonçalves-Vidigal et al. (2008)
	Co-12		Jalo Vermelho	Gonçalves-Vidigal et al. (2007)
	Co-13 *Co-17*	3	Jalo Listras Pretas SEL1308	Lacanallo and Gonçalves-Vidigal (2015) Trabanco et al. (2015)
Rust	*Ur-3, Ur-6, Ur-7, Ur-11, Ur-Dorado53, Ur-BAC6*	11	P94207 P94232 Beltsville DOR 364 BAC6 BelNeb-RR-1	Stavely (1998), Miklas et al. (2002)
	Ur-5, Ur-14, Ur-Dorado108	4	DOR 364 Ouro Negro Mexico309	Miklas et al. (2000), Souza et al. (2011)
	Ur-4	6	BAT93	Miklas et al. (2002)
	Ur-9,Ur-12	1, 7	PC50	Miklas et al. (2002)
	Ur-12	8	Kranskop	Mienie et al. (2005)
White mold (WM)	*WM1.1, WM1.2, WM2.4, WM7.1 WM8.2, WM8.3, WM9.1*	1, 2, 7, 8, 9	G122	Miklas et al. (2001)
	WM2.1, WM4.1, WM5.1, WM8.1	2, 4, 5, 8	PC-50	Park et al. (2001)

(continued)

Table 1.6 (continued)

Disease	Gene symbol	LG	Resistant parent	References
	WM2.2, WM2.3, WM5.2, WM7.2,WM8.4	2, 5, 7, 8	Bunsi	Kolkman and Kelly (2003), Ender and Kelly (2005)
	WM2.2, WM5.4, WM6.1, *WM7.5*	2, 5, 6, 7	I9365-31 VA19	Soule et al. (2011) Vasconcellos et al. (2017)
	WM3.3, WM7.5, WM9.2, WM11.1	3, 7, 9, 11	Tacana PI 318,695 PI 313,850	Mkwaila et al. (2011)
	WM1.3, WM3.1, WM6.2, WM7.1, WM7.4	1, 3, 6, 7	Xana	Vasconcellos et al. (2017)
Common Bacterial Blight (CBB)	*D2, D5, D7, D9*	2,5,7,9	BAT93	Nodari et al. (1993)
	CBB-2LL, CBB-2S, CBB-2P, CBB-2FL, CBB-1LL,	1, 2, 3, 4, 5, 6	BAC 6	Jung et al. (1996)
	Bng40, Bng139	7, 8	XR-235–1-1	Yu et al. (1998)
	FT-1, FT-2, LDT-2, Pod-1, Pod-2, Seed-1, Seed-2	1, 4, 5, 9	PX	Jung et al. (1997)
	CBLEAF, CBPOD	1, 2, 9, 10	BelNeb-RR-1	Ariyarathne et al. (1999)
	CBB-GH-leaf, CBB-GH-pod, CBB-GH-field	7, 10	DOR 364	Miklas et al. (2000)
	SU91, SAP6, Xa11.4^{OV1,OV3}	8, 10, 11	VAX1, VAX3	Viteri et al. (2015)
	Xa3.3^{SO}	3	BOAC 09–3	Xie et al. (2017)
Halo Blight (HB)	*HB83, HB16*	2, 3, 4, 5, 9, 10	BelNeb-RR-1	Ariyarathne et al. (1999)
	Rpsar-1, Rpsar-2	8,11	BAT93	Fourie et al. (2004)
	Pse-1, Pse-2, Pse-3, Pse-4, pse-5, Pse-6	2, 4, 10	UI-3 ZAA12 BelNeb-RR-1	Miklas et al. (2011, 20142009), Fourie et al. (2004)
	HB4.1, HB6.1	4, 6	Cornell 49–242	Trabanco et al. (2014)

(continued)

Table 1.6 (continued)

Disease	Gene symbol	LG	Resistant parent	References
	PDC^{3-}2, PDC^{4}-2, PDC^{5}-2, $SAUDPC^{3}$-2, $PLAUDPC^{3}$-2, $PAUDPC^{3}$-2, $PAUDPC^{4}$-2, SDC^{7}-6	2, 6	P1037 PHA1037	González et al. (2016)
	HB4.2, HB5.1	4, 5	PI 150,414 Rojo CAL 143	Tock et al. (2017)
BCMV/ BCMNV	*bc-1^{2},bc-u*	3	Olathe Sierra	Strausbaugh et al. (1999)
	bc-3	6	BAT93	Johnson et al. (1997)
	I	2	BelNeb-RR-1	Ariyarathne et al. (1999)
ClYVV	*cyv, desc*	3	Black Knight	Hart and Griffiths (2013)
WMV-2	*Hsw*, *Wmv*	2	Black Turtle-1 Great Northern 1140	Provvidenti (1974), Provvidenti (1987)
CpSMV	*Anv*, *Lnv*	2	Iguaçu Pitouco	Morales and Castano (1992)
BPMV	*R-BPMV*	2	BAT93	Thomas and Zaumeyer (1950), Pflieger et al. (2014)
CMV	*PvCMR1*	10	Othello	Seo et al. (2006), Meziadi et al. (2016)

Recently, co-segregation analysis inoculating $F_{2:3}$ families from the Rudá × Ouro Negro cross with of *C. lindemuthianum* (ANT) and *U. appendiculatus* (rust) races reported the genetic linkage between *Ur-14* and *Co-3*^{4} genes (Valentini et al. 2017). Hurtado-Gonzales et al. (2017) evaluated an F_2 population of Pinto 114 (susceptible) × Aurora (resistant *Ur-3*) for its reaction to four different races of *U. appendiculatus* and bulked segregant analysis using the SNP chip BARCBEAN6K_3 placed *Ur-3* on the lower arm of chromosome Pv11. Specific SSR and SNP markers and haplotype analysis of 18 sequenced bean varieties positioned *Ur-3* in a 46.5-kb genomic region from 46.96 to 47.01 Mb on Pv11. The authors identified in this region the SS68 KASP marker that is tightly linked to *Ur-3*, and validation of SS68 using a panel of 130 diverse common bean cultivars containing all known rust resistance genes showed SS68 to be highly accurate.

Genetic resistance to white mold (WM), caused by the fungus *Sclerotinia sclerotiorum*, is quantitatively inherited, and several QTLs have been identified thus far (Schwartz and Singh 2013). A comparative map composed of 27 QTLs for WM resistance and 36 QTLs for disease-avoidance traits was developed by Miklas et al. (2013). Recently, Vasconcellos et al. (2017) identified 37 QTLs condensed into 17 named loci (12 previously named and five new), nine of which were defined as meta-QTLs WM1.1, WM2.2, WM3.1, WM5.4, WM6.2, WM7.1, WM7.4, WM7.5, and WM8.3; these are robust consensus QTLs representing effects across different environments, genetic backgrounds, and related traits.

Bacterial Diseases

Common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* Smith (Dye) [synonymous with *X. axonopodis* pv. *phaseoli* (Smith) Vauterin et al.] recently reclassified by Constantin et al. (2016) as *X. phaseoli* pv. *phaseoli* is a severe disease of common bean worldwide (Singh and Schwartz 2010). CBB resistance is an inherited-quantitatively trait and, to date, 26 minor and major effect QTLs, that are responsible for resistance to CBB, have been reported across 11 linkage groups (Singh and Miklas 2015; Viteri et al. 2015). Among these, Viteri et al. (2015) identified the major QTL *Xa11.4*^{OV1,OV3} which explained up to 51% of the phenotypic variance for CBB resistance in leaves. Recently, a new isolate-specific QTL underlying CBB resistance was identified on Pv03 which showed an additive effect with SU91 QTL (Xie et al. 2017).

For halo blight (HB), caused by the bacterium *Pseudomonas syringae* pv. *phaseolicola* (Burkn.) Downs, qualitative and quantitative resistance genes have been reported (Ariyarathne et al. 1999; Fourie et al. 2004; Miklas et al. 2014; Trabanco et al. 2014; González et al. 2016; Tock et al. 2017). Five dominant (*Pse-1*, *Pse-2*, *Pse-3*, *Pse-4* and *Pse-6*) and one recessive (*pse-5*) genes were identified on chromosomes Pv02, Pv04, and Pv10 (Miklas et al. 2009, 2011, 2014). Two independent genes, *Rpsar-1* and *Rpsar-2* that confer AvrRpm1-specific resistance were located near genes that confer resistance to the *C. lindemuthianum* fungus (Fourie et al. 2004).

Furthermore, 76 main-effect QTLs that explained up to 41% of the phenotypic variation for HB resistance, and 101 epistatic QTLs were identified by González et al. (2016). Additionally, Trabanco et al. (2014) observed two minor-effect QTLs (*HB4.1* and *HB6.1*) that explained 11 and 12% of phenotypic variation. In the last years, Tock et al. (2017) reported a major QTL of race-specific resistance (*HB5.1*) from cv. Rojo and a major QTL of race-nonspecific resistance (*HB4.2*) from PI 150,414.

Viral Diseases

The dominant *I* gene is located at the end of chromosome Pv02 and imparts a broad resistance to at least 10 potyviruses infecting common bean, including Bean common mosaic virus (BCMV), Bean common mosaic necrosis virus (BCMNV), Clover yellow vein virus (ClYVV), Bean yellow mosaic virus (BYMV), Soybean mosaic virus (SMV), and Watermelon mosaic virus-2 (WMV-2), among others (McKern et al. 1992; Fisher and Kyle 1994; Berger et al. 1997; Hart and Griffiths 2015; Meziadi et al. 2017).

The *bc* recessive genes, which confer resistance to the Potyviruses BCMV and BCMNV in common bean, have been widely studied (Miklas et al. 2000; Meziadi et al. 2017; Feng et al. 2018). These genes either together or combined with the*I* locus, protect plants from common mosaic disease and from black root systemic necrosis (Meziadi et al. 2017). Recessive resistance is controlled by four genes that include three strain-specific genes *bc-1*, *bc-2*, and *bc*-3 and one strain-nonspecific gene *bc-u*, which seems to be required for the expression of the other *bc* genes (Drijfhout 1978; Strausbaugh et al. 1999). Moreover, there are two alleles for *bc-1* (*bc-1* and *bc-1*^{2}) and *bc-2* (*bc-2* and *bc- 2*^{2}). The *bc-u* and *bc-1* genes have been positioned at one end of Pv03, while *bc-2* has no defined genomic position (Miklas et al. 2000; Meziadi et al. 2016). Feng et al. (2018) reported that *bc-1* and *bc-2* recessive resistance genes affect systemic spread of BCMV in common bean. Moreover, the efficiency of the restriction of the systemic spread of the virus was greatly enhanced when the alleles of both genes were combined (Feng et al. 2018). On the other hand, the *bc-3* gene located on Pv06, has been identified as a gene belonging to the eIF4E gene family (Naderpour et al. 2010; Meziadi et al. 2016).Two recessive genes called *cyv* and *desc* located on Pv06 were reported to be allelic forms of *bc-3*, and confer resistance to other Potyvirus: ClYVV, encoding eIF4E factors (Hart and Griffiths 2013; Meziadi et al. 2016).

Two dominant *R* genes, named *Hsw* and *Wmv*, confer resistance against the Potyvirus WMV-2 and were mapped in the same region of the *I* locus. The *Hsw* gen was identified in genotype Black Turtle-1 while *Wmv* in Great Northern 1140 common bean genotype (Provvidenti 1974, 1987). These genes induce two distinct resistance phenotypes to WMV-2 viral strain (Meziadi et al. 2017).

R genes conferring resistance to virus have also being positioned at the*I* locus. This is the case of the *Anv* dominant resistance gene present in Iguaçu common bean genotype and *Lnv* in genotype Pitouco which confer resistance to the Bean rugose mosaic virus (CpSMV) (Morales and Castano 1992). Other *R* gene, called

R-BPMV, which is in the region of the*I*locus, confer resistance to Bean pod mottle virus (BPMV) and was described in BAT93 common bean genotype (Thomas and Zaumeyer 1950; Pflieger et al. 2014).

An *R* gene against Cucumber mosaic virus (CMV), called *PvCMR1* encodes a TNL protein and was located on chromosome Pv10 (Meziadi et al. 2016). *PvCMR1* was identified in Othello common bean cultivar (Seo et al. 2006). For resistance against Alfalfa mosaic virus (AMV), two monogenic genes, named *PvAmv* and *PvAmv-2* mediate local necrosis in Idaho common bean genotype and extreme resistance in Corbett Refugee genotype, respectively (Wade and Zaumeyer 1940; Provvidenti 1987). There are other resistance genes against viruses, with no defined genomic position, that have been described in common bean (reviewed by Meziadi et al. 2017).

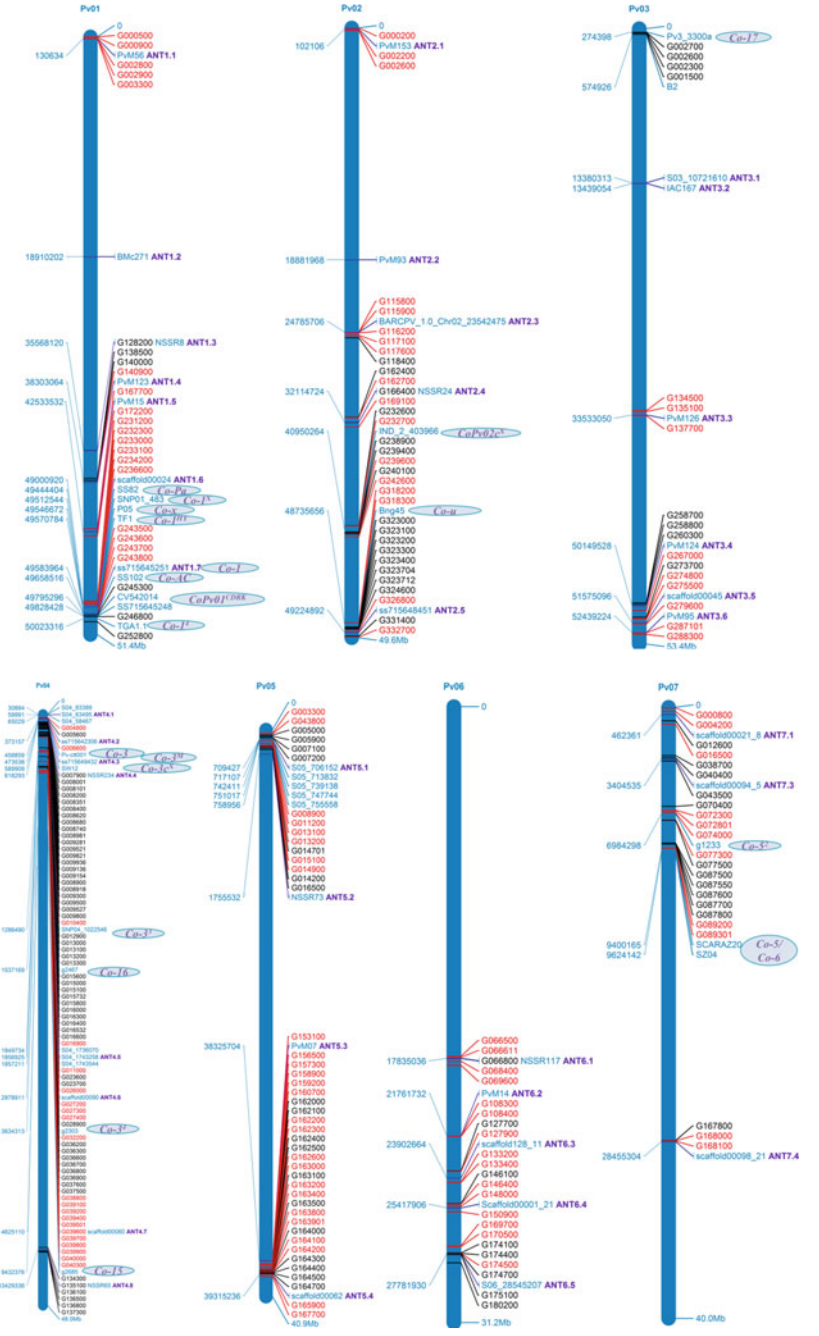
1.6.7 Framework Maps and Markers for Mapping CS QTLs

Previous studies have provided evidence for the existence of more than 20 ANT resistance genes that have been identified and mapped in common bean chromosomes Gonçalves-Vidigal et al. 2020). Furthermore, quantitative resistance loci (QRLs) have been described through genome-wide association studies (GWAS).

Identifying pathogen-responsive genes and proteins on a molecular level provides a better understanding of metabolic pathways involved in ANT resistance. Proteins with NBS-LRR domains are known to be encoded by most resistance genes. In addition, proteins with kinase domains are known to operate as pattern-recognition receptors that recognize pathogen-associated molecular patterns (PAMPs) and activate an immune response (Oblessuc et al. 2015; Meziadi et al. 2016; Vaz Bisneta and Gonçalves-Vidigal 2020).

Vaz Bisneta and Gonçalves-Vidigal (2020) reported a typical resistance protein located close to ANT resistanceloci in the common bean reference genome (Version 2.1). As typical resistance proteins, the authors investigated the ones with nucleotide-binding and leucine-rich repeat (NBS-LRR) domains and kinase domains. For this, first the authors collected available data in the literature about *C. lindemuthianum* resistance genes and Quantitative Resistance Loci (QRL). The physical position of ANT resistanceloci in the reference genome was identified performing a BLASTn search using the sequence of the molecular marker (linked to the ANT resistance gene) described in the literature. Additionally, model genes encoding proteins with NBS-LRR domains, kinase domains and tyrosine kinases that are located 500 kb upstream and downstream of the physical position of each ANT resistancelocuswere searched in phytozome.org.

Moreover, a chart with the selected candidate genes and ANT resistance loci located on the 11 chromosomes (Pv01 to Pv11) was built using the MapChart (Voorrips 2002). As a result, they obtained an integrated map containing candidate genes for all ANT resistance genes and Quantitative Resistance Loci previously described in the literature (Fig. 1.7). The integrated map contains a total of 256 NBS-LRR proteins and 200 protein kinase detected for anthracnose resistance. The authors


Pv01
Pv02
Pv03
Pv04
Pv05
Pv06
Pv07

◀**Fig. 1.7** An integrated map of common bean chromosomes with candidate genes encoding nucleotide-binding sites with leucine-rich repeats (NBS-LRR) and kinases proteins. Genetically characterized anthracnose resistance genes are displayed in circles. Genes that do not have a standardized name are represented by the symbol *Co* and an abbreviation of the cultivar name. Genome-wide association studies for anthracnose resistance loci are colored in purple, with the ANT symbol followed by the chromosome, it was mapped. Candidate genes are represented by the last seven digits from the annotation. For example, G000500 in Pv01 corresponds to *Phvul.001G000500*. Genes encoding NBS-LRR proteins and kinases are represented in black and red, respectively. Molecular markers are represented in blue (Vaz Bisneta and Gonçalves-Vidigal 2020)

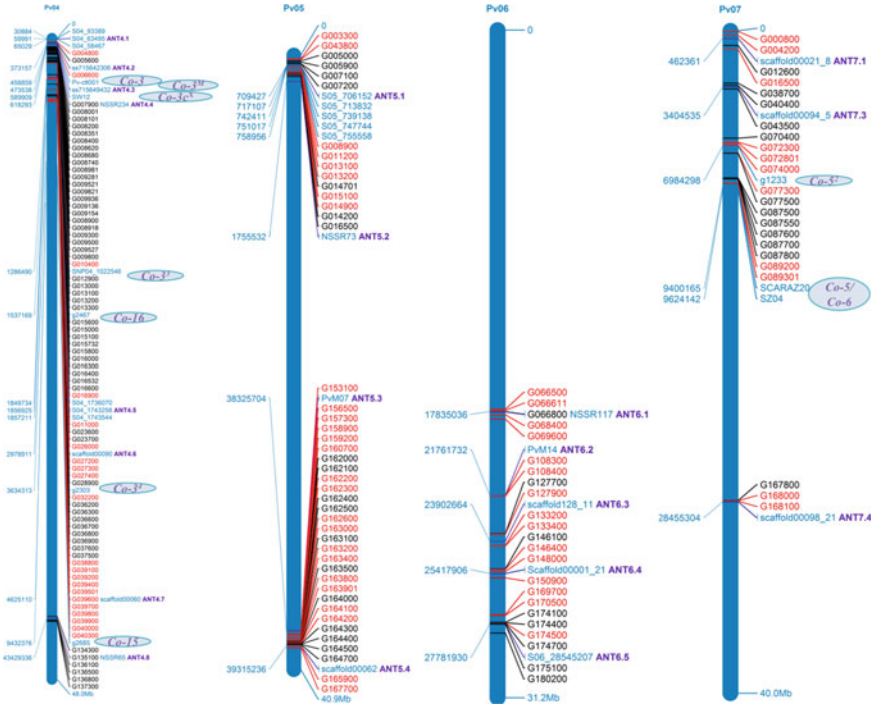


Fig. 1.7 (continued)

reported that the physical position and the molecular markers linked to these genes will be helpful to common bean breeders worldwide. Future validation of these candidate genes would be helpful to understand their function in anthracnose response and how they interact with metabolic pathways.

1.6.8 QTL Mapping Software Used

QTLNetwork is a software package for mapping and visualizing the genetic architecture underlying complex traits. It can simultaneously map QTLs with individual effects, epistasis, and QTL-environment interaction. Population data from F_2, backcross, RILs, and double-haploid populations, as well as populations from specific mating designs (immortalized F_2 and BC_nF_n populations) can be used. The Windows version of QTLNetwork was developed with a graphical user interface. Alternatively, the command-line versions have the facility to be run in other prevalent operating systems, such as Linux, Unix, and macOS (Yang et al. 2008).

Windows QTL Cartographer maps QTLs in cross populations from inbred lines. WinQTLCart includes a powerful graphic tool for presenting mapping results and can import and export data in a variety of formats. WinQTLCart implements different statistical methods as Single-marker analysis, Interval mapping, Composite interval mapping, Bayesian interval mapping, Multiple interval mapping, Multiple trait analysis, and Multiple trait MIM analysis (Wang et al. 2012).

QTL IciMapping is software capable of building high-density linkage maps and mapping QTLs in biparental populations. The following functionalities are integrated within this software package: BIN (binning of redundant markers); MAP (construction of linkage maps in biparental populations); CMP (consensus map construction from multiple linkage maps sharing common markers); SDL (mapping of segregation distortion loci); BIP (mapping of additive, dominant, and digenic epistasis genes); MET (QTL-by-environment interaction analysis); CSL (mapping of additive and digenic epistasis genes with chromosome segment substitution lines); and NAM (QTL mapping in NAM populations). Some examples of output files generated by MAP include a summary of the completed linkage maps, a Mendelian ratio test of individual markers, estimates of recombination frequencies, LOD scores, genetic distances, and the input files for using the BIP, SDL, and MET functionalities. In BIP functionality, more than 30 output files are generated, including results at all scanning positions, identified QTLs, permutation tests, and detection powers for up to six mapping methods. Three supplementary tools have also been developed to display completed genetic linkage maps, to estimate recombination frequency between two loci, and to perform analysis of variance for multi-environmental trials (Meng et al. 2015).

1.6.9 Details on Traitwise QTLs

The objective of QTL mapping is to determine the loci conditioning variation in complex quantitative traits. Because environments highly influence characteristics controlled by multilocus, it is necessary to evaluate the response of QTLs in different environments. Through QTL analysis it is possible to determine the number, the

location, the interaction of loci, as well as the actual genes and their responsive function.

Studying QTLs for important agronomic traits (e.g., yield) can lead to the development of improved crop varieties through plant breeding. Once a QTL is identified, regression analysis (R^2) can be performed to infer the proportion of phenotypic variance explained by the QTL. A large proportion of a quantitative variation explained by a significant QTL is designed for major QTLs. Usually, major QTLs exhibit R^2 from 10 to 30%, whereas significant QTLs with lower R^2 are called minor QTLs.

The LOD score compares the likelihood of a dataset exhibiting r crossovers out of a potential N between a pair of markers under the hypothesis of linkage (i.e., $\theta < 0.5$, where represents the recombination fraction) versus the same observation under the hypothesis of independent segregation (i.e., $\theta = 0.5$): $\text{LOD} = Z(\theta) = \log10((1\text{-}\theta)^{N-r} \times \theta r) /0.5^{N}$.

The LOD function is maximized at $\theta = r/N$, the maximum likelihood estimates of θ, and the convention that $Z(\theta) > 3$ lends strong support for linkage between the two markers is used frequently in mapping analysis. This value corresponds to a likelihood of observing the dataset, given that the two markers are unlinked, of < 1/1000. Given a prior probability of linkage for two markers chosen at random of 0.02, this likelihood corresponds to a probability $P < 0.05$ of a false positive (Cheema and Dicks 2009). The QTL confidence interval is located around the max LOD. The confidence region corresponds to a decline of 1 LOD from the peak.

The genetic regulation of quantitative traits is often complex due to their polygenic nature. However, QTL analysis is a useful approach for identifying chromosomal regions harboring genes that control quantitative traits. Besides mapping QTLs of the main effect, understanding epistatic interactions between QTLs is important. González et al. (2015) studied the genetic basis of quantitative resistance to two races of *C. lindemuthianum* of a segregating common bean RIL population from the cross PMB0225 × PHA1037.

Using a multi-environment QTL mapping approach, the authors identified race-specific anthracnose resistance QTLs showing significant main additive effects and observed epistatic interactions that explained phenotypic variation beyond those controlled by the main effects of individual loci. Another study (Yuste-Lisbona et al. 2014) identified single-locus and epistatic QTLs, as well as their environment interaction effects for six common bean pod traits (width, thickness, length, size index, beak length, and color). For this, Yuste-Lisbona et al. (2014) used an Andean intragene pool RIL population from a cross between a cultivated common bean and an exotic lima bean. Five QTLs with only individual additive effects and six with only epistatic effects were identified, and 12 QTLs showed both effects. Overall, the results obtained showed that additive and epistatic effects are the major genetic basis of pod size and color traits. The mapping of QTLs including epistatic loci provides support for implementing marker-assisted selection toward the genetic improvement of common bean.

Oblessuc et al. (2012) studied QTLs controlling resistance to angular leaf spot (ALS) using 346 RILs from the IAC-UNA × CAL 143 cross. The experiments were performed two years in the field and one year in the greenhouse, and data was analyzed

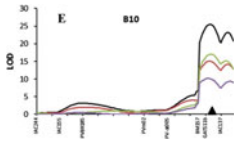


Fig. 1.8 QTL graph indicating the LOD score values for each marker position. LOD scores obtained via joint CIM analysis (y) using the molecular marker distances of the IAC-UNA × CAL 143 cross genetic map for each experiment (dry season, red; wet season, green; and greenhouse, purple) and joint analysis (black). Black triangles indicate the position of maximum LOD values for significant QTLs. Linkage group 10 is indicated as B10 (Oblessuc et al. 2012)

by joint composite interval mapping for QTL x environment interaction. As a result, the authors identified seven QTLs mapped on five linkage groups. Among these, *ALS10.1*^{DG,UC}, found linked to the GATS11b marker on linkage group B10, presented major effects (R^2 between 16 and 22%). This QTL could be important for bean breeding, as it was stable in all environments, and the GATS11b marker is a potential tool for marker-assisted selection for ALS resistance. A QTL graph indicating the LOD score values for each marker position for linkage group 10 is shown in Fig. 1.8. The QTL *ALS5.2* showed an important effect (9.4%) under inoculated conditions in the greenhouse. *ALS4.2* was another major QTL, under natural infection in the field, explaining 10.8% of the variability for resistance reaction. The other QTLs showed minor effects on resistance.

Elias (2018) studied 160 RILs derived from the cross between IAPAR 81 × LP97-28 held under conditions of drought stress and non-drought stress for two years, for QTL mapping. For this, 773 SNP markers were used to construct linkage groups covering 815.9 cM of the bean genome, with distance of 1.34 cM between markers. As a result, the authors identified 16 QTLs on chromosomes Pv01, Pv02, Pv03, Pv05, Pv07, Pv08, Pv09, Pv10, and Pv11 (Fig. 1.9).

1.7 Association Mapping Studies

1.7.1 Extent of Linkage Disequilibrium

Linkage disequilibrium (LD) is the non-random association of alleles at two loci (Mackay and Powell 2007) and constitutes the base of gene identification by association mapping. Association mapping is based on the detection of quantitative trait loci (QTLs) by evaluating the patterns of genome-wide LD in a diverse panel and studying the association between relevant phenotypes and genomic variants. An example of linkage disequilibrium observed in common bean chromosome Pv04 using 115 accessions genotyped with 5,398 SNP markers on the BARCBean6K_3 Illumina BeadChip can be observed in Fig. 1.10.

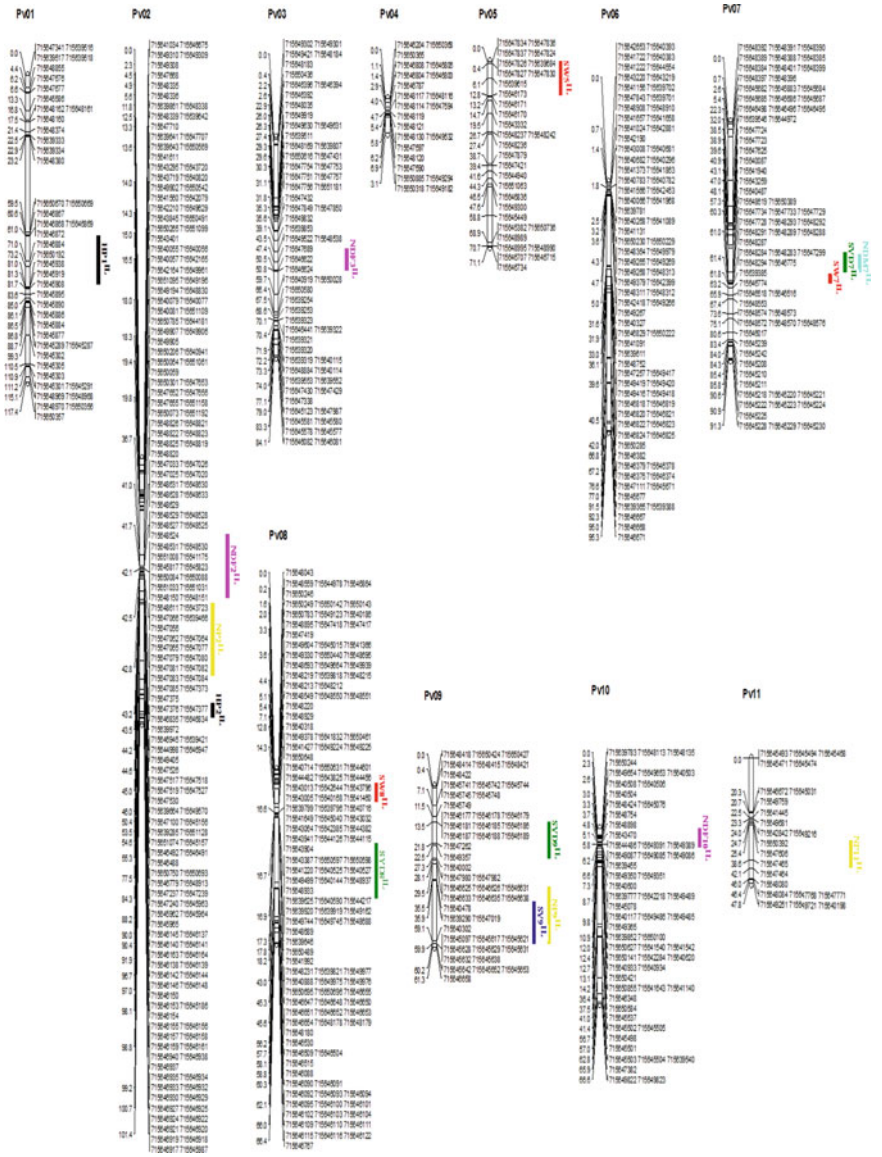


Fig. 1.9 Genetic mapping for the RIL population Iapar81 × LP97-28 cross using 773 SNPs markers assigned to the 11 common bean linkage groups. QTL locations were mapped using the Composite Interval Method (CIM) of the Win cartographer software and the LOD thresholds calculated based on 1000 permutations. A total of 16 QTLs were associated with the yield per day, the weight of 100 grains, number of pods per plant, the height of the plant, number of days for flowering, and number of days for maturation under water stress conditions (Elias 2018)

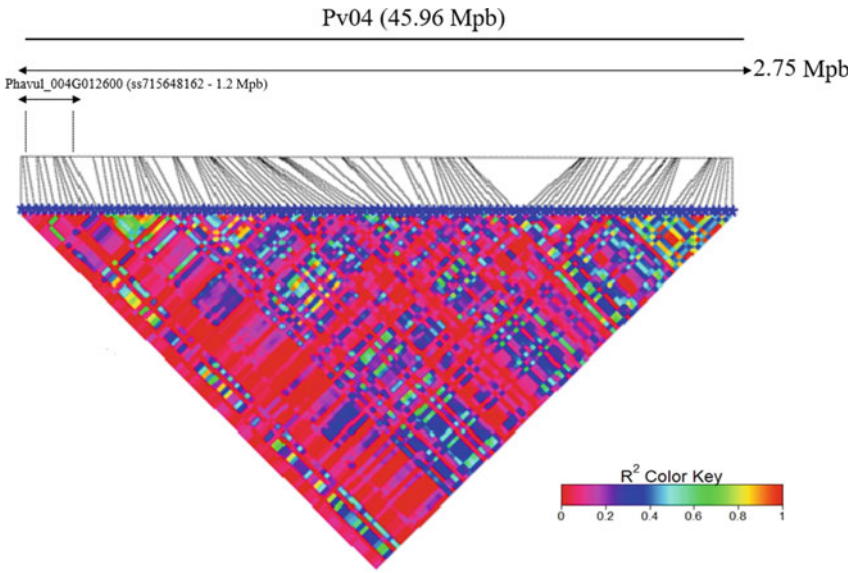


Fig. 1.10 Linkage disequilibrium observed in common bean chromosome Pv04 using 115 accessions genotyped with 5,398 SNP markers on the BARCBean6K_3 Illumina BeadChip (Vidigal Filho et al. 2020)

With the development of a reference genome for the common bean (Schmutz et al. 2014; Vlasova et al. 2016), and the availability of high-throughput genotyping platforms (Hyten et al. 2010; Goretti et al. 2014; Gujaria-Verma et al. 2016), genome-wide association study (GWAS) mapping has become a powerful and efficient tool for discovering novel genes of complex agronomic traits. However, in association mapping, population structure and kinship among individuals must be considered to avoid the emergence of false associations. If not considered as part of the analysis, structure in the population used in association mapping can lead to spurious associations, since in a structured population the LD increases if the allele frequencies between loci vary between the subpopulations that comprise it (Oraguzie et al. 2007). Vidigal Filho et al. (2020) reported that the ss71568162 marker, positioned at 1,224,240 bp, encompasses the Phvul.004G012600 candidate gene, which encodes a serine-threonine protein kinase conferring resistance to race 73 of *C. lindemuthianum* (Fig. 1.8).

1.7.2 Target Gene-Based LD Studies

Association mapping is a powerful tool that allows the identification of loci whose contribution explains part of the observed phenotypic variation. The advantage of association mapping over conventional biparental QTL mapping lies in improving

the resolution of association studies between markers and phenotypes, achieved by using a larger population that involves a greater number of alleles. Mapping based on biparental populations relies on creating experimental populations derived from controlled crossing and allows only a limited amount of genetic variation to be analyzed. Alternatively, association mapping, based on population-scale samples, allows the analysis of a wider range of natural variation (Burghardt et al. 2017). Furthermore, the smaller number of recombination events during biparental population generation makes it harder to locate a QTL with high resolution, whereas association mapping, by taking advantage of recombination events over multiple generations in a lineage, allows a finer resolution for the location of QTLs (Han and Huang 2013; Burghardt et al. 2017). Thus, association mapping further improves genetic resolution, includes a greater number of alleles and traits, and reduces research time (Korte and Farlow 2013; Xu et al. 2017).

Association mapping approaches can be classified into two types: (1) candidate gene (CG) association mapping and (2) genome-wide association study (GWAS) mapping. The first is based on selecting genes potentially involved in controlling the phenotypic variation of the trait under study. This approach aims to identify mutations and causal genes in a small number of CGs within a specific genomic region and requires a good knowledge of the genetics and biochemistry of the trait. The second approach, GWAS mapping, is a broad genome-wide study attempting to identify variation associated with phenotypic diversity and requires large, highly diverse association panels and a great number of well-distributed molecular markers (González et al. 2017). Considering that GWAS requires extensive genotypic and phenotypic information, it is more usefully applied in major crops, which may already have available resources and in which a wide research community may be interested in developing future resources (Mousavi-Derazmahalleh et al. 2019). Thus, factoring in its great genetic diversity, the common bean is a good target for GWAS (Blair et al. 2009).

1.7.3 Genome-Wide LD Studies

GWAS mapping has become an emerging approach to discover QTLs associated with agronomic and phenological traits (Galeano et al. 2012; Kamfwa et al. 2015a), symbiotic nitrogen fixation (Kamfwa et al. 2015b), drought tolerance (Hoyos-Villegas et al. 2017), and disease resistance (Shi et al. 2011;Hart and Griffiths 2015;Perseguini et al. 2016;Zuiderveen et al. 2016;Tock et al. 2017; Oladzad et al. 2019a). Shi et al. (2011) were the first to apply GWAS to identify disease resistance loci in common bean. A population of 395 dry bean lines of different market classes were evaluated for CBB resistance and genotyped using 132 SNPs evenly distributed across the genome. Twelve significant SNP markers co-localized with or close to previously identified CBB-QTLs. Moreover, eight new resistance loci were identified. Hart and Griffiths (2015) conducted a case–control GWAS approach to identify SNPs associated with resistance to bean yellow mosaic virus (BYMV), which is conditioned by the *By-2*

allele. They genotyped a set of recombinant inbred lines (RILs), derived from an introgression program, with 7,530 SNPs and identified 44 GBS SNPs associated with the resistance phenotype, which mapped onto the distal portion of chromosome Pv02. Seven of these SNPs were converted to KASP assays and shown to be tightly linked to BYMV resistance in an F_2 population of 185 individuals (Hart and Griffiths 2015).

Quantitative resistance loci (QRL) controlling resistance to both ANT and ALS diseases of 180 common bean accessions were identified by Perseguini et al. (2016) using GWAS. A total of 17 SSR and 21 SNPs associated with resistance to ANT Race 4 were detected. Moreover, 11 SSR and 17 SNPs associated with resistance to Race 0–39 of *Pseudocercospora griseola* were detected. The greatest number of loci associated with ANT resistance were in chromosomes Pv03 and Pv08, while chromosome Pv04 was the most saturated one, with six markers associated with ALS resistance. The authors reported three markers that were associated with both diseases, SSR-IAC167 and PvM95, both located on chromosome Pv03, and the SNP scaffold00021_89379 located on chromosome Pv07. Fritsche-Neto et al. (2019) performed a GWAS using 60 inbred elite lines, developed by the Embrapa (The Brazilian Agricultural Research Corporation) common bean breeding program across 22 years, to identify markers linked to ANT and ALS resistance. The lines were evaluated under field conditions and genotyped with 5,398 SNPs. Two SNPs associated with ANT resistance loci on chromosome Pv02 and one SNP associated with ALS resistance loci were observed. Recently, Vidigal Filho et al. (2020) conducted a GWAS approach using 115 accessions from five Brazilian states, revealing new sources of ANT and ALS resistance. The authors reported SNP markers associated with resistance to ANT races 9 and 73 that were positioned on chromosome Pv04; resistance to race 65 on chromosomes Pv01, Pv04, and Pv08; and resistance to races 2047 and 3481 on chromosomes Pv10 and Pv05, respectively. Furthermore, SNPs associated with resistance to race 63–39 of *P. griseola*, were mapped on chromosomes Pv03, Pv06, and Pv08, whereas for race 31–23, SNPs were mapped on chromosomes Pv02 and Pv04.

Nine disease resistance loci for ANT and seven for CBB have been detected by Wu et al. (2017) using NBS-SSR markers and GWAS. Three of these loci (NSSR24, NSSR73, and NSSR265) were located at new regions for ANT resistance, while the other two (NSSR65 and NSSR260) were located at new regions for CBB resistance. Furthermore, the SSR marker NSSR65, located on chromosome Pv04, was associated with both diseases, suggesting a possible pleiotropic effect.

Diversity panels that capture variation among a defined population are essential for discovering the genome-wide effects that control specific phenotypes (McClean and Raatz 2017). Recently, common bean geneticists and breeders developed the Andean Diversity Panel (ADP) (Cichy et al. 2015) and the Mesoamerican Diversity Panel (MDP) (Moghadam et al. 2016), which were assembled to represent the genetic diversity of cultivated beans within each major gene pool and to facilitate gene pool-specific genetic analyses. Each panel consists of modern genotypes commonly used by growers in production fields, and they are useful for GWAS mapping since they have been SNP genotyped with approximately 200,000 SNPs (McClean and

Raatz 2017). After genomic characterization, because of the homozygous nature of common bean varieties, the genetic data can be used many times to evaluate different phenotypes across different environments (González et al. 2017). The ADP consists of 504 Andean accessions, whose descriptions are available on the USDA-ARS Feed the Future-Bean Research Team website (http://arsftfbean.uprm.edu/bean/?p=472). The ADP has been used in recent GWAS mapping to identify disease resistance loci, including resistance to halo blight (Tock et al. 2017), anthracnose (Zuiderveen et al. 2016), and to root pathogens like Fusarium solani (Vasquez-Guzman, 2016; Zitnick-Anderson et al. 2020), *Pythium* spp. (Soltani et al. 2018; Dramadri et al. 2020), and *Rhizoctonia solani* (Oladzad et al. 2019b).

Despite the potential that association mapping presents for identifying complex genes, its limitations include the tendency for spurious association, identification of small-effect variants, missing genotypes, and genetic heterogeneity (Korte and Farlow 2013). Further, association mapping resolution depends on the rate of LD decay, so a better foundation could be using wild relatives of crops (Mousavi-Derazmahalleh et al. 2019). In this way, a total of 317 plant introductions (landraces and wild bean accessions) from the USDA core collection was used to conduct a GWAS to identify markers linked to the soybean cyst nematode (SCN, *Heterodera glycines*) resistance loci (Jain et al. 2019). Analyses were conducted separately for the Andean and Mesoamerican groups, using 3,985 and 4,811 SNP markers, respectively. Significant SNPs on Pv07 and Pv11 in the Mesoamerican group, and Pv07, Pv08, Pv09, and Pv11 in the Andean group, were found to be associated with SCN resistance. Moreover, homologs of soybean *rhg1*, a locus which confers resistance to SCN in soybean, were identified on chromosome Pv01 in the Mesoamerican group and on Pv08 in the Andean group.

Another study of genotyping-by-sequencing analysis and 19 climatic characteristics obtained through the WorlClim site was carried out by Elias (2018), in which a set of 110 common bean accessions previously genotyped using a sequencing genotyping methodology was evaluated, producing 28,823 SNPs. Through associative mapping, it was possible to detect loci of quantitative characteristics, for a total of 135 associations between characteristics vs. markers (Bonferroni test <0.5%). Of the 19 bioclimatic traits, eight exhibited significant associations, and associations for seasonality of temperature and precipitation in the driest quarter were found on Pv09, with $R^2 = 36.26\%$ and 36.46%, respectively. Associations between markers and climatic variables were distributed throughout common bean LGs, except for Pv08. The results show a correlation between markers and climatic characteristics on a national scale, helping to identify candidate genes for regional adaptation. These considerations are of great relevance for the conservation and exploration of genetic diversity between and within common bean accessions in Brazil (Elias 2018).

The SNP markers and candidate genes found associated with the resistance should be validated in segregating populations, which could further be used for marker-assisted selection. As a result, breeding programs might be able to develop resistant common bean cultivars to several diseases.

1.8 Marker-Assisted Breeding for Resistance/Tolerance Traits

1.8.1 Germplasm Characterization and Distinctiveness, Uniformity, and Stability (DUS)

Germplasm characterization is the recording of distinctly identifiable and highly heritable characteristics. Germplasm evaluation refers to the agronomic description of GenBank material, including traits that are generally important to breeders and researchers in crop improvement.

Germplasm characterization enables quick and easy discrimination among phenotypes and is essential to provide information on accessions' traits, assuring the maximum utilization of the germplasm collection for the final users. Evaluating genetic diversity and population structure is necessary to improve a population through plant breeding because it informs decisions such as parental selection and long-term conservation of important germplasm (Acosta-Gallegos et al. 2007; Bitocchi et al. 2012; Piñón et al. 2020).

Molecular markers are replacing morphological descriptors for some purposes, such as evolutionary studies, assessment of interrelationships among accessions and geographic groups of accessions, estimating genetic diversity, and identifying duplicates. However, the evaluation of visible descriptors will remain important for identifying landrace accessions at the field level as an adjunct using molecular markers. A descriptor can be a numeric value such as weight, length, or output from a sensor; a code within a scale, such as a 1 to 9 rating for disease severity, or a rating for shade and color intensity; or a qualifier, such as a trait's absence or presence. The main aims of germplasm characterization are to: describe accessions and establish their diagnostic characteristics; classify accessions into groups using sound means; assess interrelationships among accessions or traits, and among geographic accession groups; estimate the extent of variation in a GenBank collection; and identify duplicates in a collection.

In accordance with the UPOV (2015), varieties can be considered distinct where they have a different expression in a grouping character, such as growth type in plants and pigmentation of the hilum in seeds. Distinctness must be statistically evaluated, with a significant difference at 1% ($P = 0.01$) significance level in at least one character, in a combined over years distinctness analysis of variance. Where the number of tested varieties is too small (below 15), giving insufficient degrees of freedom for the COYD analysis to be valid, then a standard of significant differences using the one-year "t" criterion at 5% is used. Where varieties are grown nearby, under the same conditions, and direct comparisons can be made, distinctness can be determined via visual observation. In these circumstances, the basis for distinctness will be recorded with clarity. If the visual observation shows the two varieties are clearly distinct, then a case will be presented to APHA and the NLSC with any supporting evidence.

Uniformity is assessed for all characteristics used to establish distinctness and is based on the assessment of off-types (variants). Off-type plants in field-sown plots are identified by visual assessment and marked for a decision on recording omission depending upon incidence across replicates. Care is taken to ensure that the plants that are counted are not the result of any non-genetic factors, such as environment, pest, and disease. The assessment of off-types is undertaken in both test cycles, and the total combined should not exceed the following: population standard = 2%; acceptance probability = 95%. (For example: 6 off-types in a population of 160.) After all variants have been excluded, characteristics listed in distinctivenessare used to assess the uniformity of the remaining plants. Uniformity is based on the assessment of general variation where measurements are recorded. Provided a variety meets the off-type standard, it can be considered sufficiently uniform after two test cycles, if, for all measured characters necessary for distinctness, the Combined Over Years Uniformity (COYU) analysis is not significantly greater than that of the reference varieties at the 0.2% (P = 0.02) significance level.

A variety is considered sufficiently stable when there is no evidence to indicate that it lacks uniformity or fails to conform to the essential characteristics of its description in different submissions or in different tests.

The following 23 characteristics are recorded in distinctiveness,

Uniformity, and stability tests:

Foliage: intensity of green color (1 = light; 2 = light to medium; 3 = medium; 4 = medium to dark; 5 = dark).

Foliage: **greyish hue of green color** (1 = absent; 9 = present).

Time of flowering: (50% of plants with at least one open flower) (1 = very early; 3 = early; 5 = medium; 7 = late; 9 = very late).

Wing: melanin spot (1 = absent; 9 = present).

Wing: colour of melanin spot (1 = yellow; 2 = brown; 3 = black).

Standard: extent of anthocyanin coloration (Only varieties with Wing: melanin spot: present) (1 = small; 3 = medium; 5 = large).

Standard: intensity of anthocyanin coloration (Only varieties with Wing: melanin spot: present) (1 = weak; 2 = medium; 3 = strong).

Flower: length (1 = very short; 3 = short; 5 = medium; 7 = long; 9 = very long).

Standard: width (1 = narrow; 2 = narrow to medium; 3 = medium; 4 = medium to broad; 5 = broad).

Flower: ratio flower length/standard width (1 = low; 3 = medium; 5 = high).

Leaflet: length (basal pair of leaflets at second flowering node) (1 = very short; 3 = short; 5 = medium; 7 = strong; 9 = very strong).

Leaflet: width (1 = very narrow; 3 = narrow; 5 = medium; 7 = broad; 9 = very broad).

Stem: anthocyanin coloration (Only varieties with melanin spot) (1 = absent or weak; 3 = medium; 5 = strong).

Plant: growth type (1 = determinate; 2 = indeterminate).

Plant: length (1 = very short; 3 = short; 5 = medium; 7 = tall; 9 = very long).

Stem: number of nodes (up to and including first flowering node) (1 = very few; 3 = few; 5 = medium; 7 = many; 9 = very many).

Pod: length (without beak) (1 = very short; 3 = short; 5 = medium; 7 = long; 9 = very long).

Pod: width (from suture to suture) (1 = very narrow; 3 = narrow; 5 = medium; 7 = broad; 9 = very broad).

Pod: intensity of green color (1 = light; 2 = medium; 3 = dark).

Seed: shape (1 = circular; 2 = non-circular).

Seed: color of testa (immediately after harvest) (1 = light yellow brown; 2 = grey; 3 = green; 4 = black).

Seed: black pigmentation of hilum (1 = absent; 2 = present).

100 seed weight (1 = very low; 3 = low; 5 = medium; 7 = high; 9 = very high).

1.8.2 Marker-Assisted Gene Introgression

Molecular mapping and tagging of important genes have contributed to significant advances in marker-assisted selection (MAS) of crop breeding. Since molecular markers are related to nucleotide sequence variations, many tags have been developed for different types of plant crops. They also have several advantages over the traditional phenotypic markers (Mohan et al. 1997; Kole and Gupta 2004). In general, this method is faster, cheaper, and more accurate than traditional phenotypic assays. Consequently, it may provide higher effectiveness and efficiency in terms of time, resources and efforts. Besides that, MAS is not affected by the environment, which allows the selection to be conducted under any environmental conditions. In traditional phenotypic selection, an individual gene or loci might be masked by the effect of others. In contrast, MAS can simultaneously identify and select single Genes/QTLs in the same individuals, when traits are controlled by multiple Genes/QTLs. For that reason, it is particularly feasible for gene pyramiding.

The usage of MAS enables introgression of resistance genes into a cultivar, decrease of population size, and time required to develop a new variety. Methods to characterize disease resistance genes have changed over time. Initial work with RFLP, AFLP and, RAPD markers were followed by a series of SSR, SCAR, and SNP marker systems, providing suitable markers for breeding purposes. These markers linked to single-gene traits have been successfully used in MAS (Singh and Schwartz 2010). Thus, gene introgression using MAS allowed the development of numerous common bean lines with resistance to angular leaf spot (Oliveira et al. 2005), anthracnose (Alzate-Marin et al. 1999; Miklas et al. 2003), rust (Stavely 2000), common bacterial blight (Miklas et al. 2006b) and bean gold yellow mosaic virus (Miklas et al. 2002). In addition, two major white mold resistance QTLs have been successfully introgressed using MAS with a positive asset in the target traits (Ender et al. 2008). The use of MAS in breeding for resistance to biotic and abiotic stress in common bean has been widely reviewed by Miklas et al. (2006a). The latest publication about the common bean reference genome (Schmutz et al. 2014) allowed mapping and

comparison of several SSR, SCAR, and SNP markers' positions. Some of them were mapped in different chromosomes than the ones originally reported. In the last few years, GBS, GWAS, and WGS techniques improved plant breeding by making it quickly and efficiently through the usage of MAS.

1.8.2.1 Gene Tagging and Marker-Assisted Selection for Bean Diseases

Conventional breeding methods used depend on visual screening of genotypes to select for traits of economic importance. Nevertheless, success using this method depends on its reproducibility and heritability of the characteristic. MAS is an excellent methodology for common bean breeders who also work to improve disease resistance. On behalf of MAS to be highly effective, a high association and tight linkage must exist between the genes for resistance to diseases and molecular markers and easy to evaluate (Yu et al. 2004). As mentioned in the previous section, associations between resistance genes and molecular markers are widely used for mapping genes to specific linkage groups. Since the last century, several studies have used molecular markers to select qualitative resistance to anthracnose (ANT), angular leaf spot (ALS), common bean mosaic virus (BCMV), and rust diseases.

Anthracnose

Garzón et al. (2007) were the first to evaluate the efficiency of marker-assisted selection (MAS) to detect anthracnose resistance. For that purpose, a series of backcross plants, using PCR-based markers SAB3 and SAS13 linked to *Co-5* and *Co-4*^{2} genes, were used. The authors concluded that *Co-5* is associated with SAB3, whereas *Co-4*^{2} is linked to SAS13. Likewise, Vidigal Filho et al. (2008) evaluated backcross F_2BC_3 lines using a $SAS13_{950}$ marker and observed that it was linked to a *Co-4*^{2} allele. Two hundred and thirty-three BC_3F_2 near-isogenic lines containing a *Co-4*^{2} resistance allele in various combinations were developed through MAS for the resistance genes and phenotypic selection for anthracnose. The BC_3F_2 NILs having a *Co-4*^{2} resistance allele showed a wider resistance spectrum and manifested increased levels of resistance to race 2047 of *C. lindemuthianum*. Out of the 233 BC_3F_2 lines analyzed by molecular markers, 80 of them revealed the presence of $SAS13_{950}$ linked to a *Co-4*^{2} allele. Moreover, Brazilian cultivars Awauna UEM and Flor Diniz UEM, both resistant to anthracnose, were obtained by five backcrossings with a $SAS13_{950}$ marker through MAS (Gonçalves-Vidigal, personal communication). Different anthracnose and common bean mosaic genes were pyramided by Ferreira et al. (2012) using the pedigree method from a single cross between lines obtained in the introgression step: lines A1699 (derived from cross A1258 × A1220), A2438 (A1220 × A1183), A2806 (A1878 × 2418), and A3308 (A1699 × A2806). Additionally, seven molecular markers known to be linked to resistance loci were used, and it was possible to differentiate 11 fabada lines. As a result, the authors reported a new fabada line A3308

containing resistance to three anthracnose races controlled by genes included in clusters *Co-2* and *Co-3/9*, and to common bean mosaic genes with genetic resistance controlled by genotype *I* + *bc-3*.

Rust

On the subject of rust, the first resistance gene tagged in common bean was *Ur-4* gene (Miklas et al. 1993), using the molecular marker $OA14_{1100}$. This marker was used to perform assisted selection of plants containing *Ur-4* (Kelly et al. 1993). However, its usage is restricted to Mesoamerican cultivars, since progenies from a cross between Early Gallatin and Andean cultivar do not segregate for $OA14_{1100}$ marker (Miklas et al. 1993). Previous studies reported limitations of molecular markers linked to *Ur-3* gene (Haley et al. 1994; Nemchinova and Stavely 1998; Stavely 2000). However, Valentini et al. (2017) developed several SSR markers linked to *Ur-3, Ur-4, Ur-5, Ur-11, Ur-14,* and *Ur-PI310762* genes. For that, accurate phenotyping for the inheritance of resistance studies, bulk segregant analysis (BSA) combined with high-throughput genotyping using the SNP chip BARCBEAN6K_3, were used. Following the same line of experiments, further SSR and SNP markers closely linked to *Ur-3* were developed based on BSA, SNP assay, and whole-genome sequencing methodologies (Hurtado-Gonzales et al. 2017). Interestingly, KASP SNP marker SS68 reliably distinguished cultivars containing *Ur-3* alone or in combination with other genes (Hurtado-Gonzales et al. 2017). Recently, co-segregation analysis inoculating $F_{2:3}$ families from the Rudá × Ouro Negro cross with of *C. lindemuthianum* (ANT) and *U. appendiculatus* (Rust) races reported the genetic linkage between *Ur-14* and $Co\text{-}3^4$ genes (Valentini et al. 2017).

White Mold

QTLs for white mold on linkage groups Pv02 and Pv07 from an ICA Bunsi × Newport Middle American dry bean population were identified by Kolkman and Kelly (2003). In ICA Bunsi × Raven Middle American dry bean populations, QTLs were also detected and mapped on linkage groups Pv02, Pv05, Pv07, and Pv08 (Ender and Kelly 2005). Later, Miklas et al. (2007) found two QTLs in a Pinto 3 navy beans (Aztec/ND88–106–04), which were mapped on linkage groups Pv02 and Pv03. Interestingly, the QTL described on Pv02 was identified previously in populations of ICA Bunsi 3 navy and ICA Bunsi 3 black bean RIL.

Further, a comparative study revealed the presence of QTLs in two separate populations, 'Benton'/VA19 (BV) and 'Raven'/I9365-31 (R31) crosses (Soule et al. 2011). For the first one, WM2.2 and WM8.3 were described for the greenhouse straw test and field resistance. In contrast, WM 2.2, WM4.2, WM5.3, WM5.4, WM6.1, WM7.3 were found in the Raven/I9365 -31 (R31) for greenhouse straw test and field resistance.

In addition, two QTLs were characterized in 'Tacana' × PI 318,695 (linkage groups Pv04 and Pv07) and Tacana × PI 313,850 (linkage groups Pv02 and Pv09) inbred backcross lines, using the greenhouse straw test (Mkwaila et al. 2011). Recently, an evaluation of the RIL population from the AN-37 × P02630 cross demonstrated the presence of 13 QTLs for agronomic and disease-related traits (Hoyos-Villegas et al. 2015).

Fusarium Root Rot

Resistance to FRR is quantitatively inherited and is strongly affected by environmental factors. QTLs associated with this disease varied between studies and populations. Due to limited genomic coverage of the available markers, a comparison of the physical positions of those QTLs was not suitable (Schneider et al. 2001; Chowdhury et al. 2002). In 2005, Román-Avilés and Kelly identified nine QTLs in crosses 'Negro San Luis' × 'Red Hawk' and 'Negro San Luis' × C97407. Later, five regions on linkage groups Pv03, Pv06, and Pv07 associated with QTL for FRR in an Eagle/Puebla 152 population were identified (Navarro et al. 2004). Most recently, two QTLs associated with FRR for greenhouse straw test and field resistance were mapped on Pv02 (Wang et al. 2018).

Common Bacterial Blight

In the early 2000s, important historical research steps towards MAS were taken. PI 319,443 resistance was introgressed into the common bean breeding line XAN 159. By doing that, two major QTLs for common bacterial blight resistance were defined: SCAR marker SU91 (Pedraza et al. 1997) found in Pv08, and BC420 marker detected in linkage group Pv06 (Yu et al. 2000a). Yu et al. (2000b) evaluated cosegregation of two polymorphic markers and only the $BC420_{900}$ revealed a significant association with a major QTL, which conferred resistance in HR67 to CBB. Following that, another major resistance QTL in OAC Rex was mapped on Pv05 (Bai et al. 1997; Tar'an et al. 2001; Michaels et al. 2006). Recently was reported the full genome sequence of the common bean OAC-Rex with introgression from the tepary bean, *P. acutifolius* (Perry et al. 2013). However, a negative association of seed yield with the SU9 marker linked with CBB resistance QTL derived from tepary bean was reported (O'Boyle et al. 2007). Furthermore, Miklas et al. (2009) addressed the presence of SH11.800, SR13.1150, and ST8.1350 markers linked to *Pse-1* and mapped on Pv10.

Bean Common Mosaic Virus

Since BCMV resistance genes are independent in the common bean, it contributes to the use of gene pyramiding as an approach for durable resistance (Zuiderveen et al. 2016). In 1994, Raven was released as the first common bean cultivar resistant

to BCMV (Kelly et al. 1994). The aforementioned cultivar carries two genes: one dominant hypersensitive *I* and one recessive *bc-3,* both confirmed by RAPD markers. This combination has been recognized for its durability over single gene resistance to both BCMV and BCMNV (Kelly 1997). SCAR markers based on OC11350/420 (ROC11) and OC20460 RAPD markers linked to *bc-3* gene were also developed (Johnson et al. 1997). However, the use of these markers in MAS have been limited in common bean because of a lack of polymorphism and reproducibility across different genetic backgrounds and gene pools (Kelly et al. 2003).

Pedigree selection through the F_7 generation based on superior agronomic features (early maturity, erect plant architecture, and good pod set) and commercial seed type, Bella cultivar was created. Derived from cross 'Verano'//PR0003-124/ 'Raven', Bella combines resistance to BCMV, BCMNV, BGYMV and web blight (Beaver et al. 2018).

1.8.3 Gene Pyramiding

Conventional breeding methods involve the complex selection of several genotypes harboring different resistance genes, which can affect the accuracy and efficiency of the process. However, gene pyramiding, developed from a single cross between lines obtained during introgression, using either a pedigree or backcross method, is a good strategy for durable resistance and can facilitate a MAS approach (Ashikari and Matsuoka 2006). Gene pyramiding combines multiple desirable genes from multiple parents into a single genotype for a specific trait. Thus, this method enhances genetic resistance of bean cultivars.

Marker-assisted gene pyramiding: pyramiding aims to assemble multiple genes or QTLs into a single genotype (Ashikari and Matsuoka 2006). Pyramiding and the introgression of multiple genes/QTLs affecting the same phenotypic trait remains a daunting challenge due to complexity in phenotypic selection methods, and more often, is exacerbated by epistatic interactions. However, large-scale genotyping facilities revolutionized MAS in the breeding system by considerably reducing the span of breeding cycles and facilitating gene pyramiding (Xu and Crouch 2008). Gene/QTL pyramiding can be achieved through either multiple-parent crossing or complex crossing, backcrossing, and recurrent selection; and marker-assisted pyramiding of multiple QTLs will be a promising approach to enhance the stability of crops under stress (Richardson et al. 2006). The success of marker-assisted gene pyramiding depends on multiple factors, like the number of gene/QTLs involved, the distance between the QTLs, the number of parents involved or required, MTAs, and the relative cost. Currently, several resistant common bean cultivars were developed to improve levels of resistance to anthracnose, angular leaf spot, rust, and BCMV (Ragagnin et al. 2009).

Souza et al. (2014) used a marker-assisted gene-pyramiding approach to develop elite carioca bean lines harboring three different rust resistance genes, which was

only possible because the Rudá recurrent parent has a high-yield performance. Likewise, focusing on anthracnose and Pythium root rot resistance, Kiryowa et al. (2015) pyramided genes in four susceptible market class varieties using SCAR markers. They also demonstrated that higher numbers of selected pyramided genes may indirectly affect yield by reducing the number of seeds per plant. Further, through MAS, Ragagnin et al. (2009) developed pyramided lines resistant to rust, anthracnose, and angular leaf spot. These demonstrated resistance spectra equivalent to those of their respective donor parents, and yield tests showed that these lines were as productive as the best carioca-type common bean cultivar.

1.8.4 Limitations and Prospects of MAS and Marker-Assisted Backcrossing Breeding (MABCB)

MAS is an important tool for supporting plant breeders in crop improvement. It considerably increases the efficiency of breeding, when markers tightly linked to genes of interest are used. Despite its advantages, MAS might not be as successful as expected when QTL introgression is necessary (Kumar et al. 2011), and MAS is not always better or more cost-effective than direct disease resistance (DDS), especially for quantitatively inherited resistance to diseases. Efficient comparison of these two techniques, regarding pyramiding and transfer of CBB resistance into dark red kidney bean, showed that DDS was significantly more effective than MAS (Duncan et al. 2012). Under greenhouse conditions of high disease pressure, DDS produced more resistant breeding lines with greater levels of resistance than MAS. MABCB is considered smart breeding for different reasons. First, it is a nontransgenic biotechnological approach to plant improvement and is not subjected to rules/regulations that restrict its use. Secondly, disease resistance selection without the use of a pathogen is feasible, and off-season screening is possible. Finally, it is suitable for combining multiple sources of disease resistance for distinct pathogens.

MABCB represents a rapid and precise molecular breeding technique that assumes superior individuals can be precisely isolated based on genotypes at particular marker loci. In practice, MAS can be exercised in various ways, for example: the marker-assisted evaluation of breeding material, MABC, pyramiding, early generation selection, and combined MAS (Collard and Mackil 2008). Fortunately, molecular markers have many more applications beyond this scheme. To execute MAS more efficiently, DNA markers must have some key features, like greater reliability, quantity, and quality of DNA required, the ease of technical procedure to assay, a high level of DNA polymorphism, and a low cost of assay designing (Mohler and Singrun 2004). Remarkably, Toenniessen et al. (2003) noted a greater efficacy and accuracy of MAS over conventional plant breeding.

Three different types of MABC used for selection have been reported: foreground selection, recombinant selection, and background selection (Tanksley 1983; Young and Tanksley 1989; Holland 2004). In foreground selection, genotype of markers

linked to a target gene or QTL can be used either in combination with a phenotype or to replace screening for the target gene or QTL, particularly for useful traits that are generally measured through laborious and time-consuming phenotype screening procedures (Hospital and Charcosset 1997). In recombinant selection, backcrossed progenies are selected with the target gene, and recombination events are selected between the target locus and linked flanking markers. The fundamental idea that underlies recombinant selection is to reduce the size of the donor chromosome segment at the target locus (Collard and Mackill 2008). Nevertheless, the marker-assisted backcrossing MAB (Ribaut et al. 2010) method has been the most effective strategy employed to obtain beneficial QTLs from donor parents with a shortened time frame in both foreground and background selection (Kelly 2004; Varshney et al. 2010). Kaeppler (1997) reported that inbred backcrossed lines may facilitate QTL detection, as these *loci* have a greater probability of being identical by descent, and their interaction with other traits is more sharply detected (Teran et al. 2020).

1.9 Actual Context and Future Perspectives

1.9.1 Concerns and Compliances

The future perspective is not only about to promote legumes, including beans cultivation, it involves the effective rebalancing of farming and food, to ensure feasible support for actual and critical challenges, such as sustainable agriculture, food security, agrobiodiversity, conservation, zero pesticide and human health. The environment, people, and procedures through which agricultural and farmed goods are produced, processed, and delivered to customers without jeopardizing the health of the ecosystems and essential cultures that provide food, make up the sustainable food system. The world's population is expected to rise. We will face global problems, the most crucial of which are attaining food security, minimizing the danger of climate change by reducing net greenhouse gas emissions into the atmosphere, and meeting the growing need for energy. Climate change, as well as biotic and abiotic stresses to which agricultural systems will be increasingly exposed, will have severe consequences for world food production.

According www.pulsesincrease.eu there is a huge potential of *Phaseolus sp.* among other legumes to deliver benefits for food security and clean environment, its exploitation is limited, mainly due to the modest breeding investment and limited research activities on some constraint aspects of species cultivation. The genetic potential remains largely unexplored making the marginal return of investment in legume research likely to be much higher than in other species where research has been much more intensive but where crop improvement is now stagnating.

International studies reflect the fact that performance of beans continues to request significant investment in plant breeding and improving cropping systems. There is a

vast amount of leguminous genetic resources (GenRes) that requires evaluation, characterization, conservation to be superior exploited in various industries. According to IPCC report 2019 titled—Climate Change and Land (https://www.ipcc.ch/report/srccl/) the move to new plant-based diets might provide considerable potential for adaptation and mitigation while also providing significant health advantages. At the same time, there is an increased need to make this genetic material and the knowledge about it, visible and accessible to various groups of stakeholders as: farmers, breeders, processors in the food industry, nutrition specialists, technologists, health care actors, gene banks curators, crop specialists, policy makers. Technologies that have as its basic principle the functionality and access to information and genetic material to facilitate the beneficial exploitation in the agricultural and non-agricultural sector need to be developed. Sustainable development of agriculture is the core of agricultural policy in Europe, and common bean research can ensure new value chains, niche markets, scaling-up of plant breeding efforts, quality of life reflected in safety food and clean environment. The context of COVID 19 highlights the need of reconciling our food system with the demands of the earth, as well as responding constructively to genuine desires for healthy, fair, and ecologically sustainable practices. The moisture contents of all the dry legumes are in the range of 9–13% making them favorable for long storage. Food legumes and legume-inclusive agricultural systems can play a significant role by providing various services while adhering to sustainability standards. Featured by high variability, valuable for food, (inter)cropping, potential medicinal effect, highly required by market, *P. vulgaris,* is one important species to be exploited byEU new protein plan and H2020 funded and currently developed projects. Currently the breeding efforts can open significant opportunities to ensure development of new resources featured by improved resilience and superior qualitative traits, aimed to support the competitive shifts to health diets and to implement friendly cultivation technics. To achieve these ambitious goals and to expand food security, the new breeding materials must be efficient when grown under water, temperature, and nitrogen constraints, as well as resistance to pest and disease.

Grain legumes are the most important source of plant protein worldwide, particularly in many African and Latin American regions, although they have several challenges in production, including poor adaptability, pest and disease problems, and inconsistent yield production.

Screening the limits and trends surrounding bean production and commercialization offers background for determining prospective research priorities based on regional and national constraints and needs. Related the strong variability in yields along years more important focus concerning the reliable food supply and income of smallholder farms is needed. Nassary et al. (2020) recommends as valuable specific investigations related intercrop system for a certain altitude, during long periods.

1.9.2 Opportunities and Challenges

Related adaptation for development of sustainable agriculture– some legumes are adaptable to cultivation under unfavorable ecological conditions, nutritious and stress tolerant, possessing characteristics for enhancing the sustainability of different agricultural systems. Modern cropping methods are designed to decrease the need of external inputs and to take use of legumes' ability to fix atmospheric nitrogen, release high-quality organic matter into the soil, and improve soil nutrient circulation and water retention. The aim of these practices and cultivation scheme is to reduce the negative impact of agriculture on environment; Currently, the European Union devotes only 3% of its arable land to protein crops, and imports more than 75% of its plant protein. The main reasons are low yield capacity and lack of breeding efforts for adaptation of legumes to European agro-ecosystems; The low level of European plant protein self-sufficiency is due to the late development and adaptation of protein plants in Europe. Better use of genetic resources represents a precondition to increase sustainability. Common bean (*Phaseolus vulgaris*) was the most extensively grown grain legume in Europe until around 1970. Field pea and soybean became the most widely grown grain legumes when governmental support for soybean and protein feed crops was introduced in the 1970s. Field peas and faba beans are the most common pulses, while lentils and chickpeas are only cultivated in limited areas.

Pea is the most widely grown grain legume in Europe, but it suffers from poor standing ability, poor ground coverage, and low competitive ability against weeds, along with relatively low protein (20%–24%) and, on many soils, low productivity. Faba bean is the second in area, the first in yield per hectare, and on account of its higher protein content (28–32%), the highest in protein yield, but is adapted to heavy or clay-rich soils and too sensitive to water deficit on sandy soils. Lentil, chickpea, and common bean all have protein contents in the same range as that of pea and have relatively low yields, but high values as they are primarily food rather than feed crops. Since 2013, production in the EU has nearly tripled, reaching 6 million t (2.6 million ha) in 2018.

Thanks to their capacity to fix nitrogen in soils by synergic relationships with *Rhizobia* and mycorrhizal fungi, legumes help reduce the need for fertilisers and avoid economic inputs and environmental impacts. The quantity of nitrogen fixed by legumes is influenced by environmental factors such as temperature and water availability, in addition to species and cultivar. This nitrogen is used by the plant to make protein, which is then made available to humans. Reduced insect and weed incidence, as well as better soil quality, are further advantages of legumes that should not be ignored. To cover the increasing amounts of nitrogen requirements during development and filling, pods attract nitrogen from the nodules. If the nodules cannot cover their N requirements, pods attract nitrogen from older leaves, thereby reducing the photosynthetic capacity of the plants and determining rapidity of ripening. Therefore, the selection of rhizobia strains with increased nodulation capacity may improve N

availability to pods, thereby increasing pod size, which is an important quality characteristic. Selection of efficient bacteria requires specific selection processes based on efficiency and competitiveness for nodulation of the associations. In agriculture, salinity is a widespread and severe environmental stressor that is substantially increased by irrigation. Furthermore, incorrect fertilization methods contribute to salt accumulation in the roots zone of plants.

Under these conditions, the usage of commercial inoculants containing arbuscular mycorrhizal fungus is rapidly increasing, and it is being praised as an environmentally benign technique that helps to mitigate the detrimental impacts of soil irrigation water salinity. Intercropping is common in low-input, low-yield farming methods in underdeveloped countries. Despite several well-known benefits of intercropping, such as improved pest control, competitive yields with lower inputs, pollution mitigation, reduced fertilizer-N use, increased utilization efficiency of available nutrients and water, and more stable aggregate food or forage yields per unit area, intercropping is not widely used in modern agriculture due to a number of constraints, like the demand for a single and uniform product, as well as the appropriateness for mechanization or the use of additional inputs. As a result, optimizing intercropping systems is required to improve resource efficiency and crop output while also increasing numerous ecosystem services.

The possibility of intercropping in sustainable productions and grain legumes that can fix nitrogen through biological mechanisms have been the focus of current study. Legumes (top 10 most frequently used intercrop species, seven are legumes) can contribute up to 15% of the N in an intercropped cereal, thus increasing biomass production and carry-over effects, reducing synthetic mineral N-fertilizer use, and mitigating N_2O fluxes.

When maize and beans are intercropped, their yields are generally lower than those of maize or beans grown in monoculture. Studies have found that maize yielded 5.3 t ha^{-1} when monocropped, 5.2 t ha^{-1} when intercropped with bush beans, and 3.7 t ha^{-1} when intercropped with climbing beans. Maize-legume rotations help to keep soil fertilityCereal–legume intercrops can be used for forage or grain depending on growing conditions and farm management and using them for whole-crop silage is a way of boosting the forage protein content of livestock diets. The cereals are generally better than legumes at taking up mineral N. Legume root exudates released phosphate and a variety of cation species, whereas cereal roots released other minerals, resulting in higher P absorption in cereals and Fe and Zn uptake in legumes when compared to single crops.

In systems where nitrogen fertilizer is used rarely or not at all, cereal–legume combinations outperform pure cereals. Chemical weed control is difficult or impossible in intercrops, as few herbicides are tolerated by both a cereal and a legume. Intercroppinggrain legumes and cereals has demonstrated multiple agronomic and environmental benefits. Intercropping, in comparison to grain legume single crops, lowers weed density, contributes to better and/or more stable combined grain yields, reduces the severity of pest and disease issues in both the legume and cereal components, and increases biodiversity to assist pollinating insects (see LEGATO project "LEGumes for the Agriculture of Tomorrow", funded by the European Union under

the FP7 Programme, http://www.legato-project.net/). Grain legumes are poor weed suppressors, however combining species in the same cropping system might be a viable method to increase the crop's capacity to control weeds. Grain legumes substantially decreased emission factors, implying that legume-fixed nitrogen is a less emissive type of nitrogen input to the soil than fertilizer nitrogen. Nevertheless, it is important to highlight that the influence of legumes in reducing GHG depends also on the management of agro-ecosystems in which they are included. Direct reciprocal advantages in cereal-legumes intercropping entail below-ground mechanisms in which cereals improve Fe and Zn bioavailability to associate legumes while benefiting from legume-fixed N. As a result, crops following legumes have higher yields, such as wheat, maize, or rapeseed, which can be up to 10% higher than crops following cereals. Higher yields are therefore observed for crops following legumes e.g., yields of wheat, maize or rapeseed can be up by 10% compared to following a cereal. Following a legume improves also the quality of cereals (e.g., increased protein content or fewer mycotoxins contamination). The inclusion of grain legumes into cropping cycles continues to raise concerns. Cropping systems that include legume crops in farm rotations must be supported by optimal crop management methods (e.g., amount and sequencing of nitrogen fertilization, soil management, weeding, irrigation), which often differ from what farmers are used to.

Ensuring agrobiodiversity and conservation–The Common Agricultural Policy (CAP) in Europe has pushed for agricultural intensification, encouraging the simplicity and specialization of agroecosystems by reducing landscape variation, increasing chemical usage per unit area, and abandoning less productive regions. Herbicide use or monocultures, for example, are high-input agricultural methods that directly impact biodiversity and may disrupt pest management services. Over the last decades, numerous research articles and discussions have focused on the loss of agricultural genetic diversity across farmlands throughout the world, as well as the resulting loss of resistance to climatic, economic, and social severe events. In a number of situations, a lack of crop diversification has resulted in significant output losses. Crop and crop variety diversification is critical for delivering the advantages of agrobiodiversity.

Need for improvement of food legume genetic resources- To date, exploitation of genetic resources in crop breeding is limited in comparison to availability of materials, and the potential impact of their use is far from optimal (i.e., lack of comprehensive information regarding passport data and descriptors useful for users, accession heterogeneity, unharmonized data), which also affects ability to attract funds for genetic-resources conservation. These issues are more critical in food legumes, as breeding investment and research activities remain modest. Efficient genetic-resources management is required to attract further private and public investment to improve food legumes breeding. From this perspective, the availability and access to well-described and well-managed genetic-resource collections of food legume species that capture the full diversity range will be paramount to advance legume crops and to reach a competitive level in the EU regarding agronomic performance and sustainability. Indeed, without correct handling of EU legume genetic resources, the European Commission's goal of achieving the nine CAP objectives

(i.e., economic, environmental, climatic and socio-economic, including healthier diets) will be unattainable. In this context, large scale projects such as INCREASE—Intelligent Collections of Food Legumes Genetic Resources for European Agrofood Systems, recently funded through the European Union's Horizon 2020 research and innovation program (https://www.pulsesincrease.eu/), aims to improve the sustainable use of GenRes by developing efficient and effective conservation tools to promote agrobiodiversity and its use. According to INCREASE, the actual utilization of grain legumes GenRes is limited in comparison to the availability of materials and the potential impact of their use, due to several concurrent factors: (*a*) *genetic structure of accessions* - in most cases, accessions have unknown genetic structure and are heterogeneous, which impedes the projection of the phenotypic information to the genotype and vice versa. (*b*) *limited information availability on GenRes*: large numbers of accessions have only minimal, if any, information regarding biological status and geographic origin; information regarding traits of interest for breeders and users is very low and mostly limited to morphological descriptors; (*c*) *limited access to available information* (*) the heterogeneous nature and non-standardised way of data collection and integration causes that a huge amount of information is heavily under-used; (**) databases are centralized and not designed to integrate data obtained by external users strongly limiting the access to available information; (***) the available information is not easily accessible to users due to unfriendly searching and visualization tools. Accession-based collections are built and maintained, with each accession often including a mix of genotypes that reflect a population. The conservation of the population represents substantial challenges that arise from genetic drift and/or selection, which are difficult to fully address in conventional conservation management, and from the lack of knowledge of their diversity.

Beans a bridge between food and health **-** Diets throughout the world have changed dramatically; in most of the countries studied, more calories are consumed per person, and the percentage of fat and animal protein taken has grown greatly. Diet is nowadays considered as crucial not just for nutrition, but also for disease prevention and treatment, particularly when diseases are caused by insufficient, excessive, or unbalanced food consumption. One of the most controversial subjects of discussion is the establishment of an optimum human diet. Grain legumesspeciesare featured by superior quantity of protein comparing with other plant foods and have twice the dietary protein content of cereal grains, strongly having perspective to exploited against malnutrition and generally in food sector; The content of bioactive substances can be altered by genetic improvement of nutritional value. Recent investigations suggest that grain legumes may contribute to human health and wellbeing, mostly through prevention of chronic diseases like coronary heart disease, hypertension, cancer, diabetes, and obesity. Due to their satiety value, legumes contribute to regulate body weight and lower the risk of cardiovascular disease and several cancers. The influence of micronutrients (primarily folic acid and magnesium) and high fiber content, condensed tannins, phytoestrogens, and non-essential amino acids in common beans contributes to the prevention and/or treatment of degenerative-chronic diseases such as obesity, diabetes, cancer, and cardiovascular diseases. Common beans are a good

source of aromatic amino acids, lysine, leucine and isoleucine, but deficient in sulfur amino acids (methionine and cysteine), valine, tryptophan and threonine.

Pulses represent an important source of protein for vegetarians, are low glycemic index food and recognized as food choice with significant potential health benefits. They are excellent foods for people managing their diabetes, heart disease or celiac disease, and additionally can help people concerned with weight control. To improve the nutrition of many developing countries, or to combat the incidence of various chronic diseases worldwide, food technologists have developed products based on pulses, adding value thereby contributing to increase in the consumption of legumes. Legumes have appreciable quantity of all the essential amino acids excluding sulphur containing amino acids, which can be balanced to combine with cereals in daily intake. Moreover, legumes seeds also include calcium, magnesium, potassium, phosphorus, and iron. Bioavailability of nutrients can be increased by soaking, sprouting and fermentation. Grain legumes contain 20–45% protein compared with 7–17% in cereals. The protein content ranges from 20 to 25% in common bean (*P. vulgaris*). On the other hand, legumes are incomplete proteins (except soy) because they contain relatively low quantities of the essential sulphur containing amino acids cystine, methionine and cysteine (which are found in higher quantity in grains).

However, grains contain relatively low quantities of lysine, whereas legumes contain appreciable quantity. Pods and immature seeds of legumes contain less proteins than dry seeds of the same species. The nutritional value of legume vegetables as protein sources is determined by their amino acid composition and protein digestibility, as well as their protein amount. Adequate dietary fiber is vital for proper working of the gut, which is related to reduce risk of several chronic diseases including certain cancers, heart disease and diabetes. Fiber comprises pectin, mucilage, cellulose, gum, hemicelluloses and lignin. Most of the legume grains which are consumed as pulses by humans, their fiber content ranges from 0.9 to 5.3%. Legumes are mainly rich in resistant starch (RS), have low glycaemic index carbohydrates. The oligosaccharides (mainly raffinose and resistant starch) and fiber pass through the stomach and small intestine in the undigested form until they reach the colon, where they act as food (prebiotics) for the probiotic or beneficial bacteria which resides there. This bacterial fermentation leads to the development of short-chain fatty acids, such as butyrate, which possibly will improve colon health through promoting a healthier gut micro biome and reducing colon cancer risk. They also can help in weight reduction due to its satiety value. In addition, they are capable to help in moderating blood sugar levels after meals and improve insulin sensitivity.

Commonly consumed legumes having carbohydrate content in the range of 20.9–60.9%. In legume seeds, starch is the main source of accessible carbohydrate and most plentiful 22–45% along with 1.8–18% oligosaccharides and 4.3–25% dietary fiber. Legumes are excellent source of iron, calcium, zinc, selenium, magnesium, phosphorus, copper and potassium. Cereals grains generally supply the higher energy and make up the volume of diets. As sources of micronutrients legumes are superior to cereals. Most legumes, including common beans are consumed whole, resulting in conserving their mineral contents. Micronutrient deficiencies have become more common, even in developed countries. Legumes are superior source of vitamin

B-complex but are a poor source of vitamin C and fat-soluble vitamins. Legumes are normally low in fat and have no cholesterol, with soybeans and peanuts exception. Mono and poly unsaturated fatty acids decrease the possibility of coronary heart diseases. Legumes have anti-nutritional factors which affect its nutritional quality. Anti-nutritional factors can reduce palatability, protein digestibility and bioavailability of nutrients. Phytic acid, phenols, and tannins, which were once thought to be antinutritional, are now considered to be potential antioxidants with health-promoting properties. Phytochemicals reduce the digestion and absorption of nutrients or interfere with their action. The bioactive phytochemicals including enzyme inhibitors are mainly represented as phytoestrogens, oligosaccharides, phytosterols, phytates, saponins, flavanoids and phenolic acids.

Grain legumes are the main sources of lectins in human food. Beans (most species, including *P. vulgaris*) appear to be a significant source of lectins. Lectins found in certain pulses can make food proteins less digestible and biologically valuable. Lectins, on the other hand, may be beneficial by improving gastrointestinal function, decreasing tumor development, and reducing obesity. The importance of phenolic compounds has progressively been acknowledged, and various studies have recently shown that phenolic compounds have several health advantages and are essential in human nutrition. There have been reports of strong links between phenolic contents and antioxidant activity. The highest antioxidant capacity is found in pulses with the highest overall phenolic content (lentil, red kidney, and black bean). Many pulses, such as lupin, lentil, and chickpea, as well as different beans and peas, have been shown to contain saponins. Saponins may have hypocholesterolemic, anticarcinogenic, and immune-stimulatory effects, according to new research. Since excessive generation of free radicals/reactive oxygen species (ROS) and lipid peroxidation are commonly thought to be implicated in the etiology of many illnesses such as cardiovascular diseases, cancer, and autoimmunity, the antioxidant capabilities of food have been intensively investigated.

New innovative products– the market for pulses for food in the EU is benefitting from innovations in pre-cooking processes, inclusion of pulses in prepared convenience foods and the development of new pulses such as ‘edamame’. Extruded beans, which have a high protein content, might be utilized as a basic material for the production of high protein snack bars since their flavor is sufficiently neutral, allowing them to be used for both salt and sweet snacks. An added value can be given by adding functional supplements such as hemp seeds, goji berries, ginger, and others (see EUROLEGUME project, “Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed”, funded by the European Union under the FP7 Programme, http://www.eurolegume.eu). As a result, including bean flour into cereal foods can enhance protein, soluble fiber, vitamin, and mineral content.

Using selected legume genotypes, a variety of innovative products were developed, including pea and bean immature seeds with extended shelf life, pesto sauce made from legume seeds, ready-to-eat pulse spreads, extruded snacks made from dry pea and bean seeds, and protein and fiber rich legume bars in a variety of flavors (EUROLEGUME).

In 2016, the new Bean Beer was introduced as a beer made with 40% whole faba beans and 60% malted barley. The beer is marketed as a sustainable drink made as it is made from a crop that contributes to more sustainable farming practices. There are new opportunities of using legumes for food products of improved nutritional value.

Another outstanding challenge is the strong need to ensure the availability of education and training at all levels to build capacity, infrastructure, and networks to establish and maintain credibility and professionalism. To ensure the functionality and competitiveness friendly cultivation there is a need for all stakeholders, namely, government agencies, non-governmental organizations, consumers, and farmers' organizations, to work together.

1.9.3 Potential for Expansion

Industrialized agriculture has expanded during the past few decades, and along benefits a lot of negative input it has been brought into environment. Fortunately, an important opportunity also exists for the expansion of friendly environmental areas. There are many areas in that feature significant crop genetic diversity, where farmers still practice traditional agriculture and cultivate local varieties that have been selected over the course of many generations. Various programs and research projects have organized collection missions whose purposes is to collect genetic material and knowledge related to conservation techniques and cultivation methods, with the goal of to be valorized in pre-breeding and breeding. There is a strong need to detect and use the specific traits related to organoleptic qualities, and yield capacity, tolerance, and resistance to biotic and abiotic stress.

One challenge facing organic production systems all over the EU is the urgent need to provide climate-resilient cultivars. Currently, one going European project BRESOV ("Breeding for Resilient, Efficient and Sustainable Organic Vegetable Production ") started from the need to increase the plants' tolerance to biotic and abiotic stresses and adapt the varieties to the specific requirements of and low-input production processes has set out to improve the competitiveness of three important vegetable crops one being snap bean in an organic and sustainable environment. The BRESOV project aims to create a pipeline for crop improvement that will accelerate the production of high-quality organic seeds for breeders and farmers around the world. The pressure of climate changes requires the urgent need to provide climate-resilient cultivars technics and methods addressed to organic and conventional vegetable production systems and farmer's access to safe and quality seeds for resilient varieties and friendly technics. These new resources will benefit growers, seed industry, providing much needed security both under current and future scenarios of climate change.

The exploit of the genetic variation of legumes species for enhanced productivity, by exploiting up-to-date knowledge of genome structure and function for use in different directions as conservation, human health, sustainable agriculture can ensure long term benefits for human and environment.

Beans represent a valuable source of food proteins, and their exploitation is expected to increase in relation of a growing world's food need. The actual context of the need for available, healthy, long self-life food, new opportunities and challenges for the agriculture and food sector open. The value chain needs a strong improvement with new varieties with higher adaptation to different environments, better yield and improved qualities with a particular concern in the development of new products with high organoleptic and nutritional value. The availability of novel varieties will facilitate the adoption of food legumes in the agroecosystem improving the agrobiodiversity with all its related positive consequences associated to the inclusion of legumes in the cropping systems (e.g., sustainability, food security, economic returns, stable farming systems, increase of soil fertility, diversify products, improve human nutrition, etc.).

For the sustainable use of genetic resources, a coordinated, interdisciplinary, and multi-sectorial effort is needed to exploit the recent scientific and technological ground-breaking advances. Grain legumes should be reintroduced into crop rotations in the future, based on their favorable impact on production and quality attributes of following crops.

The market for meat and dairy alternatives is particularly promising, with annual growth rates of 14% and 11%, respectively. This implies huge opportunities for innovation based on added value to primary production. Strategies and plans to improve nutritional and quality traits need to be implemented to provide affordable supply for all citizens. To build these new capacities and innovative products, links to local, regional production and food tradition, which have as focus the consumer preference had to be valorized. These challenges meet citizens' needs and preferences (e.g. changing dietary habits), regarding impact on health, environment and climate change mitigation. Alternative plant proteins for food are demanded. The EU has developed a new protein plan, and its implementation will be largely based on traditional and innovative uses of food legumes and reflects the high interest of the food sector for development of products to meet consumer requests for healthful diets. In several EU states, human plant protein consumption is increasing.

Moreover, most of legume species can establish symbiotic association with nitrogen fixing bacteria, collectively known as rhizobia. Nitrogen fixation underlies the high protein content of legume seeds, and it is also of immense economic and ecologic importance, because it returns vital reduced nitrogen to the soil, thereby enhancing (agro)ecosystem productivity and sustainability. Historically, legumes were a primary source of agricultural nitrogen because they were grown in rotation with cereals. In most modern intensive agricultural systems, however, including those of Europe and North America, nitrogen fertilizer originates from industrial processes (Haber Bosch) that require immense quantities of fossil fuel to reduce N_2 to NH_4. Therefore, production of industrial fertilizers contributes ~ 3% of global CO_2 and is a primary source of pollutant NO_2. Moreover, runoff from fertilizer is among the world's most serious environmental pollutants, causing eutrophication of marine systems. Therefore, exploiting legume GenRes to improve the symbiosis between crop legumes and their rhizobia could have major impact on sustainable agriculture and the world's economic, social and environmental health.

1.10 Treaties and Conventions. Disclosure of Sources of Genetic Resources. Access and Benefit Sharing

Food safety, seed security, diversity, and clean environment are important keys, considered priority "0" at planetary level in researchers and politicians' agenda, aimed to design new strategies for the benefit of current and future generations. In this context, plant genetic resources for food and agriculture (PGRFA) are essential for achieving global food security and for sustainable agricultural development in the context of poverty mitigation and climate change. PGRFA are crucial to adapting plants to a changing and more complex environment, but their variability in current breeding, farming and forest management remains largely underused. Conservation initiatives (in-situ, ex-situ) are aimed at capturing, maintaining and making a large share of these global assets available. Access to resources, however, is also limited by the nature of the content and the knowledge provide by the different conservation sites. With growing concerns about biodiversityand genetic loss, joint efforts to extend and enhance the protection and use of PGRFA in farming and forestry has led to the development during the last few decades of numerous international instruments, treaties and conventions to ensure the efficient management of PGRFA. The Convention on Biological Diversity (CBD), the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from the Utilization (hereinafter referred to as the Nagoya Protocol), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) and various types of intellectual property rights are some examples of these.

The CBD is the first global agreement aimed on conservation and use of biological diversity to recognise the jurisdiction of states over their genetic resources in relation to their conservation and sustainable use, the traditional knowledge of the indigenous and local communities, and the allocation to these communities of the benefits derived from their use. The CBD, as an international treaty, recognizes a shared problem, establishes overarching aims and policies, as well as general commitments, and arranges technical and financial cooperation.

- National actions, the countries have a major share of the responsibility for accomplishing their objectives. Conservation and sustainable use of each country's biological diversity can be achieved in various ways. The major method of conservation, "in-situ" conservation, focuses on preserving genes, species, and ecosystems in their native habitats, such as through establishing protected areas, restoring damaged ecosystems, and enacting legislation to preserve endangered species. To save species, "ex-situ" conservationists employ gene banks. In the years and decades ahead, promoting the sustainable use of biodiversity will become more important for conserving biodiversity.
- International action, the Convention's success depends on the combined efforts of the world's nations. Individual nations are responsible for implementing the Convention, and compliance will be largely based on informed self-interest and peer pressure from other countries, as well as public opinion (text from the

document Sustaining life on Earth How the Convention on Biological Diversity promotes nature and human well-being by, Secretariat of the Convention on Biological Diversity April 2000).

The Nagoya Protocol, approved in 2010, considerably expands and fleshes out the broad framework provided in the CBD for access to genetic resources and the fair and equitable sharing of benefits arising from their use (access and benefit sharing). The ITPGRFA, adopted in 2001, established an international legal framework for the conservation and sustainable use of genetic resources for food and agriculture, as well as the equal and equitable sharing of benefits arising from their use, in accordance with the CBD and with the UN's Food and Agriculture Organization. The Nagoya Protocol's application and the ITPGRFA's application are meant to be complimentary. The Nagoya Protocol does not apply for the Parties to the ITPGRFA in respect of the PGRFA covered by and for the purpose of the Treaty. However, the Nagoya Protocol and the ITPGRFA are based on two separate models of structures for access and profit sharing. The Nagoya Protocol establishes that, in accordance with national legislation, access to genetic resources and to its associated traditional knowledge for their utilization is subject to obtaining the prior informed consent (PIC) from the provider and to the establishment of mutually agreed terms (MAT), which are to be agreed between the user and the provider. The ITPRFA establishes a "multilateral access and benefit-sharing system" whereby countries agree to practically pool and offer facilitated access to "all PGRFAs listed in Annex I of the Treaty that are under the management and control of the Contracting Parties and in the public domain". The Treaty's Annex I encompass 64 crops and forages that have been selected according to food protection criteria. Under the terms and conditions of the Standard Material Transfer Agreement (SMTA), such facilitated access under the ITPGRFA is given where the intended use of the genetic resource is its conservation and sustainable use for research, breeding and training for food and agriculture. Common bean is included in the crops mentioned in the ITPGRFA Annex I. Access to common bean genetic resources by any legal or private person from any ITPGRFA Contracting Party should therefore be facilitated under the conditions laid down in the SMTA, given that the intended uses are those cover by the ITPGRFA. The Treaty has made legal provisions to facilitate access and benefit sharing and addressed the germplasm utilization issues, which are important for crop improvement. In this context, there is a need to harness the designated germplasm in the gene banks, which includes many wild relatives (CWR) of food legumes. Without any hassle of fresh collecting, the wild relatives and other in-trust accessions held ex situ in gene banks can be used in research and varietal development programs. Molecular and classical breeding approaches can be supplemented to create diversity as well as new plant types suitable for use in different cropping systems and situations. The Treaty encourages the establishment and preservation of various farming systems while also maximizing crop usage and breeding.

Since January 2007, the Multilateral System of Access and Benefit-Sharing became live and the Secretariat created a set of SMTA users' optional information technology tools. In 2009, the Secretariat, in partnership with CIRAD, published the

first edition of Gene-IT, a user-friendly standalone software program for filling out and generating SMTAs. To make SMTA providers' reporting responsibilities easier, the Secretariat created and released an information system in November 2010 that permitted online reporting at the accession level for the specific crops mentioned in Annex 1 of the Treaty. The experience of the Secretariat of the International Treaty has allowed for modifications since then.

A set of new measurements is under implementation in frame of Green Deal. The recently released "Farm to Fork Strategy", which aims to design a strong food system capable of ensuring access to a sufficient supply of affordable products and services for all citizens, brings together international consortia to test and demonstrate systemic innovations, including leveraging legumes' multiple benefits.

1.10.1 Farmers Rights

Apart from theInternational Treatyitself—the Convention on Biological Diversity (CBD), the Agreement on Trade-related Aspects of Intellectual Property Rights (TRIPS) of the World Trade Organization (WTO), the Convention of the Union for the Protection of New Varieties of Plants (UPOV) and World Intellectual Property Organization (WIPO) of the United Nations (UN) are among the most important international agreements. These international agreements are interlinked, and they interact in various ways about Farmers' Rights, to ensure recognizing of paramount contribution of farmers to the diversity of crops that feed the world. Farmer rights are referenced also by establishing a global network with access to plant genetic materials for farmers, plant breeders and scientists.

TRIPS is an international legal agreement that all WTO members have signed. It establishes basic standards for national governments to regulate different forms of intellectual property rights (IPR) that affect citienzens of other WTO member nations. The TRIPS agreement, in particular, calls for stronger protection in areas that were previously unprotected by formal IPRs in many nations, such as genetic resources (including plant varieties). Consequently, countries around the world are gradually adopting plant variety protection legislation in line with the rules laid down by the International Convention for the Protection of New Varieties of Plants (hereinafter referred as the UPOV Convention). The UPOV Convention is a sui generis form of protection of intellectual property, specifically designed to reflect the specific characteristics of the breeding, cultivation and use of new plant varieties. The Convention was adopted the first time in 1961, and was subsequently revised in 1972, 1978 and 1991. As of February 2020, this organization had 76 countries (including the African Intellectual Property Organization and the European Union) as members (www.upov.int). UPOV's objective is to establish and support an effective system for plant variety protection, with the goal of encouraging the production of novel plant varieties for the benefit of society. The breeder's right is guaranteed for a period of not less than 20 years from the date of grant or, in the case of trees and vines, for a period of not less than 25 years. Accordingly, a breeder's authorisation is necessary for the

use of the reproduction or propagation material. However, the right of the breeder under the UPOV Convention does not apply to actions taken out privately and for non-commercial reasons, to actions taken for experimental purposes and to actions carried out for the purpose of breeding other varieties and to the exploitation of those new varieties, given that the new variety is not necessarily a variety derived from another protected variety. As of January 2021, the UPOV PLUTO database includes 12,343 varieties of genus *Phaseolus and* provided by 57 countries (last accessed in January 2021—available at http://www.upov.int/pluto/en/).

1.10.2 Participatory Breeding

International, breeding programs are often aimed at producing high-input commercial farming plant varieties that perform well in standardized environments. As a result, these varieties are typically not sufficient for the non-uniform conditions typical of marginal areas or for those farmers who are unable to buy additional inputs. In this context, participatory plant breeding and participatory variety selection will be crucial to strengthen the least productive common bean systems and to provide varieties that respond well to agro-ecological management under an integral ecology approach. Participatory plant breeding and participatory variety selection are methods in which farmers and officially qualified breeders work together during different phases of the breeding process, often locating breeding plots in the fields of farmers rather than in agricultural research stations and selecting agronomic and quality features adapted to the particular requirements of farmers. A number of successful implementations of this approach have been documented for common bean in Central Africa, Kenya, Rwanda, Uganda, Ethiopia, and in Kashmir.

With the aim to preserve, rebuild, revitalize, reinforce and develop local seed systems, with an emphasis on local varieties, community level seed-saving programs have been also developed for over 30 years. Community seed banks are run by local organizations that hold collections of seed that are maintained and administered by communities in a central facility or in a structure that is shared among a range of individuals. Community seed banks play different functions in the community such as preserving seeds, providing access to seeds for community members, and generating a degree of food security and food sovereignty, while at the same time contributing to the implementation of farmers' rights through the recognition of farmers' knowledge of local biodiversity, their participation in decision-making for its conservation and benefit sharing.

An important tool for sharing knowledge and plant genetic resources for sustainable use in breeding can be the **European Cooperative Programme for Plant Genetic Resources (ECPGR), EURISCO cathalog and AEGIS system**. This is a multi-country initiative aimed at guaranteeing the long-term conservation of plant genetic resources and making their use more accessible throughout Europe. In frame of this Program is function a working group dedicated to grain legumes species. In Europe stakeholders collaborate to conserve ex situ and in situ PGRFA, provide

access and increase sustainable use with the aim (i) to efficiently conserving and providing access to unique germplasm in Europe through AEGIS and the European Collection; (ii) to offer through the EURISCO catalogue passport and phenotypic information of actively preserved European PGRFA; (iii) to improve in situ conservation and use of crop wild relatives; (iv) to promote on-farm conservation and management of the diversity in European PGRFA; (v) to promote use of PGRFA.

1.10.3 Conclusion

It is evident that common bean improvement is an ongoing process and there is still great potential to exploit the genomic information and genetic diversity to maintain continued yield gains and to face agricultural challenges, such as climate change and food security. However, the use of genetic resources in common bean breeding to date is minimal relative to the availability of materials, and the possible effect of their use is far from optimal (i.e. lack of detailed knowledge on passport data and user-useful descriptors, accession heterogeneity, non-harmonized data). In this context, large scale projects such as INCREASE (https://www.pulsesincrease.eu/), aims to improve genetic resources use by developing efficient and effective conservation strategies to promote agrobiodiversity and its use.

Finally, it is also important to recognize the current advances in agro-biotechnology in molecular markers, functional genomics, mutagenesis, tissue culture, genetic engineering and even deep phenotyping approaches and sophisticated informatics tools, when designing new breeding programs aims to obtain new varieties with broad resistance to varied biotic and abiotic stresses. This is reflected in efforts already underway within large scale projects such as the BEAN_ADAPT Project (funded through the second ERA-CAPS call; ERA-NET for Coordinating Action in Plant Sciences), BRESOV H2020 funded project. The projects areusinga multidisciplinary approach (i.e., genomics, transcriptomics, metabolomics, plant physiology, population/quantitative genetics and biochemistry) to expand the genetic basis of phenotypic adaptation in *P. vulgaris* and its sister species *P. coccineus*across Europe and outside their origin centers.

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Chapter 2
Chickpea Biotic Stresses

L. Manjunatha, Anita Puyam, G. U. Prema, M. Sanjay Bandi, Rishikesh Kumar, M. C. Keerthi, G. P. Dixit, and T. R. Kavitha

Abstract Chickpea (*Cicer arietinum* L.) is the second most important grain legume crop of world and Indian subcontinent, contributing over 65% to the worlds chickpea production for securing nutritional and food security. The biotic and abiotic stresses in chickpea have been aggravated by the collective effect of climate change. These biotic stresses can be tactically controlled by developing effective, novel, conventional and molecular breeding technologies. Keeping this in view, we have discussed the major emerging diseases and pests, their diagnostics, epidemiological factors, breeding approaches for improved productivity, resistant/tolerant genetic resources from gene pools, disease resistant marker traits, major QTLs or genes and marker assisted breeding (MAB) technologies against major diseases and pests. Gene pyramiding for multiple disease resistances, introgressing genes from wild species with extraordinary yield potential, resistance to soil-borne (fusarium wilt, dry root rot, collar rot) and foliar (Ascochyta blight, Botrytis gray mold, rust, Alternaria blight) diseases are elaborated. The information on markers and QTLs for resistance to diseases, pests and nematodes would be useful for enhancing breeding activities in chickpea. Genetic engineering and marker assisted selection (MAS), advantages and their limitations are also highlighted.

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2.1 Introduction

Chickpea, belonging to the family Fabaceae (erstwhile Leguminosae), subfamily Papilionaceae and the tribe Cicereae Alef (van der Maesen 1987), is the only nurtured species of the genus *Cicer*. The genus *Cicer* has 44 species, of which 43 are wild (9 annual and 34 perennial wild species) and only one species is domesticated. It is a highly self-pollinated crop with an outcrossing rate of less than 1%. The Desi type, a small triangular seed, is the primogenital type, with the larger round "rams head". The Kabuli type was domesticated further in the Mediterranean region. The Kabuli type has little genetic variability than desi type, geographical distribution and morphological variation (Redden and Berger 2007). Genetic resources of chickpea are classified into primary, secondary, and tertiary gene pools based on their taxonomical identity for improving the cultivars. The primary gene pool comprises cultivated species and landraces. The secondary gene pool consists of *C. reticulatum* and *C. echinospermum*, the progenitor species that are crossable with *C. arietinum*, but with a lesser amount of fertility of the resulting hybrids and improved offspring. The tertiary gene pool involves all the annual and perennial *Cicer* species, which are not crossable with the cultivated chickpea.

2.2 Economic Importance

Chickpea contributes about 18% of the grain legume production globally (Jendoubi et al. 2017). It ranks third among the pulse crops with an annual production of 10.10 million tons (Muehlbauer and Sarkar 2017). India is the largest chickpea producer (11.38 mt) in the world with 67% share in global chickpea production (DAC and FW 2019; FAO 2019). In India, chickpea occupies about 68% of the total area under pulse crops. At present, India, Mexico and Australia are leading exporters of chickpea (Muehlbauer and Sarkar 2017). The chickpea domestic gene pool is limited than that of wheat, pea or lentil because of (i) the inadequate genetic diversity of the wild progenitor *Cicer reticulatum* found only in southeast Turkey, (ii) the genetic blockage of domestication, in which only an inadequate choice of variations was selected, and (iii) a further choice for spring habit with loss of 'vernalization. Thus, chickpea has little genetic variability for disease resistance (Abbo et al. 2007).

Chickpea is an inexpensive source of protein (24%) and it ranging from 15 to 30% (Hulse 1994) with good source of essential B vitamins and minerals, 60–65% carbohydrates and 6% fat (Muehlbauer and Sarkar 2017). Chickpea is the most common source of protein consumed by people who are vegetarian by choice throughout

the world for its nutritional value and cheap availability, especially in the semi-arid tropics. It is consumed as 'dal' or used for making flour throughout the globe.

2.3 Gene Pool

The concept of gene pool was proposed by Harlan and de Wet (1971) for their usages in plant breeding. The genus *Cicer* comprised one cultivated species, the chickpea (*Cicer arietinum*), and 42 wild species. Chickpea includes landraces, breeding material and wild species. It is estimated that more than 97,000 germplasms are conserved in more than 30 genebanks, globally. A rich genetic diversity has been observed in cultivated chickpea gene pool with less than 2% wild species. Based on size and shape of seed, two types of chickpea are recognized: Desi types and Kabuli types. Three gene pools have been proposed for *Cicer* species. Based on crossability with cultigens, gene pools of chickpeas are classified into primary, secondary and tertiary gene pools.

The primary gene pool comprises *C. arietinum*, a cultivated species and *C. anatolicum* a perennial. The *C. echinospermum* and *C. reticulatum* are two wild annual ancestor species. Some authors also group them with the primary gene pool. The secondary gene pool consists of *C. judaicum* and *C. pinnatifidum* which are annual species. The tertiary gene pool, the most distantly related cluster, comprises three annual species, *C. yamashitae*, *C. chorassanicum* and *C. cuneatum*, and 34 perennial species. The two wild *Cicer* species, *C. reticulatum* and *C. echinospermum* are cross-compatible with the cultivated species and are used in yield improvement in chickpea. All the annual and perennial *Cicer* species of tertiary gene pool are not compatible for crossing with cultivated species (Table 2.1).

2.3.1 Genetic Diversity and Its Characterization

Evaluation of genetic diversity and tagging of economically important genes can be competently carried out by using molecular markers. Due to high cost and limited availability of genomic resources, the approaches of genomic-assisted breeding in chickpea remained low until 2005. ICRISAT played an important role in generation of molecular markers and genomic resources including whole genome sequencing. At present, more than 2000 SSR markers are available in chickpea. Due to domestication of the crops, the genetic base of several legume crops including chickpea became narrow (Spillane and Gepts 2001). The wild relatives have broad range of genetic diversity followed by land races and commercial varieties. The 38 accessions of *C. arietinum*, six of *C. reticulatum* and four of *C. echinospermum* of the primary gene pool analysed using 100 SSR markers for genetic structure, diversity and relationships by Choudhary et al. (2012) and considerable diversity was reported, with a mean of 4.8 alleles per locus (ranging from 2 to 11); polymorphic information content

Table 2.1 Worldwide distribution of different *Cicer* species (Modified from Singh et al. 2014)

Annuals Cicer species	Distribution
C. arietinum	Mediterranean region to Burma, Ethiopia, Mexico, Chile
C. chorassanicum	N and C Afghanistan, N and NE Iran
C. bijugum	SE Turkey, N Syria, N Iraq
C. cuneatum	Ethiopia, SEEgypt, NE Sudan, Saudi Arabia
C. echinospermum	Turkey, E Anatolia, N Iraq
C. judaicum	Palestine, Lebanon
C. reticulatum	E Turkey
C. pinnatifidum	Cyprus, N Iraq, Syria, Turkey, USSR
C. yamashitae	Afghanistan
Perennial Cicer species	
C. acanthophyllum	Afghanistan, Pakistan, USSR
C. anatolicum	Turkey, Iran, Iraq, Armenia
C. balcaricum	Caucasus (USSR)
C. baldshuanicum, C. flexuosum, C. grande, C. incanum, C. korshinskyi, C. laetum, C. mogoltavicum, C. paucijugum, C. rassuloviae, C. songaricum	USSR
C. luteum, C. kermanense, C. korshinskyi	–
C. atlanticum	Morocco
C. fedtschenkoi	USSR, N and NE Afghanistan
C. canariense	Canary Islands, Tenerife, La Palma
C. multijugum, C. rechingeri	Afghanistan
C. montbretti	Albania, Bulgaria, Turkey
C. microphyllum	E Afghanistan, Tibet, India, Pakistan, USSR
C. macrocanthum	Afghanistan, India, Pakistan, USSR
C. kermanense	SE Iran
C. floribundum, C. heterophyllum, C. isauricum	Turkey
C. graecum	Greece
C. incisum	Greece, Turkey, Iran, Lebanon, USSR
C. tragacanthoides, C. tragacanthoides var. *turcomanicum, Cicer tragacanthoides* var. *tragacanthoides*	Iran, USSR
C. nuristanicum	Afghanistan, India, Pakistan
C. oxyodon	Iran, Afghanistan, N Iraq
C. pungens	Afghanistan, USSR
C. spyroceras, C. stapfianum, C. subaphyllum	Iran
C. tragacanthoides	–
C. incanum, C. spiroceras, C. incisum C. stapfianum, C. isauricum	–

(continued)

Table 2.1 (continued)

C. fedtschenkoi, C. *oxyodon, C. flexuosum, C. paucijugum, C. floribundum, C. pungens,* C. *graecum, C. rassuloviae, C. grande, C. rechingeri, C. heterophyllum*	–

(PIC) ranged from 0.040 to 0.803, with a mean of 0.536. The diversity was highest in wild species, which had higher values of PIC, gene diversity and heterozygosity than the cultivated species, indicating a narrow genetic base for cultivated chickpea. The information on genetic variability would be beneficial in effective identification, conservation and utilization of chickpea germplasm for allele mining, association genetics, mapping and cloning gene(s) for development of pest and disease resistant cultivars with broad genetic base with superior yield improvement for diverse environments. Genetic diversity analysis using 75 simple sequence repeats (SSRs) revealed a total of 267 alleles with an average of 3.56 alleles per marker in association mapping of the genomic loci controlling Fusarium wilt (FW) (Foc2) resistance in chickpea. The SSR markers, CESSR433, NCPGR21 and ICCM0284 could be potentially employed for targeted and accelerated enhancement of FW resistance (Jha et al. 2021). QTLs for early and late wilt on linkage group 02 (LG02) was reported in chickpea (Patil et al. 2014). Notably, chickpea LG02 harbours resistance gene(s)/QTLs for FW races 1 (Foc1), 3 (Foc3), 4 (Foc4) and 5 (Foc5) (Varshney et al. 2014; Caballo et al. 2019). The GLM and MLM analyses also detected the marker CESSR433 on LG01, showing linkage with wilt resistance in both years 2016 and 2017. Occurrence of QTLs for FW resistance in LG01 was reported earlier in chickpea (Jingade and Ravikumar 2015).

Marker-assisted breeding (MAB) can greatly improve the accuracy and effectiveness of breeding activities. It mainly focusses on the introgression of the QTL and/or genomic region associated with the trait(s) of interest from a donor parent into an elite recurrent parent using molecular markers. MABC is being used for introgressing abiotic and biotic stresses resistance in chickpea. In ICRISAT, genes from the chickpea cultivar WR315 were pyramided for resistance to two races of FW (*foc1* and *foc3*) and 2 QTLs for AB resistance from ILC3279 line into C-214. Marker-assisted recurrent selection (MARS) has also been investigated for yield improvement in chickpea and other abiotic stress tolerance. In addition to MARS, genome-wide selection (GWS) or genomic selection (GS) has been anticipated as a budding approach for improving composite traits governed by various genes/QTLs (Singh et al. 2014). In this method, equally phenotyping and genotyping data are used to calculate genomic estimated breeding values (GEBVs) of offsprings and greater progenies are selected based on GEBVs.

2.4 Biotic Constraints of Chickpea

The chickpea production is limited by several biotic and abiotic stresses. Approximately 172 pathogens have been reported to incite diseases in chickpea. For example, Ascochyta blight (AB) and botrytis could cause upto 100% grain and yield loss under favorable conditions (Nene et al. 1996). The important diseases affecting chickpea production are detailed in the Table 2.2.

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

In the present climatic scenario, those diseases which were of less or no significance previously have been gaining importance and are emerging as new threat to crop production. Climate not only affect plants but also affect the pathogens, insect pests and weeds that reduces crop yield (Anderson et al. 2004). Many researchers have been paying much efforts to study the temperature response in chickpea cultivars against FOC races. Resistance of a cultivar against the wilt is dependent upon temperature under field conditions, and as well as under artificial inoculation. Timely sowing of chickpea is reported to minimize the FW. The concept is to avoid the coincidence of susceptible host stage with the virulent pathogen. Maximum and minimum ambient temperature of soil are positively correlated with wilt incidence in the cultivar 'Pusa 212 (Mina and Dubey 2010) indicating the possible impact of the climatic conditions on the incidence of wilt disease.

There is a chance in the shift of previously less important disease becoming more threatening disease. In the recent years, due to prolonged drought spells, dry rot of chickpea has become a major threat to production. Disposing of the plants to stress condition during growth period, particularly at pre-harvesting stage, like depleting soil moisture and higher temperature predisposes the plants to the emerging threat of DRR (Sharma and Pande 2013). Pande et al. (2010) also suggested that DRR is more severe in rainfed environments. Climate change and its effect on plant diseases is the need of the time to adopt new measures to manage the pathogens like introducing new resistant cultivars and other novel techniques. Previously, researchers also proved that hot and dry environmental conditions predisposed the economically important crops to the infection and colonization of *R. bataticola* and caused drastic yield losses in chickpea (Thripathi and Sharma 1983), soybean (Pearson et al. 1984) and sunflower (Nawaz 2007). Rainfall was less severely and positively correlated with disease incidence, while maximum relative humidity was non significant and correlated negatively with the disease incidence. However, multiple correlation coefficients among weather variables and disease incidence exhibited strong association between different components of epidemics during 2001–2003. Late sowing significantly reduced the incidence of DRR (Vijay-Mohan et al. 2006).

Table 2.2 Prevalence of major diseases of chickpea and associated congenial factors for disease development

SI. no	Diseases	Causal organism	Favorable conditions for the disease development
1.1 Major soil borne diseases			
1	Fusarium wilt (FW)	*Fusarium oxysporum* f. sp. *ciceris* (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6)	Soil with 30–60% water holding capacity, high soil moisture
2	Dry root rot (DRR)	*Rhizoctonia bataticola*	Semi-arid climate, low soil moisture in February–March or high temperature (>30^{o})
3	Collar rot (CR)	*Sclerotium rolfsii*	Humid and warm climate with high soil moisture or high crop density
1.2 Major foliar diseases			
4	Ascochyta blight (AB)	*Ascochyta rabiei* (teleomorph = *Didymella rabiei)*	Humid and cool climate with high soil moisture
5	Botrytis gray mold (BGM)	*Botrytis cinerea*	High environmental humidity and cool climate
6	Stem rot	*Sclerotinia sclerotiorum*	Cool climate (20^{0}C), thick and luxurious growth and high soil moisture
7	Alternaria blight	*Alternaria alternata*	Warm and humid weather with rising temperature
8	Powdery mildew	*Leveillula taurica* (Anamorph: *Oidiopsis taurica)*	Warm windy conditions with moisture under the crop canopy
9	Rust	*Uromyces cicer-arietini*	Warm and humid conditions
10	Foot rot	*Opercullella padwickii*	Frequently irrigated field having high moisture
1.3 Minor fungal diseases			
11	Phytophthora root rot	*Phytophthora drechsleri, P. citrophthora*	Kabuli chickpeas are highly susceptible
12	Black root rot	*F. solani*	High soil moisture or heavy irrigation during December-January
13	Anthracnose	*Colletotrichum dematium*	High temperature and high rainfall condition
14	Wet root rot	*Rhizoctonia solani*	Moderate to high soil moisture and temperature
15	Stemphylium blight	*Stemphylium sarciniforme*	Extreme vegetative growth, high RH (80–90%) and cool temperature (15–20 °C)
1.4 Nematode diseases			

(continued)

Table 2.2 (continued)

16	Root knot nematode (RKN)	*Meloidogyne incognita*, *Meloidogyne arenaria* *Meloidogyne javanica*	Warm sandy loam soils
17	Root lesion (Root lesion nematode)	*Pratylenchus thornei*, *Pratylenchus brachyurus*	Heavy clay to sandy loam soils
18	Reniform nematode	*Rotylechulus reniformis* (Linford and Oliveiria)	Prevalent in hot semi-arid and hot subhumid regions of the country. The temperature of 33 °C is optimum for the nematode
19	Cyst nematode	*Heterodera cicero* Vovlas	25-30^{0}C is optimum for nematode development
1.5 Bacterial diseases			
20	Bacterial blight	*Xanthomonas campestris* pv. *cassiae*	Wet and warm condition with >30 °C temperature
1.6 Viral diseases			
21	Stunt	*Chickpea chlorotic dwarf virus* (CCDV) and *Bean leaf roll virus* (BLRV)	Presence of vectors and early sowing in September
22	Phyllody	Phytoplasma/Mycoplasma-like organisms (MLOs)	Presence of vectors and early sowing in September
23	Mosaic	*Alfalfa mosaic virus*	Presence of vectors and early sowing in September
24	Yellowing	*Pea enation mosaic virus*	Presence of vectors and early sowing in September
25	Proliferation	*Cucumber mosaic virus*	Presence of vectors and early sowing in September
26	Narrow leaf	*Bean yellow mosaic virus*	Presence of vectors and early sowing in September
1.7 Phanerogamic parasites/non parasitic diseases			
27	Dodder	*Cuscuta* spp.	Black soils and high temperature conditions favor dodder growth
28	Chlorosis	Iron deficiency	Absence of sufficient iron in the soil

2.6 Description on Different Biotic Stresses

2.6.1 Soil-Borne Biotic Stresses

2.6.1.1 Fusarium Wilt

Chickpea wilt is caused by *Fusarium oxysporum* (Schlechtend. Fr.) f.sp. *ciceris* (Padwick) Matuo & K. Sato (FOC). The fungus belongs to *Fusarium oxysporum* complex in the Gibberella clade. Based on the disease severity in set of differential chickpea cultivars, eight races were identified (Jendoubi et al. 2017). Initially, four physiological races were identified (1, 2, 3 and 4) based on 10 chickpea differentials (Haware and Nene 1980) of which three races (2, 3, and 4) in Ethiopia (Shehabu et al. 2008), two races (2 and 3) in Turkey (Bayraktar and Dolar 2012; Dolar 1997). Besides, two more races (0 and 5) were identified from Spain (Phillips 1988) and races 0, 1B/C, 4 and 5 existed in Iraq (Al-Taae et al. 2013). Race 0 and race 6 were found in California and Tunisia (Phillips 1988) and four races (0, 1B/C, 5 and 6) in northwestern Mexico (Arvayo-Ortiz et al. 2011). Altogether eight pathogenic races of *Foc* (races 0, 1A, 1B/C, 2, 3, 4, 5 and 6) were reported worldwide (Jendoubi et al. 2017). Among the eight races reported by Dubey et al. (2012) across India were, race 1(Andhra Pradesh and Karnataka), race 2 and race 4 (Uttar Pradesh), race 2 (Bihar), race 3 and 8 (Punjab), race 3 and 8 (Rajasthan), race 3, 6 and 7 (Madhya Pradesh), race 4 (Delhi and Haryana), race 4 and 8 (Punjab), and race 4 and 7 (Maharashtra), race 5 only from Rajasthan, race 6 from Chhattisgarh and Jharkhand and race 7 from Gujarat. The random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers established the uniqueness of race-1 and race-2 pathogen isolates and the close proximity of race-3 and race-4 (Sivaramakrishnan et al. 2012). Using species specific internal transcribed spacer (ITS) and random markers, races were identified in FOC (Gurjar et al. 2009). Demers et al. (2014) reported identical rDNA ITS, a xylanase gene (*xyl4*) and its transcriptional activator (xlnR), sequence characterized amplified region (SCAR)-PCR markers previously developed for identification of *F. oxysporum* f. sp. *ciceris* and of race 5 and 11 microsatellites (Jimenez-Gasco and Jimenez-Diaz 2003). The studies on the genetic lineage indicates the monophyletic origin of this forma specialis (Jimenez-Gasco et al. 2002). The seven races produced distinguished symptoms with differences in their virulence pattern. Race 0 has the least virulence as compared to races 1 to 6. The most damaging races are 1A and 2 (Landa et al. 2006; Jimenez-Diaz et al. 2015). The symptoms of progressive foliar yellowing induced by race 0 and 1B/C whereas races 1A and 2 to 6 produced early wilt with no visible yellowing of leaves, severe leaf chlorosis and flaccidity (Landa et al. 2006; Cunnington et al. 2007; Jimenez-Fernandez et al. 2011; Jimenez-Diaz et al. 2015). Wilting pathotypes are races 1A and 2 through 6, and yellowing pathotypes belonged to race 0 and 1B/C.

The FW disease causes an annual yield loss ranging from 10 to 90% (Biswas and Ali 2017). The FOC infects chickpea at any growth stage of the plant starting from seedling till the bearing stage i.e., flowering and pod forming stage. The most critical

stage for high incidence is when the susceptible stage coincides with sudden increase in temperature and water stress at flowering and podding stage. Wilt symptoms appear within 25 days of sowing in highly susceptible cultivar (e.g. JG-62). However, the symptoms appear more conspicuously at 6–8 weeks after the onset of flowering till the pod stage. So, wilt stage can be distinguished as early and late wilt. Early wilt causes more yield loss than late wilt (Chen et al. 2011).

In general wilt symptoms include, drooping of petioles and rachis, upward yellowing and drying of leaves from base of the plant, withering and death of plants, infected stem and root tissues shows internal vascular browning (Westerlund et al. 1974). The interaction of the fungus with the host plant effected in production of cell wall degrading enzymes to intrude the physical barriers of the host which in turn lead to formation of gel like substrate as a defense against the invading pathogens. This blocks the plant's transport system, leading to choking and browning of vascular system (Brayford 1998). Ultimately, from yellowing, wilting and necrosis lead to death of plant (Leslie and Summerell 2006). In wilted field, patches of affected plants exhibit uneven growth. Late-wilted plants produce rougher, lighter and duller seeds than that of the healthy plants (Haware and Nene 1980; Navas-Cortes et al. 2000).

2.6.1.2 Dry Root Rot

The second important disease of chickpea is DRR, which is gaining prominence in the changing climate scenario especially in tropical arid and semi-arid region. The *Rhizoctonia bataticola* (Taub.) Butler (Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid) is the causal agent of DRR. In the nineteenth century, Tulasne brothers (1862) merged *Rhizoctonia crocorum* DC and *Rhizoctonia medicaginis* DC to a single species *Rhizoctonia vivolaceae* Tul. based on their extensive morphological study. Before 1912, Shaw studied two species of *Rhizoctonia,* of which one was characterized by its formation of small black sclerotia. Currently, the sclerotial phase is referred as *R. bataticola* (Ram and Singh 2018) and teleomorph *M. phaseolina* is formally recognized as the right taxonomic name (CMI description of pathogenic fungi and bacteria No. 275). The systematic position of *Rhizoctonia bataticola* is as follows: Kingdom—Fungi; Division—Basidiomycota; Sub-division—Agaricomycotina; Class—Agaricomycetes; Order—Cantharellales; Family—Ceratobasidiaceae; Genus—*Rhizoctonia*; Species—*bataticola.*

Besides, morphological characterization, pathotyping of *R. bataticola* isolates and their molecular characterization provided the details about the dry rot pathogen. There has not been much report on pathotypes of *R. bataticola* of chickpea. Six pathotypes have been reported using set of chickpea differentials viz. Pusa 212, Pusa 362, Pusa 1103, Pusa 1088, BCP 17, BGD 112, ICC 12,441, ICC 11,224, ICC12450, ICC 11,332 in blotter paper and sick soil (Dubey et al. 2011). Studies also made on the relativeness of pathotype groups to agro-ecological regions of the country using different markers: RAPD, ITS-RFLP and followed by ITS sequencing (Dubey et al. 2011). Contrastingly, Sharma et al. (2012a, b) proved the diversity of

R. bataticola and un-relatedness on geographical origin based on amplified fragment length polymorphism (AFLP) analysis. They grouped 40 different isolates of central India into two pathotypes through the virulence analysis on a chickpea susceptible cultivar, BG 212 under blotter paper technique. Recently, Gupta et al. (2012) reported two pathotypes from central India on DRR susceptible chickpea cultivar BG 212. Hyphal fusion, mutation, and mitotic crossing over were anticipated to be accountable for pathogenic diversity. But, Sharma et al. (2012a) reported cultural, morphological and molecular variability in *R. bataticola* isolates of various agro-ecological zones of India.

The DRR pathogen affects both the seedlings as well as reproductive phase. However, older plants are highly susceptible to the infection than younger plants (Sharma and Pande 2013). Most acute stage for infection is flowering and podding as that of the wilt (Sharma et al. 2016). If the 30 ºC temperature coincides with the susceptible stage, the plants become straw colored with characteristic brown colored stem and lower leaves. The dry rot symptoms resemble the FW. However, leaflets and petioles drooping are limited at the top of the plant in dry rot disease (Sharma et al. 2016). But the symptoms are not as much unique to the disease as in some cases, different pictures are seen. The top most leaves become chlorotic and remaining parts stay dried. The root tip is simply broken seperating the lower portion of the tap root inside the soil. When the diseased plants were uprooted, there is appearance of black color tap root accompanied with reduced lateral and fine roots. Later, the roots turn into fragile, shredded and ultimately dies. At advanced stage, the characteristic symptoms appear as dark colored tiny sclerotial bodies inside the stem when split open (Nene et al. 1991).

Disease can occurre in varied soil types, cultivars and cropping systems and about 5–50% or more disease incidence may inflict in badly infected soils (Sharma et al. 2016). An estimated loss ranging from 10 to 25% due to DRR have been reported (Pandey and Singh 1990; Vishwa and Chaudhary 2001). DRR has been emerged as a crucial disease that occurs irregularly, both spatially and temporally causing massive destructions (Savary et al. 2011). From the surveys (2010 to 2013), an extensive rise of incidence of DRR have been reported in the central and southern India (Ghosh et al. 2013).

2.6.1.3 Collar Rot Disease

Collar rot is an emerging threat for chickpea cultivation as a result of the changing climatic variables. CRis a serious soil borne disease caused by *Sclerotium rolfsii.* It is a fast spreading fungal pathogen causing a considerable seedling mortality, varing from 54.70 to 95.00% in the initial stage and causing a yield loss of 22–50% (Shrivastava et al. 1984).

The disease affects all stages of the crop. Stem discoloration is the first visible symptom in the field. A cottony, white mold occupies in the collar region near the soil level. Later, the stem girdles above ground, followed by drying of branches and ultimate death of the whole plant. The characteristic symptom of the disease is

appearance of chlorotic or drying branches or whole plants, scattering in the field. The common symptom of this disease with that of the dry root rot is the sclerotia production in stems when splitted open in the infected plants. Like the other two diseases, losses caused by this disease is also high. Yield loss due to the disease ranged from 7.10 to 10.50% from four chickpea cultivating states of India i.e. Andhra Pradesh, Chhattisgarh, Karnataka and Madhya Pradesh (Ghosh et al. 2013).

2.6.2 Foliar Diseases of Chickpea

2.6.2.1 Aschochyta Blight (AB)

The AB of chickpea is caused by the fungus *Ascochyta rabiei*, which survives on plant trash left in the soil (Pande et al. 2005). This is the major foliar diseases in West Asia, Northern Africa and Southern Europe. Its occurrence has been reported from cooler region the world (Chongo et al. 2003). The disease symptoms can be observed on all above-ground parts of the plant which causes severe loss in quality and quantity. The fungus is a hemi-biotroph, described by an initial biotrophic phase followed by a necrotrophic stage. Hence, characteristics symptoms of the disease may appear after an incubation period of 3–4 days.

Seed-borne infection leads to brown lesions at stem bases on emerged seedlings. Consequently, the lesions enlarge in size; stem girdling leads to its breakage and collapse of the plant. On leaf surface, numerous dark minute submerged pycnidia are produced but are scattered irregularly on stems and pods of infected tissues. On infected stem, the lesions are oval shaped with brown centres and a darker margin (Manjunatha et al. 2018a). Stem lesions results in girdling and weakening which consequently break off. The leaves and pods, the lesions are circular whereas elongated lesions are seen on stems and petioles. Infected pods often fail to form seed or pod infection may lead to infection of testa and cotyledons. Infected seed are discolored, possess deep, round, or irregular cankers, occasionally pycnidia may be visible to the naked eye. The infection during pod maturation stage often results in shrivelled and infected seed. Flowering to early podding stage of the crop is the susceptible stage in the field condition. Disease spreads mainly through infected seeds produced by infected crop (Kaiser and Kusmenoglu 1997). Fungus grows on the surface of seed coat of infected seed (Luthra and Bedi 1932), cotyledons (Lukashevich 1958) and on the emerging seedlings (Kaiser and Hannan 1988).

The disease is seed and soil-borne in nature. Diseased host plant debris left over in the fields serves as a source of primary inoculum. Cool (night temperatures of 10 °C and, day temperatures 15–23 °C), cloudy and wet weather favours the disease development. Winter rains causes epidemics in north western India especially in Punjab and parts of Himachal Pradesh. The disease builds up in February and March after winter rains (Pande et al. 2005).

2.6.2.2 Botrytis Grey Mould (BGM)

Shaw and Ajrekar (1915) first time reported Botrytis grey mould on chickpea caused by *B. cinerea* Pers. ex. Fr in India followed by Butler and Bisby (1931). However, first epidemic of this disease which caused crop loss of about 95% was reported by Carranza (1965) in Argentina. Generally, disease occurs in the area where, there is persist cool, cloudy, and humid weather. In the off season, this pathogen survives on the infected crop debris and seeds. The asexual stage of this necrotrophic fungus is dominant on chickpea. On the potato dextrose agar (PDA) media, it appears as white, cottony, which turns light gray after few days. Young hyphae of *B. cinerea* arethin, hyaline, and 8–16 μm wide, which become septate with age. Conidiophores are light brown, septate, and erect ramified pseudodichotomically. They have slightly enlarged tips, bearing small pointed sterigmata which bear 1–2-celled, hyaline, oval, or globose conidia forming clusters. The size of conidia is 4–25 × 4–18 μm and 4–16 × 4–10 μm on infected chickpea plants and PDA, respectively (Nene and Reddy, 1976; Pande et al. 2002). The disease has caused serious concerns in India, Bangladesh, Nepal, Pakistan and Australia. BGM is reported on chickpea globally. Numbers of reports from Argentina (Haware and McDonald 1992; Pande et al. 2002; Davidson et al. 2004) have showed almost complete yield loss (about 100%) under conducive environmental conditions. During sexual stage, *B. cinerea* fertilized sclerotia germinate by the emergence of apothecia that release sexually produced ascospores (Faretra and Grindle 1992). Formation of apothecia requires either two sexually compatible isolates (MAT 1–1 and MAT 1–2) or a pseudohomothallic isolate (MAT-1/2) (Faretra and Grindle 1992). Natural occurrence of sexual structure was not known, however, its occurrence is reported in India under laboratory conditions (Singh 1997).

During 1978–79, the disease had become epidemic in India and destroyed about 20,000 hectares of chickpea (Grewal and Laha 1983; Grewal et al. 1992; Haware 1998). However, this disease occurs in Nepal, almost each year of cropping causing average yield loss of 15% (Joshi 1992). Pod yield depends upon the onset of disease, its growth, disease severity, weather conditions and level of inoculums of the pathogen.

2.6.2.3 Rust Disease

The basidiomycete fungus, *Uromyces ciceris-arietini* (Grogn.) Jacz. & Beyer causes rust disease in chickpea. The pathogen belongs to the Phylum- Basidiomycota, Class-Urediniomycetes, Order-Uridinales and Family-Pucciniaceae (Singh 2009). The pathogen was first detected and described in France in 1863 as *Uredo ciceris arietini*. Later Boyer and Jaczevski reported the telial stage in 1893 (Mehta and Mundkur 1946). The fungus was subsequently renamed as *Uromyces ciceris-arietini* var. *aetnensis* by Scalia in 1899 and then to *Uromyces ciceris-arietini*. *Uromyces ciceri-arietini* is a hemiform, the pycnial and the aecial stages are being unknown. Rust is prevalent in several parts of India including Maharashtra and Tamil Nadu. In northern India, disease is common in Bihar, Uttar Pradesh and Punjab. Asthana (1957)

reported the severe outbreaks of rust on chickpea in Seoni-Malwa, India on local cultivar with 100% infection within 8 h in few genotypes. Patil et al. (2016) reported 80–100% yield loss in chickpea due to rust. Spread of the disease occures primarily by airborne uredospores. The rust perpetuates on Lucerne, *Trigonella polycerata* in the uredial stage during summer in the hills where climatic conditions are favorable (Saksena and Prasad 1955).

Althought, chickpea is the main host for *U. ciceris-arietini*, other important crops and wild species have been reported. *Trigonella polycerata* grows at high altitudes of up to 5500 m is infected by urediniospores of rust and serve as a reservoir of the fungus (Saksena and Prasada 1955; Payak 1962). *Lathyrus* spp. including *L. aphaca* and *L. odoratus* were recorded as new collateral hosts for gram rust in India (Bahadur and Sinha 1967). Rust was also observed infect *Vicia biennis*, *V. ervilia* and *V. faba.* Eleven host plants including chickpea were tested to know the host range of *U. ciceris-arietini,* however, only three host plants viz., *Cicer arietinum* (Chickpea), *Trigonella polycerata* (Lucern) and *Lathyrus odoratus* (grasspea) produced rust symtoms on inoculation by *U. ciceris-arietini* (Sunilkumar 2015).

Usually, the symptoms visible in the early February, i.e. late in the season. Rusty appearance of infected crop is due to rust pustules and urediniospores on coatedon foliage. First symptoms appear on the leaf surface as small, round or oval, cinnamon brown, red or orange powdery pustules. These pustules inclined to coalesce. Sometimes a ring of tiny pustules around larger pustules can be seen on both leaf surfaces but more frequently on lower surface. Sporadically pustules may be seen on stems and pods under severe infection. Advanced stage of infection of the plants may dry up prematurely which results in drastic reduction of yield. Cool and moist weather favors rust buildup, rainfall does not seem to be essential for the infection to spread. In many cases, the individual sori covered the entire leaflet and measured from 1–5 × 1–3 mm. These sori gave a brick red color to the infected plants which could be identified from distance. Rust occurrence and development preferred moderate warm weather (Deshmukh et al. 2010; Khedekar 2012; Nene et al. 2012; and http://agropedia.iitk.ac.in).

Temperature is the key factor influencing both viability and germination of uredospore. The uredospres remain viable for 48 weeks when stored at 6–7 °C but are killed within two weeks, if exposed at 30–35 °C. Fresh spores can be germinated in tap water at 18 °C and germination commences in 2.5 h. However,spores lose their viability and germination even on host plant at temperature above 35 °C (http://agropedia.iitk.ac.in). Sharma (2014) reported that the uredospores lose viability within 2–4 weeks and 11–20 ºC temperature is optimum for germination but failed to germinate at 35 ºC and above. The spores were killed when they were exposed to 45 ºC for 72 h and at 40 ºC for 96 h. The uredospores were not likely to survive during hot summer months in plains of northern India. Saksena and Prasad (1955) revealed that chickpea rust appeared in the last week of February when the plants were four months old at Delhi, whereas at Karnal the rust outbreak was a month earlier causing heavy infection. The highest infection levels of rust were observed on uni-imparipinnate (A) compared to multipinnate (B) leaf type.

2.6.2.4 Chickpea Phyllody (Phytoplasma)

Phyllody is a minor disease of chickpea, which is negligible in northern India but can cause more damage in southern India. The conspicuous symptoms are excessive auxiliary proliferation of branches with smaller leaflets, giving a bushy appearance and excessive stunting of the plant by reduced intermodal length. At the time of flowering and podding stage, affected plants scattered in the field which can be easily spotted from the distance. The floral parts are converted to small leaf like structures instead of normal flowers and look conspicuously green (Phyllody) even after complete maturity of crop.

The pathogen has a broad host range and survives on many collateral hosts like *Brassica campestris* var. *toria*, *B. rapa*, *Crotalaria* sp., *Trifolium* sp., *Arachis hypogaea* which serves as a source of inoculum to the succeeding crop. The disease is transmitted by leaf hopper, *Orosius orientalis*, earlier described as *Orosius albicinctus.* Nymphs are incapable in transmitting the phytoplasma.

2.6.3 Viral Diseases of Chickpea

2.6.3.1 Chickpea Stunt

Stunt disease is prevalent in all chickpea growing tracts of India, which occurs every year in the month of January and February. It causes 100% yield loss, if it occures before flowering and 75–90% losses when infection occurred during flowering (Horn et al. 1995). Chickpea stunt disease was first reported from Iran, it also occurs in North Africa, the Middle East, South Africa, Australia, Indian subcontinent, Spain, Turkey, and the United States. Chickpea stunt disease incidence was reported to about 10–20 and 5–20% from Patna and Nalanda district (AICRP on Chickpea, 2011–12). Yield loss to an extent of 90–100 and 80–95% was reported by Kaiser and Danesh (1971) and Kotasthane and Gupta (1978), respectively.

Occurrence of three different viruses are reported in chickpea stunt disease viz., the leaf hopper transmitted virus *Chickpea chlorotic dwarf virus* (CpCDV) consisted of 32 kD coat protein with circular, ssDNA of 2900 nucleotides, *Chickpea luteovirus* (CpLV) is the most predominantly associated virus and *Bean leaf roll virus* (BLRV) spread by aphid vector (*Aphis craccivora*). The CpCDV and CpLV occurrs widely, whereas the BLRV is found at isolated places. The CpCDV belongs to the genus *Mastervirus*, family Geminiviridae) (Kumari et al. 2006) is transmitted by leafhopper *Orosius orientalis* (Matsumura) (Cicadellidae: Hemiptera) (Horn et al. 1993). CpCDV and CpLV are widely distributed in India and Pakistan, whereas BLRV and Beet western yellows virus are of minor significance (Horn et al. 1996).

Molecular and serological characterization of the *Mastrevirus* associated with stunt disease was reported in many countries (Nahid et al. 2008; Kanakala et al. 2012). The *Mastrevirus* is linked with severe stunting, leaf sizereduction, drying and death of plants in Delhi isolate (Kanakala et al. 2012). Recently, five other *Mastrevirus*

species, *Chickpea red leaf virus*, *Chickpea yellows virus*, *Chickpea chlorosis virus*, *Chickpea chlorosis Australia virus*, and *Tobacco yellow dwarf virus* were reported in Australia (Hadfield et al. 2012). All the *Mastrevirus* isolates infecting chickpea in Asia, Africa, and Australia were reclassified on the basis of 78% nucleotide similarity in the genomic DNA and were clustered into one species, CpCDV (Muhire et al. 2013). CpCDV also infects faba bean, lentil, french bean, pigeonpea, and lablab bean (Makkouk et al. 2003). Based on molecular characterization, chickpea stunt disease caused by CpCDV (ssDNA), belongs to the genus *Mastrevirus* which is also responsible for the lentil stunt disease (Manjunatha et al. 2021).

This disease is characterized by leaf chlorosis or leaf reddening (depending on the chickpea types) of infected plants. Infected plants can be easily spotted from the field by their yellow, orange, or brown discoloration with stunted growth. Leaf reddening in desi type and yellowing in kabuli chickpea are the charactersitcs visual symptoms of the stunt disease. The most characteristic symptom of stunt is phloem browning in the collar region, internode shortening and stunting is observed in both the types. Plant decline and premature death of the diseased plants can dramatically reduce the production. A transverse cut reveals a brown ring or a split through the collar region reveals brown streaks of discolored phloem vessels. Chickpea plants that become infected with CpCDV at an early stage of development normally do not produce any pods. The above described symptoms are followed by rapid plant decline, and very few early infected plants survive. CpCDV can cause stunting, internode shortening, phloem browning in the collar region and leaf reddening in desi type while yellowing in kabuli type chickpea.

2.6.4 Other Minor Viral Diseases

Bean common mosaic virus (BCMV): BCMV infected plants shows mosaic mottling of leaves, stunting with bushy appearance of plant. The virus is transmitted by aphid vectors, *Aphis gossypii* and *A. craccivora.*

Alfalfa mosaic virus (AMV): The virus occurs in all the chickpea growing areas of India. The symptoms of AMV are twisting of terminal bud followed by tip necrosis and initiation of secondary branches. Newly emerged branches are erect, stiff and very small leaves with mild mosaic mottling. The virus is transmitted by aphid vectors, *A. craccivora* and *A. gossypii.*

Cucumber mosaic virus (CMV): The main symptoms include yellowing and stunting of the shoots. CMV is seed borne and thin plant population is also responsible for virus susceptibility.

Bean *yellow mosaic virus* (BYMV): It produces narrow and lanky leaves in affected plants.

Lettuce necrotic yellows virus (LNYV): Necrosis of leaves is evident on infected plants.

2.6.5 Nematodes Iinfecting Chickpea

2.6.5.1 Root-Knot Nematodes

The root-knot nematodes (RKN) are important groups of plant parasitic nematodes. Three species are known to infest chickpea in different climates. *Meloidogyne artiella* is found in the Mediterranean region, whereas *M. incognita* and *M. javanica* are common in the subtropics of Asia, Africa and South America (Thompson et al. 2000). During warmer seasons, reproduction is known to be higher, therefore spring-sown crops in the Mediterranean region are more affected than winter sown crops (Di Vito et al. 1988; Thompson et al. 2000). Chickpea is grown in warmer climatic region and consequently is more exposed to RKN. Common and durum wheat are significantly better hosts for *M. artiella* than chickpea and produce higher rates of RKN multiplication (Hernandez Fernandez et al. 2005). RKN infected chickpea field bears uneven patches of plant growth in field condition. Heavily infected plants show stunted growth, less branching, and leaves with pale green to yellow color. Tissues surrounding the feeding sites of RKN usually swell, giving rise to large, characteristic galls on the roots of infected plants.

RKN's parasitism involves establishment of permanent feeding sites in the plant roots where the nemotodes stimulate the creation of giant cells that act as sinks for plant photosynthates that the nematode favorably access. Blockage deformation of vascular tissues due to nematode feeding can limit the translocation of water and nutrients which inturn suppress plant growth and result in yield reduction. Affected plants are often stunted, pale green to yellow colored leaves (Castillo et al. 2008). Root-disease complexes are commonly associated with RKN attack in plants. The wilt /root rot diseases caused by fungal pathogens, *Fusarium oxysporum* and *Macrophomina phaseolina* is aggravated by the damage caused by RKN. Nematode infection can break down the plant defenses and cause wilt resistant genotypes to become diseased (Maheshwari et al. 1995). Rao and Krishnappa (1995) reported about 28% incidence of wilt disease linked with the complex of *M. incognita* and *F. oxysporum* f. sp. *ciceris* in chickpea crops.

2.6.5.2 Root Lesion Nematodes

Lesion nematodes (*Pratylenchus* spp.) are a major limitation in chickpea production and ranked second after RKN, worldwide (Castillo et al. 2008). They cause lesions on root, which results in reduction in crop growth and yield. They can also escalate *F. oxysporum* infection (Castillo et al. 1998). Chickpea is the second most susceptible crop to *Pratylenchus thornei* after wheat under rainfed conditions. *P. neglectus*, *P. mediterraneus* and possibly *P. penetrans* also infest chickpea (Thompson et al. 2000). Chickpea genotypes differ in resistance and tolerance level to *P. thornei* (Castillo et al. 1998). However, few modern chickpea cultivars display adequate resistance to *P. thornei* or *P. neglectus*. In recent times, resistant chickpea lines to

root-lesion nematodes have been developed from hybrids of desi chickpea cultivars with resistant germplasms of *C. reticulatum* and *C. echinospermum* (Thompson et al. 2011). The resistance levels in wild relatives are far greater to the levels identified in *C. arietinum.* Though, several backcrosses are necessary to produce progeny that retain acceptable agronomic and seed quality factors for commercial production of chickpea, the quantum leap in resistance gained depends on the breeding methods.

2.6.5.3 Cyst Nematodes

Heterodera ciceri, is a destructive parasite of chickpea found in the eastern Mediterranean region (Thompson et al. 2000). Cyst nematodes infested chickpea plants appear stunted, chlorotic with condensed flowers and pods, along with poorly developed roots and less number of Rhizobium nodules, which lead to reduced seed protein (Greco et al. 1988). Resistance breeding against cyst nematodes is the best option for reduction of cyst populations and yield loss. Nevertheless, the cultivated chickpea, *C. arietinum* exhibited no resistance to *H. ciceri* (Singh and Ocampo 1997). Providentially, the accession, Ladiz of *C. reticulatum* had good resistance level, and chickpea cultivars developed from this line with extensively improved and high-yielding Kabuli cultivar have been released for cultivation (Malhotra et al. 2002).

2.7 Insect Pests of Chickpea

Among the legume crops, chickpea is damaged by a relatively few insect pests. Presence of dense glandular trichomes on all green tissues of the chickpea plant attributed to the presenece fewer herbivores than other legume crops (Ranga Rao et al. 2013). The glandular trichomes excrete some acidic substances mainly of malic and oxalic acids. *Kabuli* genotypes are generally more susceptible to insect pests than *desi* types. Nearly 60 insect species are known to infest chickpea, globally. The important insect pests damaging chickpea are mentioned in Table 2.3. The gram pod borer, *Helicoverpa armigera* (Hub.) is considered as a dominant pest of chickpea in South Asia (Reed et al. 1987). The pod borer, *H. armigera* and the aphid, *Aphis craccivora* Koch are the major pests of chickpea in the Indian Subcontinent (Sharma et al. 2006).

Table 2.3 Insect-pests infesting chickpea

S.N	Insect pest	Scientific name	Family	Order	Distribution	Nature of damage
1	Gram pod borer	*Helicoverpa armigera* (Hub.)	Noctuidae	Lepidoptera	Worldwide	Feeds on leaves, flowers, bore into pods and feeds on seeds
2	Semilooper	*Autographa nigrisigna* Walker	Noctuidae	Lepidoptera	Asia	Feeds on leaves
3	Cutworm	*Agrotis ipsilon* (Hfn.)	Noctuidae	Lepidoptera	Worldwide	Cuts at the base of the plant and feeds on the leaves
4	Termite	*Microtermes obesi* (Holm.) *Odontotermes* sp.	Termitidae	Isoptera	Asia	Damages tap root system
5	Aphids	*Aphis craccivora* Koch; *Acyrthosiphon pisum* (Harris)	Aphididae	Hemiptera	Worldwide	Sucks sap from leaves, tender parts, growing tips, flowers, pods
6	Bruchids	*Callosobruchus* sp.	Bruchidae	Coleoptera	Worldwide	Feeds on seeds during storage
7	Leaf miner	*Liriomyza cicerina*	Agromyzidae	Diptera	Worldwide	Feeds inside the mesophyll, creating characteristic serpentine mines. Heavy infestations can cause leaf desiccation and premature leafdrop

2.7.1 Gram Pod Borer, Helicoverpa Armigera *(Hub.) (Lepidoptera: Noctuidae)*

The moths of *H. armigera* lay the eggs singly on the leaves, flowers, flower buds and pods. Eggs are initially greenish yellow, shiny, sculptured and change to brown

as they grow older. Egg period lasts for about 2–4 days. The larva completes five to seven instars in about 16–30 days or more in cooler months. The larvae are generally green in color on chickpea crop, however, the color may vary from light brown to dark brown depending on the crop or the environmental factors. Pupation occurs in the soil. The gram pod borer is known to pass through about four generations in northern India, while seven to eight generations in South India (Yadav et al. 1991).

Gram pod borer, *H. armigera*, is undoubtedly the most serious pest of chickpea throughout the tropics and subtropics in Asia causing severe crop losses. Larvae of *H. armigera* feed on chickpea crop right from seedling to crop maturity stage. The early instar larva feeds by scratching the tender portions of the leaves or shoots. Second and subsequent instar larvae feed on leaves, flower buds and flowers. The third instar larvae make circular holes in the pod walls and feed the grains, whereas the fully grown larvae feed on the sedds with their head inside the pod and rest of the body outside. A full-grown larva is reported to damage about 7–40 pods. The damage at the early vegetative stage of the crop does not cause much damage as plant exhibits tolerance and with stands up to 60% defoliation. Infestation at podding and seed setting stage results in more damage to plant.

2.7.2 Aphids, Aphis Craccivora *Koch;* Acyrthosiphon Pisum *(Harris) (Hemiptera: Aphididae)*

Two species of aphids, *A. craccivora* and *A. pisum* infest many cultivated pulses including chickpea (Saxena 1978). However, *A. craccivora* is reported as one of most severe species infesting both major and minor pulses (Singh and Van Emden 1979).

Both adults and nymphs of *A. craccivora* are shiny black with brown to yellow legs and nymphs covered with light waxy growth on body. Females are apterous and viviparous (Patel and Srivastava 1989). Aphids remain active throughout the year; however, incidence is greater when drought conditions prevail. They reproduce through parthenogenesis in the tropics and are viviparous. A gravid female can produce over 100 nymphs in 15 days. Nymphs undergo four moults and life cycle from nymph to adult stage is completed in 5–8 days. Adult lives for 6–15 days and one generation can be completed in less than two weeks under favorable conditions. Nymphs and adults of aphid species congregate all along the tender parts of the plant and causes direct damage by sucking the plant sap. This initiates twisting of leaves followed by stunting of affected parts and during severe infestation they may cause wilting of plants, poor root or nodule development and death of plants (Reed et al. 1989; Sharma et al. 2010). Apart from direct feeding damage, this aphid is transmitting pea leaf roll virus in chickpea (Kaiser et al. 1990; Nene and Reddy 1976).

2.7.3 *Semilooper,* Autographa Nigrisigna *Walker (Lepidoptera: Noctuidae)*

Several species of semiloopers have been reported to feed on chickpea in Asia, but *A. nigrisigna* appears to be important. Moths have typical forewing pattern. Eggs are deposisted in clusters up to 40 on the foliage. The larvae are green in color and full-grown larvae pupates in soil. A single generation is completed in 18–52 days (Yadava et al. 1991). The semilooper is reported to cause economic damage to chickpea in Haryana and Uttar Pradesh. The newly hatched larvae scratch epidermis of the leaves, and as a result the whole leaf becomes whitish and skeletonized. Grown-up larvae feed on leaves, buds, flowers and pods by nibbling, leaving the basal part of pod with peduncle. Semilooper damage produces ragged, irregular damage to chickpea pod walls.

2.7.4 *Cutworm,* Agrotis Ipsilon *(Hfn.) (Lepidoptera: Noctuidae)*

Adult moths are having brown forewings and white hind wings. *Agrotis ipsilon* completes about four generations in October–May. Cream colored eggs are placed singly or in small clusters of 2–3 on plant parts or on soil surface. The full grown larvae are brown-black in color and pass through 6–7 instars. Larvae are nocturnal and hides in the soil during the day time. Pupation takes place in the soil. Single generation can be completed in 4–5 weeks under favourable conditions (Ranga Rao and Shanower 1999). Cutworms appears sporadically on chickpea crop. The caterpillars remain hidden within cracks or clods throughout the day and become active during night time. The caterpillars cut the tender plants or seedlings at ground level, besides branches or stems of growing plants. The cut plants or twigs are dragged and partially buried into the soil. The presence of partially buried plants or stems is the indication of larval existence in the soil which feed on leaves and plant parts.

2.7.5 *Termites,* Microtermes Obesi *(Holm);* Odontotermes *Sp. (Isoptera: Termitidae)*

The termite incidence is usually sporadic nature, but in the years of low rainfall, they assume considerable importance. They are predominant in red and light soils. Among the several species of termites infesting legumes in Africa and Asia, *Microtermes* and *Odontotermes* are the most damaging in chickpea (Reed et al. 1989). The sexual forms of winged termites (alates) emerge out of territoria at the onset of the monsoon. Mating occurs after a short dispersal flight. After the wings are shed, they enter into nest sites in pairs. Seven to 10 days after swarming, the female lays the first

batch of eggs, numbering 100–130, which hatch in 40–42 days. The female termite then enlarges itself to form the queen in due course, which can lay thousands of eggs per day. Termites are social insects composed of workers, soldiers, king and queen. Termites damage plants soon after sowing and continue till late growing stage. Termites damage the root system by entering and burrowing the root and stem. The leaves of the damaged plant droop down. Such plants, if pulled, are easily uprooted and termite cuts or galleries are seen. As plants progress towards maturity, the termite damage becomes more pronounced.

2.7.6 Bruchids: Callosobruchus *Sp. (Coleoptera: Bruchidae)*

Bruchids are the major pests of legumes in storage. The bruchid species, *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (Fab.) are widespread across the globe while *C. analis* (Fabricius) is limited to Southeast Asia (Ranga Rao et al. 2013). Though they are widely distributed, they are considered as most important in tropical and subtropical regions. The spotted brown beetles lay the eggs on seed surface. Eggs period lasts for 5–7 days, white colored grub burrows into the seed through the base of the egg and then feeds and develops into a pupa inside the seed. The adults emerge through a neatly cut cylindrical exit hole which do not feed and live for an average of 12–14 days. One generation can be completed in about 4–5 weeks or more, depending on the temperature and humidity. Bruchids cause both quantitative (loss of edible grain and loss of marketable weight) and qualitative (presence of insects and other contaminants; consumer appeal and market value) losses during storage. The damaged grains are completely hollowed and have poor germinating ability and are unfit for consumption.

2.8 Traditional Breeding Methods for Biotic Stress Resistance

The main objectives of chickpea breeding are the improvement for higher yield, extended adaptation, resistance to biotic stress, resistance to abiotic stress and identification of stable form of male sterility through development of homozygous genotypes with desirable traits. Breeding for multiple resistance with wide adaptation is a cumbersome job, comprising a number of different breeding approaches. Biodiversity evaluation for identification of parental materials is a crucial step, followed by development of segregating populations through different approaches like gene pyramiding from geographically divergent sources. Yadav et al. (2004) developed new genotypes through introgression from wild sources with improved yield potential, FW resistance and abiotic stress like drought, which was attributed to new allelic combinations created by crosses between the cultivated and wild species.

Breeding for resistance to biotic stresses would stabilze chickpea production. The biotic stresses viz., FW, AB, dry rootrot, stunt, rust (diseases), pod borer and leaf minor(pests), cyst, root knot and lesion nematodes (soil microorganisms) are the major yield limiting factors in chickpea.

Generation of genetic variation becomes vital for successful breeding programme, through: 1. Introduction of cultivars and segregating material from within or outside the country; 2. Hybridization; 3. Mutation. The finest instance of successful introduction in chickpea is bruchid resistant line (G109-1), a selection from an exotic variety introduced to India from Turkey (Saxena and Raina, 1970). Through hybridization, many cultivars like JG130 ([PhuleG 5 × Narsinghpur bold] × JG74, JG11 ([PhuleG 5 × Narsinghpur bold] × ICCC37) were developed for Wilt-resistant, DRR and pod borer-tolerant (Dua et al. 2001), cold-tolerant and AB-resistant chickpea mutants (Toker and Cagirgan 2004).

2.8.1 *Rapid Generation Advancement* (RGA) *Breeding Method*

The growing need for food and nutrition, increasing pressures of climate change and the demand for enhanced cultivars is more challenging today than ever. A breeding protocol has high potential to generate new varieties of chickpea in short-time. The RGA permits creation of six to seven generations of chickpea/year under controlled conditions. Generation time is a crucial for achieving maximum genetic gains in chickpea. Generally, it takes seven to eight years to develop homozygous (identical) lines after hybridization with one crop generation produced per year. "RGA can significantly lessen the number of years necessary to reach homozygosity and develop a cultivar or material for research purposes. The RGA breeding protocol developed at ICRISAT for fast breeding of chickpea. The generation advancement can rapidly be attained by providing extra source of light (60 W incandescent bulb) in greenhouses with extended photoperiod (light) during seedling stage that induces early flowering within 20–25 days after sowing, against normal flowering duration of 40–50 days. The duration of crop cycle is further reduced by germinating immature green seeds. These findings were described by Samineni et al (2019). This RGA method of breeding is very simple and cost-effective and can be implemented easily by any breeding program with a greenhouse facility.

2.8.2 *Biotic Stress Resistance Breeding*

AB resistance is governed by dominant and monogenic in *desi* cultivars of chickpea (Vir et al. 1975; Taleei and Homayoun 2009). A resistant *Kabuli* genotype had amalgamation of a recessive and a dominant gene (Singh and Reddy 1983). Resistant

genotypes and fungicide spray are continuously used for the disease management. However, changes in genetics of pathogen populations (Vail and Banniza 2009) and fungicide resistance reports in the pathogen offer continuing challenges for breeders. Recently, Labdi et al. (2013) reported that AB resistance was governed in different genotypes through epistatic interaction by a single recessive gene, two recessive complementary genes, two dominant complementary genes and two recessive genes. Resistance in ILC 3279, ILC 3856 and ILC 4421 was controlled either by three recessive genes or two recessive duplicated genes, whereas in ILC 72, ILC 182 and ILC 187, resistance was by polygenes.

Among the eight annual wild *Cicer* species, only *C. reticulatum* is readily crossable with cultivated chickpea resulting infertile hybrid. But *C. judaicum*, *C. bijugum* and particularly *C. pinnatifidum* are good resistance sources against various stresses of chickpea (Singh et al. 1998a, b). Modern techniques like tissue culture methods such as embryo rescue techniques have been used to break crossability barriers and produced interspecific hybridizations (Badami et al. 1997). The sources of resistance in wild species are identified by various researchers (Tables 2.4 and 2.5).

The search for resistance sources from landraces, wild species, screening germplasm, cultivated varieties are a continuous process and researchers show extensive interest to find the resistance gene and incorporate in the breeding programs. However, introgression of resistant genes into chickpea elite genotypes through traditional or conventional breeding program is a cumbersome task consuming lots of resources and time. Evaluation of wild species and landraces, even though have no remarkable impact on the yield potential, is needed. The primary gene pool has been extensively and successfully utilized in chickpea breeding programs for genetic enhancement of chickpea. However, its difficult in exploiting the resistance source present in secondary and tertiary gene pools of chickpea where hybridization with

Table 2.4 Wild *Cicer* species for resistance to different biotic stresses of chickpea

Disease/pest	Resistant sources found	References
Fusarium wilt	*C. bijugum, C. judaicum, C. pinnatifidum, C. reticulatum,* and *C. echinospermum and C. cuneatum*	Singh et al. (1998b) and Nene and Haware (1980)
Gray mold	*C. pinnatifidum, C. judaicum,*	Singh et al. (1982)
Phytophthora root rot	*C. echinospermum*	Singh and Weigand (1994)
AB	*C. echinospermum, C. pinnatifidum, C. bijugum, C. judaicum* and *C. montbretii*	Singh et al. (1998a)
Cyst nematode	*C. pinnatifidum, C. bijugum and C. reticulatum*	Singh et al. (1998b)
Leaf miner	*C. chorassanicum, C. cuneatum, C. judaicum, C. judaicum*	Singh and Weigand (1994), Singh et al. (1998b)
Bruchids	*C. echinospermum, C. bijugum* and *C. judaicum*	Singh and Weigand (1994) and Singh et al. (1998b)

Table 2.5 The most widely used resistance sources against major biotic stresses of chickpea

Chickpea genotypes	Reaction	Diseases	References
ILC72, ILC191, ILC3279, ILC3856	Resistant	AB	Singh et al. (1984)
ICC4475, ICC6328, ICC12004, ILC200, ILC6482,	Resistant	AB	Singh and Reddy (1993)
Sanford	Resistant	AB	Muehlbauer et al. (1998a)
Dwelley	Resistant	AB	Muehlbauer et al. (1998b)
Myles	Resistant	AB	Muehlbauer et al. (1998c)
HOO-108 and GL92024	Resistant	AB	Dubey and Singh (2003)
Ambar, RIL58-ILC72/Cr5	Resistant	AB	Rubio et al. (2006)
CH-2007–22	Resistant	BGM	Khan et al. (2010)
FLIP 97-121C	Resistant	AB	Kaur et al. (2012)
EC 516,934, ICCV 04,537, ICCV 98,818, EC 516,850 and EC 516,971	Resistant	AB	Pande et al. (2013)
ICC1069	Moderately resistant	BGM	Laha and Khatua (1988)
ICC5035	Moderately resistant	BGM	Rewal and Grewal (1989)
GL84195, GL84212	Resistant	BGM	Singh and Kaur (1989)
GL86094	Moderately resistant	BGM	Singh and Kaur (1989)
ICCL97322	Resistant	BGM	Haware et al. (1997)
ILWC182, ILWC 188, ICC 17,151, ILWC 31, ICC 17,207 and ILWC185	Resistant	BGM	Manjunatha et al. (2019)
C. judaicum 182, ILWC 19–2, C. pinnatifidum 188	Highly resistant	BGM	Singh et al. (1998a, b)
ICCV2, UC15, FLIP85-20C,FLIP85-29C, FLIP85-30C	Highly resistant	FW	Ali et al. (2002)
ICC14194, ICC17109, WR315	Highly resistant	FW	Gaur et al. (2006)
GL84012, GL88223, GLK8824, GF89-75	Partial resistant	Sclerotinia stem rot	Singh et al. (2007)
RIL58-ILC72/Cr5	Resistant	rust	Rubio et al. (2006)

(continued)

Table 2.5 (continued)

EC-267154, EC-267308, EC-489845, EC-489882, EC-489919, EC-489991, EC-498818, IC-83523, IC-83538, IC-83539, IC-83748 and IC-83757	Resistant	FW	Saabale et al. (2020) Saabale et al. (2019)

the cultivated species is commonly restricted by reproductive hurdles. Singh et al. (2008) also suggested the importance of diverse gene pool for breeding biotic and abiotic stresses by methodical assortment and assessment of wild species for potential or targeted traits. Determining crossability, karyotype and molecular markers among the genus *Cicer* to overcome the reproductive barriers encountered during interspecific hybridization is a painstaking task (Singh et al. 2008). This brings out the difficulty in relying only on the conventional breeding process for improved cultivars. Molecular markers becoming very popular with an advantage to overcome the problems of conventional breeding. It can screen the gene introgression from the wild species having high degree of resistance to multiple stresses to facilitate marker-assisted breeding activities to transfer the desirable traits, which may be agronomical or resistance source into desirable cultivar for development of trait specific cultivars with durable resistance.

The three genotypes (ICC 1915, ICC 6306 and ICC 11,284) moderately resistant to AB, 55 genotypes (ICC 2990, ICC 1180, ICC 4841, ICC 4533, ICC 4872) to BGM, six genotypes (ICC 1710, ICC 2277, ICC 2242, ICC 11,764, ICC 13441and ICC 12,328) to DRR, 21 asymptomatic (ICC 1205, ICC 637, ICC 1396, ICC 1356, ICC 2065) and 24 resistant (ICC 67, ICC 95, ICC 867, ICC 1164, ICC 791) to FW have been reported (Pande et al. 2006). Multiple disease resistance to AB and BGM was identified in ICC 11,284 genotype; for BGM and DRR in two genotypes (ICC 11,764 and ICC 12,328); for BGM and FW in 11 germplasms (ICC 4533, ICC 2990, ICC 6279, ICC 7554, ICC 7819); and for DRR and FW in four genotypes (ICC 1710, ICC 2277, ICC 2242 and ICC 13,441) (Pande et al. 2006).

2.9 Resistance to Insect Pests

The five chickpea accessions viz., ICC 5878, ICC 16,903, ICC 6877, ICC 11,764, and ICC 18,983 had least leaf-feeding score in detached leaf assay method; five accessions viz., ICC 12,537, ICC 9590, ICC 7819, ICC 2482 and ICC 4533 had minimum larval survival rate against pod borer (*Helicoverpa armigera* L.) and five accessions viz., ICC 3946, ICC 16,903, ICC 6877, ICC 11,746 and ICC 18,983 are the best genotypes for lower larvae weight than resistant control cultivar ICC 506-EB from the mini-core collection (ICRISAT Archival Report 2009). Similarly, 13 genotypes (ICC 1230, ICC 3325, ICC 4567, ICC 2263, ICC 6874, ICC 10,466,

ICC 5135, ICC 11,198, ICC 15,406, ICC 15,606 ICC 12,307, ICC 14,831, and ICC 16,524) with low *H. armigera* damage and plant mortality were reported under unprotected environments (ICRISAT Archival Report 2010). Additionally, ICC 4969, as a resistant source identified for pulse beetle (*Callosobruchus maculatus* F.) in both free-choice and no-choice method (Erler et al. 2009).

Conventional breeding is basically based on phenotypic traits and its main limitation is that discrete morphological traits with high heritability character are very limited in number and finding that effective gene source is very difficult. Here, comes the role of DNA markers/molecular markers that will contribute in decreasing linkage drags and increasing the introgression efficiency in pre-breeding activities to identify the linked gene governing for the desirable trait (Sharma et al. 2013).

2.10 Marker-Assisted Breeding (MAB)

Molecular markers have emerged lately for any genome related studies and are gaining popularity due to the effectiveness in genome analysis related specially to diversity studies. Identification of resistance gene analogues (RGAs) and expressed sequence tag (EST) markers help in identifying promising candidates for interspecific hybridization programs. Molecular markers showing high levels of polymorphism can be used for allele-mining of germplasm collections for candidate accessions for resistance to pests and diseases, and tolerance to abiotic stresses. (Hutokshi et al. 2005). RGAs of *Cicer* identification by using molecular techniques and mapping of interspecific cross segregation for the targeted pathogen or disease is indispensable for genome designing. Cloning and sequencing of amplified products from diverse *Cicer* species will help in identifying potent genome for a targeted pathogen. Identification and mapping of candidate resistance genes help in marker-assisted selection in *Cicer* species. Eight RGAs from *Cicer arietinum* and five RGAs from *C. reticulatum* were isolated by Huettel et al. (2002) using cleaved amplified polymorphic sequences (CAPS) and RFLP analysis against FW disease (Huettel et al. 2002). Yadav et al. (2004) selected four *desi* genotypes BG 1063, BG 1075, BG 1079 and BG 112 (green seed coat) and four *kabuli* (BG 1088, BG 1089, BG 1093 and BG 72) on the basis of resistance against two or more diseases through pyramiding of resistance genes. The line BG 1063 was resistant to FW, wet root rot, DRR, CR, stunt virus and nematodes and also BG 1075, BG 1079, BG 1088, BG 1089, BG 1093 and BGD 112 genotypes were all resistant to multiple diseases (Yadav et al. 2004).

2.10.1 Markers and QTLs for Disease Resistance

2.10.1.1 Morphological Markers for Disease Resistance

An effective breeding for disease resistance based on morphological markers depends on screening techniques, source of infection, resistance mechanisms, inheritance of resistance and techniques used for resistance breeding. Among the soil-borne pathogens attacking chickpea, only breeding programs for resistance against FW are performed successfully (Van Rheenen et al. 1992). Development of a sick plot in slightly alkaline vertisol (pH 8.85) is recommended for FW resistance screening (Nene et al. 1981). Gaur et al. (2007) also reported the resistance screening on wilt sick-plot for the field and hot-spot location screening, greenhouse and laboratory procedures for successful breeding programs. No reports are found for development of uniform and effective DRR-sick plots. Pot culture techniques for greenhouse screening and a paper towel technique for laboratory screening were suggested for DRR (Nene et al. 1981) and CR. In both the cases, artificial inoculation methods for creating a vulnerable environment for infection has been reported (Amule et al. 2014; Wagh et al. 2018). Equally, desi and kabuli genotypes can be explored for resistance source and to develop improved variety. Desi parents and Kabuli parents can be explored for developing improved variety considering the positive aspects of the plant as former can be used for transferring the resistance genes into Kabuli-type breeding programs and later, can be explored for its qualitative traits like large seed size and seed quality in Desi breeding programs (Gaur et al. 2007). Desi germplasm and wild *Cicer* spp. has been identified as resistance sources to *Foc* races. *C. bijigum*, *C. cuneatum*, and *C. judaicum* were reported to be resistant against race 0 and 5 whereas *C. pinnatifidum* accessions were susceptible to race 5, but some were resistant to race 0, accessions of *C. canariense* and *C. chorassanicum* were resistant to race 0 but susceptible to 5 (Kaiser et al. 1994). Compared with FW, studies on the resistance to DRR at different locations are limited. The inheritance of resistance to soil-borne diseases have been reported only for *F. oxysporum* and *R. bataticola* (Van Rheenen et al. 1992). Studies conducted under field conditions indicated that resistance to FW is controlled by a single recessive gene (Haware et al. 1980). For self-pollinated crops like chickpea, fixing genes in breeding lines through development of pure-line cultivars is a mandatory step and previously mass or pure-line selection from landraces was used. But lately, crossing programs and various modifications of pedigree and bulk methods were used to handle the segregating generations (Gaur et al. 2012).

Desi germplasm accessions of 165 and 110 lines Kabuli germplasm accessions were identified for resistance to FW (Haware et al. 1992). On the basis of GGE, Sharma et al. (2019) reported six genotypes viz., ICCVs 07,105, 07,111, 07,305, 98,505, 08,113, and 93,706 with high resistance and reliability across the locations and eight moderately resistant (<20% mean incidence) genotypes viz., ICCVs 08,123, 08,125, 96,858, 07,118, 08,124, 04,514, 08,323, and 08,117. Talekar et al (2017) screened 520 chickpea germplasm and identified PG 06,102, BG 2094 and

IC 552,137 as DRR resistant. DRR resistance governed by monogenic inheritance was reported from the phenotyping of the mapping population of 129 $F_{2:3}$ progeny derived from the cross L550 × PG 06,102. The four polymorphic markers distinguished the resistant and susceptible bulks based on the reaction of $F_{2:3}$ progenies. Out of them, two markers ICCM 0299 and ICCM 0120b were reported to be co-segregating with resistance with an additive effect on resistance and potential source for dry root resistance breeding program. Wagh et al. (2018) reported BG 3033 as moderately resistant and three entries RKG 2020 22, NDG 11- 12 and H 09–90 are tolerant against DRR.

2.10.1.2 Molecular Breeding and Mapping for Disease Resistance

Since beginning, molecular markers have become important strategies for plants in determining hybridity, diversity/relatedness, developing linkage maps, mapping genes and QTLs, and monitoring introgression of genes into elite genotypes including gene pyramiding, because the chickpea has narrow genetic base and consequently many DNA-based markers were unsuccessful in revealing desired polymorphism. The markers have subsequently been used to map genes or identify QTLs for biotic stress (FW, AB, Phytophthora root rot, and BGM) resistance. The efficacy of masrker assisted selection (MAS) determined by linkage strength flanked by the marker and the gene locus governing the trait of interest, and genetic control (Hayward et al. 1994; Kole and Gupta 2004).

Differentiation of physiological races using differential lines are difficult and needs minimum of 40 days. The identification of pathotypes or races using molecular markers are more rapid, less labor-intensive and cheaper. Molecular markers can also be used for differentiation of species, formae speciales, races and even fungal isolates. Barve et al. (2001) distinguished the four races of *Fusarium oxysporum* f. sp. *ciceris* viz., 1, 2, 3 and 4 prevailing in India using oligonucleotide. Race specific markers for continuous monitoring of racial distribution in different regions, could not to be identified. Resistance to race 1 of FOC is controlled by a single recessive locus and RAPD markers linked to the resistance gene were identified (Mayer et al. 1997). The two RAPD markers UBC-170550 and CS-27700 are associated with the FW resistance and susceptible alleles, respectively, and are located on the same side of the locus. Ratnaparkhe et al. (1998a) reported an inter-simple sequence repeat (ISSR) marker linked to a gene governing resistance to race 4 of FOC using RILs. The ISSR marker, UBC-855 500 is linked in repulsion phase of wilt resistance gene at a genetic distance of 5.2 cM, which is highly suitable for practical application. Near-isogenic lines have been developed for the resistance to race 5, that can be used for fine mapping and map-based cloning (Tanksley 1983; Castro et al. 2015). Collard et al. (2001) recognized resistant wild *Cicer* germplasms in controlled environment in *C. bijugum*. QTL associated with seedling and stem resistance were detected in the F2 population in cross of *C. echinospermum* and a susceptible accession of cultivated chickpea (Collard et al. 2003a, b). Numerous QTL were clustered in LG 4, but there is also proof for QTL on other linkage groups (Santra et al. 2000).

A considerable number of QTLs have been identified through molecular mapping (Table 2.5). Several linkage groups (LG 2, 3, 4, 6, and 8) have been identified for AB resistance (Varshney et al. 2013). More recently, 14 microsatellite markers that were linked to seven QTLs viz., Ar2a, Ar2c, Ar3c, Ar4a, Ar4b, Ar6b and Ar8a on the five linkage groups (LG 2, LG 3, LG 4, LG 6 and LG 8) in chickpea were identified against AB (Hamwieh et al. 2013). Two major QTLs on LG 2, adjacent to the GA16 and TA37 loci controls the resistance to pathotype I of AB (Cho et al. 2004). Two QTLs for pathotype II is located on LG 4, one is linked to CaETR or GAA47 and the other is linked to TA72/ScY17(Winter et al. 2000). The marker-assisted backcrossing (MABC) has been successfully employed recently to introgress AB resistance with double-podding traits in chickpea cultivars CDC Xena, CDC Leader, and FLIP98-135C (Taran et al. 2013). Sabbavarapu et al. (2013) recorded two major QTLs on LG06 that revealed up to 18% PV for FW resistance (race 1) in chickpea (Table 2.6).

2.11 Genetic Transformation

Overcoming the chickpea production constraints through traditional breeding are challenging due to inadequate genetic diversity. Novel regeneration is essential for genetic transformation to overcome hybridization obstacles and present novel genes for resistance. Though direct gene transfer via direct DNA transfer has been described (Anwar et al. 2010). *Agrobacterium* mediated transformation is the preferred technique. Only few cases have been reported using genetic transformation/ transgene(s) against biotic stress tolerance transgenic chickpea plants. Transgenic chickpea using bacterial *cry1Ac* gene tolerance against pod borer have been developed (Sanyal et al. 2005). The first successful genetic transformation in chickpeas was reported using the cry1Ac gene (Kar et al. 1997). Genetic transformation can be carried out using any one of several methods accessible. Indirect methods comprise the use of a vector, which may be a bacterium; *Agrobacterium* or a virus; CaMV or Gemini virus. Direct methods of gene transfer are particle bombardment, electroporation, PEG mediated DNA uptake and microinjection (Potrykus 1990).

The genetic engineering permanently permits alteration of living organisms with unique specificity and permit a qualitatively different level of genetic makeover. It offers an opportunity of altering plants through incorporation of genes providing resistance to broad spectrum herbicides, pests, diseases and abiotic stresses. In genetic engineering, the selected useful genes from one living organism can be transferred into a preferred crop plant and gaining a proper expression. Hence, genetic engineering of plants helps in developing novel varieties with a new combination of genes with more effectiveness than conventional breeding methods. In this technique, the gene transformation and protoplast fusion permit to bypass sexual reproduction and move desirable gene of interest between entirely distinct organisms, while conventional breeding depends upon sexual reproduction to transfer genetic materials. Recently, genetic engineering gaining importance in developing transgenic chickpea that are resistant to *Helicoverpa armigera* (Pod berer) and FW resistance.

Table 2.6 List of QTLs/mapped genes for disease resistance in chickpea

Trait	Mapping population	QTL/gene	References
Ascochyta blight resistance loci	*C. arietinum* (ILC1272 × ILC3279)	SSR	Udupa and Baum (2003)
FW resistance for race 4 and 5	RIL, interspecific (*C. arietinum* × *C. reticulatum*)	RAPD and ISSR	Winter et al. (2000)
FW resistance, race 0	RILs, Intraspecific (*C. arietinum*)	RAPD	Rubio et al. (2006)
FW resistance, race 0	RILs, Intraspecific (*C. arietinum*)	RAPD and STMS	Cobos et al. (2006)
AB	RIL, interspecific (*C. arietinum* × *C. reticulatum*)	2 QTL	Tekeoglu et al. (2002)
AB Pathotype I & II	Intraspecific (*C. arietinum*)	3 QTLs	Cho et al. (2004)
AB	F_2, interspecific (*C. arietinum* × *C. reticulatum*)	2 QTLs	Collard et al. (2003a, b)
AB	Intraspecific (*C. arietinum*)	3 QTLs	Flandez-Galvez et al. (2003)
AB	–	2 QTLs	Millan et al. (2003)
AB	RIL, interspecific (*C. arietinum* × *C. reticulatum*)	1 QTL	Rakshit et al. (2003)
AB Pathotype I & II	*C. arietinum* (ILC1272 × ILC3279)	3 QTLs	Udupa and Baum (2003)
AB	*C. arietinum* (Kabuli × Desi)	2 QTLs	Irula et al. (2006)
FW resistance for race 1	Interspecific (*C. arietinum* × *C. reticulatum*)	ASAP	Simon and Muehlbauer (1997)
FW resistance for race 3	BAC library	–	Rajesh et al. (2004)

The α-Amylases (α-1,4-glucan-4-glucanohydrolases) are hydrolytic enzymes present in microorganisms, animals and plants (Strobl et al. 1998) and the most important digestive enzymes of many insects which feed exclusively on seed products. Inhibition of α-amylase impairs the digestion in an organism. α-Amylase inhibitors (α-AIs) are found in in cereals and legumes as a part of the defense system (Iulek et al. 2000). The alpha-amylase inhibitor gene isolated from *Phaseolus vulgaris* was introduced to chickpea for resistance to bruchid weevil by Agrobacterium-mediated transformation method (Ignacimuthu and Prakash 2006).

The entomopathogenic *Bacillus thuringiensis* (*Bt*) produces proteinaceous crystalline-Crytoxins. Cry proteins are toxic to insects (mainly against lepidopteran,

coleopteran, dipteran, and nematodes), but non-toxic to human and animals (BANR 2000). The specificity of insecticidal activity of *Bt* on a particular insect species is determined by the form(s) of the cry gene(s) carried by the bacterium. Recently, chickpea lines expressing pyramided Bt genes, *cry1Ac* and *cry1Ab*, have also been developed; however, the previous reports have suggested that *Cry1Ac* is more effective against *H. armigera*, and pyramiding two or more genes with different mode of action is preferred for effective pest management. Transgenic chickpea conferring resistance against pod borer contains cryIAc gene (Sanyal et al. 2005). The genetic transformations of a plant have to be achieved only if the transgene/s is stably inherited and expressed in succeeding progenies of plant. The vegetative growth (Vips) proteins also possess toxic effects toward insects (Estruch et al. 1997). Vip3 is highly toxic to Agrotis and Spodoptera (Ratnaparkhe et al. 1998a, b) and *H. armigera* (Zhu et al. 2006). Later, transgenic chickpea expressing *Cry1Ac* (Sanyal et al. 2005) and *Cry2Aa* genes were also generated. Wide molecular analyses and insect bioassays showed high expression of the transgene and high insect mortality (up to 100%) under detached leaf bioassay at T4 generation in transgenic chickpea plants harboring codon-optimized synthetic *Cry1Aabc* gene (Das et al. 2017). Nevertheless, the Government of India allowed the field trials for six transgenic crops, namely, rice, cotton, maize (corn), mustard, brinjal, and chickpea, in 2015 (Nester et al. 2002).

2.12 Future Concerns

- Efforts on modeling for crop loss estimation which can provide the reference values between theoretical, attainable and actual yield. Hot spot of each disease should be identified.
- Identification, isolation of disease resistant, drought and salinity tolerance traits and their incorporation in desire legume crops.
- Research efforts to integrate resistance genotype, moisture conservation practices that reduce disease incidence, inter and mixed cropping, soil amendments, biological control, use of trap or decoy crop, early and late sowing varieties etc. are very much required. Dedicated efforts of multi-disciplinary team of scientists in different agro-climatic zones can meet this constraint.
- Adequate attention on epidemiological studies should be undertaken for diseases that are prevalent in chickpea growing countries. The information generated will help in the development of sound disease forecasting models.
- Development of multiline cultivars and gene deployment, identification of predisposing factors avoids the possible outbreak of epidemics.
- Cropping combination and cropping sequence may enhance yield and reduce the disease incidence
- The timely supply of quality critical inputs viz., fertilizer, pesticides and seed material (breeder, founder and certified seed) is required.

- Public–Private partnership for sustaining value chain for pulses to minimizing post-harvest losses is essential for sustainable production.

The chickpea yield is often reduced by various biotic stresses. On the other hand, most of the wild species possessing high degree of resistance to multiple diseases are present in the secondary and tertiary gene pools where breeding with the cultivated species is regularly narrow down by reproductive hurdles. Introgression of gene from the wild species can be supervised efficiently with the help of molecular markers, which will accelerate the disease resistant gene transfer for the development of varieties with durable resistance through MAS. Molecular markers such as SSR and SNP are useful for construction of high density genetic maps of chickpea in identification of genes/QTLs associated with biotic stress resistance in chickpea for undertaking widespread molecular breeding. Organized pathogen inspections to identify new virulence and their regional distribution will help in planning suitable management approaches for controlling main fungal diseases.

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Chapter 3
Development of Biotic Stress Resistant Pea in the Post-genomics Era

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Abstract Pea (*Pisum sativum* L.) is an annual pulse crop which is eaten fresh and dried form around the world. It is widely accepted as a culinary best due to its protein rich nature. Pea crop is devastated due to various abiotic and biotic factors, which sometimes leads to complete crop loss worldwide. Biotic stresses in pea are mainly characterized by pathogens like virus, fungus, bacteria, insect pests, and nematodes. Infestation of insect pests not only damages the pea crop but also acts as vectors to spread viruses. These pathogens cause severe productivity loss if proper control measures and integrated disease management (IDM) strategies are not implemented. Various measures like cultural, chemical and biocontrol methods help reduce the crop damage. Severe damage by these pathogens leads to more than 80% crop loss in pea. Utilization of the available genetic resource for resistant sources from the *Pisum* genera will make the introgression of novel traits to develop disease resistant lines. It mainly includes primary, secondary, and tertiary gene pool sources which

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could be included for developing lines resistant to various biotic stresses. Classical genetics and conventional breeding techniques have doubled the pea productivity during the last six decades. Various molecular markers like SSR, STMS and SNP have been developed in pea for various biotic stresses which have led to marker assisted breeding. QTLs for various fungal, bacterial and insect resistance have been detected though mapping studies in pea. Whole genome sequencing of pea has been accomplished and this has opened a plethora of opportunities to carry out genomics-assisted breeding for developing resistant varieties against various pests and diseases in pea. Functional genomics techniques by reverse genetics approach like TILLING by sequencing (TbyS) has increased the relevance of development of non-GMO by utilizing conventional mutation breeding techniques along with next generation sequencing (NGS) technologies for obtaining variability in pea germplasm. Development of transgenics has been done in pea by *Agrobacterium* mediated transformation techniques for insect and fungal resistance. Gene editing techniques with CRISPR-Cas9 has been used in pea for precisely editing genes of importance for developing resistant lines for various biotic stresses. Bioinformatics tools with the development of various databases have increased the knowledge of genomics, proteomics and metabolomics in pea for biotic stresses. With the advent of modern tools like gene editing the conservation of wild type and landraces has raised concern with regulatory framework drafted in different countries. A combined effort from the conventional breeding with utilizing the modern biotech tools along with nanotechnology and speed breeding will help molecular breeders to design climate resilient pea varieties with resistance to biotic stress.

Keywords Biotic stress · *Pisum sativum* L. · Fungus · Virus · Insects · Nematodes · Molecular breeding · Resistance genes · QTL

3.1 Introduction

Pisum sativum L. ssp. *sativum*, commonly known as 'pea' or 'garden pea', is an annual, cold-season legume crop which belongs to Family the Fabaceae (Elzebroek and Wind 2008; Mahajan et al. 2018). There are mainly two types of peas grown—fresh green peas and dry peas. Green peas are mainly produced in China, India, and USA, whereas in case of dry peas, majority of the production happens in Canada, Russia, China, USA and India (Anonymous 2018). Pea is one of the major legumes consumed in both fresh and dry forms. It is also among the top five pulses in the world, i.e., dry beans (32%), chick pea (17%), peas (14.6%), cowpea (9%) and lentils (6%) (Rawal and Navarro 2019).

Peas have very high nutritional value with typically carbohydrates, proteins, minerals (Iron and Potassium) vitamins (Thiamine, Pantothenic acid, Folate) and fibers (USDA 2019). Culinary use of peas, mainly from fresh garden peas in various dishes throughout the world shows the wide dietary acceptance as nutritious food.

In fact, the dry pea production has doubled during the past six decades due to rising demand for consumption (Rawal and Navarro 2019).

Global climate change scenario shows greenhouse house gas (GHG) emissions and land use by dairy beef are about 36 and 6 times greater than peas (Poore and Nemecek 2018). This shows the relevance of pulse crops like peas in the future climates with challenges like global warming, rising sea levels, new virulent strains of viruses, fungus and bacteria in crops.

The impact of biotic and abiotic stresses on pea yield has been drastic; the data show that there has been a decline in both yield and area under pea cultivation in France. During 1990–2015, the cultivated area of pea has declined from a peak of more than 700,000 ha to 1,000,000 ha. In fact the total pea productivity has declined from 55 q/ha in 1999 to 38 q/ha in 2015 (Bénézit et al. 2017). This has not been a local feature, but such drastic effects due to climate change have been observed in other countries as well. The overall global production of dry pea has tremendously increased from 10.7 to 13.5 M tons during 2000 to 2018, whereas in case of green pea 12.2 to 21.2 M tons in the same period (FAOSTAT 2018) (Fig. 3.1). These have been mainly because of the adoption of improved varieties with productivity management practices.

Another major challenge is the increasing population, which is putting pressure on both farm and the field with more and more land getting used for housing, and fertile

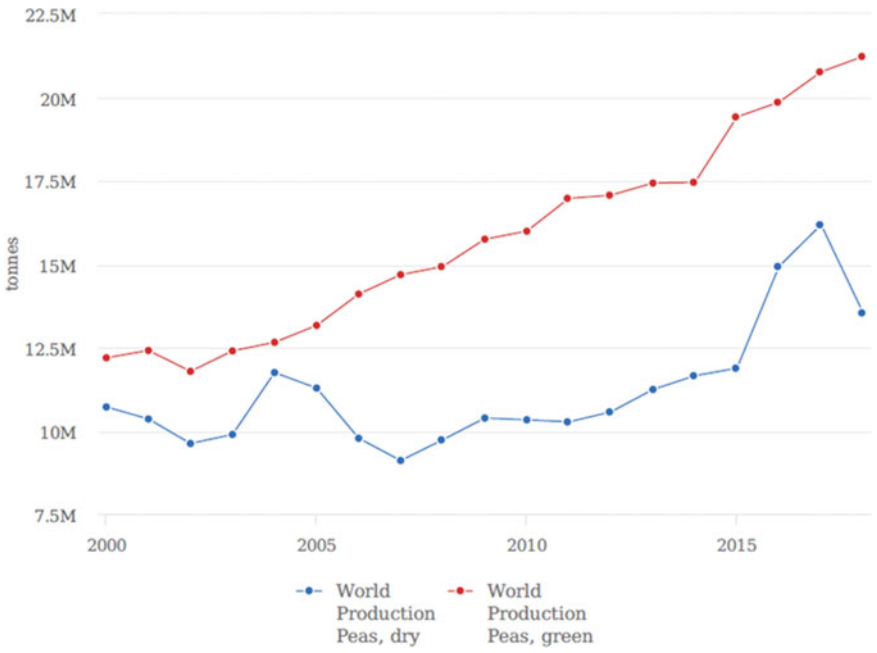

Fig. 3.1 World pea production from 2000 to 2018; dry pea (blue dotted lines) and green pea (red dotted lines)

fields turning infertile due to continuous use of pesticides and chemical fertilizers. By the end of 2050 the world population will reach about 9.7 billion and by the end of 2100 to 10.9 billion (World Population Prospects 2019). Challenges like climate change and population explosion will exert mounting pressure on governments across the world to work towards sustainable crop production. Abiotic and biotic stresses in crops have put a lot of pressure on the productivity of crops thereby leading to nonfulfillment of sustainable development goals (mainly in case of Goal 13—Climate action) laid down by the United Nations (UNDP 2017).

Changes in the environmental conditions lead to severe pressure on crop productivity. Disease and insect pest incidence increases tremendously due to climate change. Biotic stresses lead to complete loss of crops when combined with abiotic stresses like drought, heat, salinity, waterlogging, etc. Traditional breeding has been utilized for biotic stresses by crossing wild species with biotic stress resistance with elite parents with quality traits like better yield, nutritive traits, etc. But due to continuous breeding and development of new pests and diseases the resistance build up is broken. Utilizing modern biotechnological tools like marker assisted selection (MAS), transgenic development, high through-put 'omics' approaches and gene editing can help to overcome the drawbacks of conventional breeding in pea. Integrated pest and disease management (IPDM) strategy with biotechnological interventions for improving various pea cultivars will help growers to maximize pea productivity (Smýkal et al. 2012).

Modern biotechnological tools are helping the breeders to design new varieties which will be more resilient towards climate change challenges and various biotic stresses. This chapter will give an overall enumeration of various fungi, bacteria, virus, insect pests and nematodes infecting and infesting pea. Various aspects included like genetic resources for resistance genes, mapping studies, diversity analysis in germplasm, and strategies for development of improved varieties of pea in the future. Novel biotech tools to develop resistant pea lines, utilizing artificial intelligence for precision farming in pea have been discussed.

3.2 Biotic Stresses

Biotic stress is a condition created by various microorganisms and pests including bacteria, viruses, fungi and insects, infecting and infesting crops leading to complete crop loss in some cases. Biotic and abiotic stress together aggravate the crop damage and will have a grave impact towards food security in the future. Crop productivity of pea is impacted by infections by various diseases and pests, which mainly lead to abnormal growth, non-productive crop and permanent damage. In this section we discuss in detail regarding various insect pests and diseases affecting pea crop with details of taxonomy, races, isolates, biotypes of pathogens, insect pests and nematodes. Further the stages and extent of crop damage along with various methods of controlling the incidence and proper management by IPM strategies have been detailed.

3.2.1 Viruses

3.2.1.1 Main Groups of Viruses Affecting Pea

Garden pea is prone to a large number of vector transmitted viruses, leading to individual disease or in multiple combinations. There are three distinct groups of viruses infecting different pea species—first group with pea enation mosaic virus, second group with pea streak and red clover vein mosaic virus and the third group with bean yellow mosaic, clover yellow vein and pea seed borne mosaic virus (Zitter 1984).

***Pea Enation Mosaic Virus* (PEMV)**

Pea enation mosaic virus (PEMV) (Fig. 3.2a–c) generally infects pea in temperate part of the world, with unique features of symptoms and is spread by aphid (*Acrythosiphon pisum*). It also infects other legumes including broad bean, sweet pea, and *Medicago*. It is a single stranded RNA virus from family Luteoviridae. Main symptoms include mainly veinal enations in the abaxial side of leaves with chlorosis, necrotic lesions, and stipulated leaves with distortion in plants. PEMV infection leads to accumulation of abscisic acid and salicylic acid and have enhanced accumulation of nitrogen dioxide near the veins, which causes leaf enations (Kyseláková et al. 2013). Controlling PEMV infection in pea is mainly through control of aphid spread by spraying insecticides. Alternative hosts from legume family can be removed to check the spread of the disease (Zitter 1984).

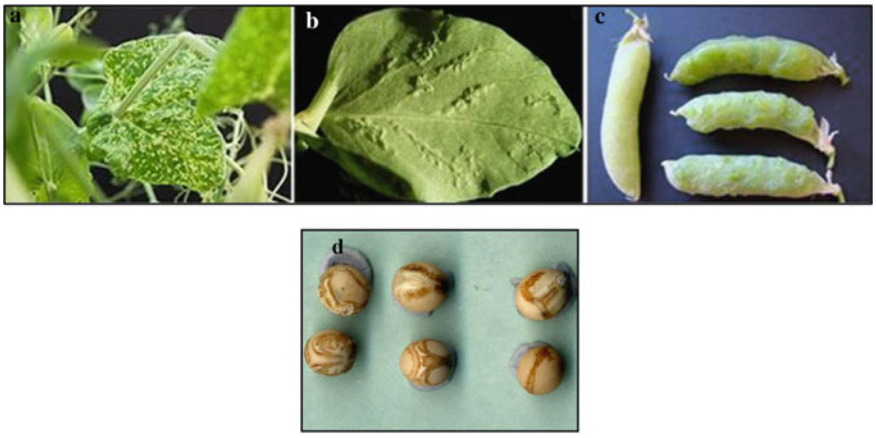

Fig. 3.2 *Pea enation mosaic virus* (PEMV) infection symptoms in pea (*Pisum sativum*). **a** Leaf flecks on leaves. **b** Enations in the abaxial side of leaf. **c** PEMV infected pea pods compared with healthy pod (left) (Porter 2014). **d** PSbMV infection in pea seeds showing symptoms of necrotic rings and line patterns (tennis-ball' like symptoms) on seed coat (https://www.agric.wa.gov.au/field-peas/pea-seed-borne-mosaic-virus-field-peas).

***Pea Seed-borne Mosaic Virus* (PSbMV)**

Field pea crops have been severely affected by *Pea seed-borne mosaic virus* (PSbMV) (Fig. 3.2d), which is generally a seed-borne and aphid-borne virus from family Potyviridae. It mainly spreads from infected field pea seed to healthy seedlings and further to the next generation and causes yield loss and defective seeds (tennis-ball like symptoms) (DPIRD GOWA 2018). Seed-born PSbMV infection in field pea causes yellow, stunted plants. When young plants are infected with PSbMV aphids, the symptoms of the leaf include slight moth and low rolling, and the affected plants show moderate inhibiting and delayed maturity and mild malformation. Infection observed in seeds includes seed discoloration, deformed pods and malformed terminal rosette, splitting of seed coats (Wunsch et al. 2014; DPIRD GOWA 2018).

***Bean Yellow Mosaic Virus* (BYMV)**

Bean yellow mosaic virus (BYMV) infects many leguminous plants like peas, red clover, and lupin. BYMV is a single-stranded, positive-sense, RNA (Group IV) virus from family Potyviridae. It is mainly found in most pea producing regions of the world and the main vector is the aphid**.** General symptoms are distinct dark and yellowish green areas on leaves and infected plants mostly have bright yellow spots which intensifies in color with plant age.

***Clover Yellow Vein Virus* (ClYVV)**

Clover yellow vein virus is from family Potyviridae and infects pea and other legumes generally transmitted by aphids (pea aphid). Symptoms of ClYVV disease in pea are intense vein chlorosis, white patches to extreme yellowing on leaves, accompanied by apical necrosis and early death. Plants appear to be stunted and deformed pods are observed during severe infections of ClYVV.

***Alfalfa Mosaic Virus* (AMV)**

Alfalfa mosaic virus (AMV) has damaging affect in pea and also other pulse crops like faba beans, lentils and chickpeas. AMV belongs to family Bromoviridae and it is transmitted from plant to plant through aphids. Damp conditions aggravates the spread of aphids whereby leading to increase in AMV infections in pea. Chlorosis and necrosis in new shoots are general symptoms of AMV infection. Older leaves show necrotic spots or streaks and generally plants are stunted as compared to healthy plants. Pods are malformed and seed set is poor thereby leading to lower production. Chemical control is not so effective to control AMV and pesticides for aphid control will indirectly help to lower the incidence of AMV. Other methods to control AMV are cultural methods like using healthy seeds, and proper weed management to contain the spread (Trebicki 2020a)**.**

***Cucumber Mosaic Virus* (CMV)**

Cucumber mosaic virus (CMV) causes severe disease in lupin, chickpea, lentil besides pea. CMV belongs to family Bromoviridae and is transmitted by more than 80 different aphid species (Trebicki 2020b).

3.2.1.2 Strategies for Viral Disease Control

Integrated disease management (IDM) strategies with integrating cultural, chemical and other control measures will facilitate to minimize the extent of crop damage in pea. Seed fractionation technique could be used as a phytosanitary method for lowering the levels of PSbMV infection in seeds and thereby lowering the spread to other pea crop (Congdon et al. 2017). Selection of tolerant and resistant pea lines helps to lower the incidence of virus. PSbMV resistant lines with recessive *eIF4e* gene have been developed from utilizing resistant source available in numerous pea accessions. These lines hold potential to improve productivity in pea (Smýkal et al. 2010). Resistance gene, *wlv* for *Bean yellow mosaic virus* (BYMV) and potyviruses were identified from pea genotypes, which are mapped to linkage group VI in pea. The *wlv* gene corresponds to the *sbm1* allele of *eIF4E* gene, which also confers resistance towards PSbMV in pea (Gao et al. 2004; Bruun-Rasmussen et al. 2007). Protein-wide analysis in pea PSbMV resistant (B99) and susceptible (Raman) lines showed that 116 proteins were differentially expressed in resistant lines. These data could be utilized for advancement of the development of specific gene markers for pea breeding programs (Cerna et al. 2017).

3.2.2 Fungi

3.2.2.1 Types of Fungi

Powdery Mildew *(Erysiphe pisi)*

Powdery mildew disease (Fig. 3.3a) caused by *Erysiphe pisi* is the major disease of pea. Morphological characterization of *E. pisi* done by scanning electron microscope revealed hyaline colored conidia, with shape varying from oblong (young) to cylindrical (matured) (Fondevilla et al., 2012; Parthasarathy 2017). Typical symptoms of powdery mildew in pea are white powdery spots on the upper surface of the leaves, stipules, stems and pods. Disease spreads quickly during warm dry days and cool night weather conditions (Beck and Mathew 2019).

Ascochyta Blight (*Aschochyta pinodes, A. pinodella, A. pisi, Phoma kooolunga*)

Ascochyta blight (Fig. 3.3b) is spread by a complex of *Ascochyta pinodes*, *Ascochyta pinodella*, *Ascochyta pisi*, and/or *Phomakoolunga*, which has devastating effects in field peas growing regions (Skoglund et al. 2011). IPM strategies for Ascochyta blight control includes mainly use of fungicides such as tebuconazole, boscalid, iprodione, carbendazim, and fludioxonil, which displayed more than 80% disease control efficacy under the recorded conditions (Liu et al. 2016). Other control strategies are use of biocontrol such as strains of *Bacillus* sp. and *Pantoeaagglomerans*, which were isolated from pea-related niches and had significantly reduced the severity of disease under greenhouse and field conditions (Jha et al. 2016; Liu et al. 2016).

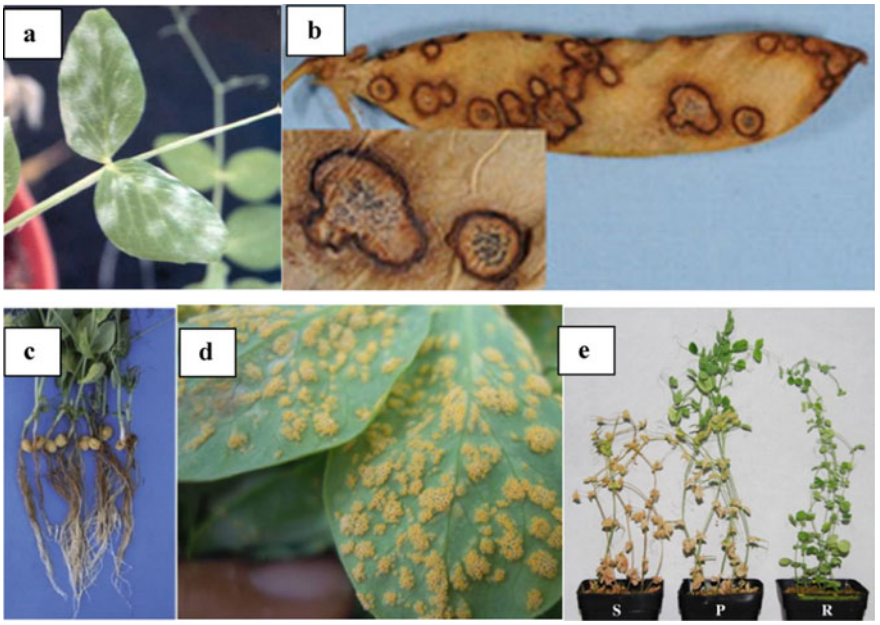

Fig. 3.3 **a** Powdery mildew in pea. **b** Ascochyta blight in pea pods. **c** Aphanomyces infection on pea pods with honey-brown dicoloration. **d** Pea Rust (Skoglund et al. 2011; Fondevilla and Rubiales 2012; Barilli et al. 2014; Castillejo et al. 2015; Wu et al. 2018). **e** Pea fusarium wilt Castillejo et al. (2015)

Root Rot *(Aphanomyces euteiches)*

Aphanomyces euteiches Drechs. causes damping off and root rot diseases (Fig. 3.3c) in pea and leads to more than 80% yield loss. It is a soil-borne pathogen which survives in soil for many years and till now there has been no efficient chemical control available. To control this disease, it is recommended not to grow pea in fields where legumes or pea has been grown earlier. The genus *Aphanomyces* comes under the order Saprolegniales and family Saprolegniaceaes. l. or Leptolegniaceae. Generally root rot is quite widespread in legumes like alfalfa, snap bean, clover and vetch including field pea (Gaulin et al. 2007; Wu et al. 2018). Glossary of information has been documented in European Union: Grain legume Integrated project areas http://www.indexfungorum.orr/Names.asp and http://www.medicago.org.

Pea Rust *(Uromyces viciae-fabae* Pers. de Bary*)*

Pea rust (Fig. 3.3d) is caused by *Uromyces pisi* and *U. viciae-fabae*, mostly at the reproductive stage of the crop during mid-spring season. In pea rust, severe infections lead to leaf and pod drop immaturely, as a result yield loss upto 30% is observed (Barilli et al. 2014). Resistance towards this pathogen is mostly partial and is influenced a lot by environmental conditions. *U. fabae* is an autoecious and heterothallic fungus, which forms all types of spores (pycniospores/ spermatiospores,

aeciospores, urediospores and telliospores) in pea (Das et al. 2019; Upadhyay et al. 2019). Common symptoms of pea rust are appearance of yellow aecia (powdered spots) under the leaf surface, with browning during severe infections (Chand et al. 2006; Das et al. 2019).

Fusarium wilt

Fusarium oxysporum f. sp. *pisi* causes Fusarium wilt (Fig. 3.3e) in pea with 30–40% yield loss. Generally, the symptoms are yellowing of leaves and stunted plant growth. There are various strains and races of *F. oxysporum*based on the virulence. It is a soil borne fungi which can even survive for more than 10 years in soil due to the presence of thick walled chlamydospores. Different cultural practices, use of resistant varieties and biological and chemical methods could be used to control Fusarium spread in pea fields (Castillejo et al. 2015; Gupta and Gupta 2019).

3.2.2.2 Management of Fungal Diseases

Management of powdery mildew is mainly by utilizing resistant varieties. Other method includes utilization of fungicides like treatments with carbendazim (0.1%), followed by Neem Seed Kernel Extract (NSKE) (5%) and nimbicidin (0.3%) (Abhishek and Simon 2017). Other management strategies are use of biocontrols such as mycolytic bacteria, mycophagous arthropods, and yeasts (Fondevilla and Rubiales 2012). Severe conditions of powdery mildew could be controlled by foliar sprays of *Trichoderma* or Karathane, while the former is more beneficial as compare to the toxic impact on health and environment of the latter (Maharjan et al. 2015). IPM strategies have to be strictly followed for control of this complex disease, chemical control up to 80% could be achieved by the use of fungicides like tebuconazole, boscalid, iprodione, carbendazim and fludioxinil are recommended (Liu et al. 2016).

Pea rust could be controlled by altering sowing dates of pea seeds during different seasons. Three different factors which affect the disease development are host, pathogen and environment. Crop rotation with other crops like mustard, wheat and linseed helps the containment of leaf rust in pea (Upadhyay et al. 2019). Due to the lack of durability to manage and wide host range in pea rust disease, chemical control is not recommended (Barilli et al. 2009; Das et al. 2019). Partial resistance to pea rust is available which shows reduced disease severity and low necrosis in host cells (Xue and Warkentin 2001; Chand et al. 2006; Barilli et al. 2009).

Resistant germplasm of pea for Fusarium wilt disease expressed differential proteins, which were taken from roots of susceptible, partially resistant and resistant varieties. This knowledge will help molecular breeders to utilize such resistant sources for developing better breeding materials (Castillejo et al. 2015).

3.2.3 Bacteria

Bacterial diseases are very common threat for major crop loss in legumes. Pea bacterial blight, bacterial pustule and bacterial wilt are more commonly found bacterial diseases in pea.

3.2.3.1 Pea Bacterial Blight (*Pseudomonas syringae* pv. *pisi*)

Pseudomonas syringae pv. *pisi* is the major causative agent for field pea bacterial blight. It is a gram-negative, aerobic, non-spore-forming rod shaped bacteria (Fig. 3.3a) (CABI 2019). Bacterial blight is most noticeable when necrotic patches arise inside the crop. Around the edge of the dead parts exhibit the characteristic water-soaked and the fan-shaped lesions of bacterial blight. In dry weather with occasional frost, symptoms on stems occur as elliptical water-soaked regions, which will be olive-green and eventually brown. Sometimes, these lesions circle the stem and can spread a few centimetres and infect the stem with both the stipulations and the leaflets. Severe infection of the stem may lead to plant death. Infection on the pod also shows the lesions on the pods which are normally sunken and become dark brown and shiny (Hollaway et al. 2007).

3.2.3.2 Pea Brown Spot (*Pseudomonas syringae* pv. *syringae*)

Pea brown spot is caused by *Pseudomonas syringae* pv. *syringae* which typically shows brownish spots on pea leaves and leaf sheath area and looks similar to infections of *P. syringae* pv. *pisi.* Bacteria can live on seed or pea trash, while *P. syringae* pv. *syringae* can live on a variety of host plants. Spread of bacterial blight is enhanced by rainfall, heavy precipitation, strong winds and cold temperatures (Victoria 2020). Acibenzolar-S-methyl (ASM) spray on plants significantly controls the bacterial blight disease (Akköprü 2020).

3.2.3.3 Bacterial Wilt Disease (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*)

Bacterial wilt and tan spot of dry beans (family Fabaceae), are caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, which, a gram positive bacteria, has also been reported to be infecting field pea (Fig. 3.3b). It is a common pea disease in USA, Brazil, Canada, eastern Australia, and Iran (Tegli 2011; Harveson et al. 2015; Harveson et al. 2015). Copper oxychloride, copper hydroxide, and copper sulphate are some of chemicals for controlling bacterial wilt by either field spray or seed treatment. Antimicrobial chemical like Kocide (copper hydroxide) and MasterCop (copper sulphate) are other anti-bacterial controls. Further, antibiotic

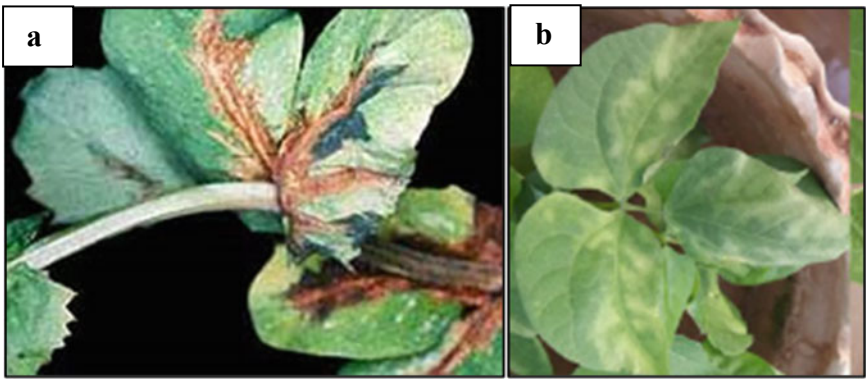

Fig. 3.4 **a** Bacterial blight infection in field pea. **b** Bacterial wilt in dry beans. https://agriculture.vic.gov.au/biosecurity/plant-diseases/grain-pulses-and-cereal-diseases/bacterial-blight-of-field-peas

seed treatment can reduce surface contamination of seeds. Slurry seed treatments using StreptomycinAgri-Strep 500 can give effective results on pea plants (Osdaghi et al. 2020) (Fig. 3.4).

3.2.4 Insect Pests

Insect-pests are biotic stresses which affects yield of pulses severely. It damages crop by chewing plant parts like leaves, stem, fruits and roots. They also act as carrier for virus, bacteria and fungi. They affect crop directly by damaging plant tissues or indirectly by damaging quality of the harvest and increased crop production cost (Bardner and Fletcher 1974). There are many pests which affect pea plants at different stages of growth and are as following.

3.2.4.1 Early Risk Pests

Pea and bean weevil (*Sitona lineatus*)

Pea weevil (Fig. 3.5a) belongs to family Curculionidae and genus *Sitona.* It causes damage when plants are small and growth is slow. Plants affected by weevil show 'U' shaped cut at the edges of leaves. Major damage is caused by larva nurturing on the root nodules (Processors and Growers Research Organisation—https://www.pgro.org/pests-diseases-peas/). Nitrogen availability for plant is highly reduced as *S. lineatus* larvae uses nitrogen fixing bacteria from root nodules for feed (Cárcamo et al. 2015, 2018).

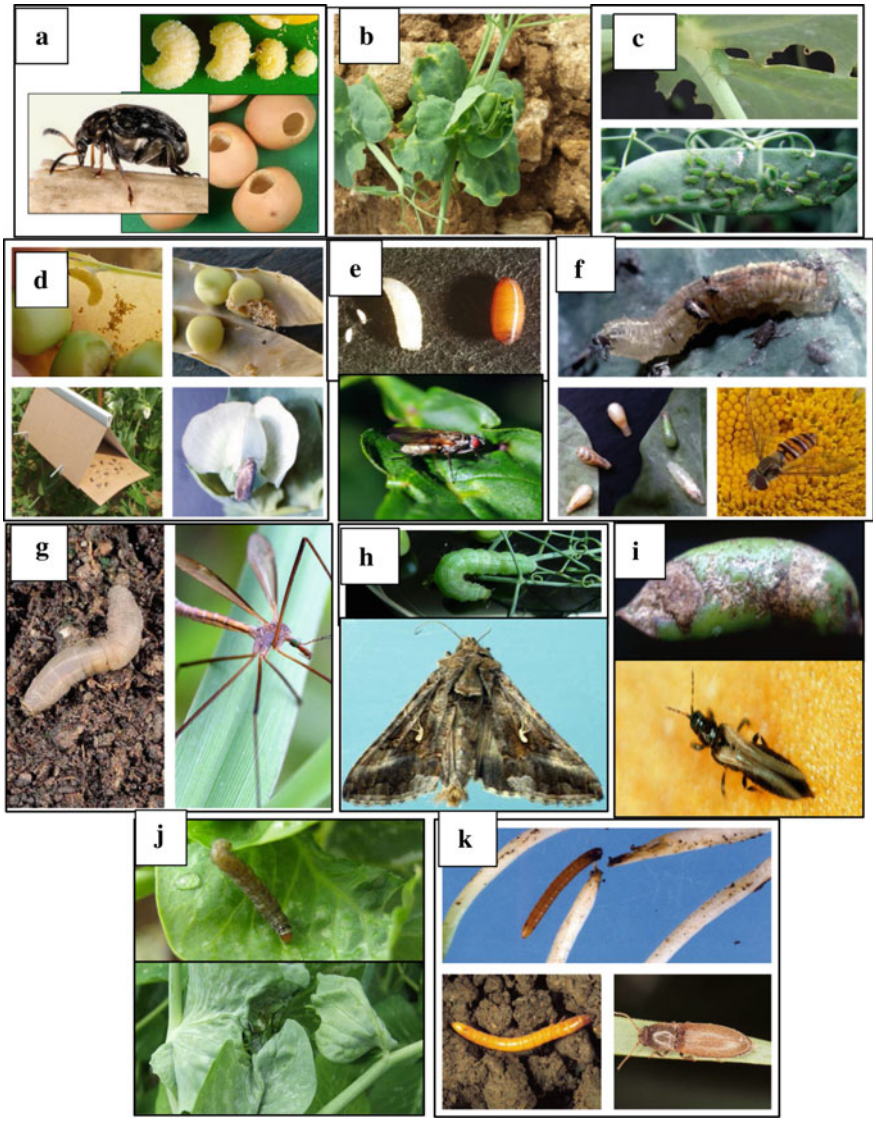

Fig. 3.5 Different pest infesting in pea. **a** Pea and bean weevil. **b** Field thrips. **c** Pea aphid. **d** Pea moth larvae. **e** Bean seed fly. **f** Hoverfly. **g** Leatherjacket. **h** Silver Y moth. **i** Thrips. **j** Tortrix moth and **k** Wireworm (Pea and Bean Crop Walkers' Guide 2018)

Field thrips (*Thrips angusticeps*)

Field thrips (Fig. 3.5b) belongs to family Thripidae and genus *Thrips*. It targets emerging plant leaf surface and make it thicker and wrinkled. Pale colored seedlings appeared (Processors and Growers Research Organisation—https://www.pgro.org/pests-diseases-peas/). *Thrips angusticeps*is considered as most dangerous pest on pea in parts of France and England (Pobozniak 2011).

3.2.4.2 Pre/Early Flower Pests

Pea aphid (*Acyrthosiphon pisum*)

Pea aphid (Fig. 3.5c) belongs to family Aphididae and genus *Acyrthosiphon*. Yield loss in pea is caused by large number of Aphids. It affects crop by acting as career for *Pea enation mosaic virus* (Processors and Growers Research Organisation—https://www.pgro.org/pests-diseases-peas/). Pea aphid *Acyrthosiphon pisum* causes crop damage of hundreds and millions of dollars every year. It is also used in laboratory. Many aphid populations became resistant from conventional pesticides (Sadeghi et al. 2009).

3.2.4.3 Late Flower/Early Pod Pests

Pea moth larvae (*Cydia nigricana*)

Pea moth (Fig. 3.5d) larvae belong to family Tortricidae and genus *Cydia*. Pea moth larva affects developing seeds in pods. It does not affect yield but reduce the quality of harvest. It reduces the value of harvest by reducing quality (Huusela-Veistola and Jauhiainen 2006).

3.2.4.4 Other Pests

Bean seed fly (*Delia platura*)

Bean seed fly (Fig. 3.5e) belongs to family Anthomyiidae and genus *Delia*. Flies put eggs on soil. Larvae from eggs attack seeds and seedlings during germination. It damages seeds, stems and upper root system. Damaged seeds cannot form seedlings (Valenciano et al. 2004).

Leatherjacket (*Tipula* spp.)

Leatherjacket (Fig. 3.5g) belongs to family Tipulidae. Larvae of crane flies are known as leatherjacket. It lives in soil and feeds on grassroots. During spring larvae feeds

on germinating seedlings and damage it. It damages stem at soil level. Presence of larvae in large number causes crop loss (Pea and Bean Crop Walkers' Guide 2018).

Silver Y moth (*Autographa gamma*)

Migratory moth *Autographa gamma* (silver Y moth), is a pest which affects wide range of crops including pea (Ingeborg Menzler-Hokkanen 2020). Silver Y moth (Fig. 3.5h) belongs to family Noctuidae and genus *Autographa*. It is a migratory pest which damages foliage and pods. Adult fly is having silver grey forewings with silver Y in the middle of each. Larvae vary from bright to dark green with white strips at sides and back (Pea and Bean Crop Walkers' Guide 2018).

Thrips (*Kakothrips pisivorus*)

Thrips (Fig. 3.5i) belong to family Thripidae and genus *Kakothrips*. Heavy presence of thrips in large pea production area is observed more in humid condition which damages the pods. Damage does not affect yield and pod quality but damaged pod shows silver and rutted surface (Pea and Bean Crop Walkers' Guide 2018).

Tortrix moth (*Cnephasia asseclana*)

Tortrix moth (Fig. 3.5j) belongs to family Tortricidae and genus *Cnephasia*. Tortrix feed on foliage but does not affect crop yield. It webs leaves together giving hooded appearance to plant. It leaves the crop before harvesting (Pea and Bean Crop Walkers' Guide 2018).

Wireworm (*Agriotes* spp.)

Soil dwelling pests causing high yield loss in various crops are known as wireworms (Barsics et al. 2013). Wireworm (Fig. 3.5k) belongs to family Elateridae. Click beetle larvae are known as wireworms. It is of yellow or brown color with smooth and rigid bodies. It affects on small area. It attacks on shoot and germinating seeds below ground level which fails to recover (Pea and Bean Crop Walkers' Guide 2018).

3.2.4.5 Beneficial Pests

Hoverfly (*Syrphidae*)

Hoverfly (Fig. 3.5f) belongs to family Syrphidae. Hoverflies benefits pea by predating aphids. It is present in infected crops. Larva and pupa contaminate vining peas during harvesting (Pea and Bean Crop Walkers' Guide 2018). Hoverfly is an efficient aphid-specific predator in natural eco-system which plays important role in population dynamics of their prey (Almohamad et al. 2007).

3.2.4.6 Integrated Pest Management Strategies for Managing Insects and Pests

Integrated pest management is very critical for optimum productivity, which includes cultural, biological and chemical control measures. Pea weevil is very active during spring and fall season. Cultural control mainly includes by keeping enough spacing between host and infested fields. Proper weeding and removal of infested plants are the best methods for temporary relief for the spread. Timely irrigation of pea field also helps to keep the pea weevil larval survival in check (Cárcamo et al. 2018). Pheromone traps are a useful technique which includes pheromone-baited pitfalls and ramp traps for capturing adults of pea weevil (Reddy et al. 2018). Main strategy to lower the aphid incidence in pea field is to clear out infested hosts. Utilization of aphid resistant pea lines could be beneficial for effective control to reduce production loss (https://ipmdata.ipmcenters.org/documents/pmsps/2016PulsePMSP_FINAL.pdf). Crop rotation is also an effective strategy for controlling *Thrips angusticeps* infestation in pea field. Resistant or tolerant varieties of pea could be grown in highly infested fields (https://www.plantwise.org/KnowledgeBank/datasheet/53727).

Chemical method includes synthetic pyrethroids spray on adult pea weevil before small pods are visible. Insecticides are effective on adults only. Border spray will control pea weevil from entering into crop. If heavy infection occurs, whole paddock shall be sprayed (https://www.agric.wa.gov.au/pest-insects/management-pea-weevil). Aphicides partially reduce the spreading of secondary infection by controlling *Acyrthosiphonpisum* (Pea aphid) (https://ipmdata.ipmcenters.org/documents/pmsps/2016PulsePMSP_FINAL.pdf). Chemical control of *Thrips angusticeps* in pea can be done by using insecticides pyrethroids and neonicotinoids. Treatments are mostly done during blooming stage and the same is repeated at 7 days interval (Pobożniak and Leśniak 2015).

Biocontrol methods with natural products and biotic agents also help to control pea insect pest infestation. Parasitoids, predators, entomopathogenic fungi, and entomopathogenic nematodes act as a biological control agent for *Sitona lineatus*. From laboratory experiments it is observed that some general predators also act on *Sitonalineatus*. It includes the large, adventive ground beetle, *Pterosticusmelanarius* (Illiger; Coleoptera: Carabidae) which eats *Sitonalineatus* adults and small species *Bembidionquadrimaculatum* (L.; Coleoptera: Carabidae) eats its eggs (Vankosky et al. 2011; Satyagopal et al. 2015). Some parasitoids species like *Hyperapostica* (Gyllenhal) Coleoptera: Curculionidae were released in North America for management of *Sitonalineatus*. *Anaphesdiana* (Girault; Hymenoptera: Mymaridae) is considered promising species to control *Sitona lineatus* which eats eggs of *Sitona* weevils which is established in the eastern United States (Cárcamo et al. 2018). Use of *Beauveria bassiana* (Mycotrol Ois listed by the Organic Materials Review Institute (OMRI) to control *Acyrthosiphon pisum* (O'Neal 2017). Implementation of IPM strategies will make sure the production loss due to insect pest is reduced to minimal. Timely decisions to counter the spread of the insects is the only way to efficiently implement the IPM strategies in pea crop production.

3.2.5 Nematodes

Nematodes cause severe problem for pea crop productivity with tremendous revenue loss. Nematodes can interact with other organisms like fungi, bacteria and viruses to form a disease complex (Singh et al. 2013). Nematodes mainly attack the root system leading to lowering of nutrient uptake and drastic decrease in plant growth and yield. More than 100 species of plant parasitic nematodes are published to be related with pea and other legumes, of which root-knot nematode (*Meloidogyne* spp.), lesion nematode (*Pratylenchus*spp.), reniform nematode (*Rotylenchulus* spp.), cyst nematode (*Heterodera* spp.), spiral nematode (*Helicotylenchus* spp.), stunt nematode (*Tylenchorhynchus* spp.) and lance nematode (*Hoplolaimus* spp.) are common. Needle nematode (*Longidorus* spp.) and dagger nematode (*Xiphinema* spp.) also act as vectors in transmitting viruses to the crops (Askary 2017). Most pea varieties are susceptible to root-knot nematode, which is the major cause of crop loss, so resistant pea lines need to be used for production in areas where the nematode infection is severe (Youssef and El-Nagdi 199). Nematode infection can be confusing to be diagnosed as it shows similar symptoms with fungal diseases and nutritional disorders (Askary 2015). Large, circular patches of stunted pea plants in field are often linked with nematode-infested areas in pea fields. Another common symptom is presence of only primary roots and no secondary roots in nematode infected pea plants. Severe nematode infection shows chlorotic leaves which discolors from the base to the top of the plant (Northwest et al. 2012).

3.2.6 Nematode Management Practices

Cultural methods used to minimalize root-knot nematode damage is soil tilling for two to three times and crop rotation. Optimal soil pH, fertility and soil moisture requirements for plant growth would reduce nematode pressures. Avoiding alternative hosts like faba bean crop near pea field will be good to keep a check on nematode spread (Inglis 1998). Common nematicides used for nematode control are methyl bromide, methyl isothiocyanate and chloropicrin treatment of the soil. Timely soil test from the pea field helps to know the rate of infection and for timely decision (PNW-VEG 2012). Intercropping of green manure crops such as sundan grass, sesame, rapeseed, white mustard and ryegrass in pea field helps to minimize the nematode population in general. Root galling of *M. incognita* in pulses could be reduced by a combined use of cow dung manure and egg shell powder and *Rhizobium* sp. during soil preparation before sowing (Rizvi et al. 2015).

3.3 Genetic Resources of Resistance Genes

Pisum sativum is a diploid species ($2n = 14$) and has a large genome size of 4.45 Gb, as compared to other legumes with smaller genome like *Medicago truncatula*, *Lotus japonicas* or *Glycine max* (Kreplak et al. 2019). About 82.5% of the genome is repetitive sequences as estimated after the complete pea genome sequencing. There are three distinct species of *Pisum* genus, first one is *Pisum sativum* subsp. *sativum* and wild subsp. *elaitus*, which is native in Europe and northwest Asia, second one is *Pisum fulvum* from middle-East Asia and the third one is *P. sativum* subsp. *abyssinicum* or *P. abyssinicum* from Ethiopia and Yemen (Smýkal et al. 2017; Trněný et al. 2018; Coyne et al. 2020).

3.3.1 Primary Gene Pool

Primary gene pool in pea predominantly includes wild pea *P. sativum* subsp. *elaitus* (var. *elaitus*, var. *brevipedunculatum* and var. *pumilo*) along with the cultivated garden pea (*P. sativum* subsp. *sativum*–var. *sativum* and var. *arvense*) (Zong et al. 2019). The intercrosses between the subspecies are difficult due to nuclear-cytoplasmic incompatibility which has hampered the gene flow in some cases (Bogdanova et al. 2014; Nováková et al. 2019; Coyne et al. 2020). But mostly the cross compatibility and rich genetic diversity have led to efficient use of the primary gene pool for genes imparting tolerance to both biotic and abiotic stresses (Zong et al. 2019).

Promising genotypes for pea weevil resistance with less damage were obtained by screening 602 pea accessions from Ethiopia. These germplasm lines from the primary gene pool of *P. sativum* subsp. *sativum* could be utilized in pea breeding program for obtaining enhanced resistance (Teshome et al. 2014). Expressed sequence tag (EST)—simple sequence repeat (SSR) marker analysis showed high differentiation among 46 *P. sativum* accessions and pathological screening of these accessions led to the identification of few less susceptible accessions towards pea weevil infestations (Teshome 2015).

Powdery mildew (resistance in pea has been characterized and markers have been developed with close link to *er1*, *er2* and *Er3* genes. These have been good candidate genes for conferring resistance and are widely utilized in pea breeding programs. As new pathotypes and strains of *Erysiphe* species have emerged, the efficacy of the resistance genes is challenged (Ghafoor and McPhee 2012). Two pea lines resistant to powdery mildew were identified, ILS657 (foliage and pod resistance) and UN6651 (pod resistance only). The study showed that the *er1* gene showed Mendelian recessive segregation pattern. The new sources were confirmed to be allelic to *er1* in case of foliage and pod resistance and another unique allele for pod resistance only (León et al. 2020).

3.3.2 Secondary Gene Pool

Secondary gene pool in the genus *Pisum* includes *P. fulvum* and *P. abyssinicum. P. abyssinicum* exhibits a distinct diversity and karyotype without being found in the wild (Trněný et al. 2018; Weeden 2018). Pea weevil resistance was searched in the *Pisum* secondary gene pool (*P. fulvum* Sm.) due to low detection of seed resistance in *P. sativum* and subspecies. Study was carried out to account for the extent of pod and seed damage in *P. fulvum* accessions and about 26 moderately resistant and resistant lines were obtained after the screening (Clement et al. 2002; Coyne et al. 2020). These lines could be utilized for introgression of these traits into cultivated species for obtaining resistant cultivars. Fusarium root rot leads to significance yield loss in North America where dry pea production has increased significantly over the last few decades. Partial resistance to *Fsp* was identified by developing recombinant inbred line (RIL) population and three associated quantitative trait loci (QTLs) were detected by using three disease criteria i.e. root disease severity, ratios of diseased versus healthy shoot heights and dry plant weights. *Fsp-Ps 2.1* QTL has been observed to be promising for partial resistance to *Fsp* and the single nucleotide polymorphisms (SNPs) associated with the same could be utilized for pea breeding program (Coyne et al. 2019). Rust resistance in pea could not be achieved due to lack of variability in pea germplasm. *P. fulvum*, wild relative of *P. sativum*, is an important source for allelic diversity mainly for rust resistance. Diversity arrays technology sequencing (DArT-Seq) was used for genotyping the RIL population, developed by crossing two *P. fulvum* accessions, IFPI3260 and IFPI 3251. Seven linkage groups with 12,058 markers were assembled, equivalent to both the parents haploid chromosome number. Three QTLs, *UpDSII*, *UpDSIV* and *UpDSIV.2* revealed by composite interval mapping were distributed over two linkage groups linked with the percentage of rust disease severity. These QTLs were found to be closely linked to pea rust disease resistance (Barilli et al. 2018).

3.3.3 Tertiary Gene Pool

The tertiary gene pool of *Pisum sativum* L. includes *Vavilovia Formosa* (Stev.) Fed. which is the common ancestor of tribe Fabeae and shares close phylogenetic relationship. Pea and Vavilovia are close relatives with similar chromosome number of 14 (2*n*). Utilization of Vavilovia germplasm for pea breeding activities will help to introgress essential traits for fighting against various biotic stresses (Zong et al. 2019; Coyne et al. 2020). Along with Vavilovia, grasspea (*Lathyrus sativus* L.) is also a part of the tertiary gene pool of pea. Previously various interspecific crosses were achieved between these two close relatives that has been reviewed earlier. Resistant source of fungal pathogens (*Erysiphe pisi*, *Uromyces pisi*, and *Mycosphaerellapinodes*) of pea has been observed in Lathyrus and could be utilized for obtaining fungus resistant

lines in pea. Some grasspea germplasm also have resistance to pea rust which is caused by *Uromyces pisi-fabae* (Kankanala et al. 2019; Zong et al. 2019).

3.4 Classical Genetics and Traditional Breeding

Peahas been utilized as one of the main crops for studying the inheritance of traits or genes. Further research in pea led to the development of classical genetics and this became the landmark study for development of traditional breeding. From Mendel's work on pea to the whole genome sequencing of pea has seen a remarkable change in the understanding of the inheritance of various traits (Smýkal et al. 2016, 2020; Kreplak et al. 2019). Crop improvement in pea mainly includes tending to biotic and abiotic stresses which significantly affect the crop productivity. It requires utilization of numerous crosses of diverse germplasm as parental lines and selection of important traits which are climate resilient and effective towards biotic stresses as well. Biotic stress in crops has been varied in different geographical areas and various abiotic stresses aggravate the rates of infection of pathogens. Biotic stress in pea is mainly due to fungi, virus, insects and in some cases parasitic plants (Tayeh et al. 2015b; Zong et al. 2019).

Conventional breeding have achieved significant increase in the production of pea consistently with approximately 2% increase per year (Warkentin et al. 2015). Limitation of the classical breeding are brought out by the increased challenges of climate change and new strains of various pathogen infections in pea, mainly due to unavailability of variability.

Availability and inclusion of resistance sources for pea breeding program is the key technique for pea improvement for biotic stresses (Hussain 2015). With increasing requirement for better quality and yield the classical breeding has transformed in to molecular breeding with the advent of new biotechnological tools for introgressing of new traits. Abiotic and biotic stresses have put more constraints on the crop improvement strategies in pea, but utilization of germplasm diversity in pea and genome edited lines will be utilized to overcome some of the challenges in future (Tayeh et al. 2015b; Ali et al. 2018; Zong et al. 2019). SRAPs (sequence-related amplified polymorphisms) were utilized for making linkage maps in pea by crossing cultivars, DDR11 and Zav25. The F_2 mapping population was screened with 25 SRAP markers and about 208 polymorphic markers were generated. Linkage map constructed had seven linkage groups with length ranging from 47.6 to 144.3 cm (mean 75.54 cm). These linkage groups could be utilized for mapping studies in pea (Guindon et al. 2016). Morphological and SSR-based marker approaches were studied to characterize pea germplasm at the Aegean Agricultural Institute of Turkey. About 40 cultivars collected from different geographical regions of Turkey were used for the study according to UPOV criteria and with 10 SSR markers. A total of 61 alleles were detected at 10 loci from the SSR markers and a UPGMA dendrogram was constructed with the morphological characterization. These studies could be utilized for better management of pea germplasm for breeding activities (Sarikamiş

et al. 2010). Resequencing of gene fragments in pea was done with 384-SNP set in different genotypes and genome sequence data were compiled using Illumina GoldenGate assay. About 92% allelic diversity and 37 new gene markers was obtained from the pea germplasm collection which included species and subspecies of *Pisum sativum* ssp. *Sativum* (Deulvot et al. 2010).

Isozyme electrophoresis analysis was carried out to find protein markers for reproducible characterization of individual genotypes of *Pisum sativa* L. Seed and leaf tissues from 45 cultivars were utilized to obtain isozyme profile, mainly with six enzyme systems i.e. acid phosphatase, amylase, esterase, leucine amino peptidase, shikimate dehydrogenase and phosphoglucomutase (Pošvec and Griga 2000).

Pea seed-borne mosaic virus resistant gene *sbm-4* in pea was found to be closely linked to the isozyme marker plastidic glutamine synthetase GS 185. Isozyme and restriction fragment length polymorphism (RFLP) mapping study also revealed that another allele, the *Prx-3* allozyme was loosely linked to the PSBMV resistance gene (Dhillon et al. 1995). These markers could be utilized for pea breeding program and a gene conferring PSBMV resistance could be introgressed in elite pea lines. Isozymes like amylase, esterase and glutamate oxaloacetate transaminase were examined in pea seeds from F_2 population with different crosses. Isozyme characterization and morphological markers were developed and linkage studies were also carried out in pea germplasm (Mahmoud et al. 1984).

Over the last decades conventional plant breeding has resulted in development of various open-pollinated (OP) and hybrid varieties but due to some limitations the biotechnological interventions was required. Limitations of conventional breeding were mainly the non-compatibility for interspecific crosses limiting the novel trait introgression and multiple undesirable traits get passed to the next generation which have harmful impact on other characters (ISAAA 2006).

Conventional plant breeding mostly focused on yield and resistance to pest and disease, by introgressing genes from various wild sources. Recent molecular genetic and biotechnological innovation for developing better crop varieties has tremendously enhanced in both quality and quantity. Conventional breeding techniques led to Green Revolution from 1960 to 80 in cereal crops mainly wheat and rice. Utilization of biotechnological tools such as marker-assisted selection and genetic engineering are the remarkable advancements in crop improvement strategies for sustainable agricultural development (Bhargava and Srivastava 2019).

Developing improved crop varieties quickly is the only way of alleviating food scarcity problems and increasing food security. Genetic variability has come down drastically in crops like pea, where novel molecular techniques are needed to make it more climate resilient as well as biotic stress resistant. Conventional breeding techniques have been improved by the inclusion of genetic selection, molecular breeding, somaclonal variations, genetic engineering, next-generation sequencing (NGS) approaches for whole genome and functional genomic tools. Recent advances in genome editing tools like clustered regularly interspaced short palindromic repeats with associated proteins Cas9 (CRISPR/Cas9) have helped to develop traits which were difficult to develop with conventional breeding. Speed breeding techniques and gene editing tools will help the molecular breeders to develop varieties which are

resistant to both abiotic and biotic stresses along with addressing concerns in case of yield and nutrition (Ahmar et al. 2020).

3.5 Genetic Diversity Analysis

Pisum sativum L. is a protein-rich legume crop with diverse germplasm distributed through the geographical region in the world. According to use there are mainly three different variants of *Pisum* species i.e. garden peas, fodder peas and field peas.

Various marker techniques has been utilized to document the diversity in pea germplasm. Marker techniques like SSR, retrotransposon based insertion polymorphism (RBIP) along with SNP markers have also been utilized for diversity analysis (Burstin et al. 2015). SSR marker results showed that in pea germplasm evolution with fastest rate but RBIP showed the slowest rate showing dissimilarity in mode of evolution. About 331 SNP markers were utilized to analyze to predict phenotypes like flowering, number of seeds and seed weight (TSW).

The origin and domestication of Abyssinian pea (*Pisum sativum* ssp. *abyssinicum*) and its phylogenetic relationship with other subspecies and species were studied with the help of 54 genes. A close relationship of Abyssinian pea with *P. sativum* subspecies i.e. *P. s.* ssp. *sativum* and *P. s.* ssp. *elaitus* was observed after the allele affinities were studied (Weeden 2018).

Identification of stable and high yielding field pea lines from about 12 pea genotypes in seven environments were carried out in Eastern Amhara, Ethiopia. Experiments showed that different genotypes exhibited different levels of productivity in different environments. Some genotypes like EH-03-002 (Yewaginesh) were identified with higher yield than normal checks and were released in drought areas (Kindie et al. 2019). Interspecific crosses between similar species had been done for introgressing traits for biotic stresses in pea. Increased yield by 30% was observed by crossing dwarf and *afila* plant type in field pea.

About six structural types were observed after diversity studies in mitochondrial genomes from 38 accessions of pea (*Pisum* sp.) from different geographical areas, ranging from wild relatives to elite lines. Six events of hybridization in the past were revealed by topology study of the phylogenetic trees. Discordant inheritance of organelles, both plastids and mitochondria was also observed and this has resulted in the plastid-nuclear incompatibility, which is very common in pea (Bogdanova et al. 2020).

Genome-wide analysis shows that *P. sativum* subsp. *elatius* was clustered into five geographical clusters and *P. fulvum* has been identified as well-supported species. Spatial and environmental patterns of these two species of Mediterranean pea, alongwith the genetic diversity study does not correlate much with the origin of these two species (Smýkal et al. 2017). To identify SNPs associated with Ascochyta blight resistance in pea, allele diversity analysis was conducted with about 54 *P. sativum* accessions from Australia, Canada and Europe. Genotyping and phenotyping for the disease reaction was conducted and 15 SNPs were detected within the candidate

genes for Ascochyta blight resistance. Two SNPs, PsDof1p308 and RGA-G3Ap103 showed significant associations with Ascochyta blight resistance in pea, which could be utilized for pea breeding program for Ascochyta blight resistance (Jha et al. 2019). Nematode (*Meloidogyne incognita*) resistance was studied in about 23 field pea selections in greenhouse conditions and root-knot indices (RKI) were analyzed after inoculation of nematodes. Selections HFP-990713, Pant P-25 and HFP-0129 were found to be resistant and no root-knot was observed as compared to other susceptible lines (Sharma et al. 2006).

Fusarium solani f. sp. *pisi* (*Fsp*) is the causative agent of Fusarium root rot resulting in drastic yield loss in pea. Pea accessions and commercial varieties were screened for *Fsp* resistance by analyzing the root disease severity (RDS) and pigmented lines under greenhouse conditions. Physiological data like plant height, shoot dry weight and root dry weight were compare with RDS and it was observed that plant height was negatively correlated with the RDS value (Bodah et al. 2016).

3.6 Association Mapping Studies

Pisum germplasm is varied and has been widely studied and documented through various bureaus of genetic resources. Association mapping studies is the mapping of QTLs by linking phenotypes to genotypes for the detection of genetic associations.

An annotated pea genome has now been has been published with chromosome-level genome assembly for the Cameor cultivar. Pea genome shows intense gene dynamics which is associated with divergence from other Fabeaesister tribes due to genome size expansion (Kreplak et al. 2019). Genome sequencing results shows that across pedigrees differential occurrence of translocation and transposition was observed during pea evolution.

Field pea agronomic traits, seed morphology, seed quality was analyzed to identify gene loci with genome-wide association study (GWAS) by utilizing a germplasm collection of 135 pea accession from 23 different breeding programs form Africa, Asia, Europe and North America (Gali et al. 2019a). Genotyping by sequencing (GBS) gave linkage disequilibrium (LD) decay across chromosome varying from 20 to 80 kb and about nine sub-populations were grouped after population structure analysis.

To determine the genetic loci associated with stress responsive traits of physiological and agronomic importance in pea, GWAS was carried out. Three different environments were considered for identifying 32 marker–trait associations. For developing heat resistance varieties of pea, these markers could be used for crop improvement strategy in pea (Gali et al. 2019a). GWAS study was carried out to understand pathogen-legume interaction and about 52 QTLs were identified associated with resistance to root rot caused by *Aphanomyces euteiches*, by screening about 175 *Pisum sativum* lines which were genotyped using 13,204 SNPs from the GenoPea Infinium BeadChip (Desgroux et al. 2016; Kankanala et al. 2019).

3.7 Molecular Mapping of Resistance Genes and QTLs

Extensive mapping studies in QTLs for various agronomic traits as well disease resistance were carried out in pea by various researchers but were poorly documented earlier (Smýkal et al. 2012). Genome-wide SNPs and genetic linkage maps were developed for identifying QTLs in pea for seed mineral nutrients, which could be utilized for marker assisted selection (MAS) for improving nutritional qualities of pea (Ma et al. 2017a). A total number of 1609 polymorphic SNPs were found in pea parental lines from an F_6-derived RIL population, which generated a linkage map size of 1310.1 cm. About 46 seed mineral concentration QTLs, 37 seed mineral content QTLs and 6 seed weight QTLs were detected in total showing phenotypic variance of 2.4–43.3% (Ma et al. 2017a, b). QTLs in pea for yield traits were identified by SRAP, SSR, and SNP markers and a total of 873 polymorphic markers for linkage mapping was identified. About 45 QTLs were detected by composite interval mapping (CIM) method and a map constructed with 9 linkage groups (LGs) covering 655.5 cm was obtained with 128 genetic markers. About 10% phenotypic variance was observed in most of the QTLs detected through different generations and environments (Guindon et al. 2019).

3.7.1 QTLs in Pea for Fungal Resistance

QTLs for resistance to various pathogens have been identified in various pulse crops including pea. ***Fusarium solani*** f. sp. *pisi* (Fsp) is an infectious fungus that leads to severe symptoms like root rot in pea. To identify resistance associated QTL for Fusarium root rot in pea genome CIM was carried out. About three disease reaction criteria were taken into consideration i.e. root disease severity, ratios of diseased to healthy shoot heights and dry plant weights, for identifying the QTLs. These QTLs could be utilized in pea breeding programs for obtaining partial resistance to Fusarium root rot (Coyne et al. 2019). QTL for partial resistance of root rot caused by ***Aphanomyces euteiches*** in pea accessions were screened by meta-analysis using 244 QTLs reported earlier from mapping populations and about 27 meta-QTLswere identified for resistance to *A. euteiches* (Hamon et al. 2013). Similarly QTLs for partial resistance and management strategies have been reviewed for developing better varieties of pea cultivars (Wu et al. 2018). ***Aphanomyces euteiches*** causes severe root rot in pea and there have been several QTLs associated with partial resistance in pea for root rot caused by the same. Linkage maps were developed from different crosses with different parents. RIL population was developed from a cross of pea genotypes, Puget and 90–2079 lines and about three stable QTLs—*Aph1, Aph2* and *Aph3* were identified. These QTLs were located on the linkage groups IVb, V and Ia in the pea genome (Pilet-Nayel et al. 2002; Wu et al. 2018).

Highly significant and reproducible QTLs by genotyping-by-sequencing (GBS) were developed for various traits in three RIL populations. High-density linkage

maps were constructed and 375 QTLs were identified for traits like flowering, crop maturity, resistance to lodging, *Mycosphaerella* blight (caused by *Mycosphaerella pinodes*) resistance, seed weight and yield (Gali et al. 2018). Mapping populations were created from the crosses of partially resistant lines (3147-A26 and 3148-A88) with susceptible lines (Rovar population) in Western Australia and New Zealand, and about 11 novel putative QTLs for Ascochyta blight resistance (caused by a complex of three fungal pathogens—*Mycosphaerella pinodes* (Berk. and Bloxham) Verstergren, *Phoma medicaginis* Malbr. & Roum var. *pinodella* (L. K. Jones) Boerema, and *Ascochyta pisi* Lib.) in pea were identified (Timmerman-Vaughan et al. 2004). QTL mapping was done by mapping different linkage groups and QTLs were detected in Linkage groups II, III, IV, V and VII. These mappings of the QTLs were also measured for plant reproductive maturity as Ascochyta blight is severe when plants start maturing. Six and five plant maturity QTLs in case of A26 x Rovar population and A88 x Rovar population respectively were associated with the linkage groups found earlier. Different linkage groups of the QTLs were linked with different phases i.e. repulsion and coupling phases, which was found to be the result of pleiotropic effects of plant-maturity genetic loci (Timmerman-Vaughan et al. 2004). A total nine QTLs were identified for Ascochyta blight resistance, which individually explained 7.5 to 28% of the total phenotypic variation. These QTLs could be useful for development of molecular markers associated with Ascochyta blight resistance (Jha et al. 2016). A total six QTLs associated with resistance to *M. pinodes* were found in linkage groups II, III, IV and V, which collectively explained between 31 and 75% of the phenotypic variation (Fondevilla et al. 2008).

3.7.2 QTLs in Pea for Bacterial Resistance

Bacterial blight disease caused by *Pseudomonas syringae* pv. *syringae* causes a yield loss of upto 70% in pea. TwoQTLs for bacterial blight resistance were identified in pea. RIL population created from a cross between P665 x 'Messire' was used for QTL identification associated with bacterial blight resistance genes *psy1* and *psy2* and 21 SSR markers in pea (Rubiales and Caminero 2012). In pea, a total of two and four QTLs for resistance to infection of *P*. pv. *syringae* were detected in case of crosses of Kaspa (Susceptible genotype) x PBA (Resistance genotype) Oura and Kaspa x Parafield populations, respectively. QTLs for resistance to both *P. syringae* pv. *syringae* and *P. syringae* pv. *pisi* race are co-located on Ps III cross of Kaspax PBA Oura, which is an important area for developing resistance to bacterial blight and also provides the basis for co-selection in genomics-assisted breeding activities (Sudheesh et al. 2015).

3.7.3 QTLs in Pea for Insect Resistance

QTLs in pea have been identified for resistance against pea weevil (*Bruchus pisorum*) from a high-density integrated DArT-Seq SNP-based genetic map developed from a RIL population (RIL F8:9). Both pea weevil larval development and seed infestation were screened in five different environments. Genetic linkage mapping allowed to identify three QTLs from the study associated with the pea weevil resistance. Expression of the QTLs varied with different environmental conditions. Seven markers co-located with QTLs are potential markers which could be utilized for MAS in pea breeding program (Aznar-Fernández et al. 2020). Similarly with DArT-Seq SNP-based QTL mapping, candidate genes were identified for aphid tolerance in wild relatives of pea (*Pisum fulvum*). A total of eight QTLs linked with eight linkage groups were identified which were associated with tolerance towards aphid infestation (Barilli et al. 2020). Genomic studies with the help of published pea genomic sequence will help molecular breeders to generate more resistant and tolerant genes associated with various pests in pea (Kreplak et al. 2019).

3.8 Marker-Assisted Breeding for Biotic Stress Resistance

Marker-assisted breeding for introgressing genes into elite commercial pea lines have been done by molecular breeders in various pea breeding programs happening in various institutes and commercial companies to address various biotic stresses.

3.8.1 Pea Markers Developed for Virus Resistance

Mutagenesis has helped to develop various gene markers for resistance towards various biotic stresses, mainly pathogens like viruses in pea. Mutation in eukaryotic initiation factor 4E (eIF4E) confers resistance in both for *Pea seed-borne mosaic virus* (PSbMV) with *sbm-1* gene and *Clover yellow vein virus* (ClYVV) with *cyv-2* gene in pea. About 202 pea lines were screened by sequencing *eIF4E* gene and the resistant lines were generated for ClYVV (Andrade et al. 2009). PSbMV resistance is conferred by a single recessive gene of *eIF4E* which is localized on LG VI i.e. *sbm-1* locus. Resistant donors were obtained from 43 different pea varieties by sequencing the *eIF4e* genomic sequence. Markers for *eIF4E* allele were developed and PSbMV infection data were used to confirm resistance in 60 pea accessions (Smýkal et al. 2010). The RFLP marker (GS185) for PSbMV resistance, which is closely linked to *sbm-1* locus, was developed from screening different pea accessions. These markers could be used for introgressing the PSbMV resistance gene into the elite pea varieties (Timmerman et al. 1993). KASP (Kompetitive allele-specific PCR) markers were developed for resistance to PSbMV with endpoint genotyping for speedy testing new

breeding lines without requirement of greenhouse facilities for screening or ELISA testing. Breeders will benefit with such KASP markers for obtaining varieties with resistant lines, which in turn could be utilized for pea breeding programs (Grimm and Porter 2020). Other diagnostic assays such as tissue blot immunoassay (TBIA) has been used for virus diagnosis in pulses, which are manly reliable, fast and cost-effective methods for screening of large numbers of plant samples (Kumari et al. 2001).

3.8.2 Pea Markers for Fungus Resistance

A novel genomic region was identified for controlling cellular mechanism involved in pea resistance to Ascochyta blight which is useful for marker associated screening (Carrillo et al. 2014). Validation for Ascochyta blight resistance in pea was carried out with SNP markers in 36 cultivars of pea from Saskatchewan, Canada. KASP assays and SNP marker association studies were carried out and SNP makers like RGA-G3Ap103, PsC8780p118, and PsC22609p103 were found to be associated significantly with the Ascochyta blight scores (Jha et al. 2019). These markers could be further utilized in pea breeding programs for improved lines with Ascochyta blight resistance in pea. MAS for developing cultivars for resistance to powdery mildew (caused by *Erysiphe pisi*) resistance has been done with *er1, er2* and a new dominant *Er3* gene. A new pathogen, *E. trifolii,* for pea powdery mildew has been reported and for ita new resistant source has been searched in pea germplasm (Ghafoor and McPhee 2012). A total of 24 pea lines were evaluated for high yield and resistance to powdery mildew. ANOVA results showed that grain yield of 24 lines ranged from 22.87 to 102.54 g and were also highly resistant to powdery mildew (Iqbal et al. 2017). Mutagens like methylnitroso urea (MNU) and ethylnitroso urea (ENU) were utilized for obtaining variability in pea (*Pisum sativum* L.) germplasm. Two novel mutations related to powdery mildew (*Erysiphe pisi* Syd.) resistance were obtained by ENU treatment, which could be utilized for marker development for screening a large number of pea germplasm (Pereira and Leitão 2010). These *er1* mutants have inheritance as monogenic recessive trait, which exhibits Mendelian mode of inheritance. Several powdery mildew resistant lines were developed by mutation in two novel *er1* alleles. The first such allele is *er1-8*; germplasm accession G0004839 has which a 3-bp (GTG) deletion of the wild-type *PsMLO1* cDNA, that affects the *PsMLO1* protein sequence. Another mutation in accession G0004400 was caused by a 1-bp (T) deletion of the wild-type *PsMLO1* cDNA sequence, resulting in a truncated *Ps*MLO1 protein. Theses result concluded that *E. pisi* resistance in pea germplasm could provide a powerful tool for MAS in pea breading (Sun et al. 2019). The KASPar assay is useful tool for development of powdery mildew resistance line (accession PI 142775) in pea by phenotyping and genotyping to carry the allele *er* 1-1 (Ma et al. 2017b).

3.8.3 Pea Marker Developed for Insect Resistance

Pea weevil (*Bruchuspisorum* L.) infestation is a global problem for the pea crop production, various resistance sources from wild pea (*Pisum fulvum* Sibth. & Sm.) were introgressed into cultivated field pea (*Pisum sativum* L.). F2:3 families showed mortality rates of larva on pods similar to resistant parents, but complete resistance to pea weevil was not observed in the progenies (Clement et al. 2009).

3.9 Map-Based Cloning of Resistance Genes

Pulses are relatively minor crops on a global scale when compared with cereals and others when global production and field area sown are taken into consideration. The nutritive value of pulses are immense but fewer studies are being conducted in various legume species as compared to other major crops. Traits from wild relatives have been utilized in conventional breeding for introgressing into elite parents. Bacterial artificial chromosome (BAC) libraries of pulsed crops are essential genetic resources that will quicken gene discovery and augment molecular breeding in pulse crops especially in pea. Various BAC libraries in pulses like mungbean (*Vigna radiata* L.), cowpea (*V. unguiculata* L.), pigeonpea (*Cajanus cajan*L.), field pea (*Pisum sativum* L.), Lima bean (*Phaseolus lunatus* L.) and common bean (*P. vulgaris* L.) has been reviewed by Yu (200). *Hind* III BAC libraries in pea (Pea plant inventory (PI) accession 269818) were developed to isolate genes involved in plant disease resistance and other economically important traits. About 65,280 clones were obtained from a single-copy oriT-based T-DNA vector (pIndigoBAC-5) library. Two replication methods was developed to analyze the usefulness of the library, one by probing high-density filters with low copy number sequences and the second by amplifying 7 of 9 published pea resistance gene analogs (RGAs) with BAC plate-pool DNA (Coyne et al. 2007).

3.10 Genomics-Aided Breeding for Biotic Stress Resistance

Crop improvement in pea has been done by utilizing multiple numbers of genomics aided breeding techniques. Breeding tools like genome selection (GS) are being utilized for achieving novel trait development, where trait measurements are difficult due to environmental influence and occurrence of multiple pathogens during infection, especially in case of biotic stresses. Due to climate change and adaptation of insect pests the crops are on high risk of biotic stresses. To overcome this challenge there is a high requirement of availability of genomic resources which help researchers to improve crops.

3.10.1 Genome Sequencing

Individual and consensus genetic maps were constructed by genotyping of 12 pea (*Pisum sativum* subsp. *pisum*) RIL populations (French cultivar Cameor) by GenoPea 13.2 K SNP array. High resolution consensus map consisting of 12,802 transcript-derived SNP markers was constructed thereby revealing the duplication sites in pea genome. These SNP array data helps breeders to analyze genetically and physiologically for crop improvement strategy in pea (Tayeh et al. 2015a). Transcriptome analysis was done in pea by generating full length de novo assembly of RNA sequencing data from 20 cDNA libraries produced from plant tissues collected at various developmental stages from plants grown under different nitrogen levels. CameorUnigene set of a total number of 46,099 contigs were identified and further online search engine was developed (http://bios.dijon.inra.fr/FATAL/cgi/pscam.cgi) for annotation of candidate genes, transcript expression study, identifying uncharacterized genes and gene ontology study (Alves-Carvalho et al. 2015). Physical mapping in pea was assembled by constructing BAC contig libraries from about 295,680 BAC clones. Whole genome profiling (WPG) sequence tags were utilized to assemble 220,013 BACs into contigs which helped to construct a robust physical map of pea (Gali et al. 2019b).

An international consortium was formed to sequence the whole genome of *Pisum sativum* L. with the inclusion of latest NGS technologies, which are both time saving and cost-effective. The inbred pea cultivar used for sequencing was 'Cameor' which was released by French Breeding company Seminor in 1973 (Kreplak et al. 2019). The first annotated chromosome-level reference genome assembly for pea was reported by Kreplak and team (Kreplak et al. 2019). About 82.5% (3.23 Gb) of the estimated pea genome size (about 4.45 Gb) was assembled into seven pseudomolecules and about 14,266 unassigned scaffolds (685 Mb), with the size gap being highly repeated sequences (Fig. 3.5).

As pea production and storage is prone to be affected by several pests, development of resistant variety is required to match the increasing demand. As the pea draft genome sequenced, identification and annotation of agronomical important genes boosted programs of resistance breeding. In pea, gene annotation was done by combined ab initio and homology based methods. Using these methods 44,756 complete and 29 truncated genes were predicted. The average gene length is 2,784 bp. The average coding sequence length is 1,016 bp. The average exon numbers are 6.33 exons (Tayeh et al. 2015a; Kreplak et al. 2019).

Evaluation of Ascochyta blight resistance in pea germplasm was evaluated with GBS, Bayesian Reproducing Kernel Hilber spaces regression (RKHS) and genome based linear unbiased prediction (GBLUP) modeling techniques. Ascochyta blight disease score (ASC) of 0.56 was obtained in case of GBLUP analysis, after screening of 215 pea accessions originating from The New Zealand Institute for Plant and Food Research Limited (PFR) pea breeding program and other commercial cultivars from other sources (Carpenter et al. 2018).

3.10.2 Application of Functional Genomics

Identification and isolation of genes underlying functional genomics studies were carried out in pea by developing a fast neutron (FN)-mutagenized population. Mutant population developed will give a pool of variability due to deletions in associated gene for various functions which are not found in wild relatives of pea. Forward genetics screening with NGS helps molecular breeders to identify genes with deletions rapidly and steadily (Domoney et al. 2013).

Agrobacterium-mediated transformation is a challenge in pea, as it is a species which is recalcitrant to the same. Mutant populations of peas were created by treating the seeds with EMS (ethyl methane sulphonate) mutagen and TILLING (targeting induced local lesions in genomes), a high throughput reverse genetic tool, was developed. UTILLdb, a phenotypic peaphenotypic database was created from the mutant populations with sequence information on the mutant genes. UTILLdb is an online searchable database developed by INRA, France, which gives a platform where mutant gene sequence information can be searched through BLAST tools and also associated phenotypes (Till et al. 2007; Dalmais et al. 2008). TILLING has been applied for obtaining numerous mutants in different model plants and crops like *Medicago truncactula* (Carelli et al. 2013), *Phaseolus vulgaris* (Porch et al. 2009), and *Cicer arientinum* L. (Amri-Tiliouine et al. 2018), including other crops (Kumar et al. 2013). Screening for mutants with NGS technology to mutagenized TILLING populations as a tool for functional genomic study is known as TILLING by sequencing (TbyS). TbyS could be utilized in pea breeding program for screening large mutant population to identify and characterize induced mutations in gene of interest. TbyS will accelerate the functional genomics platform together with rapid increase in genome editing capabilities and enhance the quality and number of genome sequencing (Kumar et al. 2017). These mutant populations developed will allow plant breeders to utilize the resources for pea breeding program to obtain enhanced variability for various traits not only for yield and nutrition but also for biotic and abiotic stresses.

3.11 Recent Concepts and Strategies

Conventional breeding in pea has contributed significantly for improving yield and nutritional traits, but addressing issues pertaining to biotic stresses have been lagging behind (Warkentin et al. 2015). Pulses are generally climate smart, as they adapt to and mitigate the effects of abiotic stress like salinity, drought and heat. Low genetic diversity in pulses has been the main drawback for crop improvement efforts. Biotech techniques like transgenics by genetic transformation, gene editing, and nanotechnology could be utilized for accelerating the effort for developing climate smart pulses (Kumar et al. 2019).

3.11.1 Gene Editing

The process of delivering site-directed nucleases (SDNs) and single guide RNA to explants in culture for editing specific region of gene is known as gene editing (van de Wiel et al. 2017; Maher et al. 2020). Further these gene-edited cells are grown in plant growth medium with plant hormones (Cytokinins and auxins) for cell differentiation by tissue culture. SDNs mainly lead to small deletions/insertions (indels) and modification or replacement of genes. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALEN) and CRISPR/Cas9 nucleases are SDNs which could be used for gene editing in plants. CRISPR (Clustered regularly interspaced short palindromic repeat) and Cas9 (associated protein 9) gene editing system is an effective technique which has been applied for targeted trait development in plants. There are different techniques of gene editing, viz. targeted gene mutagenesis, cytosine-based editing (CBE) and adenine-based editing (ADE), which are very efficient for obtaining gene-edited plants with desired phenotype (Fig. 3.6) (van de Wiel et al. 2017; Mao et al. 2019; Maher et al. 2020). Gene editing has provided a new cutting-edge strategy for development of traits against biotic and abiotic stress mainly because it is simple, specific, consistent, and very have very high efficiency, which was not feasible with conventional breeding techniques (Fig. 3.7).

Innovative modes of gene editing techniques are being developed for effective editing of complex traits in various crops. Virus-mediated gene editing is one such techniques which overcomes drawbacks of conventional transgene-mediated CRISPR-Cas reagent delivery method, such as unexpected genome changes,

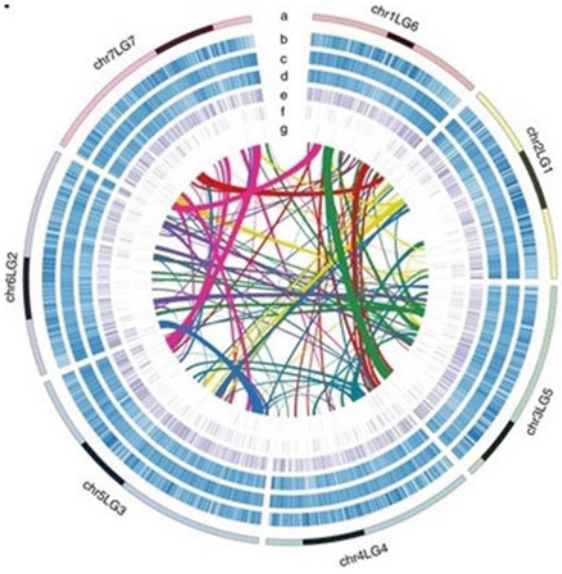

Fig. 3.6 Pea genome showing all the seven chromosomes; centromere position is colored in black. Circular representation of pseudomolecules in the lane (**a**), density of retrotransposons (**b**), transposons (**c**), genes (**d**), ncRNA (**e**), tRNA (**f**) and miRNA. Synteny-selected paralogues are linked in the inner circle with lines (Kreplak et al. 2019)

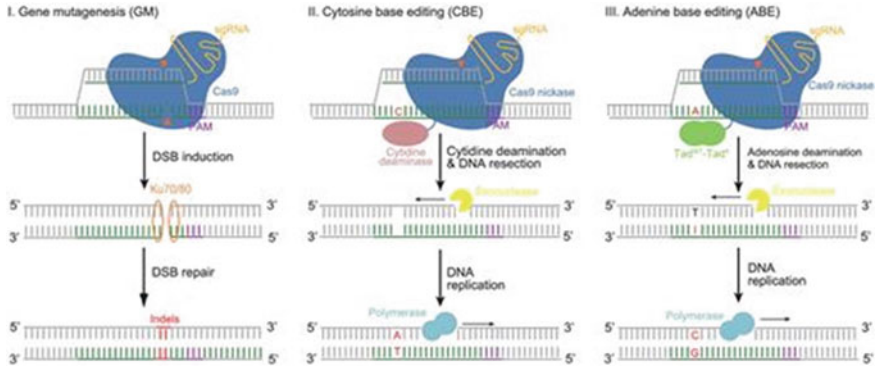

Fig. 3.7 CRISPR/Cas-mediated gene mutagenesis and base editing models. I—Gene mutagenesis, II—Cytosine base editing and III—Adenine base editing (Mao et al. 2019)

prolonged breeding cycles comprising foreign DNA segregation along with regulatory restrictions. Virus-mediated gene editing utilizes plant negative-strand RNA virus-based vector for DNA-free plant delivery of CRISPR-Cas9 cassette in tobacco. More than 90% of the plants contained targeted mutations, in which about 57% had tetra-allelic, inheritable mutations (Ma et al. 2020). *Tobacco rattle virus* (*TRV*) and *Pea early browning virus* (*PEBV*) were engineered to deliver multiple ssRNAs into *Nicotiana benthamiana* and *Arabidopsis thaliana* (Col-O) plants for inducing targeted mutations (Ali et al. 2018).

CRISPR/Cas9 gene editing and other latest techniques have enormously enhanced the characterization of complex genes mainly for biotic and abiotic stresses and other traits. Utilization of targeted mutagenesis with CRISPR/Cas9 helps to obtain novel lines with improved traits, gene regulation, breeding virus resistance and high-throughput editing mutant libraries (Chen et al. 2019).

Clover yellow vein virus (*ClYVV*) (Family: Potyviridae) infections in pea leads to crop damage in pea, mutated eIF4E proteins confers potyvirus resistance. CRISPR-Cas9–cytidine deaminase technology was used to edit *eIF4E* gene and *eIF(iso)4E* to develop various mutants (W69L, T80D, S81D, S84A, G114 and N176K substitutions) which gave *ClYVV* and potyvirus resistance in pea (Bastet et al. 2019) (Fig. 3.8).

Pea aphids (*Acyrthosiphon pisum*) are major pest in pea and other crops which also act as vectors for plant viruses which leads to a large extent of crop loss. Gene editing by CRISPR-Cas9 in pea aphid was carried out to obtain stably edited line. *Stylin-01* gene in aphid was edited, which mainly controls the transmission of *Cauliflower mosaic virus* (*CMV*), which will help in controlling crop loss due to the aphid transmitted viruses (Le Trionnaire et al. 2019).

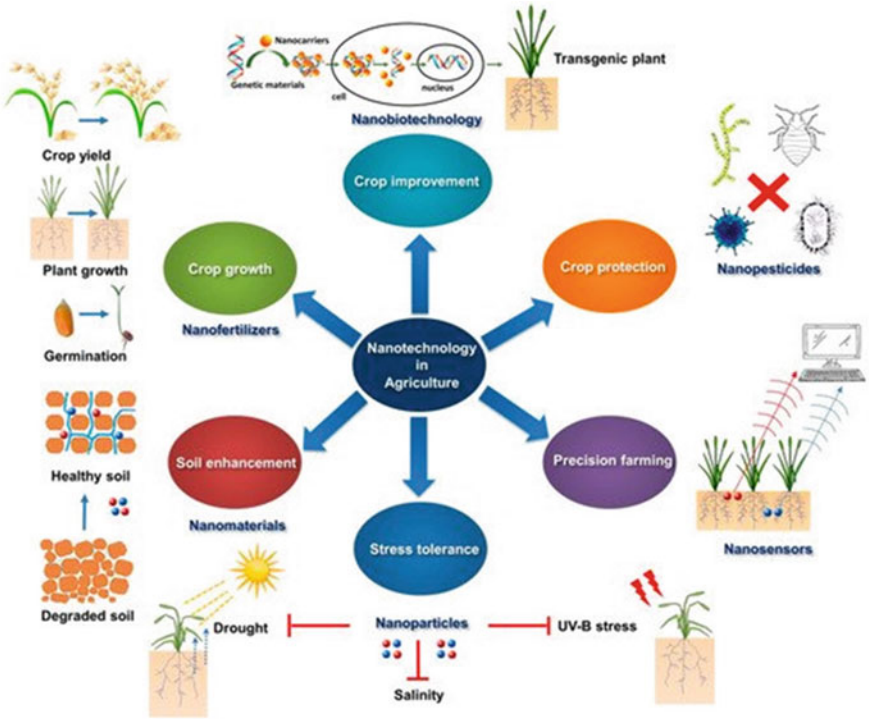

Fig. 3.8 Applications of nanotechnology in agriculture. Nanotechnology is in applied various sectors of agriculture mainly for crop improvement (nano carriers for genetic engineering), crop protection (nanopesticides), precision farming (nanosensors), stress tolerance (nanoparticles), soil enhancement (nanomaterials), and crop growth (nanofetilizers) (Yifen et al. 2019)

3.11.2 Nanotechnology

Another major inclusion of modern technology in sustainable crop development is nanotechnology. Nanotechnology enhances the efficiency by improving the agriculture productivity and by lowering the yield loss. Nanomaterials are those materials which efficiently site-directly delivers the pesticides and fertilizers and help to improve the crop output. Nanotools such as nanobiosensors help to collect intricate crop developmental data which could not be collected otherwise. Nanotechnology enhances crop care mitigation by sensing and identifying environmental impacts both from biotic and abiotic stress (Shang et al. 2019). A detailed diagrammatic representation of all the main areas were applications of nanotechnology in agriculture has been shown in Fig. 3.7 (Shang et al. 2019).

Nanotechnology in crop science is still in infancy, mainly in areas like plant-nanomaterials (NM) interactions, uptake of NM in cellular level, mobilization of NMs into target sites and accumulation in cellular vesicles. Enhanced research in crop improvement with NM is required to fill the void of information in this area.

Apart from NM, development of nano carriers with gene editing capability is also revolutionizing the genome variability of desired biotic and abiotic traits. This will help future molecular breeders to develop climate resilient pea genotype along with addressing challenges of biotic stresses (Sanzari et al. 2019).

3.12 Brief on Genetic Engineering for Biotic Stress Resistance

Genetic engineering have been utilized to improve pea crop by various techniques. Transgenic techniques like direct and indirect transformations, anti-sense RNA, RNAi and VIGS have been utilized for attaining biotic stress resistance in pea.

3.12.1 Transformation in Pea

Transgenic pea for resistance against wax moth (*Galleria mellonela*) was developed by screening small inhibitors with antibacterial and antifungal activities (gmSPl-2) which were isolated from labial glands of wax moth. Czech and French pea cultivars were used for *Agrobacterium tumefaciens* mediated transformation with construct EHA105::pWell11 bearing GUS and GFP reporter genes (Svabova et al. 2010).

Transgenic pea was developed by inserting *cry1Ac* gene from *Bacillus thuringenesis* for tobacco budworm resistance and *Bar* gene from *Streptomyces hygroscopicus* for herbicide resistance. The transgenic was taken up to T4 generation and results of insect bioassay showed complete mortality of tobacco budworm (Negawo et al. 2013). *Agrobacterium tumefaciens*-mediated transformation was carried with antifungal genes coding for polygalacturase-inhibiting protein (PGIP) from raspberry and stilbene synthase (*Vst1*) from grapes in pea. Stable transgenics were developed and antifungal activities were detected by various experiments (Richter et al. 2006). Similarly, transgenic lines with antifungal genes (1–3 β glucanase (G), endochitinase (C), polygalacturonase inhibiting proteins (P) and stilbene synthase (V) were created for Fusarium root rot (*Fusarium avenaceum*) resistance in pea. Transgenic lines developed could be utilized for gene stacking and field trials for overall crop development in pea (Kahlon et al. 2018). But drawbacks of transgenic development such as regulatory cost, time requirement for trials and labor required have to be taken for consideration for efficient implementation.

Transgenics expressing multiple genes for various biotic and abiotic stresses will enhance and broaden the resistance of crops. Transgenic pea was developed by transforming the cultivar with antifungal gene i.e. chitinase and glucanase (Amian et al. 2011).

3.12.2 Gene Silencing

Gene silencing techniques like virus-induced gene silencing (VIGS) have been utilized for different crop species for characterizing genes controlling abiotic stresses like drought, salt, nutrient deficiency related stresses (Ramegowda et al. 2014). VIGS is an efficient method to change the expression of genes in host plants. *Pea white clover mosaic virus* (WClMV) was utilised as base virus vector and VIGS was demonstrated with *phytoene desaturase* (PDS) in pea. PDS mRNA and subgenomic RNAs of WClMV were reduced and photo-bleached tissues were obtained (Ido et al. 2012). VIGS is also an effective reverse genetic tool for silencing genes in certain plants that are difficult to transform. Pea early-browning virus (PEBV) has been developed as a VIGS vector and has been used in pea for functional analysis of several genes. Genes involved in symbiosis with mycorrhizal fungi (AMF) were the targets for gene silencing and results obtained showed early and late stages of AMF symbiosis (Grønlund et al. 2010).

Powdery mildew (PM) in pea caused by *Erysiphe pisi* (*Ep*) is a fungus which secretes a plethora of effectors, primarily through specialized infection structures termed haustoria, to establish a dynamic relationship with its host. To identify *Ep* effector candidates, a cDNA library of enriched haustoria from *Ep*-infected pea leaves was sequenced. The functional roles of EpCSEP001, EpCSEP009 and EpCSP083 were probed by host-induced gene silencing (HIGS) via a double-stranded (ds) RNA-mediated RNA interference (RNAi) approach. Foliar application of individual EpCSEP/CSP dsRNAs resulted in a marked reduction in PM disease symptoms. Microscopic and molecular studies also shows similar results, suggesting that these Ep CSEP/CSPs play major roles in pea PM infections. This study also recognizes and functionally validate candidate effectors from the agriculturally relevant pea PM, and highlights the utility of transcriptomics and HIGS to elucidate the key proteins associated with Ep pathogenesis (Sharma et al. 2019).

RNAi techniques like miRNA, siRNA and piRNA pathways have been utilized for developing pea aphid resistant lines in pea. The role of the genes were studied by targeting 25 core RNAi genes, which in turn were expressed at various developmental stages and in various tissues during aphid infestation (Yang et al. 2020). Pea pathogen resistance to mainly virus, fungus, bacteria and insects have been attained by genetic engineering techniques but due to prevalence of new strains and biotypes of these pathogens the resistance built-up gets broken. Continued research in developing molecular tools like gene-editing will help to overcome such challenges.

3.13 Role of Bioinformatics as a Tool

3.13.1 Gene and Genome Databases

Climate change indirectly affects pea yield by biotic stresses like insect-pests and diseases. Genomic resource for pea is very crucial for developing resistant varieties. Several genomic resources generated for pea include pea gene atlas (Alves-Carvalho et al. 2015), whole-genome polymorphism data for multiple genotypes, and BAC libraries developed for the genotype Cameor (http://cnrgv.toulouse.inra.fr/fr). Databases developed to access genome data includes NCBI (National Centre for Biotechnology Information), The Legume Information System (LIS) (Dash et al. 2016), URGI (INRA) and Pulse Crop Database (PCD).

3.13.2 Comparative Genome Databases

Comparative analysis helps to develop novel varieties using knowledge of known resistant genes for several biotic stresses like disease resistance and insect resistance in other species. Comparative studies have been reviewed in detail earlier and have revealed conservation between various pulses (mainly alfalfa, chickpea, pigeonpea, lentil and soybean) and pea (Tayeh et al. 2015a). Comparative analysis of known powdery mildew resistance between pea and other species supports loss of function mutation in *MLO* (*Mildew Resistance Locus O*) gene (Humphry et al. 2011). Alignment tools such as BLAST (Basic Local Alignment Search Tool) from NCBI database, help to identify gene orthologs in other species (Altschul et al. 1990). There are several other comparative genomic analysis tools likeInParanoid (Remm et al. 2001) and OrthoMCL (Li et al. 2003).

3.13.3 Gene Expression Databases

To study the expression of genes related to Biotic stresses transcriptome data provide good resource. The high quality pea transcriptome data was generated and raw reads were submitted to NCBI Sequence Read Archives (SRA) (http://www.ncbi.nlm.nih.gov/sra/). Assembly created for some data was submitted to NCBI Transcriptome Shotgun Assembly (TSA) database. This data can be used to do BLAST analysis to find candidate genes related to biotic stresses (Zhukov et al. 2015).

3.13.4 Protein or Metabolome Databases

PlantPReS (Plant stress proteome database: www.proteome.ir) was developed by Agricultural Biotechnology Research Institute of Iran (ABRII) which presently contains more than 35,086 entries from 577 articles which are manually curated and more than 10,600 unique proteins related to stress response (Mousavi et al. 2016). PSPDB: Plant Stress Protein Database was developed by inserting data which are manually curated proteins from UniProt. It involves experimentally validated plant proteins related to biotic and abiotic stresses. It is useful for predicting function of proteins related to stresses (Anil Kumar et al. 2014) (http://www.bioclues.org/pspdb/index.php). Functional analysis of protein can be done using InterPro. It classifies protein into families and predicts domains and other important sites by using predictive model which is known as signatures (Mitchell et al. 2019). Pfam is a protein family database used to study protein domains which provides information about protein function. Latest release of Pfam 33.1 contains 18,259 entries as per May 2020 data (El-Gebali et al. 2019). Protein Information Resource (PIR) provides resource for protein informatics which supports proteomic research. It maintains mainly three databases which are the Protein Sequence Database (PSD), the Non-redundant Reference (NREF) sequence database, and the integrated Protein Classification (iProClass) database (Wu et al. 2003). PROSITE database is used to analyze protein domain, families and functional sites. It contains patterns and profiles for protein families and domains which gives information like structure and function of proteins (Sigrist et al. 2013). RCSB PDB (Protein Data Bank) is a database which contains structure information for proteins that helps researchers to visualize 3D structures of experimentally determined proteins. Recently PDB has become more users friendly by developing high-speed NGL Viewer which helps to visualize 3D molecules in any web browser (Rose et al. 2017). Universal Protein Resource (UniProt) is used to analyze protein sequence and annotation data. It is collaboration between three major databases European Bioinformatics Institute (EMBL-EBI), the SIB Swiss Institute of Bioinformatics and the Protein Information Resource (PIR) (Magrane and Consortium 2011). KEGG (Kyoto Encyclopedia of Genes and Genomes) is the pathway database which contains cellular processes like cell cycle, signal transduction, metabolism, membrane transport represented in graphical format (Kanehisa and Goto 2000).

3.14 Social, Political and Regulatory Issues

3.14.1 Concerns and Compliance

Genetic engineering and genome editing in various crops have opened up possibilities of manipulating a plethora of traits which were difficult earlier to handle. Genome sequencing of pea has been published (Kreplak et al. 2019) and will help molecular

breeders to precisely edit the genes mainly dealing with biotic stresses. Biotic stress in pea is very complex which gets more complicated with climate change scenarios. Breeding of gene edited crops will be ascertained to the necessity which has arisen due to such situations, which will lead to more and more social, political and regulatory issues pertaining to utilization of genome edited crops. Gene editing techniques has its own advantages and drawbacks in crop breeding, which needs to be taken into account for better production of pea (Arora and Narula 2017). Gene editing has led to issuance of fresh guidelines of use and marketing of gene-edited crops or genetically modified organism (GMO) worldwide. As gene edited crops are more or less like naturally occurring mutants or artificially induced mutants, concern arises for better management of germplasm (Zong et al. 2019). As different countries have different regulatory mechanisms for GMOs, it is high time for evolving an integrated global regulatory mechanism for genome edited crops (Schmidt et al. 2020).

3.14.2 Intellectual Property Rights, Treaties and Conventions

Novel pea varieties developed needs to be registered and protected in international consortium for protection of breeder's rights. New crop mutants are now patented for various traits and are being registered under several regulatory mechanisms in different countries. Worldwide the food security with plant variety protection is overseen by International Union for the Protection of New Varieties of Plants (UPOV). UPOV oversees an international system of intellectual property (IP) rights that guards plant breeders' rights and reassure innovation in agriculture through the invention and development of novel varieties (Rivoire 2019). In India, plant varieties are registered under PPVFR Act 2001 (The Plant Variety and Farmers Rights Act), in which New variety, Extant variety, Farmer's variety and Essentially derived variety are the four varieties which get registered (PPVFR 2003). The authority has received around 1,200 applications for registration, with 284 new varieties application, 900 for existing varieties application and 9 farmers' variety applications. These includes pulse crops like garden pea, chick pea, pigeon pea, French bean, lentil, black gram, green gram; cereals like rice, maize, wheat, pearl millet, sorghum and other crops like cotton and jute (Kumaran and Sridharan 2009) (Fig. 3.9).

Farmer's rights towards their traditionally grown varieties and traditional knowledge are protected in India by Biodiversity Act 2002, which has been promulgated by National Biodiversity Authority (NBA), an autonomous and statutory body under Government of India. It provides equitable sharing of benefits arising from traditional biological resources not limited to plants only but other organisms as well (NBA 2002). NBA, India comes under multilateral United Nation Conventions on Biological Diversity (UNCBD) in which 196 countries are signatories. The national legislation has to be followed by all the parties—farmers, breeders and the marketing companies, which are very crucial for protecting farmer's rights. Participation of all the parties are essential to make sure the conservation and sustainable use of plant

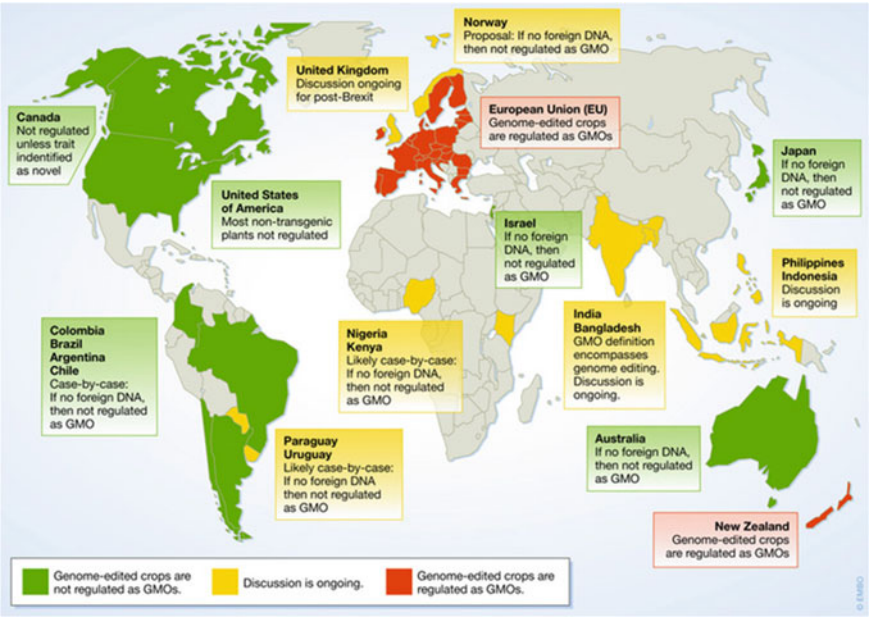

Fig. 3.9 Current situation of gene-edited crop regulations in various countries (Schmidt et al. 2020)

genetic resource are widely done and protected for sustainable development of the food and agriculture in the world (FAO 2009).

Mutual system of germplasm access and benefit sharing in food and forage crops has been established under the International treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). India is one of the signatory under ITPGRFA since 10th June, 2002. The main objective of the treaty are the protection and sustainable use of plant genetic resources for food and agriculture and the equal and equitable distribution of benefits resulting from their use for sustainable agriculture and food security, in accordance to UNCBD. ITPGRFA protected legumes covers about 29 food crops and 35 forage crop are referred in the treaty. Eight genera of pulse crops are included under these, namely *Cajanus, Cicer, Lathyrus, Lens, Phaseolus, Vigna, Vicia and Pisum* (Kochhar 2008).

3.15 Future Perspectives

Global warming and increasing human population has put a doublewhammy on the whole humanity but with the advent of modern biotechnological tools and improvement in the sector of artificial intelligence (AI) in agriculture, we could overcome these challenges faced today by the growers. Legumes are considered to be an affordable plant-based source for proteins, mainly among vegetarians. Pulse crops are

considered to be environmental friendly due to soil fertility enrichment due to its symbiotic nitrogen fixation capabilities. Importance of pulses has grown a lot as United Nations designated 2006 as the International year of Pulses, declaring the critical role of legumes in improving global food security. Various gaps and opportunities in pulse genetics research and relevance of pulse crop improvement in face of challenges like anthropogenic and climate change has to be addressed in future (Sahruzaini et al. 2020).

Lowering crop productivity and the increasing attacks of diseases and pest has made us to think more deeply with the help of precision agriculture. AI in agriculture is now being utilized in the area of weather forecasting and predictions, soil health (temperature and pH), water utility for healthy crop production, and detection of pest and diseases and application of pesticides and herbicides for maintaining better quality of crop produce (Talaviya et al. 2020). Utilization of drones for continuously monitoring the disease and pest spread helps by utilizing better IPM strategy in early phases.

Advent of novel software and hardware development in the field of AI will help the future farmers to be more climate change resilient. New-age Agri-entrepreneurs are making the most of AI and machine learning and taking the precision agriculture to new level. The number of modern age farmers becoming more tech-savvy will enhance the crop efficiency, crop productivity, and speeding up the agricultural finance for better outcome from agriculture (Faggella 2020). Agriculture Data analytics is an emerging area which helps growers to address the farm management issues by implementing improved IPM strategies for sustainable agriculture development (Coble et al. 2018).

Machine learning (ML) and deep learning is a new age concept for improving crop productivity by utilizing robotics through conventional learning process. It includes set of attributes or characteristics which are uploaded to software for analyzing the data for particular trait in crops or policy decisions for better crop management (Mcqueen et al. 1995; Raj et al. 2015; Rana and Miller 2019). Modern robotics for monitoring and analyzing the crop data for improved crop productivity will be new norm in the future farms which will effectively tackle both abiotic and biotic stress challenges (Kamilaris and Prenafeta-Boldú 2018).

Various types of morpho-physiological traits for both abiotic and biotic stress tolerance together were observed in different crop varieties including pea (Pandey et al. 2017). Combined adverse effect on crop productivity was observed due to abiotic stress circumstances like drought, salinity and extreme temperatures impacting the incidence and spread of microbes and insects (Scherm and Coakley 2003). Effects of combined stress from both abiotic and biotic stresses are mostly deleterious for crop survival itself, but sometimes genetic response curtailing both stresses are observed (Atkinson et al. 2013). Introduction of new species in non-conventional production areas are good for climate change responses but long-term repercussions in agronomy and ecology is expected (Peters et al. 2014).

Speed breeding is another recent advancement in the crop improvement research, mainly in case of shortening the breeding cycle and accelerating crop research through rapid advancement in generation time. Methods by speed breeding could

be achieved are, increasing the daily exposure of plants to light, early seed harvest, speedup cycle of seed to seed, thereby generation time is decreased for day-neutral or long-day crops. Crops like wheat, barley, chickpea, pea, and quinoa were raised under speed breeding conditions and enhanced productivity and increased generation cycles were achieved. Inclusion of next generation sequencing, genome editing and genetic selection, with speed breeding will speed-up the crop improvement rate (Watson et al. 2018; Ghosh et al. 2018).

Overall the impact of climate change has aggravated the infection levels of various pathogens. A combined effort from the conventional breeding with utilizing the modern biotech breeding tools along with nanotechnology and speed breeding will help modern day molecular breeder to design climate resilient pea varieties biotic stress ready.

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Chapter 4
Development of Biotic Stress Resistant Cowpea

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Abstract Cowpea (*Vigna unguiculata* L. (Walp)) is one of the world's major food legumes produced on almost every continent, although concentrated in Sub-Saharan Africa from where it originated. In its native range and in Asia and the Americas where it spread to and is widely grown, it has a series of disease pathogens and insect pests which constitute the most important biotic stresses of the crop. Although fungal pathogens are more numerous; bacterial, nematode and viral pathogens cause serious yield losses. This chapter reviews the biotic stresses found in cowpea and their management and control strategies along with the germplasm sources for resistance and tolerance of them as well as the genes and QTL needed to develop new varieties for them. Among the fungal diseases affecting leaves and stems are: anthracnose, Cercospora leaf spot, powdery mildew, southern blight, stem rot and rusts. Roots and plant vasculatures are affected by charcoal rot, damping off and Fusarium wilt. Bacterial blight is the main pathogen of this class; while many viruses affect cowpeas some of which are seed borne and others just insect vectored. The most important insects are aphids and leafhoppers especially as vectors, bean fly as a seedling pest; flea beetles, leaf miners, pod borers and pod bugs or leaf defoliators as mature plant pests; as well as pulse beetles or bruchids as insects of stored seed. Implications for crop improvement are provided especially given the availability of genome sequence

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and multiple molecular marker systems for the plant species. Various molecular markers like RFLPs, SSRs and SNPs have been identified and tagged for various insect-pests and disease resistant genes. These markers have played a significant role in accelerating various cowpea molecular breeding programmes like QTL mapping; Marker-assisted selection (MAS), Marker-assisted back cross breeding, association mapping etc. Host plant resistance has been an important component of integrated pest management for all these disease pathogens and insect pests and is based on collections of cowpea accessions and their screening. Sources of resistance to major constraints are listed in this review as well as how their deployment can best be managed. In addition, the future of cowpea breeding and germplasm utilization is commented upon.

Keywords Biotic stress resistance and breeding · Molecular marker · Bacterial · Fungal and Viral pathogens · Breeding for disease resistance and insect resistance

4.1 Introduction

Cowpea, (*Vigna unguiculata* [L.] Walp), is one of the important legume crops cultivated by subsistence farmers in the developing world, especially Sub-Saharan Africa, where it is used for both human and livestock consumption (Singh 2005; Timko et al. 2007). Cowpeas constitute the cheapest source of dietary protein and energy for many poor people in the tropical world and is an important marketable crop for developing countries. It is generally not grown in temperate regions such as Europe and certainly not suitable for cooler climates but is found in sub-tropical North America. Overall, cowpea is most important in West Africa, but it has taken hold in some parts of Asia as well as in East and Southern Africa; the Caribbean and South America. Within these regions most of the cowpea grown is in areas with limited access to management and resources to curb the effects of plant pests and diseases, the subject of this chapter.

The genus *Vigna*, has multiple species and several sub-species particularly in cowpea that make it a multipurpose food legume. Cowpeas (*V. unguiculata*) are eaten as green seeds and dry grains (spp. *unguiculata*), tender leaves or green pods (spp. *sesquipedelis*) or used as a vegetable (Boukar et al. 2019). In addition, subsp. *cylindrica* and some accessions of subsp. *unguiculata* provides feed, forage, hay and silage for livestock. The whole species is characterized by a high degree of resistance to drought and high temperatures. It is therefore grown mainly in arid and semi-arid regions where cultivation is an important tool for small and resource-poor farmers to protect against drought (Peksen and Gulumser 2014). Even though there is an increasing trend towards monocropping, intercropping of cowpea with cereals like maize, sorghum and pearl millet remains widespread (Monyo and Gowda 2014). The cowpea can fix atmospheric nitrogen, thereby increasing soil fertility and thus helping to increase cereal crop yields when grown in rotation/intercropping and contributing to the sustainability of cropping systems.

Unfortunately, cowpea yields are generally much lower than for cereals. This lower grain productivity of cowpea is due to number of biotic and abiotic stresses. The abiotic stresses that cause yield reduction include poor soil fertility, drought, temperature extremes, excessive moisture, late maturity, acidity and stress due to intercropping with cereals. The biotic stresses include insect pests, diseases, and parasitic nematodes. At every growth stage in life cycle of the crop there is at least one disease or insect pest that may cause economic yield losses.

Among the biotic stresses, pests pose the greatest threat to cowpea production, because the crop is heavily attacked by insects and nematodes at every stage of its development. Above all, insect pests' infestation and damage have the most negative impact on cowpea productivity in all cropping locations worldwide (Singh and Jackai 1985). Average grain yield losses range between 50 and 80% in untreated fields (Singh and Allen 1980) and can reach 90–100% under high pest infestation conditions (Jackai and Daoust 1986; Singh 2014). The extent of insect pests' infestation and the severity of their attacks and damage in cowpea field vary from one location to another and depend on the plant developmental stage. All parts of the plant can be affected by herbivorous insects and losses can occur from seedling to harvest stages. Post-harvest losses due to insects in storage are another primary constraint for most of the world's cowpea production and limit the time in storage after dry seed has been collected.

Another important biotic stress responsible for lower yield is disease. Cowpea is susceptible to many fungal diseases such as damping off in seedling stage, southern blight, root rot and leaf spot at vegetative growth. Additional important diseases of adult plants include rust, anthracnose, powdery mildew and bacterial blight diseases inflicting heavy losses (Emechebe and Lagoke 2002). Many viruses also infect cowpea and are often seedborne, such as *Cowpea mosaic virus* (CMV) which cause disease symptoms that vary with variety (Singh and Allen 1987). Meanwhile root knot nematodes affect overall growth of the plants (Das et al. 2008). The balance of different diseases varies depending on location. For example, India's production of cowpea is often beset by rust, powdery mildew, and blight as the most common diseases (Raju and Anilkumar 1990). In most cases, Indian farmers grow cowpea during high humidity of the monsoon season so incidences of these diseases are high; and the management the farmers put into the cowpea fields is minimal because they are grown as a secondary crop compared to a main cash crop of cereal. Another area of cowpea production is in Eastern Africa. In this particular region the scale of cowpea production is relatively small scale, around 1–7 ha per field with about 5–76% of that land being devoted to cowpea (Edema et al. 1997). Again, the use of management practices is limited and the instances of disease can range depending on season and farming systems, based on the intercrop, the planting time and the weather conditions. Below we summarize the biotic stresses in major production regions of Asia, Africa and the America and describe their causal agents, symptoms and management strategies.

4.2 Biotic Stresses in Cowpea

As mentioned above, biotic stresses in cowpea can be divided into disease, insect and nematode stresses. The relative important of each is discussed below as this depends on the specific causal agents of disease and or insect species/complexes that infect or infest the plants.

4.2.1 Diseases in Cowpea

Further division of diseases affecting cowpea is made between those caused by bacterial, fungal and viral pathogens with nematodes included amongst these. The symptoms to the plant and the chemical or cultural management strategies for each are given.

4.2.1.1 Fungal Diseases of Cowpea

Perhaps the most common among disease causing organisms found on cowpea, fungi can attack the plant at all growth stages and all plant parts. They include the following major fungal groups and species described below. Note that only the true fungi are considered in this section.

Anthracnose

Anthracnose is an important disease constraining the efficient production of cowpea in tropical and subtropical regions of the world. Anthracnose accounts for up to 50% yield reduction. Cowpea anthracnose was first reported in 1985 (Prasanna 1985) in India. It has been variously advanced and reported as a form of *Colletotrichum lindemuthianum*, *C. gloeosporoides*, *C. dematium* and recently as *C. destructivum* O'Gara. However, most commonly considered to be initiated by both *C. dematium* (Fr) Grove var. *truncatum* and *C. lindemuthianum*, the causal agent of the anthracnose disease in many bean (*Phaseolus vulgaris* L.) growing regions of the world (Masangwa et al. 2013). All above ground parts can be affected but anthracnose is chiefly a stem disease in cowpea. Individual lesions are lenticular to sunken, and tan to brown in colour. Lesion size and distribution depend on varietal susceptibility. Highly susceptible lines develop large spreading lesions which rapidly merge to girdle stems, branches, peduncles and petioles. The disease may be controlled by using clean seed, application of carbendazim or mancozeb (0.2% a.i.).

Powdery Mildew

Powdery mildew is one of the serious diseases of cowpea especially in the southern parts of India. Cowpea powdery mildew is important in Zambia (Kanniyan et al. 1987), Zimbabwe (Stoffella et al. 1990) USA, Puerto Rico, and other cowpea growing countries of Latin America. It is particularly important in climates with warm, dry days and cool nights. The causal agent of disease was identified as *Erysiphe polygoni* (Braun 1987). It is an obligate pathogen that establishes long lasting interactions with their host tissues. For management of disease, foliar application of Trifloxystrobin 25% + tebuconazole 50% @ 350 g/ha twice at 10 days intervals found effective in controlling powdery mildew of cowpea (Banyalet al. 2019). Varietal resistance has been investigated in Peru but few sources of resistance were found and more work is needed in germplasm screening.

Rust

Cowpea rust is caused by *Uromyces phaseolis* var. *vignae* (Barclay) and occurs in all cowpea growing areas of the world. The serious disease is reported from at least 20 countries causing 10–15% annual losses (Anilkumar et al. 1989). Rust disease in South Asia is observed in severe form only in the July sown crop during southwest monsoon resulting in considerable yield loss (Chandrashekar et al. 1989). The disease is not found in the United States to any large extent, but future spread from Africa and Latin America may occur as has for other rusts. *Uromyces vignae* is autoecious rust responsible for causing rust disease in cowpea. The fungus forms brown or black pustules on both leaf surfaces, sometimes in small concentric circles. The pustules also occur on leaf stalks and pods. The pustules are at first light green, later reddish-brown, with distinct yellow haloes. For management of this disease some of the promising Indian cowpea lines were identified against *Uromyces phaseoli* var. *vignae* Viz., IC206240, PKB6-2, V-16, EC458483, PKB6-4, EC458480, KBC-2, IC402180, 21-2, IC58905, IC249593 (Jayashree et al. 2019). Arafa et al. (2016) reported that foliar application spore suspension of *Trichoderma harzianum* and *T. hamatum* mixture @ 2 lit/100 lit of water significantly reduced rust severity. Foliar application of mancozeb @ 0.25% found effective in controlling rust caused by *Uromyces phaseoli* var. *vignae* (Kale and Anahosur 1989).

Charcoal Rot (Damping off)

A principal root-rot of cowpea is called charcoal rot. It is caused by the pathogen *Macrophomina phaseolina.* Being widely distributed in tropical and sub-tropical countries, it is a devastating disease occurring immediately on seedlings and throughout the establishment of the crop. Besides charcoal rot, the pathogen also induces symptoms of dry root rot, wilt, leaf blight and in adult stages is often referred to as ashy stem blight disease (Singh et al. 1990a, b). Epidemicsof and yield losses

from charcoal rot of cowpea have been observed in many bean growing areas around the world, including notably in Nigeria (Singh et al. 1990a, b). The disease is also important in the Southeastern United States, with the effects are more important about two weeks after the onset of a drought event, even if this occurs mid-season instead of at early or terminal growth stages. Stem rot or root rot caused by *Macrophomina phaseolina* (Tassi) Gold. has been rated as one of the most devastating disease in cowpea. The fungus invades the host both inter and intracellularly, it grows rather fast covering large areas of the host tissue and eventually killing it in short order. Often the conidial or pycnidial stage is produced on the host. Symptoms include leaf withering along with whole plant drying and roots rotting followed by sudden decline and death of the whole plant. The infected roots usually show brown to black lesions on stem. The cotyledonary leaves can appear completely blighted and necrotic. Stem decay in advanced stages of the disease destroys young seedlings to adult plants. *M. phaseolina* produces sclerotia in root and stem tissues of its hosts which enable it to survive adverse environmental conditions. Microsclerotia in soil, infected seeds or host tissues serve as primary inoculum. They can survive for 2–15 years in the soil depending on environmental conditions (Baird et al. 2003). Most of the described control methods aim to reduce the number of sclerotia in soil or to minimize the contact of the inoculum and the host, and include breaking a crop cycle with a non-legume rotation. Soil moisture content greatly affects the sensitivity of resting structures to heat treatment and one summer irrigation was sufficient to reduce the population of *M. phaseolina* by 25–42% (Lodha 1995). Good sanitation of field equipment, their tires and even shoes can help to limit the spread of microsclerotia.

Management: Sources of resistance to some soilborne pathogens have been identified, but highly resistant cultivars are often not available for polyphagous and unspecialized pathogens like *M. phaseolina.* Ahmed et al. (2012) reported that cowpea cultivar ITO4K-217-5 from Nigeria resistant to *M. phaseolina.* Screening in the Southern USA of germplasm from the core collection of USDA cowpea entries has resulted in a few sources of resistance, although levels of resistance tend not to be very high. Management strategies to control charcoal rot also include the use of biocontrol agents to prevent host infection or to suppress the growth of the pathogen. Sendhilvel et al. (2005) found that soil application of talc formulation of SVPF2 isolate of *Pseudomonas fluorescens* reduced the root-rot incidence significantly in green house condition. Seed treatment of Pencycuron @ 0.7 ml/Kg as along with two spays of tebuconazole (0.7%) at thirty days interval was most effective in managing the root rot disease of cowpea.

Southern Blight or Stem Rot

Southern blight, is a common stem disease of cowpeas worldwide. Fery and Dukes (2002) observed that the impact of southern blight on cowpea yield may be more attributable to reduced plant vigor than to plant mortality per se. They reported that the disease can cause dry-seed yield loss of up to 53.4%. The disease is caused by the fungal pathogen *Sclerotium rolfsii*. According to Stephen and Rebecca (1992)

Sclerotium rolfsii grows, survives and attacks plant at or near the soil surface. The first observed symptoms are usually a general wilting and yellowing of plants, followed by drying of foliage and plant death. Plants with advanced disease development characteristically exhibit tan to brown sclerotia and white mycelial growth on the stem epidermis at the soil surface (Karat et al. 1985). The disease is best known for its girdling of the stem with a large set of lesions found on the hypocotyl near the soil line. Apart from causing basal stem rot, the pathogen is known to cause pod and branch rot.

Management: Tanimu et al. (2018) evaluated five cowpea varieties (L-25, Ife brown, IT89-KD-374, IT89-KD-434 and IT86-D-715) for resistance to basal stem rot disease and found that three varieties viz., L-25, IT89-KD and IT86-D-715 were immune to infection by the pathogen. Presently, many chemicals can inhibit *S. rolfsii*, includes Carboxin + thiram, Captan, Carbendazim and Mancozeb etc. Addition of antagonistic fungi to soil, is an interesting method for management of *S. rolfsii*. Several researchers have reported the inhibition of soil-borne pathogen by *Trichoderma* species such as *T. virens*, *T. harzianum*, *T. atroviride* and *T. asperellum* etc. (Wijesinghe et al. 2010).

Fusarium Wilt

Fusarium wilt is a major constraint to cowpea production throughout the world and particularly in the Indian subcontinent and the Mediterranean basin (Nene et al. 1989) and parts of the USA, especially in the areas with intermittent drought stress or low rainfall during the season and requirements for supplemental irrigation (Fery 1985). The Fusarium wilt of cowpea is thought to be caused by *Fusarium oxysporum* f. sp. *tracheiphilum*. Fusarium wilt was first reported in cowpea from USA quite some time ago. In India it was first reported by Singh and Sinha (1955). The pathogen is soil-borne and probably also seed transmitted. Infection by *F. o.* f. sp. *phaseoli* is also possible in cowpea but uncommon because common bean its main host is not usually grown in the same areas as cowpeas and therefore specialization of the *Fusarium oxysporum* has occurred. The fungus can remain endophytic in the seeds as dormant mycelium or chlamydospores without causing disease. Fusarium wilt usually causes the lower leaves on one side of the plant to turn yellow. Infected plants usually are stunted and wilted as the organism develops in the sap and water conducting tissues of phloem and xylem. Brick red tissue can be observed as streaks along the vasculature inside the stem when it is split lengthwise.

Variants of the pathogen: Currently, three races of *F. oxysporum* f. sp. *tracheiphilum* are known: Races 1 and 2 were described in South Carolina. Race 1, in addition to cowpea also attacks soybean [*Glycine max* (L.) Merr.] and chrysanthemum (*Chrysanthemum morifolium* Ramat), according to Armstrong and Armstrong (1950), and Race 3 was described in Mississippi (Hare 1953). Resistance to the three races has not been well studied in cowpea varieties, so most are considered susceptible.

Management: *Rhizobium* species are symbionts with an important role in plant productivity and nutrition but also in plant disease resistance, namely against soil-borne diseases. *Bradyrhizobium japonicum* isolates S2, S3 and S4 from cowpea showed inhibition of 11, 15 and 19 mm of inhibition respectively (Kannan et al. 2019).

Cercospora Leaf Spot

This leaf spot has a widespread distribution and occurs all over the world. It causes leaves to fall off and serious yield losses of up to 40% in cowpea. The disease occurs on other legumes, including closely related plants such as mungbean (*Vigna radiata* L.), common or 'true' beans (*Phaseolus*) and soybean (*Glycine max* L.). Two species of *Cercospora*, *C. cruenta* and *C. canescens* have associated with leaf spot diseases in cowpea. Bird and Maramorosch (1975) reported that *C. cruenta* and *C. canescens* caused severe leaf spotting and defoliation in cowpea at Ibadan. Cercospora leaf spots of cowpea begin as small, lighter colored areas, almost yellow. Later they become bronze to dark grey, roughly circular to more elongated and up to 10 mm across. The fungus produces masses of wind-borne spores on the lower surface of the leaf giving the spots a distinctive grey to dark powdery appearance. When held up to the light the older leaf spots are darker, more reddish and often with a distinct ring. Dead areas fall out, giving a shot-hole appearance. The leaf withers as the spots join together. Leaves die and fall off.

Management: Mancozeb should be applied after the crop has flowered and pods are starting to develop, with a maximum of 2–3 applications per planting season. However, foliar application of Trifloxystrobin 25% + tebuconazole 50% @ 350 g/ha twice at 10 days intervals found effective in controlling the disease (Banyalet al. 2019). Unfortunately, no resistance sources for defense against Cercospora leaf spot are known in cowpeas.

4.2.1.2 Bacterial Diseases

Bacterial Blight

Bacterial blight of has been identified as the most important biotic constraint to cowpea production worldwide. Okechukwu and Florini (2000) have reported yield depressions of 42–71% in pod, 43–68% in seed and 29–53% in fodder. Bacterial blight is caused by *Xanthomonas campestris* pv. *vignicola.* Initial symptoms are tiny, water-soaked dots under the leaf. These vary from pinpoint size to more than 1.25 cm in diameter, with a yellow halo. They often expand, join up and develop into large necrotic lesions. The pathogen also invades the stem, causing cracking with brown stripes, and the pods, where they manifest as dark green, water-soaked areas. Infected seeds are discolored and shriveled. In a severe infestation, pod development is poor and most of the seeds are shriveled and unable to germinate. The bacteria

can remain viable for nearly 400 days in infected seed and debris at temperatures of 5–10 °C, and for 250 days at temperatures between 10 and 40 °C. The pathogen can survive in the soil for 260 days at 10 °C and 100 days at 40 °C (Alina 2017).

Management: A combination of extracts of pawpaw, neem and red acalypha reduced bacterial blight disease incidence by 73.68% and improved yield by 1.58 tons/ha (Ganiyu and Akinola 2017). Nandini and Kulkarni (2015) reported that hot water treatment + seed treatment with *Pseudomonas fluorescens* (0.5%) + foliar spray of *Pseudomonas fluorescens* at 25 days and streptocycline + copper oxychloride (0.05 + 0.3%) spray at 45 days, reduced the severity of cowpea bacterial blight significantly and improved both the germination per cent and yield.

4.2.1.3 Viral Diseases

The diseases caused by viruses, many of which are seed-borne, have been responsible for great damage in cowpea crops around the world, causing serious losses in crop yield in several countries. Viral diseases have been considered one of the most important sanitary problems in cowpea causing serious reductions in crop productivity. Worldwide, up to 20 viruses have been reported to occur in cowpea. In India, a total of 8 viruses have been identified: namely, *Alfalfa mosaic virus* (AMV), *Cowpea chlorotic mottle virus* (CCMV) *Cowpea mosaic virus* (CPMV), *Cowpea mild mottle virus* (CPMMV), and *Cowpea yellow mosaic virus* (CPMV) and *Southern bean mosaic virus cowpea strain* (SBMV-CS), as well as two important potyviruses: *Blackeye cowpea mosaic virus* (BLCMV), and *Cowpea aphid-borne mosaic* (CABMV). Each of these viruses can be distinguished only based on molecular diagnostics, serology, transmission tests and/or symptomatology reactions on diagnostic hosts. CABMV is one of the most damaging viruses in West Africa (Neya et al. 2015). Cowpea severe mosaic virus is found in the Caribbean (Booker et al. 2005). Potyvirus infections are important where there is no seed certification program or where seed is saved by farmers.

Cowpea Aphid-Borne Mosaic Virus

Cowpea Aphid-Borne Mosaic Virus (CABMV) is the most important viral disease of cowpea in South Asia and is responsible of important crop losses ranging from 15 to 87% depending on cowpea varieties and the plant age at infection (Thottappilly and Rossel 1992). *Cowpea mosaic virus* (CPMV) is the type member of the Genus Comovirus (Pouwels et al. 2002). It infects various legume species but multiplies most in its natural host, *V. unguiculata.* The genome of CPMV consists of two separately encapsidated positive-strand RNA molecules of 5,889 nucleotides (RNA-1) and 3481 nucleotides (RNA-2), both of which are required for infection.

Symptoms and transmission: Initial symptoms include mottling, interveinal chlorosis and vein-banding. As the disease develops, leaf cupping occurs. Later, leaves became further distorted and developed necrotic lesions. Infected plants

remained stunted and bushy, and flowering is retarded or inhibited. CABMV is readily transmitted by aphid vectors but also by sap inoculation. The aphid species reported to be vectors of CABMV are *Aphis craccivora, A. gossypii, A. spiraecola, A. fabae, A. sesbaniae, Macrosiphum euphorbiae, Myzus persicae, Rhopalosiphum maidis and Acyrthosiphon pisum* (Atiri et al. 1984). *Aphis craccivora* is a widespread and common pest of cowpea in many countries of Africa and in India (Singh and Allen 1979). Seed transmission depends mainly on gamete infection by the virus and plays an important role in the epidemiology of CABMV. Seed transmission occurred only when the mother plants were inoculated with CABMV at least 20 days before flowering whereas CABMV was recovered from pollen only when the mother plants were inoculated at least 17 days before flowering (Tsuchizaki et al. 1970). Seedborne infection is expressed in the primary leaves which will show vein-clearing or yellowing, diffused chlorotic spots or patches on leaves, and finally intense chlorosis on all foliage. In trifoliate leaves, the symptoms are usually more distinct and include vein-yellowing/banding or variable degrees of yellow mosaic with or without dark-green or somewhat irregular blistering (Bashir and Hampton 1996).

Management: Being a seed borne pathogen, that is easily transmitted by aphids, several control techniques are in practice and vary from the planting of resistant varieties to cultural methods of control. The virus can be controlled through cultural practices which include early sowing and intercropping of cowpea with cereals, possibly leading to decreased virus incidence. The use of virus-free seed grown in quarantine areas such as under cooler temperatures unfavorable to aphids is important, particularly in preventing spread to new areas (Zettler and Evans 1972). Excellent sources of resistance to CABMV have been identified among cowpea germplasms. In West Africa, Cisse et al. (1997) reported an extra early maturing cowpea line PI 596353 that was not only resistant to CABMV but also to the aphid vector (*A. craccivora*). Bashir and Hampton (1996) tested 51 cowpea lines by mechanical inoculation under greenhouse conditions against seven CABMV geographical diverse isolates, and identified TVU-410, TVU-1582 and TVU-1593 as immune to all seven isolates. IITA and the University of California at Riverside have had programs to identify resistance and to characterize the genes underlying resistance to multiple cowpea viruses.

Control through seed certification: Certification against seedborne viruses such as CABMV is one of the methods which minimizes their spread and it must be used in the production of certified seed. The seed certification program should be started at the basic level of the germplasm collection available to the plant breeders and continue through the subsequent development of varieties. Moreover, such programs must also take into consideration other means by which a seed-borne virus may be disseminated in the standing crop. The major method of monitoring the presence of seedborne viruses, i.e., visual inspection, should be followed in the standing crop.

4.2.1.4 Balance of Viral and Fungal Diseases

Prevalence of viral and fungal pathogens varies and are affected by predominant cropping patterns, agroecologies and climates. Two case examples are discussed below.

East Africa

In Uganda, as representative of East Africa, some of the most common diseases are cowpea mosaic virus, cowpea bacterial blight, Cercospora leaf spot, and pod mold (Edema et al. 1997). Differing factors go into the spread and severity of each of these biotic stresses in Ugandan cowpea. Depending on the season and number of rains the incidence of fungal diseases sees higher levels earlier with wetter rains. Rust sees higher levels later with drier rainy seasons. Additionally, growing practices influence the severity of disease occurrence. For viruses, the presence of the disease is not affected by intercropping as much as many of the other diseases like rust or powdery mildew (Edema et al. 1997). Vectors are key (Whitney and Gilmer 1974).

South Asia

In India, cowpea is often grown after rice. This practice, of growing cowpea as a secondary crop, limits the farmers in their ability to manage diseases in cowpea. One area in which resistance can be managed more easily, without the need for intensive farmer intervention or additional techniques outside of planting is to use different varieties (Raju and Anilkumar 1990). The deployment of cultivars could provide resistance for cowpea against many stresses. As mentioned earlier, anthracnose, powdery mildew and CABMV are major pathogens.

4.2.2 Insect-Pests of Cowpea

4.2.2.1 Black Bean Aphid-*Aphis craccivora* (Aphididae: Hemiptera)

Geographic distribution: Cosmopolitan, India, Argentina, China, U.S.A., Europe, Australia, Philippines, Thailand, and throughout much of tropical Africa and Latin America.

Host range: The aphid is polyphagous and reported hosts are *Cajanus cajan* (pigeon pea), *Arachis hypogaea* (groundnut), *Medicago sativa* (alfalfa), *Vigna radiata* (mung bean), *Capsicum* (peppers), *Chenopodium quinoa* (quinoa), *Cicer arietinum* (chickpea), *Citrus* (oranges and limes), *Gossypium* (cotton), *Lablab purpureus* (hyacinth bean), *Lupinus* (lupins), *Lycopersicon esculentum* (tomato), *Phaseolus vulgaris* (common bean), *Sesamum indicum* (sesame), *Solanum*

tuberosum (potato), *Theobroma cacao* (cocao), *Trifolium* (clovers) *Viciafaba* (broad bean), and *Vigna mungo* (black gram).

Biology: *A. craccivora* is aholocyclic almost everywhere, with only females (winged and wingless) normally encountered and parthenogenetic reproduction occurs all year round. The aphid is ovoviviparous and the females retain eggs inside their bodies and give birth to small nymphs. Young colonies of these small aphids are found on growing points of plants in association with ants (Soans and Soans 1971; Patro and Behera 1991). The female may produce 8–30 young ones in a life span of 10–12 days. The nymphs transform into adult in 5–8 days after passing through four nymphal instars.

Damage symptoms: The aphids, both nymphs and adults suck sap from leaves and stems which are mostly confined to lower parts of the plant and ventral surface of leaves. Under heavy infestation the plant to turn yellow, die and drop off. The seedling stage is more prone to aphid damage even under light infestations. The aphid secretes honeydew on which sooty mould, grows which deteriorate the quality of fodder.

4.2.2.2 Pod Bugs

Riptortus pedestris, R. dentipes, Clavigralla gibbosa, C. tomentosicollis Anoplocnemis phasiana (Coreidae: Hemiptera).

Geographic distribution: *R. pedestris* prevalent in Asian countries like India, China, Myanmar, Sri Lanka, Pakistan, Japan, Malaysia, Cambodia, Vietnam South Korea, and Taiwan. *R. dentipes* restricted to African countries like Benin, Ghana, Kenya, Malwai, Nigeria, Tanzania and Uganda. *Clavigralla tomentosicollis* is widespread in sub-Saharan Africa and the *Clavigralla gibbosa*, is limited to India and Sri Lanka (Sharma et al. 2010).

Biology of *R. pedestris*: Pod bugs are the most prominent post-flowering insect pests of cowpea at the podding stage. The adults paired 2–3 days after emergence and preoviposition period ranges between 12–13 days. Each female laid an average of 115 eggs preferably on the pods. The oviposition period lasted about 30 days, and adults lived for a total of about 45–47 days.

Damage symptoms: The nymphs and adults of pod sucking bugs suck sap from the shoots and unripe seeds from the green pods. The shoots fade, pods shrivel and seeds with dark patch loose germination capacity due to the feeding of bugs. Under severe infestation, the tender parts get shriveled and later dry up. The bugs are seen clustered around on the pods.

4.2.2.3 Leaf Miner, *Phodoryctis caerulea*

Geographic distribution: Widespread in Afrotropica region include: Cape Verde, Réunion, Uganda, West Africa (De Prins and De Prins 2019); Mauritius (Mamet and Williams 1993) and Madagascar; in the Oriental region recorded from India, Japan,

Malaysia, Taiwan, Indonesia and from Oceania recorded in Fiji, Guam and Solomon islands. In the last twenty year *P. caerulea* reported as major pest on cowpea.

Host range: De Prins and De Prins (2019) documented more than 20 hostplants records for this pest, mostly from Asia. Most of host plants belong to Fabaceae, major host plants are cowpea (*Vigna unguiculata*), black gram (*Vigna mungro*), mungbean (*Vigna radiata*), broad bean (*Vicia faba*), French bean (*Phaseolus vulgaris*), soybean (*Glycine max*), *Centrosema*, *Pueraria* and *Crotalaria.*

Biology: The adults are small and dirty white color with smoky forewings. The antennae longer than the length of the body and end of the wings are fringed and upturned. The immature larva is flattened, carrot-shaped. The larvae were greyish-pink to light reddish in color when reared on Fabaceae plants, but the larvae are light green in color when reared on other host plants. The pupa develops inside a flat silky cocoon on crop plants and survives as pupae in plant remains, in soil after harvest or on weeds.

Damage symptoms: The larva does the damage by burrowing in the upper surface of the leaf. Upon hatching, the larva makes a narrow winding snake-like mine, and size of mining increase with age of larvae. Among Fabaceae, cowpea was most susceptible, and damage increases with age of the crop.

4.2.2.4 Asian Pulse Beetle, *Callosobruchus chinensis* (Bruchidae: Coleoptera)

Geographic distribution: *C. chinensis* has been first described in China during 1758. The pest is commonly found in Africa, China, Burma, USA, Philippines, Japan and India. In India, it is prevalent in almost every part of the country. *C. chinensis* originated in tropical Asia where it is still the dominant species.

Host range: The beetle reported to damage all whole pulses, beans and grams. It majorly feed on *Cajanus cajan* (pigeon pea), *Cicer arietinum* (chickpea), *Glycine max* (soyabean), *Vigna mungo* (black gram), *Vigna radiata* (mung bean) and *Vigna unguiculata* (cowpea). In addition to pulses, the beetles also known to feeds on cotton seed, maize and sorghum.

Biology: The adults are small, brownish grey beetle with characteristic dark stripes on dorsal elytra. The elytra don't cover the abdomen exposing the pygidium. The female adult is slightly bigger than the male. Females lay many eggs up to 115 eggs. The beetle lays eggs on maturing cowpea pods. The eggs are small and oval to spindle shaped, individual eggs are glued over the surface of the grain. Fresh eggs are translucent, orange cream in color, changing to greyish white with age. The egg hatches within 4–5 days after laying. Just after hatching, the grub bore onto the grain, thereafter feed inside the grain and complete its larval and pupal stage. The average larval and pupal duration vary between 10–38 and 4–28 days, respectively. It takes 117–168 days for the hibernating larvae to complete their growth.

Damage symptoms: The infestation begins before the mature pods are harvested from the main field. However, in the main field, the pest does no or least harm, but they do severe damage in storage. Both grubs and adults of pulse beetle feeds on

whole content of the grain, leaving only the shell behind. The damage due to pest is severe that every grain in a lot or bag is infested. Infested stored seed can be recognized by the white eggs on the seed surface and the round exit holes with the 'flap' of seed coat. The infested grains are unfit for human consumption and also unfit for sowing. The damaged grain is often converted into flour.

4.2.2.5 Green Leafhopper: *Empoasca kerri, E. binotata, E. flavescens* (Cicadellidae: Hemiptera)

Geographic distribution: *E. kerri* documented from Asian countries like India and Bangladesh and also reported from USA. It is one of the destructive pests in north-western region of India. Whereas *E. flavescens* reported from whole of European continent, Asian, South Asia and South-east Asian countries.

Host range: Greengram, blackgram, groundnut and cotton, pigeon pea, maize, castor, cluster bean, rice bean, faba bean, lentil, *Dolichos* bean, Sunflower, Safflower etc.

Biology: The adults are elongated wedge-shaped green insects with average life span of 21–33 days. An adult female of *E. kerri* laid an average of 15.7 eggs. The hopper lays eggs on the underside of the leaves and the incubation period of eggs varied from 4 to 7 days. The *E. kerri* passed through five nymphal instars and the total nymphal period varied from 10 to 17 days. The winged adults jump at the slightest disturbance and are positively phototactic in nature.

Damage symptoms: The nymphs and adults feed on tender leaves and other parts of the plant by sucking the plant sap. In cases of severe attack, leaves become brittle and dry. Characteristics hopper burn i.e., cupping of leaves appears. The plant may lose its vigor resulting in poor growth. Under severe infestation, the plants show stunting and resulted in pre-mature drying of the plant.

Spotted pod borer, *Maruca vitrata* (testulalis) (Geyer) (Lepidoptera: Pyralidae)

Geographical distribution: It is the most important pantropical lepidopteran pest of cowpea, reported to be present over 100 countries of Asia, Africa, South America and Australia.

Host range: It is a major pest of leguminous crops like pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), soybean (*Glycine max*), jack bean (*Canavalia ensiformis*), *Crotalaria*, *Jewelvine*, *Derris*, hyacinth bean, *Lablab purpureus*, tropical kudzu, *Pueraria phaseoloides*, lima bean (*Phaseolus lunatus*), common bean, (*Phaseolus vulgaris*), hoary-pea, *Tephrosia.*

Biology of *M. vitrata*: The adults are small and less than inch large at the wing tips. The adult moths have dark brown forewings with three white spots and a brown border on grayish white hind wings. The female moths usually deposit her eggs on flower buds, although leaves, flowers, and abscission scars also serve as oviposition sites. Each female is capable of laying about 400 eggs either individually or in overlapping group of 2–14. The eggs were white in color and dorso-ventrally flattened. The incubation period varies between 2–5 days. The larvae are cream white in color with

dark brown head and prothorax. The later instar larvae (II–V) have characteristic six rows of black spots. The larval and pupal duration vary between 8–14 and 6–9 days, respectively. The pupation may occur within the larval web or in the soil within a pupal cell made by the final instar larvae and covered it with debris. The total life cycle of *M. vitrata* is typically 18–25 days, sometimes can be as long as 57 days.

Damage symptoms: The larvae webs together the foliage including the flowers and pods and feeds on them. During vegetative growth, the young larvae feed on tender plant stems, terminal shoots and peduncles and start feeding on flowers as plants mature. The larvae feed inside a maturing pod and rendering them unmarketable. At the entrance of larval furrow larval webbing, mass excreta can be seen. The larvae cause extensive damage to floral buds and flowers, resulting in discoloration and dropping of flower. The larvae emerged from the webbed area and fed during the night, then returned to the shelter as morning approached due to their strong photonegative response. The concealed nature of larvae (the damaging stage) and pupal stage complicates management by chemicals or other conventional means.

4.2.2.6 African Pulse Beetle, *Callosobruchus maculates* (Coleoptera: Bruchidae)

Geographical distribution: Like the other important species of bruchid beetle are *C. maculatus* is widely distributed throughout the tropics and sub-tropics. *C. maculatus* originated in Africa where it remains dominant but both are reported present in India.

Host range: Bruchids are very important pest of grain legumes both in storage and field. *C. maculatus* is a major pest of cowpeas, green gram and lentils. Although the bruchid beetle has been recorded to consume a variety of beans, the developmental period can vary. The previously described *C. chinensis* is a major pest of even more legumes: chickpeas (Pandey and Singh 1997), lentils, green gram, broad beans, soybean (Srinivasacharyulu and Yadav 1997; Yongxue et al. 1998) adzuki bean and cowpeas in various tropical regions.

Biology: The adult bruchid beetles are oval in shape, chocolate or reddish brown in color with characteristic dark stripes on dorsal elytra. The elytra don't cover the entire abdomen exposing the pygidium. Each female can lay 30–130 eggs. Female bruchid attach eggs individually to the exterior surface of seeds, but they do not oviposit on broken or damaged seeds. The eggs are small and oval to spindle shaped and hatches within 4–5 days of laying. Hatching larvae penetrate grains, feed inside the grain and complete development within the seeds. The average larval and pupal duration vary between 10–38 and 4–28 days, respectively. It takes 117–168 days for the hibernating larvae to complete their growth.

Damage symptoms: Seed beetles attack cowpeas both in the field and storage condition, however the pest causes no or least damage in the main field condition. They do serious damage in storage. Both grubs and adults cause damage by eating out the entire content of the grain, leaving only the shell behind. The infested lot becomes unfit for human consumption and such infested grains are often converted into flour. In addition, the damaged seeds are also unfit for sowing. The reduction

in seed weight due to bruchids is directly proportional to the number of holes or "windows" produced in seeds. Losses in seed germination due to bruchid attack may reach 100% for grains with four holes per seed.

4.2.2.7 Bean Fly, *Ophiomyia phaseoli* (Diptera: Agromyzidae)

Geographical distribution: A tropical and subtropical species that occurs in Australia, Africa, Asia and the Middle East.

Biology: The adults are shiny black flies, 2 mm long, with clear wings, about 5 mm wide. The eggs are white, oval, laid on the lower leaf surface near the leaf stalks of the tender young leaves. Each female fly laid an average of 100–200 eggs. The egg hatches on the leaf and the small white maggot bores down through the stem. There are three stages over about 10 days, and then the maggots pupate; this occurs at the stem/root junction (or in older plants at the junction of leaf blade and leaf stalk). Pupation lasts about 10–12 days depending on temperature.

Damage symptoms: After hatching the maggots mine the leaf and bore into stem tissues to tunnel in the pith. This behavior disrupts the normal transport of water and nutrients and results in seedling mortality. The larval mines better seen on the underside of the leaves just under the epidermis and appear as silvery, curved stripes; on the upper side of the leaf only a few tunnels are visible. Under severe attack, the infested leaves become blotchy and later hang down. The later instar larvae continue to feed downwards into the tap root and returns to pupate still inside the stem, close to the soil surface. The feeding tunnels are clearly visible on the stems.

4.2.2.8 Minor Pest of Cowpea

Flower bud thrips

A small insect *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), is an important pest of cowpea in sub-Saharan Africa (Togola et al. 2019).

Flea beetle, *Pagria signata*

The adults vary in color from shiny brown to dark brown with strips on the elytra. They have stout femora with which they jump in a flea like manner. The eggs are laid in the soil near the vicinity of the host plants. The larvae feed on the roots and generally do not cause economic damage. Pupation takes place in the soil.

Cowpea Curculio Weevil, *Chalcodermus aeneus* Boheman

Originally from the Caribbean, this large weevil is unique in that as an adult it destroys whole plants especially when it emerges from the ground and attacks cowpeas in seedling stage, reducing plant stand and eventually yield. In addition, the larvae of the Curculio hatch inside pods and destroy most seeds found in it before diapause in soil over the winter or dry periods. The species has spread to the Southeastern United States and ranges from Florida to Georgia and the Carolinas, with western expansion to traditional states for cowpea production. Early season scouting programs, good sanitation and removal of crop residues along with use of pesticides have been the best control methods for curculio where the insect has become established (N'Guessan and Chalfant 1990).

Blue Butterfly: *Lampides boeticus* (Linnaeus) (Lycaenidae; Lepidoptera)

The main host of the pest includes Redgram, cowpea, lab, and other legumes. The eggs are deposited singly or in groups on flower buds, green pods, shoots and leaves. The larvae are pale green in colour, flat and slightly rounded, hairy and slug like. Upon hatching the larvae enter unopened flower bud and feed inside. Afterwards they may attack another flower or enter a pod and feed on the developing seeds. Pupation takes place on leaves, twigs and pods.

4.2.2.9 Defoliator Complex

Semi Looper, *Plusia nigrisigna*

The adults are dark having dark-brown and dirty white patches on the forewings. The moths are very active at dusk. The eggs are laid on the leaves of the plant. The caterpillars cause damage as they move and form characteristic half-loops.

Tobacco Caterpillar, *Spodoptera exigua, S. litura*

The adults are medium-size, stout bodied with pale gray to dark brown forewings having wavy white markings and whitish hind wings. The larvae are mainly defoliators. The early instars are gregarious and scrape the chlorophyll content of leaf lamina giving it a papery white appearance. Larvae feed for first few days and then disperse to feed individually. Later irregular holes are made on the leaves.

Pod Borer, *Helicoverpa armigera*

Fore wing of the adults are gray to pale brown in color with characteristic V shaped speck. The eggs are spherical and creamy white and laid individually on flower buds. The larval color varies, commonly greenish to brown with dark brown grey lines laterally on the body. The pupae are brown in color and occur in soil, leaf, pod and crop debris. Defoliation in early stages and later feed on seed larva's thrust head inside the pods and the rest of the body hanging out and make round holes.

4.2.3 Management of Insect-Pests

4.2.3.1 Cultural Methods of Control

Simple modification of a pest's environment or habitat often proves to be an effective method of pest control. The advantages of cultural control over other more intensive or dangerous methods of control such as pesticide use are evident. Cultural methods are possibly the oldest control system adopted by cowpea farmers all over the world. Moreover, it can be very efficient in cowpea pest management programs, if adopted at community level (Karungi et al. 2000; Asante et al. 2001). Cowpeas in Africa are generally grown as a companion crop with maize, sorghum, finger millet and other crops. In Asia and North America, cowpeas are grown in rotation after rice and winter crops such as canola or wheat, respectively. As a result, studies on cultural control have tended to concentrate on mixed cropping or rotations. From the farmers' point of view, the use of cowpea as an intercrop or rotation crop can be attributed to tradition, land shortage, better cereal yields, more crops at harvest, increased soil fertility and insurance against total crop failure rather than reduction in pest infestations (Isubikalu et al. 1999). Amoako-Atta et al. (1983) reported reduced incidence of *M. testulalis* when cowpea was intercropped with maize or sorghum. Nampala et al. (1999) reported lower incidence of aphids and thrips when cowpea intercropped with sorghum and green gram. However, cowpea and groundnut are usually not good companions, due to the risk of the aphid *A. craccivora* spreading from cowpea to groundnut and vice versa. Proper preparatory cultivation practices such as deep summer ploughing helps in exposes all resting stages of insects, such as larva and pupa to abiotic and biotic factors. It helps in removing quiescent pupae of *H. armigera*, *Lampides boeticus* and *M. vitrata* from the field. Changing planting dates has been used as a strategy to reduce pest damage in a number of crops (IITA 1982; Ezeuh 1991). Sowing of cowpea immediately after the first rainstorm of the season reduced aphid infestation, but had no significant effect on thrips, legume pod borers and pod sucking bugs infestation. In addition to the time of planting, population density have both positive as well as negative effect on the pest population. Edema et al. (1997) and Karungi et al. (1999, 2000) reported close spacing consistently lowered aphid infestation. The denser plants provide increases soil cover and reduce the strength of the visual contrasts between the earth and plants (Kennedy et al. 1961;

Pettersson et al. 1998). In contrast, Gethi and Khaemba (1991) reported that there were slightly more thrips, legume pod borers and pod sucking bugs in the closely spaced plants than the sparsely spaced ones.

4.2.3.2 Host Plant Resistance (HPR)

Host plant resistance is an environmental friendly, cost effective and sustainable pest management option for minimizing the pests' incidence and severity. It is the most suitable approach for the resource-poor, small-scale farmers who cannot afford to purchase insecticides. For instance, the dense and long trichomes on some cowpea cultivars were found to increase their resistance to the pod borer *M. vitrata* (Oghiakhe et al. 1992; Oigiangbe et al. 2006) and also to the pod sucking bugs (Oigiangbe et al. 2002, 2006). Pod wall strength and hardness are considered as important traits for resistance to pods borers (Oigiangbe et al. 2002) and potentially for the Cowpea Curculio as well (N'Guessan and Chalfant 1990), since predation and oviposition, respectively can be inhibited by strong thick pod walls in each case. Togola et al. (2020) documented TVu-6464, TVu-1583, and TVu-15445 genotypes resistant to cowpea aphids. They reported that both low sucrose levels in the plant, as well as high levels of kaempferol and quercetin, aglycones of phenolic compounds, were related with high resistance to aphids. Even though several workers documented multiple resistant sources for aphids, they are mostly controlled using the more highly effective method of pesticides since aphids act as frequent vectors of viral diseases.

4.2.3.3 Natural and Biological Control

The pest populations under field conditions are naturally regulated by unsuitable environmental conditions and natural enemies. For instance, aphid and thrips population scan be completely washed away by rainfall and become easy prey for soil dwelling predators. Persistent rain increases the relative humidity around the plants thereby promoting the function of entomophagous fungi. Since we cannot have control over weather conditions and rain is a density independent mortality factor that cannot be relied upon as a strategy in pest management.

Biological control of insect pest is the best alternative technique for replacing reductionist chemical pesticide control methods and their effects on the non-target organism and environment. Despite comprehensive research on cowpea pests, natural enemies and microbial agents have received little attention. Furthermore, the data that is available has yet to be successfully implemented into pest control strategies. Several predators, parasites and microbial agents of potential importance in cowpea pest suppression have been reported by various workers. Ofuya (1997) documented seven coccinellid predator were predating on cowpea aphid viz*., Coccinella undecimpunctata, Metasyrphus corolla, C. carnea, Scymnus* sp., *Orius* sp., *Cydonia vicinaisis* and *P. alfierii*. Among the reported species *C. undecimpunctata* was the dominant

predator species with a rate of occurrence reached, 30.92%. *C. carnea* represented the second one with a percent occurrence of 18.44%. Parasitization of lepidopterous and coleopterous pests (Caswell and Akibu 1980; Don-Pedro 1983), in addition to that of the bean fly (Greathead 1975) has also been reported. In Iran, Rakhshani et al. (2005) collected *A. craccivora* colonies from different host plants and documented about eight hymenopteran parasitoids emerged from the field collected aphids viz., *Aphidius colemani* Viereck, *Lysiphlebus fabarum* (Marshall), *Lysiphlebus confusus* Tremblay and Eady, *Lysiphlebus testaceipes* (Cresson), *Binodoxys acalephae* (Marshall), *Binodoxys angelicae* (Haliday), *Praon volucre* (Haliday) and *Ephedrus persicae* Froggatt. Among the reported parasitoids, *L. fabarum* was the most common parasitoid emerged from aphids collected from different host plant.

For yardlong bean (vegetable cowpea) in Taiwan, Huang et al. (2003) monitored natural enemy complex of *M. vitrata* and reported three braconids viz., *Apanteles taragamae* Viereck, *Bassus asper* Chou and Sharkey and *Dolichogenidea* sp., three ichneumonids namely *Trichomma* sp., *Triclistus* sp., and *Plectochorus* sp., two unidentified tachinids, one unidentified predatory staphylinid and three entomopathogenic fungi: *Fusarium* sp., *Paecilomyces* sp. and *Beauveria bassiana.* Several researchers documented the natural enemy complex of cowpea pests from different regions but none of the biological control of cowpea pests has failed to quantify the real impact of these agents, except few exemptions (Greathead 1975; Lima et al. 1984; Matteson 1982). Although descriptive reporting is promoted, it's also important to remember that the mere existence of a natural enemy in a cowpea system doesn't mean it will be useful as a control agent.

4.2.3.4 Chemical Control

The majority of cowpea growers in the tropics are small-scale farmers who do not use insecticides on their crops. Large-scale farmers, on the other hand, typically use insecticides as foliar applications and seed dressers, with the latter being more commonly used (Adipala et al. 2000; Jackai and Daoust 1986). Chemical pesticides are heavily used in commercial agriculture to control insect pests and diseases, resulting in widespread environmental issues. Seedling pests like bean fly, aphids, leafhoppers, foliage beetles and many others can be effectively managed by treating seeds with Imidacloprid 70 Water soluble formulation (Gaucho) @ 5 g/kg seed. Flower and pod pests can be controlled by application of systemic and contact insecticide at recommended rate.

4.2.3.5 Integrated Pest Management

The need to promote environmentally friendly agriculture practices is becoming more widely recognized around the world (Sagar 1991). Integrated Pest Management (IPM) is a globally accepted strategy for promoting sustainable agriculture and human livelihood. IPM utilizes all the available management techniques in a

compatible manner to reduce the pest populations below the injury levels through an ecologically sound, economically practicable and socially acceptable manner (Ha 2014). In IPM, various cultural, physical, mechanical and biological methods are integrated to bring down the pest populations to economically lower levels with little disturbance to ecosystem particularly to natural enemy populations.

Field operations in cultural control such as deep summer ploughing in South Asia or winter rotations in the North America, expose the resting stages of insects to predatory birds and sunlight. The use of pest free and resistant planting material offers protection against pests as well. Sowing at the correct time is important in cultural management; as early or late sowing escapes insect infestation. Finally, proper removal of diseased plants, alternate host plants and weeds, applying balanced fertilizers and irrigation at such a level that pests are not encouraged, choosing bio pesticides like NPV, *Bt* formulations and neem-based formulations not only control the pests but also encourage the activity of natural enemies and pollinators.

Applications of properly labelled insecticides must be a last resort in managing pest populations and applied only when the pest population reaches injury level. An IPM practice that combines early planting, close spacing cowpea and three insecticide applications had the highest yield with a 51% gain over farmer's traditional practice of 5–6 insecticide applications (Nabirye et al. 2003). The 3-spray treatment also provided the highest net returns for growers with a return of 3:1 (Karungi et al. 2000). The IPM measures integrating cultural practices with foliar sprays out-yielded cultural treatments suggesting that use of cultural controls alone is not effective in managing pest infestations (Nabirye et al. 2003).

4.3 Breeding for Biotic Resistance

The productivity in cowpea is affected by wide range of disease and insects, which infect or infest the crop respectively. Infestation by insects can be both in field as well as in storage conditions. About 15 major fungal diseases, 5 or more viruses and at least one bacterial disease are important in cowpeas. Out of 85 insect species attacking cowpea, 20 insects mainly affects the yield worldwide. The most widespread and damaging insects pests includes black aphid *Aphis craccivora*, leaf miner *Phodoryctis caerulea*, pod bug *Riptortus pedestris*, leafhoppers *Empoasca kerri*, hairy caterpillar *Spilosoma obliqua*, blister beetle *Mylabri* ssp., semilooper *Autographa nigrisigna*, pod borer *Maruca vitrata*, gram caterpillar and *Helicoverpa armigera* Gram pod borer, spotted pod borer, spiny pod borer, blue butterfly. Among storage pests, *Callosobruchus maculates* cause serious damage to the grains. The development of the resistant cultivars helps to overcome the problem of yield loss due to pest attack. As a result, knowledge on genetic control of resistance helps in accelerating the development of resistant varieties.

4.3.1 Traditional Breeding Approaches

The productivity of cowpea is hampered by several biotic stresses such as pests and pathogen which affect the crop at all the stages of its development and storage. Host plant resistance is sustainable management option since it is environment friendly and cost-effective. The cowpea host plant resistance mainly focuses on the development of resistant varieties through traditional breeding and biotechnology approaches.

4.3.2 Importance of Genetic Resources

The narrow genetic diversity of cowpea is mainly due to its origin in West Africa from a single species and its self-pollinating character. Cowpeas are thought to have evolved from a narrow section of the wild germplasm for the species *V. unguiculata* and related species with only partial gene flow between cultivated and wild types (Boukar et al. 2020). Three subspecies are recognized for the species and correlate with forms of utilization. *V. u.* spp. *cylindrica* is a semi-wild type that is sometimes used as forage but not for grain. *V. u.* spp. *sesquipedelis* is a cowpea types selected for long pods in Asia that is used as a vegetable type. Meanwhile, the much more common *V. u.* spp. *unguiculata* is the common cowpea types used as dry grain with some dual purpose as fodder or a source of leaves that can be stewed.

4.3.3 Population Structure of Cowpea

Some levels of out crossing have led to a mixed phylogeny and branched dendrogram for the relationships of most cultivated dry grain types from West Africa, the centre of origin for the species and the domestication centre for the crop. Apart from this primary centre of diversity, cowpea has not been in many secondary centres of diversity for very long. The cultivation of cowpeas outside of Africa is perhaps less than a millennium in age, and part of trade with the Mediterranean, Middle East and South Asia through caravan, nomadic or ship-based trade. Cowpeas arrived to the Caribbean and the mainland Americas through the slave trade about four hundred years ago and became established in Northeast Brazil, in the Southeastern USA and in the northern edge of South America (Venezuela and Guajira, Colombia). These regions are part of the dry tropics and less humid subtropical zones where cowpea adapted better than other legumes. It seems that cowpeas have not displaced common bean in Central America and the Andean region of South America.

4.4 Genetic Sources in Germplasm Banks

Sources of genes for various traits have been identified by screening of germplasm available in different countries. The principal gene bank for cowpea globally is at the International Institute for Tropical Agriculture (IITA) in Ibadan Nigeria. This is the main CGIAR centre working on cowpeas with a worldwide mandate. The germplasm bank and breeding programs for cowpea at IITA work on identification, conservation and utilization of traits important for cowpea germplasm development and crosses. IITA's genetic resources centre (GRC) maintains a total of 17,051 cowpea accessions; of which 15,100 are cultivated and more than 1,900 are wild relatives. The main cowpea wild species available there include: *V. vexillata*, *V. spontanea*, *V. tenuis*, *V. protracta*, *V. baoulensis* and *V. stenophylla* (Boukar et al. 2013). Several researchers have reported on wild accessions having novel resistance against biotic stresses, which can be shared and used in breeding programs (Singh 2002; Boukar et al. 2015).

The main objective of breeding for biotic stress resistance is finding resistant germplasm as listed in Table 4.1. Many of the genotypes showing resistance to multiple biotic stresses in cowpea have already been identified. The wild *Vigna* species such as *V. unguiculata* spp. *dekindhana*, *V. oblongifolia* and *V. vexillata* are reported to be resistant to pod borer. These wild species are potential source for transferring resistant genes to adapted cultivars. The Table 4.1 represents the resistant germplasm accessions of cowpea against various biotic stresses. This information is highly useful for the development of resistant varieties through different breeding techniques. Most of the germplasm has been from the IITA collection or its breeding work in Sub-Saharan Africa but recently some USDA accessions have been screened for constraints in the United States. These have been derived from breeding lines, core and mini-core collections of the FAO treaty germplasm.

Cowpea germplasm screening in the USA has been methodical in the approach of developing new tools for genotype identification. For example, Huynh et al. (2017) developed a MAGIC (Multi-parent Advanced Generation Intercross) population for cowpea (*Vigna unguiculata* L. Walp.) from eight founder parents. These founders were genetically diverse and carried many abiotic and biotic stress resistance, seed quality and agronomic traits relevant to cowpea improvement. The eight parents were inter-crossed using structured mating to ensure that the population would have balanced representation from each parent, followed by single-seed descent, resulting in 305 F8 recombinant inbred lines each carrying a mosaic of genome blocks contributed by all founders as confirmed by single nucleotide polymorphism genotyping with the Illumina Cowpea 60 K iSelect Consortium Array. Due to its broad genetic base, this cowpea MAGIC population promises breakthroughs in genetic gain, QTL and gene discovery, enhancement of breeding populations and, for some lines, direct releases as new varieties.

Induced mutagenesis has also been used to increase the genetic variability of cowpea. Raina et al. (2020) developed eleven high yielding mutant lines with higher protein and micro nutrient content from the genetic background of cowpea varieties,

Table 4.1 Cowpea germplasm accessions having resistance to various biotic stresses

Diseases	Resistant/Tolerant germplasm accessions	References
Fusarium wilt	TVu 109-2, TVu 347, TVu 984, TVu 1000	Singh et al. (1983)
Scab	TVu 853, TVu 1404, TVu 1433	Singh et al. (1983)
Septoria	TVu 456, TVu 483-2, TVu 486, TVu 1433, TVu 11761, TVu 12349	Singh et al. (2002)
Bacterial blight	TVu 347, TVu 410, TVu 483-2, Danilla (Nigerian landrace)	Singh et al. (1983)
BICMV	TVu 2480; TVu 2657, TVu 3433	Bashir (1992)
CABMV	TVu 401, TVu 1582	Bashir (1992)
CPMV	TVu 227, TVu 345, TVu 612, TVu 2331	Patel (1982a, b)
CPMoV	TVu 3901	Allen et al. (1982)
Striga and Alectra	B301; TVu 14676; TVNu-1070, TVNu-1083, TVNu-585, TVNu-1535, TVNu-1537, TVNu-1647, and TVNu-491	Lane et al. (1997), Ouedraogo et al. (2012)
Insect pests		
Aphid	TVu 36, TVu 62, TVu 408, TVu 410, TVu 801, TVu 2896, TVu 3000, TVNu-1158, TVu-6464, TVu-1583, and TVu-15445, TVu-801, IT97K-556-6	Singh et al. (1983), Souleymane et al. (2013), Togola et al. 2020
Flower bud thrips	TVu 1509, Sanzi (Ghanaian land race)	
Leafhoppers	TVu 59, TVu 123, TVu 662	Singh et al. (1983)
Bruchid	TVu 2027, TVu 11952, TVu 11953	Singh et al. (1983)
Pod borer	Some accessions of *Vigna Vexillata* (5), *V. oblongifolia* (10), *V. macrosperma* (2), and *V. reticulate* (1)	Singh et al. (1990a, b)
African pod bug	*V. luteola* (3), *V. vexillata* (17), *V. macrosperma* (2), and *V. angustifolia* (3)	Singh et al. (1990a, b)

(continued)

Table 4.1 (continued)

Diseases	Resistant/Tolerant germplasm accessions	References
Cowpea seed weevil	*V. luteola* (6) and *V. vexillata (27)*	Singh et al. (1990a, b)

Note TVu-tropical *Vigna unguiculata* are germplasm lines available at the GRC of IITA, TVNu-accession of wild cowpea relative, *BICMV*-Black eye cowpea mosaic virus, *CABMV*-Cowpea aphid-borne mosaic virus, *CPMV*-Cowpea mosaic virus, *CPMoV*-Cowpea mottle virus

Gomati VU-89 and Pusa-578 went through induced mutagenesis and proceeded until theM4 generation to increase the genetic variability in cultivated cowpeas. Genetic divergence of mutant lines were analyzed through sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), simple sequence repeat (SSR) and CAAT box derived polymorphism (CBDP) markers. Mutant lines showed higher polymorphism than their parental genotypes.

4.5 Genetics of Resistance

The source of resistance genes and knowledge of their inheritance are two prerequisites for developing varieties resistant to biotic stresses. Resistance to biotic stress can be governed by both polygenic as well as monogenic inheritance. Major resistance (R) genes have been the subject of countless studies in many plants where they are of monogenic in inheritance. On the other hand, polygenic inheritance or pyramiding of R genes is mostly preferred for controlling multiple pests. However, introgression of multiple and polygenes into new varieties is a complex process compared to single major gene transfer. Progress with polygenes and QTL has accelerated through the use of molecular markers as tools for selection.

Examples of gene transfer with major R genes include: the monogenic resistance established for aphid resistance in cowpea (Redden et al. 1983); and mono or digenic seed resistance to bruchids (Adjadi et al. 1985). Meanwhile, the inheritance of resistance to flower bud thrips is polygenic of (Omo-Ikerodah et al. 2009; Sidibe et al. 2019). Studies of QTL and polygenic inheritance facilitate the development of resistant cultivars to insects. Disease resistances are much more likely to be under single gene control or simple inheritance, although cowpea R genes are not well studied.

The use of genetic resistance to disease and insects is an effective and ecofriendly way to control pest incidence. The resistant sources for disease and insects are already available in germplasm resources, the effective screening which helps in identification of resistant germplasm. Apart from screening in the IITA international collection, EMBRAPA (Brazil), NBPGR (India) and USDA (United States) are examples of large national genebanks with extensive cowpea collections that have scientists within the institutes who work on identification of biotic stress resistant cowpea germplasm. Along with collaborators in academia, efforts have been made to transfer R genes from wild species to cultivated cowpeas.

Pre-breeding as well as elite line breeding, respectively, have been used to widen the genetic base of cowpea and incorporate biotic stress tolerance. Interspecific crossing incompatibility is the major challenge that limits development of resistant cultivars for pod borer. Most of the wild species have been reported as source of resistance genes to pod borer but introgression of the R genes has been problematic due to pollen-pistil incompatibility and abortion of fertilized ovules in interspecific crosses (Barone et al. 1992). As mentioned before, the world's largest cowpea

germplasm collection (11,000 entries plus) is maintained by IITA and multiple resistance germplasm has been found in its core collection (Togola et al. 2019). The wild relative *V. vexillata* is resistant to aphids but the R genes cannot be transferred without overcoming natural barriers which hinder the hybridization. Attempts to develop interspecific hybrids between *V. vexillata* × *V. unguiculata,* showed resistant traits such as leaf, stem and pod hairiness which are deterrent to insects (Gomathinayagam et al. 1998); however, embryo culture is the only feasible option for viable hybrids. In summary, R-gene transfer has been through conventional breeding as well as application of molecular techniques and some resistant lines have been developed for biotic stress resistance. These germplasm resources can be utilized in cowpea breeding programs for development of further resistant varieties.

Resistance sources for many of the soilborne pathogens have been identified, but highly resistance sources are often not available for some of the necrotrophic pathogens like *M. phaseolina, S. rolfsii* and *Rhizoctonia* spp. Moderately resistant sources were identified in five of 33 cowpea cultivars (Singh and Lodha 1986) and four of 141 cultivars (Sohi and Rawal 1983). Most resistance to root rots in legumes are quantitative and polygenic in nature.

Leaf spot resistance to *Cercospora* is controlled by single dominant gene and its heritability varied from 81 to 97% (Omoigui et al. 2019). Many authors reported different mode of inheritance of rust resistance genes in cowpea. The rust resistance in cowpea was conditioned by dominance genes with additive effects (Rangaiah 1997), recessive genes (Uma and Salimath 2004) or polygenes located at various loci (Uma et al. 2016). The resistance to southern blight is controlled by dominant single gene (Fery and Dukes 2002).

Viral resistance to Cowpea aphid-borne mosaic virus was governed by more than one recessive gene in eight populations or single recessive genes in another seven populations (Orawu et al. 2013). Barro et al. (2016) observed that resistance to cowpea aphid-borne mosaic virus is governed by two dominant genes and each parent is contributing a resistant gene. Less discrete results were found by Gumedzoe et al. (1998) in screening of germplasm to CMV. Since many different viruses infect cowpeas at the same time (Taiwo and Shoyinka 1998) multiple resistance is the key to breeding against this stress. Ogunsola et al. (2010) identified multiple virus resistance to three different virus species in breeding lines IT98K-1092-1 and IT97K-1042-3, however cowpea varieties with multiple virus resistance are yet to be found.

The inheritance of resistance against bacterial blight is quantitative in nature and segregation was decided by genetic background of parents and modifying factors, however susceptibility was dominated over resistance and segregation pattern did not fit into genetic ratio as reported by Prakash and Shivashankar (1984). Three different types of host reactions were noticed in cowpea during bacterial pustule (*Xanthomonas campestris* pv. *vignae unguiculatae*) infection (Patel 1981) that included non-hypersensitive resistant (R), brown hypersensitive resistant (BHR) and susceptible (S). The inheritance study revealed that BHR reaction was dominated over R and S reaction and R was recessive to S reaction (Patel 1982a, b). The study indicated that, BHR reaction is conditioned by two genes and R reaction is controlled by two or three recessive genes.

The use of resistant varieties in crop production and overcoming disease limitations is the most economical and eco-friendly approach of disease management. Although many management options have been evaluated for management of plant pathogens but use of resistance cultivar is practical, economical and safe for non-target organism as well as environment (Bashir and Hampton 1996). Transmissible forms of resistance have been observed in some cultivars and wild relatives (Fraser 1992).

4.6 Genomic Resources and Molecular Breeding for Biotic Stress Resistance in Cowpea

In cowpea, development of genomic resources was initiated (GCP) through the Tropical Legumes I project, initially under the CGIAR Generation Challenge Program and then as part of projects with the Bill and Melinda Gates Foundation. SNP information obtained through this program was converted to KASP (Kompetitive allele specific PCR) by LGC Genomics based on the design/sequence information provided by the University of California, Riverside, (UCR). Initial genotyping platforms were made up of customized SNP markers that were utilized in marker-assisted backcross (MABC) and marker-assisted recurrent selection (MARS) strategies in NARS and IITA cowpea breeding programs (Boukar et al. 2015). Since then Infinium SNP chips have been developed for screening larger numbers of SNPs simultaneously, resulting in effective screening of both sources and associated markers in GWAS (Genome Wide Association Studies) described below.

Informative markers associated with quantitative trait loci related to biotic stress resistance were also identified. QTLs related to aphid resistance (Huynh et al. 2015), bacterial blight resistance (Agbicodo et al. 2010), Fusarium resistance (Pottorff et al. 2012), foliar thrips resistance (Muchero et al. 2010; Lucas et al. 2012), *Macrophomina* resistance (Muchero et al. 2011), nematode resistance (Huynh et al. 2016) and virus resistance (Gioi et al. 2012) have been reported. Cowpea has a relatively small genome size estimated at 620 Mbp and therefore has been relatively easy to use in genetic mapping studies (Fatokun et al. 1993). Various researchers developed linkage map of cowpea with different types of molecular and morphological markers. These maps provide resourceful information for various downstream application including quantitative trait loci (QTL) identification, map-based cloning, diversity analysis, association mapping, and molecular breeding. Table 4.2 summarizes genetic mapping in cowpea.

Table 4.2 Timeline representing linkage map development in cowpea

Linkage map	Mapping population	Number and types of markers	References
First linkage map	F2 population (IT84S-2246-4 X TVNu 1963)	89 loci comprising 79 RFLP, five RAPD four cDNA markers and one inherited morphological marker were distributed on 10 linkage groups that spanned 680 cM of the cowpea genome	Fatokun et al. (1993)
Second linkage map	RIL population (IT84S-2049 X 524B)	181 loci comprising of 133 RAPDs, 19 RFLPs, 25 AFLPs and three each of morphological and biochemical markers were assigned to 12 LGs spanning 972 cM with an average distance of 6.4 cM between markers	Menendez et al. (1997)
Third linkage map	RIL population (TVNu110-3A X IT84S-2246-4)	80 mapped loci comprising of 77RAPD and 3 morphological loci) were assigned to 12LGs spanning 669.8 cM of the genome with an average distance of 9.9 cM between marker loci	Ubi et al. (2000)
Fourth linkage map	Six bi-parental RIL population	A consensus map comprising of 928 SNP markers distributed over 11LGs, covering a total genetic distance of 620 Mbp	Muchero et al. (2009)
Fifth linkage map	Five RILs and 2F4 population	A consensus map comprising of 19% more SNP markers and had an improved order covering a total genetic distance of 680 Mbp	Lucas et al. (2011)

4.6.1 Genome Sequencing and Next Generation Marker Development

The cowpea genome has been sequenced in various steps with an initial assembly of gene rich space by Timko et al. (2008). Most recently, Munoz-Amatriain et al.

(2017) and Lonardi et al. (2019) reported a whole-genome shotgun (WGS) assemblies, of breeding lineIT97K-499-35. In addition, WGS sequencing of 36 other diverse cowpea accessions supported the development of a genotyping assay, Illumina Cowpea iSelect Consortium Array for 51,128 SNPs. This assay was found to be very useful in three of the currently running West African breeding programmes at Institut National de L'environnement et des Recherches Agricoles (INERA—Burkina Faso), Savanna Agricultural Research Institute (SARI-Ghana), Institut Senegalais de Recherches Agronomiques (ISRA-Senegal) and IITA. Apart from these genotyping platforms, other genomic resources include, HarvEST:Cowpea, an EST database with gene function analysis and primer design (http://harvest.ucr.edu/) (Muchero et al. 2009), Cowpea Genespace/Genomics Knowledge Base (CGKB) containing information on genetic markers, gene-space, metabolic pathways, mitochondrial, and chloroplast sequences, a tool for gene discovery; enzyme and metabolic pathways (http://cowpeagenomics.med.virginia.edu/) (Boukar et al. 2015). Some of the software commonly use in molecular breeding of cowpea include 'SNP Selector', 'KBioConverter', and 'Backcross Selector' for the management of genotyping data (http://breedit.org/ and https://www.integratedbreeding.net/) (Boukar et al. 2015).

4.6.1.1 Transgenic Improvement of Cowpea

Transgenic technology also plays a key role in enriching the genetic base of any crop. It can easily overcome the limitations associated with cross compatibility of species. Nigeria is the first country to approve cultivation of the first GM insect resistant cowpea (SAMPEA 20-T, Pod Borer Resistant (PBR) Cowpea (Boukar et al. 2020) developed by scientists at the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria Nigeria, in collaboration with the African Agricultural Technology Foundation (AATF) (Mohammed et al. 2014). The transgenic Bt cowpea lines, showed high levels of resistance to *Maruca* under field conditions (Mohammed et al. 2014). It carries an insecticidal Cry1Ab gene encoding a *Bacillus thuringiensis* (Bt) toxin against legume pod borer *Maruca vitrata* Fabricius (Lepidoptera: Crambidae), which is the most damaging and economically important post-flowering insect pest of cowpea in SSA that can cause up to 80% yield loss.

RNAi transgenic lines were also developed against viral disease of the cowpea. Cruz and Aragao (2014) developed transgenic cowpea lines against Cowpea severe mosaic virus (CPSMV) and Cowpea Aphid-borne mosaic virus (CABMV) through RNAi gene silencing technology. They silenced proteinase cofactor gene of CPSMV and coat protein gene of CABMV through RNAi. Out of the ten transgenic lines generated, seven transgenic lines showed milder symptoms while three exhibits enhanced resistance against both the viruses. Similarly Transgenic lines were containing three different intron hairpin (hp) RNAi constructs, containing AC2, AC4 and fusion of AC2 and AC4 (AC2 + AC4) of begomoviruses which codes for transcription activator protein. RNAi transgenic lines were analysed in T0 and T1 generation. Transgenic lines expressing AC2 hp and (AC2 + AC4) hp RNA were

showed nearly 100% resistance against MYMIV whereas transgenic lines expressing AC4 hp RNA showed milder symptoms after 5 weeks of infection.

4.6.2 Marker Assisted Selection

To expedite the cowpea resistant breeding programs the molecular markers associated with some of the insect resistance genes have already been identified. These markers mainly help in introgression of resistance genes from resistant source to susceptible cultivars by backcrossing. As an example an early RFLP marker closely associated with aphid resistance was used in selection of a major gene (Myers et al. 1996). More recently, the single nucleotide polymorphism (SNP) markers for resistance to aphids have been identified in the USDA core collection through association mapping (Qin et al. 2017). All this information together with the genome sequence can help in developing aphid resistant varieties through marker assisted selection. For mapping aphid resistance genes both quantitative trait locus (QTL) mapping and association mapping are utilized in cowpea. The markers linked to resistance helps to overcome the environmental effects associated with insects creening of genotypes. Therefore, advances in marker assisted selection will accelerate the introgression of resistance traits in to cowpea. Moreover, it also helps to overcome tedious inoculations and screening process used for selection of resistant genotypes. The modern biotechnological tools also help to overcome the crossing barrier between wild and cultivated species and help in deployment of the resistant genes from wild species. However, to make use of resistant germplasm knowledge on the inheritance, genomic location and marker association with the resistance genes are necessary requirements.

Introgression of disease resistance genes using traditional breeding techniques is complicated and time-consuming. To overcome this problem the best alternative option is to use molecular markers for identification of resistant individuals in the early generation, which helps in the effective improvement of the breeding procedure (Torres 2010). Bulked segregant analysis reported that simple sequence repeat (SSR) marker namely RB24 differentiate the resistance and susceptibility to *Cercospora* leaf spot (Omoigui et al. 2019). Hence RB24 marker can be used for marker assisted selection for this diseases. Wu et al. (2018) reported one major and two minor quantitative trait loci (QTLs) controlling rust resistance. Major QTLs (named as Ruv1) was mapped to 12.48 cM interval between the SNP markers 2_01772 and 2_03292 on LG09 which explained 34.8% of the phenotypic variation. The minor QTLs, designated as *Ruv2* and *Ruv3*, were mapped to a 7.01 cM interval on linkage group (LG) 7 and a 6.19 cM interval on LG8, which accounted for 13.4% and 11.9% of the phenotypic variation, respectively. Interval QTL mapping was used to show 98.4% of variance for the resistance trait mapped in the region of three loci AGB1, VM31 and VM1 covering a genetic interval of 32.1 cM, in which 95% confidence was found for the CYMV resistance. In another study, three QTL against cowpea bacterial blight, namely CoBB-1, CoBB-2 and CoBB-3, were detected on linkage group LG-3, LG-5 and LG-9 respectively showing that highly potential resistance candidate genes.

QTLs such as CoBB-1, CoBB-2 were reliable confirmed (Agbicodo et al. 2010). Meanwhile for virus resistance, Gioi et al. (2012) studied linkage of SSR markers to *Cowpea Yellow mosaic virus* (CYMV) by using resistance and susceptible lines of cowpea. Three SSR markers (AG1/AF48383, VM31 and VM1) were linked to resistance in cowpea against CYMV.

4.7 Conclusions

Resistant genes and their mode of inheritance have been already established for various biotic stress resistances in cowpea. Development of varieties through conventional breeding has been complemented by marker assisted selection and genetic engineering for resistance to several pests and diseases in cowpea. Presently many new genomic resources are available to progress the development of resistant varieties for biotic stress. The molecular tools like molecular markers, genetic maps, and QTL mapping support resistance breeding. Moreover, next-generation sequencing helps in genome wide characterization of markers linked to biotic stress.

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Chapter 5
Tackling Lentil Biotic Stresses in the Genomic Era

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Abstract Lentils are already one of the main pulses of the world, as they are one of the main sources of protein for humans. As a crop, they are also gaining momentum because the rusticity and tolerance to water scarcity of some varieties are a good fit with the current global warming trend and climate change in general. However, while the harvested area and overall production have drastically increased over the last decades, yield has only experienced very modest increments. The reasons are two-fold. First, pathogens are affecting the crop as never before, likely due to not only the changing climate but also to the expansion of lentil cultivation to new geographic areas. Second, genomics-aided breeding is far behind many other crops. This is in partly due to the lack of genomic tools currently available to researchers. Progress is being made to adopt high-throughput genomic methods, and researchers will be able to tackle lentil gene discovery and breeding for pathogen resistance and other biotic stresses more efficiently in the coming years. We outline the current situation, novel findings, and prospects of lentil research for biotic stresses.

Keywords Lentil · *Lens culinaris* Medik · Biotic stress · Genomics · Resistance · Breeding

5.1 Introduction

Lentil (*Lens culinaris* Medik. subsp. c*ulinaris*) is one of the first domesticated species in the Fertile Crescent and along with barley, emmer wheat and einkorn wheat, pea, chickpea, and flax was part of the set of crops that defined the beginnings of the Neolithic transition to agriculture in this part of the World. The origin of the cultivated form is the wild *L. culinaris* subsp. *orientalis* (Boiss.) Ponert (syn. *L. orientalis* Boiss.). A recent publication by Liber et al. (2021) suggests that phylogenetics, population structure, and archeological data coincide in a lentil domestication prolonged in time in Southwest Asia, with two different domesticated gene pools. From the

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Fertile Crescent the crop expanded eastward, westward and southward in pre- and protohistoric times to almost all temperate areas of the Old World. The expansion of cultivated lentil most likely occurred simultaneously with other first-domesticated crops as the agriculture expanded fromthe Fertile Crescent to the rest of the Old World. The diffusion of lentils occurred during the earliest period of agriculture expansion since lentil remains have been recovered in several archaeological sites corresponding to the earliest agricultural sites in Old World geographical areas. For instance, in the ancient western end of its distribution, the Iberian Peninsula, there are lentil archaeological remains since the early Neolithic (Cubero et al. 2009; Pérez de la Vega et al. 2011). In the cave "de les Cendres" (Spain), there are remains of several crops (*Triticum monococcum*, *T. dicoccum*, *T. aestivum*, barley, pea, grass pea, lentil and faba bean, i.e. a typical Near East crop complex) dated by ^{14}C to 7540 ± 140 BP (Buxó 1997). Archeological data indicate that lentil reached the Atlantic Canary Island though North-African colonizers in prehistoric times, long before the first contact with Europeans in the XIV century (Henríquez-Valido et al. 2019). From the sixteenth century it was introduced in America and later in Australia.

Lentil has been grown and/or consumed in most temperate areas of the World during centuries. An indication of the extent of its diffusion among cultivated plants is that according to the compilation by Mikic (2019) there are more than 180 languages with a word of their own to designate lentil. According to Cubero et al. (2009), if lentils have been maintained by farmers through ages, it is most likely because they grow in poor soils, rough climates, and harsh conditions for humans, animals and crops. In many cases, they may be the only source of protein available to them.

The binary scientific name of lentil is attributed to Friedrich Kasimir Medikus (1736–1808), a German physician and botanist, hence the standard abbreviation of Medik. Medikus was a younger contemporary of Linnaeus (1707–1778) and reviewed some of the specific assignments of Linnaeus. Linnaeus included lentils into *Cicer* and later in *Ervum*. Thus, among the synonyms of *L. culinaris* are *Cicer lens* (L.) Willd., *Ervum lens* L., *Lens esculenta* Moench, *Lens lens* Huth, *Lentilla lens* (L.) W. Wight ex. D. Fairchild; *Vicia lens* (L.) Coss. & Germ. (Cubero et al. 2009; Mikic 2019). Among these binary names, only *L. esculenta* Moench is found in relatively recent scientific papers, or even *L. culinaris* Moench. Although the genus *Lens* had been recognized by earlier scientists, the authorized name of the genus is *Lens* Miller (Cubero et al. 2009).

The C (unreplicated haploid) genome size of lentil was determined by flow cytometry in an amount of 4.41 pg equivalent to 4,063 Mbp (Arumuganathan and Earle 1991), or by means of Feulgen's microdensitometry in 4.6 pg (Bennet and Smith 1976), a size similar to the size estimated to pea; and like in the pea genome, the lentil genome seems to be rich in transposable elements (Rey-Baños et al. 2017). It is worth mentioning that due to the repetition of transposon and other sequences the complexity of the lentil genome must be much lower than its size.

Lentil is the only cultivated species of the genus *Lens* in which all species have the same chromosome number, $2n = 14$, and share similar karyotypes (Ladizinsky 1993), but there are chromosomal rearrangements between species and sometimes intraspecific. Chromosomal rearrangements are observable partly by differences in

karyotype, but mainly by the occurrence of multivalents (translocation) at the first meiotic metaphase, or a bridge and fragment (paracentric inversion) at the first meiotic anaphase in pollen mother cells of intraspecific hybrids (Ladizinsky and Abbo 2015). The genus *Lens* is a relatively small genus that includes no more than six biological species *L culinaris* Medik., with two subspecies (*culinaris* and *orientalis*), *L. ervoides* (Brign.) Grande, *L. lamottei* Czefr., *L. odemensis* Ladiz., *L. nigricans* (M. Bieb) Godr., and *L. tomentosus* Ladiz. (Ladizinsky and Abbo 2015). The species or subspecies status of some of these taxa has been widely discussed. In part this is due to the use of two different species concepts: morphological and biological. On the basis of the biological species concept (a group of individuals that actually or potentially interbreeds and forms one genetic pool that is isolated by various reproductive barriers from individuals belonging to other species). Ladizinsky and Abbo (2015) rejected the subspecies status of *odemensis, lamottei* and *tomentosus.* According to the criterion of reproductive isolation, the only taxa that show high reciprocal crossability are *L. culinaris* and *L. orientalis*, hence the wide acceptance that *L. c. culinaris* and *L. c. orientalis* are two subspecies of a single biological species. Obtaining hybrids among the other taxa (in the vast majority of cases it has been tried between the cultivated lentil and some wild taxon) is difficult and sometimes it is only achieved through embryo rescue techniques; in addition, in many cases the existence of hybrid breakdown is evident (Fratini and Ruiz 2006, 2008; Singh et al. 2013, 2018). Therefore, it is very likely that in nature the reproductive isolation is total between these taxa.

According to a comparative analysis of DNA sequences, Alo et al. (2011) concluded that *L. nigricans* and *L. ervoides* are well-defined species at the DNA sequence level, while *L. odemensis*, *L. tomentosus*, and *L. lamottei* may constitute a single taxon pending verification with crossability experiments. Phylogenetic tree and STRUCTURE analysis of the genus *Lens* using genotyping-by sequencing (GBS) identified four gene pools (GP), namely *L. culinaris-L. orientalis-L. tomentosus*, *L. lamottei-L. odemensis*, *L. ervoides* and *L. nigricans* which form primary (GP1), secondary (GP2), tertiary (GP3) and quaternary (GP4) gene pools, respectively (Wong et al. 2015). However, Ladizinsky and Abbo (2015) included only subsp. *orientalis* in GP1 (likely limited to the accession with the same chromosome arrangement than subsp. c*ulinaris*), *L. odemensis, L. ervoides*, and *L. tomentosus* in GP2, and *L. nigricans* and *L. lamottei* in GP3. Phylogenetic analysis clustered carried out by Dissanayake et al. (2020) grouped the six traditional *Lens* taxa into four groups, namely, *L. culinaris/L. orientalis, L. lamottei/L. odemensis, L. ervoides,* and *L. nigricans*. Liber et al. (2021) confirmed previous studies proposing four groups within the genus *Lens.*

The genus *Lens* is included in the tribe Fabeae (formerly Vicieae) which comprises about 380 legume species, including some important grain legume crops such as pea, grasspea, and faba bean, in addition to lentil. In this tribe are also included the genera *Lathyrus* and *Vicia* (with around 150 species each one), *Pisum* (three species) and the monotypic genus *Vavilovia* (*V. formosa* (Stev.) Fed.). Phylogenetic analyses of the species in the tribe show that the genera *Vicia* and *Lathyrus* in their current circumscription are not monophyletic: *Pisum* and *Vavilovia* are nested in

Lathyrus, the genus *Lens* is nested in *Vicia* (into the Ervoid group of *Vicia*). According to ancestral character state reconstruction results, ancestors of Fabeae had a basic chromosomenumber of $2n = 14$, an annual life form, and evenly hairy, dorsiventrally compressed styles (Smykal et al. 2011; Schaefer et al. 2012). The close relationships between lentils and other Fabeae species ensure a good transferability of genetic and genomic information between species.

Some other taxa from close genera such as *Vicia* or *Lathyrus* have been assigned to *Lens*, for instance *Vicia montbretii* has been classified as *L. montbretii*, but there is a general agreement that they do not belong to *Lens* (Cubero et al. 2009; Ladizinsky and Abbo 2015; Smykal et al. 2015; Leht and Jaaska 2019).

Lentil has never been a model species in basic research; perhaps its greatest contribution is its inclusion among the species used by Vavilov (1922) in his seminal work on the Law of Homologous Series in Variation. Lentil (as *L. esculenta* Moench) was included in the legume species list of Vavilov's comparative study, together with pea (*Pisum sativum* L.), vetch (*Vicia sativa* L.), fava bean (*V. faba* L.), grass pea (*Lathyrus sativus* L.), chickpea (*Cicer arietinum* L.), and other legume species (Pérez de la Vega 2016). Ultimately, the law of homologous series indicates that the variation displayed among related species entails similar characteristics (morphological and also molecular) and that the equivalent characters are controlled by homologous genes (orthologs or paralogs). This law is in fact the basis of the comparative genetics and genomics (Pérez de la Vega 2016). From the practical point of view, what this law indicates is that any genetic or genomic information obtained in one species is always the first clue to be used in the research in any other phylogenetically close species.

Since lentils are cultivated in more than 70 countries this crop is subjected to different climatic conditions and culture practices such as winter or spring sowing; likewise, the biotic factors which affect yield and production are diverse. For instance, while Ascochyta blight (AB), a seed-borne disease, has been described in at least 16 countries in five continents making it the likely most widely distributed and devastating lentil disease, Stemphylium blight (SB) caused by *Stemphylium botryosum* Wallr. was once a minor disease with local significance in South Asia (its first outbreak was reported in Bangladesh in 1986), but is becoming a serious threat to lentil cultivation in many parts of the world such as Canada where it has become more prevalent (Mwakutuya and Banniza 2010; Das et al. 2019).

5.1.1 Economic Importance

Lentil is a predominantly self-pollinated diploid ($2n = 14$) annual grain legume species adapted to growth in dry-temperate climates, traditionally as a rainfed crop. Lentil (*Lens culinaris* ssp. *culinaris*) is a bushy annual herb with erect, semi-erect or spreading growth habit ranging from 25 to 30 cm in height for the majority of genotypes. The legume fruits usually contain one, two or rarely three seeds. They are lens shaped and weigh between 20 and 80 mg and are a rich source of protein and

dietary fiber. Seed diameter is the main characteristic of Barulina's classification of lentil genotypes into the large seeded macrosperma type (6 to 9 mm) or small and medium sized microsperma (2 to 6 mm) (Muehlbauer et al. 1995).

Lentils are consumed almost exclusively in the form of dry seeds and for human consumption, unlike some other nearby species that are also consumed as vegetables or are also used for animal feed (garden/field peas and faba beans). Normally, only damaged lentil grains, not suitable for human consumption, are destined for animal feed. Lentils are traditionally valued as a source of energy, proteins and iron in human nutrition. In addition, they are an important dietary source of fiber, minerals, vitamins and antioxidants (Pérez de la Vega et al. 2011). The amounts of these components vary among cultivars or accessions, thus the ranges for different components per 100 g of raw lentil dry matter are: energy 1483–2010 kJ, protein 20.6–31.4 g, fat 0.7–4.3 g, carbohydrates 43.4–69.9 g, fiber 5.0–26.9 g, ash 2.2–4.2 g (Urbano et al. 2007), although these values can vary depending on the lentil material and the cooking or precooking (e.g., dehulling) treatments (Petterson et al. 1997; Cuadrado et al. 2002; Almeida-Costa et al. 2007; Wang et al. 2009).

The average lentil production of the last five years (to minimize annual fluctuations) of which there are statistics (2014–2018) is 5.9 million tons, harvested in 5.2 million hectares; with an average yield of 1.1 t/ha (Table 5.1). The interest in the consumption of the lentil is shown in the constant and gradual growth of the production of this crop, although that growth is mainly due to the increase in the sown area. The lentil world production is now more than double that of 25 years ago, increasing since 1994 to 2018 from 2,818,469 tons to 6,375,732 tons (126.2%) (Fig. 5.1), but while its yield has moderately increased during this period (from 0.81 to 1.04 tons/ha; 28.4%) the harvested area has increased from 3,456,492 ha to 6,119,509 ha (77.0%). The key year in this change was 2009, in the previous 15 years the average yield was 0.85 t/ha while in the following 10 years it was 1.12 t/ha. Likewise, almost simultaneously the harvested area increased during these ten last years from roughly 3.5 million hectares to approximately 6 million hectares. According to FAOSTAT data, although there is a gradual increase in the surface sown with lentils in many countries and areas, such as the European Union, the most significant contribution to this increase is due to Canada and to more recently to India (Fig. 5.2).

Table 5.1 World lentil harvested area, yield and production from 2014 to 2018[1]

Year	Harvested area (ha)	Yield (hg/ha)	Production (Tons)
2014	4,017,683	11,697	4,699,562
2015	4,710,991	11,673	5,499,290
2016	5,444,686	12,055	6,563,805
2017	5,886,665	10,932	6,435,369
2018	6,119,509	10,419	6,375,732

[1]Data from FAOSTAT

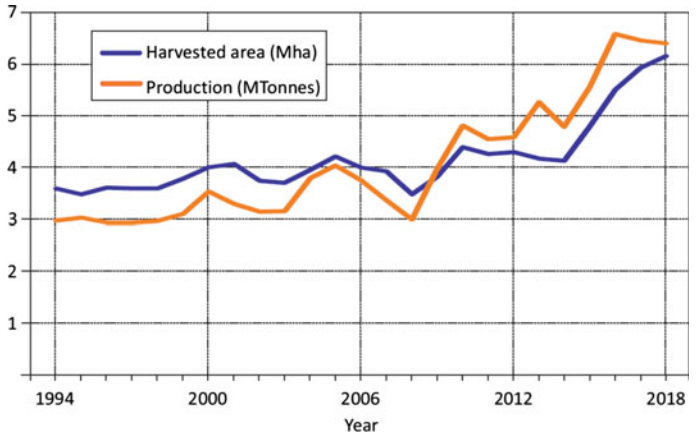

Fig. 5.1 Lentil production and harvested area from 1994 to 2018. Source FAOSTAT

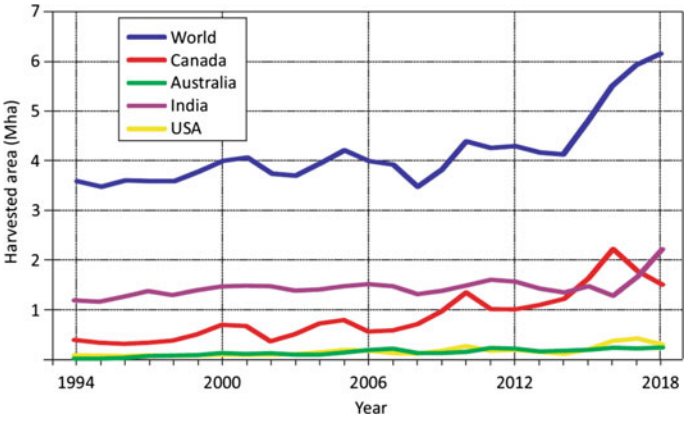

Fig. 5.2 Lentil production in some representative countries from 1994 to 2018. Source FAOSTAT

5.1.2 Reduction in Yield and Quality Due to Biotic Stresses

Yield losses caused by the different biotic stressors in lentils, as in other crops, are highly variable. They not only depend on the causative agent, but also on the environmental circumstances of the region and the year. Ascochyta blight (AB), caused by *Ascochyta lentis*, is probably the most generalized disease in lentil, and it has been reported to be the major lentil disease in many lentil-producing countries. The disease has considerable effects on both seed quality and yield. Yield losses have been estimated to reach of up to 40%, but inCanada economic losses from infected seed may reach more than 70%. In some cases, seed infection is so severe that the lentils are unmarketable (Gossen and Morrall 1983, 1984; Ye et al. 2002).

Virus diseases incidence and the losses they caused varied widely from extremely low (less than 1% incidence) to almost 100% with complete crop failure (Makkouk and Kumari 2009). A major lentil pest *Bruchu*s spp. also causes significant losses. Mean seed loss under organic farming was 15% and mean yield loss was 0.13 t/ha. Seed and yield losses were 2.6- and 8.4-fold higher, respectively, under organic than conventional farming. Valuable genotypic variability was observed with respect to both seed and yield losses. Farming system was the main source of variation for both losses, while early flowering and small seed size were traits associated with low losses (Vlachostergios et al. 2018). Lentil yield loss from the competition with weeds can range as high as 80% (Pala 2019).

Tests carried out in Australia showed that aphids and aphid-transmitted viruses cause appreciable yield reduction in pulse crops. Lentils were most affected by viruses, followed by faba beans, lupins (narrowleafed) and field peas, with yield reductions averaging 85% in lentils. Feeding damage on lentils averaged 4.5% (Valenzuela and Hoffmann 2015). Tests in the Palouse region of northern United States with the aphid *Acyrthosiphon pisum* and the associated *Bean leafroll virus* (BLRV) and *Pea enation mosaic virus* (PEMV) predicted lentil yield loses up to 100% by early virus infections (10 days after emergence) (Paudel et al. 2018), and a previous publication (Elbakidze et al. 2011) with these three species showed that aphid outbreaks have historically decreased pea and lentil yields by approximately 5% and 7% on average, respectively in the Palouse region (See also Sect. 5.2).

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

Global warming and other climatic changes associated with it are having and will have a clear effect on agriculture. The most evident direct effect of higher temperatures is on the growth and yield of crops, and the indirect effect more clearly associated with the increase in temperature is the increase in drought risk. Gupta et al. (2019) stated that it is anticipated that climate change is likely to exert a substantial effect on various insect pest management programs including host-plant resistance, natural plant products, bio-pesticides, natural enemies, and efficacy of synthetic chemicals. Several works have addressed the effect of global warming and climate change in lentil and other pulses (Cutforth et al. 2007; Bueckert and Clarke 2013; Bhandari et al. 2016; Bourgault et al. 2018). But the change in the geographical distribution in which crops and their possible pests and pathogens can grow is also important. The new challenges for crops in relation to biotic stresses are the spread of new diseases or pests, such as the recent spread of *Stemphylium* from a practically regional disease to extend to several main areas of lentil production (Mwakutuya and Banniza 2010; Das et al. 2019); or the ability to grow, and therefore infect, in areas that were relatively cold but are warmer now. An example of this is the northern enlargement of the

area where the parasitic weed *Orobanche* has been observed in lentil fields in Spain (Rubiales et al. 2008).

5.1.4 Limitations of Traditional Breeding and Rational of Genomic Designing

Breeding productive and resistant genotypes to diseases, pests and weeds is considered the most feasible and environmentally friendly method to manage major stressors (Rubiales et al. 2015; Keneni and Ahmed 2016). The use of resistant varieties against biotic stresses provides a number of comparative advantages particularly in reducing the use of environmentally unfriendly agrochemicals. The latest definition of the fundamental theorem of natural selection by Fisher (1941) says that the rate of increase in the average fitness of a population is equal to the genetic variance of fitness of that population. This is the reason why genetic variability is an indispensable initial condition for any selective breeding procedure. Natural genetic variability can be found in crop landraces and old varieties or can be gained or increased by sexual crosses, within the cultigene or through wide crosses with wild relatives, so that crossing is usually one of the ways to start a breeding program. Genetic maps and markers are invaluable genetic tools to advance in the rational use of variability in breeding, hence the advantage provided by the dense maps and the thousands of markers provided by the new genomic technologies (See Sect. 4.2).

Resistance genes to biotic stresses are often preferably or exclusively found in wild relatives, but transfer of genes from wild relatives to cultivated varieties can present interspecific cross-incompatibility hindering the use of this genetic variability. There is generally agreement in which landraces are sources of initial breeding materials since they have breeding values under suboptimal production as they contain valuable adaptive genes to different circumstances. Effective resistance against biotic stresses may be achieved from genetic improvement of the host species but genes for complete resistance to pests or disease agents may not exist in cultivated species of crop legumes as opposed to wild relatives which have coexisted with pests on an evolutionary time scale (Keneni and Ahmed 2016). Hence the frequent need to draw on wild materials. Another variable to consider is the genetic control of the resistance in question, monogenic, oligogenic or polygenic, which largely determines the breeding method to be followed (bulk, pedigree, backcrossing, etc.). When designing a breeding program, it is also appropriate to consider the convenience/possibility of pyramiding resistance genes for the same or for different pests or pathogens. When no broad spectrum resistance mechanisms are known in a host–pathogen-pest interaction, the convenience of pyramiding genes against the same biological agent arises from the fact that resistance in many cases is strain specific (or gene-for gene, see Flor 1971), and/or, therefore several resistance genes are needed to obtain a relatively broad resistance. On the other hand, pathogens and pests, as living beings, constantly evolve in such a way that new strains or pathovars can appear by mutation

or be selected between pre-existing variability by new environmental factors, which insists on the constant need to search for broad spectrum resistances, new resistance genes and/or pyramid genes already known. Nor can we forget that the globalization of the food trade helps the dispersal of pathogens and pests so that new agents or new stains can quickly colonize new territories. Another complication in breeding for resistance to biotic stresses is that the response to a biological agent may depend on other environmental factors (this could at least explain in part because many times a genotype is resistant under the controlled conditions of a greenhouse but susceptible in the field), in part because the signaling pathways to biotic and abiotic stresses are not completely independent (Atkinson and Urwin 2012; Ramegowda and Senthil-Kumar 2015). Last but not least, when improving for resistance to stresses, especially when using landraces or wild relatives, resistance genes can be linked to genes that are unfavorable for the yield or the characteristics of a domesticated crop (for example, pod shattering).

Transgenesis is a way to overcome interspecific cross incompatibility barriers and to extend gene sources to species from other biological kingdoms. Since the first transgenic crops in the 90's of the twentieth century, success has been obtained in achieving transgenic crops, in particular resistance to insects, viruses and herbicides. Legumes are a natural source of genes coding in particular for insecticidal proteins but there are only a few examples of transgenic pulses (Solleti et al. 2008; Kumar et al. 2018b; Sagar and Dhall 2018; Kumar and Jogeswar 2020), there is a lower number of examples of use in commercial production, and to date none in lentil (Gupta et al. 2020).Furthermore, as Kumar and Jogeswar (2020) stated, bio-safety issues and the possible effect of genetically modified crops on nutrition, growth, metabolism and health of people persists as a subject of public debate.

5.2 Description of Different Biotic Stresses

5.2.1 Fungi (See also Sect. 5.7)

Among the main threats to lentil production are several diseases caused by fungi. Ascochyta blight (AB), caused by *A. lentis*, is probably the most important and frequent disease of lentil throughout the world, and it can cause yield losses up to 70% in addition to seed damages (Gossen and Morrall 1983, 1984). *Ascochyta* spp. (teleomorphs: *Didymella* spp.) infect a number of legumes; including many economically crops, and the diseases they cause represent serious losses in legume production worldwide. *Ascochyta rabiei, A. fabae, A. pisi, A. lentis*, and *A. viciae-villosae* are pathogens of chickpea (*Cicer arietinum*), faba bean (*Vicia faba*), pea (*Pisum sativum*), lentil (*Lens culinaris*), and hairy vetch (*V. villosa*), respectively. Under controlled conditions *A. fabae, A. lentis, A. pisi, A. rabiei*, and *A. viciae-villosae* demonstrated to be host specific (Hernandez-Bello et al. 2006). These authors were able to obtain several interspecific hybrids between *Ascochyta* species, but

hybrids have shown a low pathogenic ability on the respective crop species. They conclude that the low pathogenic fitness of hybrids may be animportant speciation mechanism contributing to the maintenance of host specificity.

Other important diseases caused by fungi are: Stemphylium blight (SB) which is a defoliating disease in lentils, caused by the necrotrophic Ascomycete, *Stemphylium botryosum*. Rust originates by the infection of the biotrophic fungus *Uromyces viciafabae*. This pathogen is widespread and attacks the aerial parts of the plants. The root disease Fusarium wilt (FW) is caused by *Fusarium oxysporum*, a filamentous ascomycete fungus. Anthracnose is a disease caused by the hemibiotrophic fungus *Colletotrichum lentis*. Root rot disease is caused by the oomycete *Aphanomyces euteiches*.

5.2.2 Bacteria

Compared to fungal diseases, publications on bacterial diseases are very scarce. Most of the data is limited to the bacterial blight caused by *Pseudomonas syringae* pathovars. At least three pathovars have been associated to bacterial blight in lentil. Hunter and Taylor (2006) analyzed the patterns on interaction between *Pseudomonas* pathovars and several grain legume species. They found that lentil (nine accessions) showed patterns of interaction with isolates of *P. syringae* pv. *glycinea*, *P. syringae* pv. *phaseolicola,* and *P. syringae* pv. *pisi*. The minimum numbers of resistance (*R*) and avirulence (*avr*) gene pairs to account for the observed interactions were nine *R* genes in lentil, and the *avr* ranged from seven to nine among pathovars. It is likely that *P. syringae* pv. *syringae* can also cause bacterial blight in lentil since this pathovar is able to infect other close species of *Pisum* and *Lathyrus* (Martín-Sanz et al. 2011, 2012).

Phytoplasma naturally infecting lentil was first reported in 2016 (Akhtar et al. 2016). In April 2011, lentil plants were found with symptoms reminiscent of phytoplasma infection in Pakistan. Phytoplasma presence was confirmed by 16S rDNA PCR amplification, and experimental transmission was successful by grafting and by the leafhopper *Orosius albicinctus.*

5.2.3 Pest

Pests can cause a direct reduction in yield by feeding of plants and indirectly by transmitting pathogens. Many pest species inoculate pathogens such as viruses while feeding on plants, or open ways for further microbial infections through the wounds caused to plants; furthermore, these infections can reduce the plant ability to response to further abiotic stresses. For instance, the aphid *Acyrthosiphon pisum*is an infection vector of the *Bean leafroll virus* (BLRV) and the *Pea enation mosaic virus* (PEMV) in lentil. Thus, this section is related with Sect. 2.4 devoted to viruses.

Lentil is damaged by many types of insects and other pests. Among insects, major field pests are aphids (*Aphis craccivora, Acyrthosiphon pisum*), leaf weevil (*Sitona* spp.), lygus bugs (*Lygus* spp.) and the cutworm (*Agrotis ipsilon*) (Pérez de la Vega et al. 2011). Another major pest problem causing great seed losses areseed insect species: *Bruchus ervi* and *B. lentis* with *Callosobruchus chinensis* and *C. maculatus* (Stevenson et al. 2007). Rinehold et al. (2018) described as lentil pests the species bean aphid (*Aphis fabae*), green peach aphid (*Myzus persicae*), pea aphid (*Acyrthosiphon pisum*), lygus bugs (*Lygus* spp.), seed-corn maggot (*Delia platura*), western yellow-striped armyworm (*Spodoptera praefica*), and also included lentil as host of the pea moth (*Laspeyresia nigricana*). Rinehold et al. (2018) publication also includes pest description, monitoring and control. In a review on pest management in grain legumes, Sharma et al. (2010) listed the bruchid species *Callosobruchus chinensis* as a highly important pest in lentil; the pod borer *Etiella zinckenella*, weevil *Sitona* spp., the aphids *Aphis craccivora*, *Acyrthosiphon pisum*, and *Aphis fabae*, as moderately important; and pod borers of the genus *Helicoverpa*, pod sucking bugs of *Clavigralla*, defoliators of *Spodoptera*, and grasshoppers of *Empoasca* as occasional lentil pests. But the list of lentil pests is larger if local or regional pests are added to the list of global and general pests. For instance, chalky spot damage on red lentil, caused by the stink bugs *Piezedorus lituratus* and *Dolycorus baccarum*, is the most important problem waiting for a solution regarding plant health at lentil cultivation in Southeast Anatolia Region, Turkey (Mutlu et al. 2016). Although trips are less frequently mentioned as lentil pest, some trip species have been described as pea and lentil pest during spring in Eastern Europe (Pobozniak 2011).

Bruchids in particular are a legume pest that causes post-harvest damage by feeding inside the grains, decreasing their value. Although these pests are ancient, since there is evidence of bruchid infestation in lentils stored in ancient Egypt and preserved in the British Museum (radiocarbon dated 2,112 ± 48 BP, c. 162 BC) (Burleigh and Southgate 1975), little resistance has been achieved over centuries of cultivation and breeding. Clement et al. (1994), in a review on resistance to insect in cool season food legumes, indexed only three publications in relation to lentil, specifically against *Aphis craccivora*, *Bruchus lentis*, and *Sitona* spp. But, according to Clement et al. (1994), resistance to *B. lentis* was ecological, not genetics. In general, resistance against these pests seems to be scarce in all cool season food legumes. For instance, no resistance by antixenosis, antibiosis and/or tolerance was found after mass screening of 6,697 accessions of chickpea (to *Callosobruchus chinensis*) or 1,000 accession of broad bean, as summarized in the above mentioned review. In an evaluation of lentil varieties and farming system effect on seed damage and yield loss due to bruchid infestation in Greece, Vlachostergios et al. (2018) found that early flowering and small seed size were traits associated with low seed loss and yield loss. Among varieties, mean seed loss ranged from 8.5% to 29.2% and yield loss from 0.06 to 0.31 t/ha. Bruchid tolerance, revealed two types of promising varieties: varieties with high yield and low seed bruchid damage due to phenological escape, and varieties with high yielding potential despite the high seed loos and yield loos.

Other "generalist" insects, such as grasshoppers can reduce lentil yield. In field trials carried out in Canada using cages to evaluate the damage caused by grasshoppers to lentil flowers and pods, Olfert and Slinkard (1999) reported a decrease in yield from 28 to 57% in cages with two to 10 grasshoppers (*Melanoplus bivittatus*).

El-Bouhssini et al. (2008) reported the first sources of resistance to the coleopteran weevil *Sitona crinitus* in wild *Lens* taxa, namely, *L. ervoides, L. nigricans, L. odemensis*, and *L. orientalis*. Eight accessions were identified as resistant, with ≤ 10% nodule damage, compared to > 56% damage recorded on the cultivated lentil. Field evaluation and screening of lentil germplasm against black aphids (*Aphis craccivora*) resulted in the identification of 26 highly tolerant genotypes; Precoz was found to be a major source of resistance followed by LG 171 (Kumari et al. 2007).

5.2.4 Viruses

Table 5.2 summarizes the number of different virus species tested in four cool season legume species listed in the VIDE database (Brunt et al. 1996). The number of virus species tested in lentil and to which it was found susceptible (upper number in the diagonal) or unsusceptible (lower number) is clearly lower than the figures for the other three species. For instance, 27 susceptible in lentil in comparison with 124 in pea. These numbers are not absolute values because sometimes the same virus species is classified as susceptible and or unsusceptible, likely because this response is the results of particular interaction between a plant genotype and a virus strain. The table also indicate the number of common virus species; for instance, 22 virus species as been described trigging the susceptible response and four the unsusceptible one,

Table 5.2 Number of susceptible and unsusceptible virus species tested in four cool season legume species

	Lentil *Lens culinaris*	Sweet-pea *Lathyrus odoratus*	Pea *Pisum sativum*	Faba bean *Vicia faba*
Lentil *Lens culinaris*	27/14	9	22	20
Sweet-pea *Lathyrus odoratus*	7	57/25		
Pea *Pisum sativum*	4		124/164	
Faba bean *Vicia faba*	6			108/153

The diagonal indicates the virus species to which each of the four crop species has been described as susceptible/unsusceptible

Numbers above the diagonal indicate the number of common virus species to which they are susceptible

Numbers below the diagonal indicate the number of species to which the lentil is susceptible but unsusceptible the other species

in both pea and lentil. These data show that the available information on plant-virus interactions in lentil is limited in comparison to other closely related crops.

Surveys conducted in many countries in West Asia and North Africa during the last three decades established the most important aphid-borne viruses posing a significant limitation to legume production (and to cereals) (Makkouk and Kumari 2009). The list of the most important of these viruses affecting cool-season food legumes (faba bean, lentil, chickpea and pea) included: *Bean leafroll virus* (BLRV), *Beet western yellows virus* (BWYV), *Bean yellow mosaic virus* (BYMV), *Chickpea chlorotic stunt virus* (CpCSV), *Faba bean necrotic yellows virus* (FBNYV), *Pea enation mosaic virus-1* (PEMV-1), *Pea seed-borne mosaicvirus* (PSbMV) *and Soybean dwarf virus* (SbDV). All the above-mentioned viruses are persistently transmitted by aphids except BYMV and PSbMV which are occasionally transmitted by aphids. And the most important aphid species reported to transmit these legume viruses were *Acyrthosiphon pisum* (BLRV, BWYV, PEMV-1, FBNYV, SbDV), *Aphis fabae* (BLRV, FBNYV), *Aphis craccivora* (BLRV, PEMV-1, FBNYV, BWYV), *Myzus persicae* (PEMV-1, BLRV, BWYV), *Macrosiphum euphorbiae* (PEMV-1, BLRV), and *Aulacorthum solani* (PEMV-1, BWYV, SbDV) (Makkouk and Kumari 2009). This review also includes some sources of resistance to PEMV, BLRV, FBNYV and SbDV in lentil germplasm (ILL 75 had resistance to BLRV, FBNYV and SbDV, whereas ILL 74, ILL 85, ILL 213, ILL 214 and ILL 6816 were resistant to FBNYV and BLRV), in addition to procedures for the integrated management of these aphid-borne viruses. Makkouk et al. (2014) add information on Australia and listed the most important viruses reported to naturally infect lentil: *Alfalfa mosaic virus* (AMV), *Bean leafroll virus*, *Bean yellow mosaicvirus*, *Beet western yellows virus*, *Broad bean mottle virus* (BBMV), *Broad bean stainvirus* (BBSV), *Broad bean wilt virus* (BBWV), *Chickpea chlorotic dwarf virus* (CpCDV), *Chickpea chlorotic stunt virus*, *Cucumber mosaic virus* (CMV), *Faba bean necrotic yellows virus, Pea enation mosaic virus-1, Pea seed-borne mosaic virus*, and *Soybean dwarf virus.*

Sources of resistance seem to be scarce. All the 29 lentil lines tested in a pioneering work (Aydin et al. 1987) were susceptible to the PEMV strains PI 472,547 and PI 472,609 and showed significant yield reduction. The accessions of three wild *Lens* species tested were also susceptible. More promising results were obtained by Jain et al. (2014) who screened a total of 44 lentil accessions for resistance to PEMV. Two accessions (PI 431,663 andPI 432,028) were identified with resistance to PEMV in field tests while 14 accessions were found resistant or moderately resistant in greenhouse screenings. Most of the resistant accessions came from Iran. Thirty-six polymorphic simple sequence repeat (SSR) markers which produced 43 loci were used for genetic diversity analysis of this collection.

5.2.5 Nematodes

Askary (2017) listed the prominent genera of nematodes attacking pulses (including lentil): *Meloidogyne*, *Heterodera*, and *Paratylenchus*, the endoparasites,

Rotylenchulus, semi-endoparasites, and *Tylenchorhynchus* and *Helicotylenchus*, the ectoparasites. The root-knot nematode *Meloidogyne incognita*has been described as one of the major limiting factors affecting lentil growth and yield (Khan et al. 2017). Nine out of 300 lentil accessions were found to be resistant to *M. incognita.* Results suggested that the disease resistance in lentil accessions may be related to both post-infectional (nematode growth and development) as well as pre-infectional (penetration and establishment) defense mechanisms (Khan et al. 2017).

5.2.6 Weeds

Lentil growth and production are challenged by many weed species that depend in part on the region in which this crop is grown, and which are generally controlled by crop rotation and herbicides (Jurado Expósito et al. 1997). There are many weed species which largely depend on the geographic area in which the lentil is grown. In Southeastern Anatolia, Pala (2019) described as common weeds the species *Sinapis arvensis* L. (36%), *Ranunculus arvensis* L. (16%), *Galium aparine* L. (11%), *Cephalaria syriaca* L. (8%), and *Centaurea depressa* L. (8%). But one of the biggest challenges to the cultivation of lentils, and other crop legumes, in the Mediterranean and potentially dangerous in other temperate zones is broomrape, which unfortunately, and probably due to the general warming, is extending its range of distribution (Grenz and Sauerborn 2007; Rubiales et al. 2008). Broomrape could reduce the yield up to 90% in the Mediterranean region. Broomrape, *Orobanche crenata* Forsk., is a root holoparasitic weed and the main damaging weeds for temperate legumes, but other species such as *Orobanche foetida, Orobanche minor*, and *Phelipanche aegyptiaca*, can also induce high local damage (Rubiales and Fernandez-Aparicio 2012). Lentil can be severely infected by *O. crenata*, it can also be damaged although with less virulence by *O. aegyptiaca*, and can only be slightly infected by *O. foetida* (Fernández-Aparicio et al. 2009). Resistance to these parasitic weeds is difficult to access, scarce, of complex nature and of low heritability (Rubiales et al. 2009). Low infection rates seemed to be based on a combination of various escape and resistance mechanisms (Fernández-Aparicio et al. 2008). Resistance sources to broomrape have searched in cultivates and wild materials (Fernández-Aparicio et al. 2008, 2009). Fernández-Aparicio et al. (2010) proposed the use of berseem clover (*Trifolium alexandrinum*) as an intercrop with grain legumes to a significant reductionof *O. crenata* infection. Considerable internal variation within *O. crenata* populations parasitizing faba bean and lentil species was observed by molecular analyses, but significant divergence among the populations was detected (Ennami et al. 2017).

5.3 Genetic Resources of Resistance Genes

Domesticated lentil has a relatively narrow genetic base globally and most released varieties are susceptible to severe biotic and abiotic stresses. The crop wild relatives could provide new traits of interest for tailoring novel germplasm and cultivated lentil improvement (Singh et al. 2020a). There are a considerable number of global (mainly the ICARDA collection) and national collections of germplasm of land races and wild lentil relatives. GENESYS, the online platform that includes information on plant genetic resources for food and agriculture conserved in genebanks worldwide (https://www.genesys-pgr.org/), encompass records of 31,211 accessions named as lentil, although the information collected on lentil only refers to 70% of the total of 43,214 accessions conserved ex situ in all genebanks. References to lentil germplasm collections have been published in some review papers (Muehlbauer et al. 1995; Pérez de la Vega et al. 2011), and more recently extensive compilations of the cultivated and wild *Lens* germplasm collections can be found in the reviews by Singh et al. (2018) and Malhotra et al. (2019). The number of accessions varies widely between national collections, highlighting 10 collections with more than 1,000 accessions. Regarding the percentage of wild relatives, the high 70% of the Ethiopian collection stands out, and Canada, the Russian Federation, Chile, China and Spain have relevant values, around 10% up to 17%, although the number of wild relatives is unknown in approximately 50% of the collections. The percentage of land races varies between more than 90% in Pakistan, Nepal and Turkey, to 3–5% in Egypt and Hungary. The ICARDA collection maintains almost 11,000 accessions, of which 82% are local breeds and 583 wild relatives.

Three recent papers (Singh et al. 2018; Gupta et al. 2019, 2020) have reviewed the use of wild germplasm in relation to the response to stresses (cold, drought, salinity, diseases, etc.). Among the extensive amount of data compiled, these papers summarize the wild germplasm used to that date for the introgression of useful traits in cultivated lentil in relation to the response to several diseases (anthracnose, Ascochyta blight (AB), Fusarium wilt (FS), powdery mildew, and rust), to pests and parasitic plants (Sitona weevils, bruchid weevils, and orobanche), in addition to some abiotic stresses (drought, cold, and yield components). Also Ladizinsky and Abbo (2015) and Malhotra et al. (2019) summarized the potential of wild *Lens* resources as resistance sources. Likewise, Gupta et al. (2019) summarized the resistance sources found in the lentil cultivated gene from many different countries, namely to AB, anthracnose, rust, FW, Botrytis gray mold, and Stemphylium blight. Rana et al. (2016) summarized the pulse crop resources in the large national collection of India, listing lentil, and other pulses, accessions with resistance to diseases and pests and as sources of agro-morphological characters, quality, biochemical traits, and abiotic stresses.

Resistance to the pest *Callosobruchus chinensis* was evaluated in a germplasm collection of wild and cultivated *Lens* accessions (Gore et al. 2016). Accessions were categorized as highly resistant, resistant, moderately resistant, moderately susceptible, and susceptible. *L. ervoides* was highly resistant and *L. culinaris* was the most

susceptible species. Likewise, resistance against *Bruchus* spp. has been evaluated in a large collection of 571 cultivated and wild accessions from 27 countries (Laserna-Ruiz et al. 2012). A total of 32 accessions, including *L. culina*ris subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, *L. nigricans*, and *L. lamottei*, showed lower infestation rates than the check and were selected as potential sources of resistance to this pest.

Resistance to AB has been found in several germplasm screens. In a wide experiment carried out under field and greenhouse condition in Canada,Tullu et al. (2010) found resistant accessions in all *Lens* wild taxa tested except in *L. tomentosus*, using a mixture of three monoconidial isolates of A*scochyta lentis* as the inoculum. Several consistently resistant accessions were found among entries of *L. ervoides* and *L. nigricans*. Thirteen accessions, previously reported as resistant to Syrian isolates of *A. lentis* were also resistant to the Canadian isolates. Furthermore, some of the wild accessions showed resistance to anthracnose. Cultivars and germplasm accessions of cultivated lentil showed a wide range of response between the resistant and susceptible controls. In a further study Dadu et al. (2017), in a search among 30 accessions from five wild *Lens* taxa, found resistance to AB to new highly aggressive Australian *Ascochyta* isolates in several wild *Lens* taxa, particularly in *L. orientalis*.

Dadu et al. (2018) analyzed the early response to two *Ascochyta lentis* isolates (FT13037 and F13082) of two cultivated lentil genotypes, ILL7537 (resistant) and ILL6002 (susceptible), and the recently identified AB-resistant *Lens orientalis* genotype ILWL180. Both isolates had significantly lower germination, shorter germ tubes and delayed appressorium formation on the resistant genotypes compared to the susceptible genotype; furthermore, these were more pronounced on the wild ILWL180 than on cultivated ILL7537. The authors concluded that the faster recognition of *A. lentis* is likely to be a major contribution to the superior resistance observed in genotype ILWL180 to the highly aggressive isolates of *A. lentis* assessed. Likewise, Dadu et al. (2019) using the focused identification of germplasm strategy, selected a subset of 87 landraces (originating from 16 countries) with highest likelihood for *A. lentis* resistance from 4,576 accessions held by the ICARDA. Significant variation for resistance was detected within the subset using completely randomized and replicated controlled climate bioassays with a highly virulent Australian *A. lentis* isolate, FT13037. Genotype IG 207 expressed the lowest percent area of symptomatic tissue and further 12 genotypes demonstrated moderate resistance. Furthermore, IG 207 recorded lowest mean disease score against four other highly aggressive fungal isolates and performed better than the currently used best resistance sources.

A recent study (Singh et al. 2020a) evaluated, under multi-location and multi-season, performances for several agronomic traits and resistance against rust (*Uromyces fabae*), powdery mildew (*Erysiphe trifolii*) and FW (*Fusarium oxysporum* f. sp. *lentis*) under field and controlled screening conditions. Genetic material comprised 96 wild lentil accessions, including samples of all *Lens* species, and two cultivated varieties. Results describe several donor accessions for their introgression against these three biotic stresses, in addition to lentil genetic improvement of important agronomic traits. Donor accessions for disease resistance breeding were found in all wild taxa. Moreover, some of the wild accessions from Syria and Turkey showed resistance against more than one disease indicating a rich diversity of lentil

genetic resources [accessions ILWL230 and ILWL476 of *L. orientalis* (rust and powdery mildew); ILWL9 and ILWL37 of *L. nigricans* (rust and powdery mildew); IG136639 of *L. ervoides* (powdery mildew and FW) and ILWL308 of *L. tomentosus* (rust and FW)]. Further, some stable gene sources were identified: ILWL203 of *L. odemensis* for rust and high pod number; ILWL230, ILWL476 of *L. orientalis* for rust and powdery mildew; ILWL191, ILWL9, and ILWL37 of *L. nigricans* for rust and powdery mildew, IG136639 of *L. ervoides* for powdery mildew and FW, and ILWL308 of *L. tomentosus* for rust and FW. The study has also identified some trait specific accessions, which could also be taken into the consideration while planning distant hybridization in lentil; but some belong to the most distant genepool from the lentil cultigen, such as ILWL18 and ILWL19 of *L. nigricans* promising for high seed yield per plant, or ILWL191, ILWL9, and ILWL37 for resistance, which makes their real use in breeding difficult.

Partial resistance to the parasitic weed *Orobanche crenata* was detected in in a collection of 234 Spanish cultivated accessions under field conditions, scoring a wide range of responses but no complete resistance. A range from complete resistance to susceptibility was found among 23 wild *Lens* accessions. The higher levels of resistance were observed in accessions of *L. ervoides, L. odemensis* and *L. orientalis* (Fernández-Aparicio et al. 2008, 2009).

5.4 Glimpses on Classical Genetics and Traditional Breeding

5.4.1 Breeding Objectives

The main breeding objective in relation to biotic stresses is to introduce genes that confer resistance to pathogens and pests, if possible, durable resistance. The complexity of the selection and breeding process is imposed by the great variety of stressors, which implies a large number of potentially useful qualitative (single-gene resistance) and quantitative genes (oligo- or polygene resistance), and by the convenience of their pyramiding.

The single-gene resistances have both advantages and disadvantages. The advantages, in addition to the fact that dealing with single gene genetics is much simpler than polygenic genetics, are complete protection against the parasite in question, and compatibility with breeding for wide climatic adaptation. The main disadvantage of single-gene or vertical resistance (genetic resistance that is effective at preventing successful attack only by certain races of a pathogen, also called specific) is its temporary nature, since it breaks down to new strains of the parasite. Other disadvantages include a loss of horizontal resistance (resistance that is effective at preventing successful attack by most/all races of a pathogen; also called general) while breeding for vertical resistance, and the fact that single-gene resistance cannot always be found.

Thus, it has appeared impossible to breed for vertical resistance to some species of crop parasites, including many of the insect pests of crops (Robinson1997).

Although theoretical and empirical studies comparing deployment strategies of more than one resistance gene are scarce, the REX Consortium (2016) concluded that that the overall durability of resistance genes may increase by pyramiding their presence in the same plant; and that data also suggests that the pyramiding of disease resistance genes is the most durable strategy. By extension, authors suggested that the combination of disease resistance genes with other practices for pathogen control (pesticides, farming practices) may be a relevant management strategy to slow down the evolution of virulent pathogen genotypes.

5.4.2 Classical Mapping Efforts

The inheritance of morphological and agronomical traits in lentil was first described at the end of 70's and early 80's (Pérez de la Vega et al. 2011). Several traits such as seed coat color, epicotyl and flower color, and pod dehiscence, were found to be controlled monogenically and thus appropriate to be used as morphological markers. Linkage analysis in lentil started in the 80's and was initially based on morphological and isozyme markers. However, the number of morphological and isozyme markers in lentil is relatively low, which made the classic genetic maps based on them of little use for breeding. The first genetic linkage analysis based on morphological and isozyme markers in lentil was reported by Zamir and Ladizinsky (1984). Muehlbauer et al. (1989) described the allozyme polymorphisms for 18 loci and the linkage relationships among them and with four genes controlling morphological traits. The linkage analysis resulted in six linkage groups (LGs), which contained 14 of the loci analyzed. This work also contributed to the first evidence of shared synteny between *Lens* and *Pisum* since several of the LGs were conserved between both species. Tahir and Muehlbauer (1994) were the first to use recombinant inbred lines (RILs) for mapping lentil markers. Kumar et al. (2015), Ates et al. (2018), and Gupta et al. (2019) have recently summarized the mapping efforts in lentil using from isozyme and morphological markers to molecular markers including random amplified polymorphic DNA (RAPD), SSR, inter simple sequence repeat (ISSR), single nucleotide polymorphism (SNP), etc., and from 1994 to 2017. The more recent list by Gupta et al. (2019) includes 20 different maps (23 references) generated from different segregant populations obtained from intra- and interspecific crosses. Likewise, this publication lists recombinant inbred line (RIL) populations obtained in the ICARDA, the National Bureau of Plant Genetic Resources, India, and the Indians Institute for Pulse Research, India. Further, some more RIL populations have been described (Suvorova and Ikonnikov 2014; Bhadauria et al. 2017a; Polanco et al. 2019).

5.4.3 Classical Breeding Achievements

According to the recent review by Gupta et al. (2020) breeding methods for incorporation of breeding traits employed in lentil majorly included pure line selection, hybridization, backcross, bulk, pedigree and single seed descent methods. As a result of these methodologies, a total of 146 cultivars have been released until 2017 across major lentil-producing countries with targeted traits. Pure line selection was extensively used to release cultivars with adaptability to wider areas and superior yield performance and disease resistance for Ascochyta blight (AB), rust and Fusarium wilt (FW). Certainly, in relation to biotic stresses, resistance to ascochyta is the main breeding targets for the newly released cultivars in all geographical areas of the world, followed by resistance to rust, stemphylium and fusarium. Cross-breeding is the widely chosen method in the recent past by breeders particularly to introgress special traits from exotic or other popular germplasm to the locally adapted cultivars, while single seed descent has often been used to produce RILs for use in constructing linkage maps and identification of quantitative trait loci (QTLs) controlling traits of interest such as resistance to AB, anthracnose and FW. Mutation techniques have been used in lentil as a complementary breeding strategy to introduce a desirable trait which is absent in the available germplasm. Several cultivars with different traits of interest have been developed and released worldwide using irradiation and ethyl methanesulfonate as a source of mutagens (Gupta et al. 2020). If we stick to biotic stresses, most of cultivars developed through mutation breeding registered in the Indian subcontinent and outside it have been improved for resistance to diseases such as to FW, AB, botrytis, rust and anthracnose (Laskar et al. 2019).

5.4.4 Limitations of Classical Endeavors and Utility of Molecular Mapping

Marker-assisted plant breeding based on the use of morphological markers had a slow development from the pioneering work of Sax (1923) due first to the limited number of morphological markers that could be simultaneously and independently genotyped. Additional drawbacks arose from epistatic effects among genes controlling related, an even apparently unrelated, traits, or due to the lack of observable markers during the first development stages hindering early selection. Likewise, the estimates of the recombination fraction were often limited by segregations in repulsion phase. The beginning of the use of isozymes (codominant markers) in plant genetics in the 70's of the twentieth century partially solved these problems, but again the number of isozyme markers that can be analyzed simultaneously was too low to be able to accurately locate most of the genes of interest, and even more so if they were QTLs. The incorporation of the molecular markers was a great step towards the solution of obtaining sufficiently saturated genetic maps and flanking markers of the locus of interest. The number of markers that could be studied simultaneously increased

dramatically and, above all, the use of codominant markers, such as SSRs and SNPs, solved the disadvantages of the dominant ones in estimating genetic linkage distances and confidence intervals. Isozyme and molecular markers have the additional advantage that they can be considered selectively neutral. Thus molecular markers allowed analyzing large segregating populations to attain much greater marker saturation with neutral phenotypic effects. However, the precision with which QTLs with minor effects are often located was still poor. The high-throughput sequencing techniques have contributed a definitive advance since marker numbers have gone from hundreds of codominant markers to thousands, fundamentally SNPs, which allows obtaining saturated maps with a good coverage of the entire genome, that in turn allows greater precision in locating QTLs and easily finding markers flanking the locus of interest for their use in marker-assisted selection (MAS).

The molecular information gained by the 'omics' techniques removes, although only partly, some of the limitations of selection on phenotype, by allowing selection at the genotype level, which results in more accurate, faster, and cheaper selection. It also provides a high number of markers for MAS. Ultimately, the use of MAS will be determined by the economic benefit relative to conventional selection. Further applications of MAS require the redesign of breeding strategies and their integration with other technologies, such as higher-resolution genetic maps and high-throughput genotyping technologies (Dekkers and Hospital 2002).

5.5 Brief on Diversity Analysis

5.5.1 Phenotype-Based Diversity Analysis

Numerous works have been published on the phenotyping of lentil germplasm collections in relation to the responses to the main diseases and pests and in the search for resistance genes. Many of these citations can be found in previous reviews, such as those by Pérez de la Vega et al. (2011), Singh et al. (2018) and Gupta et al. (2019), Malhotra et al. (2019), and Gupta et al. (2020). Phenotyping for response to several pest species has been carried out by El-Bouhssini et al. (2008), Kumari et al. (2007), Gore et al. (2016), and Laserna-Ruiz et al. (2012). Some sources for resistance to several viruses in lentil germplasm were reported by Makkouk and Kumari (2009). Resistance sources to broomrape weed were searched in cultivates and wild materials by Fernández-Aparicio et al. (2008, 2009). Recent phenotyping screenings for AB has been carried out by Dadu et al. (2018, 2019) and Singh et al. (2020a). This last publication describes the resistance against rust, powdery mildew and FW under field and controlled screening conditions. Podder et al. (2013) carried out a screening of wild and cultivated *Lens* germplasm for resistance to SB.

Some advances in phenotyping for resistance can help in accelerating the search for resistance and in obtaining new varieties. Lulsdorf and Banniza (2018) described a rapid generation cycling technique to cut the selection period in half. The technique

was tested on an F_2 population derived from a *L. culinaris* × *L. ervoides* cross in combination with a liable technique for the screening to aphanomyces root rot (ARR). Phenotyping of an F_2 population of more than 1,200 plants resulted in scores ranging from 2.4 to 4.0 on a scale from zero to five. Plants with scores lower than 4.0 were selected for advancement for five generations using a modified single-seed descent method and optimum growing conditions. Phenotyping of the F_7 population resulted in scores ranging from 1.4 to 4.0. Marzougui et al. (2019, 2020) applied phenomics technologies to evaluate ARR resistance in 547 lentil accessions and lines using Red–Green–Blue images of roots. Models were able to classify three disease categories with an accuracy of up to 0.91. The authors concluded that the image-based phenotyping approaches can help plant breeders to objectively quantify ARR resistance and reduce the subjectivity in selecting potential genotypes. The use of such technologies to the evaluation of other biotic stresses would certainly be of great help in phenotyping and plant breeding.

5.5.2 Extent of Genetic Diversity

As it was mentioned in Sect. 1.1, genomic studies suggest the existence of four gene pools in relation to cultivated lentils. The primary gene pool would include the taxa *orientalis* and *tomentosus*, the secondary *lamottei* and *odemensis*, and finally *L. ervoides* and *L. nigricans* would be the tertiary and quaternary gene pools, respectively (Wong et al. 2015), although there are different assignations of species to gene pools (Ladizinsky and Abbo 2015). Breeding lines and recombinant inbred lines have been obtained and described in the scientific literature at least from hybrids with *orientalis*, *odemensis* and *ervoides* (Suvorova and Ikonnikov 2014; Bhadauria et al. 2017a; Polanco et al. 2019).

A recent analysis (Khazaei et al. 2016) of the primary germplasm has indicated that cultivated lentils can be grouped into three agro-ecological zones. The study was based on the use of 1,194 SNP markers which span the lentil genome, analyzing 352 accessions from 54 countries obtained from three large germplasm collections. Accessions were categorized into three major groups, namely, South Asia (sub-tropical savannah), Mediterranean, and Northern temperate, which prominently reflected geographical origin (world's agro-ecological zones). The three clusters complemented the origins, pedigrees, and breeding histories of the germplasm. The study revealed that considerable genetic diversity for breeding can still be found in this primary pool, but that the South Asia and Canadian germplasms had narrow genetic diversity.

Pavan et al. (2019) analyzed a collection of lentil accession covering one of the first areas of distribution of this crop after domestication, the Mediterranean Basin countries, which holds large part of lentil biodiversity. They analyzed 184 *L. culinaris* accessions by high-throughput genotyping by sequencing of a Mediterranean collection. On the basis of 6,693 single nucleotide polymorphisms, the analysis of no redundant genotypes highlighted the occurrence of five highly differentiated

genetic clusters, related to geographic patterns and phenotypic traits, indicating that post-domestication routes introducing cultivation in Mediterranean countries and selection were major forces shaping lentil population structure. The identification of distinctive alleles across clusters suggested the possibility to set up molecular keys for the assignment of lentil germplasm to specific genetic groups, helping in lentil conservation genetics and breeding.

Dissanayake et al. (2020) carried out a wide analysis of the genetic variation and the relationships among the *Lens* taxa using a worldwide sample of 467 wild and cultivated accessions collected from 10 diverse geographical regions and 28 countries. *L. nigricans* exhibited the greatest allelic differentiation compared to all other species or subspecies, indicating that this species is the most distantly related to *L. culinaris*. Genetic distance matrices revealed a comparable level of variation within the gene pools of *L. culinaris, L. ervoides*, and *L. nigricans*. This work will be certainly a valuable source for the use of the wild germplasm in lentil breeding and gene introgression.

Liber et al. (2021) combined GBS of 190 lentil accessions (67 wild and 123 domesticated) from the Old World with archeological information to analyze the evolutionary history, domestication, and diffusion of lentils. GBS led to the discovery of 87,647 SNPs, which allowed inferring the phylogeny of genus *Lens*. The only gene flow detected was between cultivated varieties and their progenitor (*L. culinaris* subsp. *orientalis*) albeit at very low levels. Nevertheless, a few putative hybrids or naturalized cultivars were identified. Within cultivated lentil, three geographic groups were detected.

5.6 Association Mapping Studies

In the search for genetic variants linked to phenotypic differences, association mapping (AM) exploits long-term historic recombination in natural populations. Population based AM employs a sample of individuals from the germplasm collections or a natural population. With more accumulated recombination events it is considered to be more accurate than traditional mapping based on biparental crosses. The resolution of the AM depends on the extent of linkage disequilibrium (LD) across the genome, the number of accessions considered, and the number and distribution of markers employed. Lentils display extensive LD (Lombardi et al. 2014; Singh et al. 2017a; Kumar et al. 2019a; Ma et al. 2020), likely due to their high degree of self-pollination and the narrow genetic base of the breeding material, thus shirking the need for a large number of markers. The drawback of a wide LD is a lower resolution because a significant marker-trait association does not necessarily imply that a marker is in close proximity to the gene. The future release of a reference-quality genome assembly will allow quantifying the LD decay over the physical distance, and thus estimating the number of markers that are required for a particular scrutiny of the genome through AM.

The collection of cultivars, landraces and wild genotypes has been extensively reviewed and characterized (Coyneand McGee 2013; Lombardi et al. 2014; Laskar et al. 2019; Dissanayake et al. 2020; see also Sect. 5.3). Currently the whole cultivar collection amounts to a total of 43,214 accessions of the genus *Lens*, ICARDA being the institution that holds the most (24%). While wild accessions are genetically diverse, there is a reduced gene pool in the cultivated material that dates back to the bottleneck associated with domestication (Lombardi et al. 2014; Dissanayake et al. 2020). In order to introduce new variability into cultivars, hybridization with wild genotypes and with close species has been proposed (Singh et al. 2014).

Because of their large number and scattered distribution throughout genomes, SNPs are the most-used molecular markers for AM studies. Two SNP-based high-throughput approaches have been utilized in lentil research: SNP chips (microarrays) and genotyping-by-sequencing (GBS). Within the first, the Illumina® GoldenGate® assay has been the chosen genotyping platform for several studies (Gujaria-Verma et al. 2014; Lombardi et al. 2014), but it has been superseded by other microarray-based technologies and is now discontinued. The chip was able to interrogate up to 1,536 SNPs simultaneously. More modern microarrays, such as the customizable Infinium iSelect high definition (HD) and the Infinium iSelect high-throughput screening (HTS) custom genotyping BeadChips, are expected to grow in popularity as we gain more knowledge of the lentil genome and more trait-linked SNPs are discovered. Lentil researchers can now design a custom genotyping panel that supports up to 700 k custom targets among SNPs, indels, and copy number variations. The Infinium iSelect can be deployed in two options: either the HD with 3,072 to 90,000 custom markers, or HTS, able to screen between 90,001 and 700,000 markers. Creation of these custom assays enables focused, high-throughput genotyping applications tailored to specific project needs in a cost-effective manner.

Access to a genome assembly has facilitated GBS considerably, and, at the same time, has made GWA studies affordable. GBS (Elshire et al. 2011) is a high-density genotyping approach extensively used in breeding and genetics because of its low cost, high number and uniform distribution of SNP markers, and the capacity to simultaneously perform polymorphism discovery and genotyping. It has been proven effective in crops with large and repetitive genomes (Gutierrez-Gonzalez et al. 2019). In lentils, using GBS markers in a genome-wide association study (GWAS), Ma et al. (2020) identified 38 QTLs and 15 candidate genes that could be associated with aphanomyces root rot (ARR). Two of them, ABC transporter A family protein (ABCA), and pectin esterase (PE) were found differentially expressed at the early stages of infection, likely involved in the plant-defense mechanism against ARR. We expect GBS approaches to play increasing roles in highlighting plant defense mechanisms as more polished genome assemblies are being released.

5.7 Molecular Mapping of Resistance Genes and QTLs

Since the first efforts in lentil breeding, the main goal has been similar in all countries: to obtain larger and more stable seed yields (Pérez de la Vega et al. 2011). In order to reach this objective, the development of resistant cultivars to pathogens plays a crucial role in breeding programs, and the knowledge of the genetic basis of the resistance helps to design faster and more efficient breeding approaches. Traditionally, the genes involved in the resistance were studied by crossing two parental lines differing in the response to the pathogen and evaluating the segregating descendant population. The development of lentil RIL populations provided permanent materials that can be shared by many research groups, in which new additional markers and characteristics can be added along the time. Additionally, another advantage of RILs is that because the lines have gone through several rounds of meiosis before homozygosity is reached, the degree of recombination is higher compared to F_2 populations, and consequently, RIL populations show a higher resolution than maps generated from F_2 populations, and the map positions of even tightly linked markers can be determined (Schneider 2005).

Despite the advantages of RIL populations, the gained results may be relevant only for the studied material, and the validation in new germplasm is laborious. An improvement in the detection of relevant accessions and genes related with interesting breeding traits is the development of immortalized segregant populations obtained from the crossing of multiple parents (i.e. MAGIC populations). Some populations of this kind are being developed in ICARDA at the present time (Kumar et al. 2021). In addition, the availability of a high number of markers, in special after the genomic and transcriptomic studies published in lentil, has made possible to start some GWAS, such as the published by Kumar et al. (2018a) or Khazaei et al. (2018), but there are not published information on disease resistances so far.

Several diseases in lentil are due to fungus infections, such as AB, SB, rust, FW, anthracnose, ARR, collar rot, molds (*Botrytis cinerea*, *B. fabae* and *Sclerotinia sclerotiorum*). However, the genetic basis of the response to many of these pathogens has not been analyzed in a formal research. In the following paragraphs a review of the main data available to date is presented.

5.7.1 Genetics of Lentil Resistance to Ascochyta Blight

Ascochyta blight (AB) is one of the main lentil diseases in most lentil growing countries. This disease, caused by the fungus *Ascochyta lentis* (syn. *A. fabae* f. sp. *lentis*; teleomorph *Didymella lentis*), affects all above ground parts of the plant and is characterized by necrotic lesions, which on susceptible cultivars, in favorable conditions, can lead to breakage of the stems and severe yield reduction. Seed quality may also be reduced through seed discoloration or retardation of seed development. AB can be controlled by chemicals, but besides the environmental problems related

with the use of fungicides, the development of resistant cultivars is considered a more efficient and sustainable approach (Davidson and Kimber 2007). *Ascochyta lentis* is a host-specific pathogen (Peever 2007) and considered as a necrotrophic fungus, although a short biotrophic period cannot be completely excluded (Tivoli and Banniza 2007; Sari et al. 2017). The pathogenicity of this type of fungus and the resistance of the plant could be related to the production of specific fungal toxins and plant receptors or detoxifying molecules, and Kim et al. (2016) have described the presence of a set metabolites only found in *A. lentis*. The recent publication of the *A. lentis* genome sequence by Lee et al. (2021) will provide a powerful tool in order to identify the candidate genes involved in the pathogenicity of this fungus.

In lentil, the genetic control of the resistance response to the fungus were firstly studied in a qualitative way, by crossing susceptible and resistant cultivars obtaining various results, mainly one or two genes, dominant and/or recessive (see Pérez de la Vega et al. 2011; Sudheesh et al. 2016a; Rodda et al. 2017 for reviews). The different results may be due to the different genotypes used; however, the differences in screening methods or *Ascochyta* isolates employed cannot be ruled out. These initial studies allowed the identification of some major genes that have been used in the breeding programs, such as those found in the cultivar Indianhead, or in the ICARDA lines ILL5588 or ILL7537, although the molecular mechanisms for the resistance provided for these genes is still unknown. In the last years, pathogen isolates capable of overcoming the resistance provided for the major genes have appeared in Australia (Rodda et al. 2017), making a priority the identification of new genes and sources of resistance.

Although the qualitative classification of the resistance to AB provided the detection of some major genes, most of the results did not show a clear Mendelian segregation, and consequently, a quantitative analysis of the resistance response seems more appropriate to describe this trait (Rubeena et al. 2006; Gupta et al. 2012). QTL analyses on the response to *Ascochyta* in lentil using high-density maps based on gene SNPs have been carried out, allowing the identification of several QTLs (reviewed by Rodda et al. 2017), with magnitudes varying from 3 to 89% of the phenotypic variance evidenced, although it is common to find values between 20 and 50%. An important drawback of these studies is the lack of common markers between the genetic maps, and consequently the difficulty to establish a comparable nomenclature for the linkage groups (LG) in order to determine the QTL locations. Despite of that, some limited relationships have been done based in a few markers: for instance, the QTL named *AB_NF1* in LG6 in the study of Sudheesh et al. (2016a) is comparable in position to *QTL5* in LG1 of Rubeena et al. (2006), to *QTL1* in LG1 of Gupta et al. (2012), and to the three closely linked *AS-QTLs* detected in LG6 in Polanco et al. (2019) in an interspecific cross between *L. culinaris* and *L. odemensis*.

The use of QTL knowledge in breeding programs requires of the validation of the markers associated with the QTL in a diversity panel of genotypes. So far, only the allelic identity of the QTL *AB_IH1* (Sudheesh et al. 2016a) was found to predict the resistance response in more than 85% of the diversity panel, mainly composed by

Australian lentil germplasm. This relationship was not so conserved in the international germplasm panel, which suggests that there are new resistance genes or alleles to be detected.

All the lentil genotypes which showed resistance to AB so far known show a partial resistance or it is surpassed by new and more aggressive isolates (Dadu et al. 2017, 2018), and genetic and genomic studies point to that there are several response mechanisms to this pathogen. Genetic studies suggest that AB resistance genes in several partially-resistant lentil lines are nonallelic (Sari et al. 2017). Furthermore, these authors found that the partially resistant genotypes CDC Robin and 964a-46 differed in the timing and the magnitude of salicylic acid (SA) and jasmonic acid (JA) signaling pathway activation. The SA signaling pathway was only triggered in 964a-46, whereas the JA pathway was triggered in both partially resistant genotypes. The expression of JA-associated genes was lower in 964a-46 than in CDC Robin. These observations corroborate the existence of diverse AB resistance mechanisms in lentil genotypes carrying different *R*-genes (Sari et al. 2017; Khorramdelazad et al. 2018; Garcia-Garcia et al. 2020).

From a practical point of view in breeding programs, it is interesting to remark that some regions in which QTLs conferring resistance to AB are located also contains genes for resistance to other pathogens. For instance, a QTL that explained 41% of the variation in the reaction to AB found in the LG6 (Tullu et al. 2006) showed linkage to the *LCt2* gene for resistance to anthracnose (*Colletotrichum lentis*).

5.7.2 Stemphylium Blight Resistance

Stemphylium blight (SB) has recently emerged as a new important fungal defoliating disease in lentils. It is caused by the necrotrophic Ascomycete, *Stemphylium botryosum* Walr. (Pleosporales, Pleosporaceae) (teleomorph: *Pleospora herbarum* (Fr) Rab.), and it was firstly described in 1986 in Bangladesh, but the reports on yield losses caused by this disease have been increasing in the recent years all around the world (Das et al. 2019). The host range of *S. botryosum* is wide and includes a large number of ornamentals, horticultural and crops, including lentil, pea, tomato, alfalfa, lettuce or onion (Das et al. 2019). Usually the pathogen infects the lentil plants in the first stages of pod setting, when the spores germinate on the leaflet surfaces and the hyphae penetrate through the stomata or directly through the epidermis (Pierre and Millar 1965).

The first studies on the genetic basis of the resistance were done by Saha et al. (2010b), detecting several QTLs in two different years, although only one was significant in both, explaining between a 25% and a 46% of the phenotypic variation. The quite different results obtained in posterior crosses with *L. culinaris* genotypes made the inheritance of this resistance to be in an ambiguous stage (Das et al. 2019).

Deeper information on SB resistance is available from the wild species *Lens ervoides*. Because few sources of resistance were found in *L. culinaris*, a screening was carried out in wild genotypes, and *L. ervoides* was found to show high levels of

resistance, at higher frequencies than the other species (Podder et al. 2013). In the RIL population LR-66 derived from the cross between two *L. ervoides* accessions (L01-827A and IG 72815), Bhadauria et al. (2017a) detected three QTLs in the linkage groups LG2 and LG3 that together explained the 40.5% of the phenotypic variance. Because the *L. ervoides* genetic map in this experiment could be related with the *L. culinaris* genetic map of reference, and a high level of collinearity between the two genomes, especially in the identified QTL regions, the *L. culinaris* genome can be utilized to identify the candidate genes. Cao et al. (2019) analyzed two transgressive RILs derived from the *L. ervoides* RIL population LR-66 above mentioned in a search for candidate resistance genes against SB using transcriptome sequencing. In this work, three of the genes located in the QTLs have been chosen as the more promising candidate genes because of the expression changes showed after the infection in the resistant and susceptible RILs.

Additional information comes from the research of Adobor et al. (2020) with an interspecific RIL population (LR-26) developed from a cross between the moderately resistant parent *L. culinaris* cv. 'Eston' and the resistant parent *L. ervoides* accession IG 72,815. The plant resistance to SB was tested under controlled conditions and under field conditions. Although the distribution of disease severity scores for all RILs indicated a polygenic inheritance of SB resistance in the population, no resistant transgressive segregants were observed. Across all environments, 14 RILs consistently had resistance levels similar to the resistant parent IG 72,815, which makes them a promising material to be included in future breeding programs.

*5.7.3 Rust (*Uromyces viciae-fabae*) Resistance*

Rust disease in lentil is due to the infection by the biotrophic fungus *Uromyces viciae-fabae* (Pers.) J. Schröt. This pathogen is widespread and attacks the aerial parts of the plants, producing defoliation and the plant death. Although *U. viciae-fabae* infects several legume genera such as *Vicia, Lens, Pisum* or *Lathyrus*, the pathology studies have identified three specialized groups named *U. viciae-fabae* ex *V. faba* which infects only faba bean, *U. viciae-fabae* ex *V. sativa* which infects other species of *Vicia* and *U. viciae-fabae* ex *L. culinaris* which infects *L. culinaris* only (Rubiales et al. 2013b).

Lentil rust resistance seems to be under a simple Mendelian control. The results depend on the specific cross, but generally the segregation of a single gene explained the data, being the resistance dominant over the susceptibility; however, controls based on a recessive gene or duplicate dominant genes have also been founded (Chahota et al. 2002; Mishra et al. 2007, 2008; Saha et al. 2010a; Negussie and Pretorious 2012; Mekonnen et al. 2014; Dikshit et al. 2016; Singh et al. 2021). The names proposed for these genes are *Urf1, Urf2* and *urf3* (Sharma 2009). The relatively simple genetic control of the resistance has allowed the development of some molecular markers of potential utility in breeding programs, which will be described in the Sect. 5.8.

The two types of resistance (prehaustorial and posthaustorial) reported in the lentil germplasm suggest the existence of two different genetic mechanisms for the response to rust (Rubiales et al. 2013a). Prehaustorial resistance is usually connected to a non-host resistance and is generally based in a polygenic control. This kind of resistanceis expected to be more durable than the posthaustorial one, usually controlled by single genes. But unfortunately, so far no research on the genetic control of this type of resistance has been published.

5.7.4 *Wilt (Fusarium oxysporum f. sp. lentis) Resistance*

The wilt disease is one of the most important biotic stresses affecting the stability of production in lentil. It is caused by *Fusarium oxysporum*, a filamentous ascomycete fungus that produces spores protected by thick walls, making them able to survive in the soil by long periods, reason why is usually considered as saprophytic. When some nutrients are available, such as root exudates, the spores germinate and the hyphae grow and penetrate in the plant roots, invading the inter- and intra-cellular spaces. While the plant is alive, the fungus remains strictly limited to the xylem tissues and a few surrounding cells. After the host plant is killed by the pathogen, the fungus can invade the parenchymatous tissue, sporulate on the plant surface and release spores (Pouralibaba 2017). *F. oxysporum* infects a large number of plant species, and some strains have been adapted to colonize specific hosts, giving the named *formae speciales* (ff. spp.). More specifically, lentil wilt is caused by *Fusarium oxysporum* Schlecht. Emend Snyder and Hansen f. sp. *lentis* Vasudeva and Srinivasan.

The genetic studies on FW in lentil point to a simple control by a low number of genes, usually dominant (Choudhary and Kumar 2016). Thus, Kamboj et al. (1990) identified in total five dominant independently segregating resistance genes. More recently, the segregations usually detected only one dominant gene, named *Fw* (Eujayl et al. 1998; Hamwieh et al. 2005). This locus was mapped in the LG6 (Hamwieh et al. 2005) of their genetic map, linked to some SSR markers that seem to be located in the pseudochromosome 4 of the lentil genome v1.2.

New resistance genes of utility in lentil breeding have been detected in some transgressive segregants obtained for crosses between *L. culinaris* and *L. ervoides* (Singh et al. 2017c), although their characterization has not been published so far.

5.7.5 *Anthracnose (*Colletotrichum lentis*) Resistance*

Anthracnose is a disease attributed to the hemibiotrophic fungus originally identified as *Colletotrichum truncatum* [(Schwein.) Andrus & W. D. Moore] but since 2014 is attributed to the new species *Colletotrichum lentis* Damm, sp. nov. MycoBankMB809921 (Damm et al. 2014). When the pathogen infects the plant, initially it shows a biotrophic and symptomless stage, and afterwards changes to

a necrotrophic phase causing the death of plant cells. This switch seems to be the pathogen adaptive response to the defense mechanism of the plant, based on the cell death (Bhadauria et al. 2013). This disease has been described in many countries producing minor losses in production, however in western Canada has become the most important foliar fungal disease (Gela et al. 2020). There has been identified two pathogenic races of the fungus, race 0 and race 1 (Banniza et al. 2018), and the genetic resistance to anthracnose depends on the *C. lentis* race. While a partial resistance to race 1 is quite frequent in lentil, and it has been effectively transferred to elite cultivars, resistance to the highly virulent race 0 has not been identified. To date, the only sources of high levels of resistance to race 0 seems to be restricted to wild lentil species, especially *L. ervoides* (Gela et al. 2020).

The genetic resistance to race 1 appears to be under a single dominant gene (Tullu et al. 2003, 2006; Tar'an et al. 2003), although the different levels of resistance that has been detected in some crosses points to the existence of additional genes. Thus, Buchwaldt et al. (2013) explained their results as the interaction among two recessive genes, *ctr1* and *ctr2*, and three closely linked dominant genes, *CtR3*, *CtR4* and *CtR5*.

The genetics of the resistance to race 0 and race 1 has been analyzed in the same RIL population LR-66 derived of the cross between two *L. ervoides* accessions (Bhadauria et al. 2017a) mentioned in the SB resistance section. The results showed five QTLs with a significant association with resistance to race 0 and six QTLs to race 1 resistance. Three QTL for resistance to *C. lentis* races 1 and 0 co-localized, one in LG3 and two LG5, collectively explaining 47.58% and 54.82% of the variance in resistance response to *C. lentis* races 0 and 1, respectively. This suggests that a large proportion of the resistance to both races of *C. lentis* is regulated by genes at the same loci. The joint analysis of transcriptome studies and QTL mapping has allowed the identification of two genes as main candidates to be responsible of the resistance response, *Lc23518* (in LG5) coding for an LRR receptor-like kinase protein, and *Lc09295* (in LG2) coding for a MYB transcription factor, although these genes need further evaluation (Bawa 2020).

Recently, Gela et al. (2021) have analyzed a RIL population obtained from the cross between *L. culinaris* Eston and *L. ervoides* IG 72,815 to test the resistance to race 0 and 1 in an interspecific genomic background. Two QTLs conferring resistance to both races with a significant effect were consistently detected in the experiments, one in the LG3 that explained a 20.1–30.2% of the phenotypic variance, and the other in the LG7, explaining an 8.3–18.4%. The QTL in LG3 probably coincides with that found by Bhadauria et al. (2017a) since they map in the same genomic region. Bhadauria et al. (2017a) also detected a QTL in LG7, although in their research it was associated only with resistance to race 0. The co-localization of QTLs for resistance to both races detected in these studies suggests that the same genes are controlling some resistance responses common to both races or, alternatively, the race-specific defense genes to anthracnose are closely linked, according to the genomic distribution found in *Phaseolus* by Murube et al. (2019).The analysis by Gela et al. (2021) of the CDC Redberry genomic regions (assembly v.2.0; Ramsay et al. 2019) harboring the QTLs showed at least 22 genes in LG3 and 26 genes in

LG7 annotated as disease resistance/defense-related genes, supporting the clustering of resistance genes to different races, and making them candidates for new studies.

5.7.6 *Root Rot (*Aphanomyces euteiches*) Resistance*

Root rot (ARR) disease is caused by the oomycete *Aphanomyces euteiches* Drechs. This soil-borne pathogen has a wide host range within Fabaceae, including pea, lentil, faba bean and alfalfa (Gaulin et al. 2007). Although this pathogen was well known because is considered one of the most frequent in pea fields, in lentil it was not described as the cause of the root rot until 2008 and 2012 in U.S.A. and Canada, respectively. Nowadays is considered as a widespread pathogen in the American fields (Ma et al. 2020). Germplasm analyses showed that none of the lentil cultivars are resistant, which constitutes a threat because of the possible production losses.

In order to analyze the genetic basis of the resistance, a combination of classical and image-based phenotypic tools and a deep QTL mapping study using 2,880 SNPs has been recently carried out by Ma et al. (2020) in a RIL population. This RIL was obtained from the cross between a breeding line with a high level of partial resistance and a susceptible one. The results point to a classical polygenic inheritance of the resistance, because a high number of QTLs (19) were detected located on all the chromosomes except pseudochromosome 1, each QTL explaining a 5–12% of the phenotypic variance. It is worth noting than in this same research a complementary GWA study was undertaken, detecting 38 QTLs in a sample of 326 accessions from 60 countries on four continents (Asia, Europe, America, Africa). Notably, very limited co-localizations occurred among QTL detected in the RIL population and the association mapping population. As Ma et al. (2020) state "this highlight the importance of integrating QTL mapping and association mapping for a comprehensive assessment of genetics of the resistances". Despite the complexity of the genetic basis of the resistance to *A. euteiches*, two candidate genes have been identified combining these results with transcriptomic analysis (Ma et al. 2020).

5.8 Marker-Assisted Breeding for Biotic Stress Resistance

In recent years several reviews on the status of marker-assisted breeding in lentil have been published (Kumar et al. 2019b; Rana et al. 2019). Theoretically, MAS in breeding for disease resistance has a very important advantage over traditional methods because the phenotyping with artificial infections is influenced by the specific methodology used to measure the level of resistance and some subjective classification cannot be completely ruled out. MAS overcomes these problems associated with the selection based on the response to the pathogen, and additionally allows the selection in the very early stages of the development. Besides, MAS enables the pyramiding of several genes for the same or different resistances in an

elite cultivar and speeds up the breeding programs. In order to get these advantages, it is essential to develop locus-specific and highly reproducible markers that show a tight linkage (i.e., genetic distance <1 cM) with the genes controlling the character of interest. Frequently, the markers obtained do not accomplish these requirements. Many of the markers described in the early literature including RAPDs, amplified fragment length polymorphism (AFLP) or ISSRs, although contributed to a significant improvement in the QTLs mapped, were not easily transferred from one study to another. The increase in the development and use of SSR and SNP markers has allowed the identification of candidate genes in lentil for different resistances; nevertheless, very few have been progressed to the MAS level in lentil breeding (Rana et al. 2019).

Although not optimal and with limitations, there have been described markers that could be of practical interest for MAS, at least in some genetic backgrounds. For instance, the work of Shudheesh et al. (2016) identified three markers for *Ascochyta lentis* resistance relevant for de Australian breeding program, and one of those (AB_IH1) is also predictive in more than 85% of the germplasm tested. These markers also allow the selection of the two major resistance genes found in the cultivars Indianhead and Northfield (ILL5588).

A simple genetic basis of the resistance facilitates the use of markers in the breeding programs. For instance, the marker ME4XR16c is tightly linked to the major gene responsible of the SB resistance (Saha et al. 2010b), although there are not reports about the validation of the marker in different genetic backgrounds. The marker SSR59-2B (Hamwieh et al. 2005), closely linked to the *Fw* (Fusarium wilt), or the markers F7XEM4a (Saha et al. 2010a), SSR GLLC106 (Mekonnen et al. 2014), and SSR GLLC527 (Dikshit et al. 2016), linked to genes conferring rust resistance, are in the same stage. For this last disease, two markers (LcSSR440 and LcSSR606) flanking the resistance gene have recently been validated in a small set of resistant and susceptible genotypes (Singh et al. 2021).

When the resistance genes are located in different chromosomes or no common molecular markers are available, the pyramiding must involve a simultaneous selection for them. A favorable characteristic in lentil breeding programs is the linkage among some resistance genes for different pathogens, which facilitates the pyramiding of these traits. For instance, Tar'an et al (2003) obtained resistant lines to AB and anthracnose with a 55% efficiency using three markers, two linked to alleles conferring resistance to ascochyta ($RB18_{680}$, $UBC227_{1290}$) and one to anthracnose ($OPO6_{1250}$). When the markers were used in selecting only one resistance, the efficiency was higher than 80%. Tullu et al. (2006) identified a RAPD marker ($OP\text{-}P4_{400}$) linked to the major resistance gene to AB*AbR1* and to the *LCt2* responsible for resistance to anthracnose.

The development of high-density genetic maps based on genic markers obtained from transcriptomic studies provides a high number of useful markers for different traits, including resistances to pathogens. A clear example can be found in Polanco et al. (2019), in which several markers for morphological or agronomic traits are described, besides markers for QTLs related with AB resistance. It is clear that the integration of data from high-density linkage maps and the information available for

the lentil genome will speed the number of genic markers with a real utility in MAS programs.

A different approach for MAS is the named genomic selection, in which the genotypes of a high number of markers covering the genome are used to predict the final phenotype by means of mathematical models. In this way, it is supposed that all QTLs for a trait are detected. Recently, some initial studies on the genomic selection applicability in lentil breeding programs have been done (Haile et al. 2020); although no resistance traits have been analyzed.

5.9 Genomics-Aided Breeding for Biotic Stress Resistance

Recent advances in genomics have furthered research on plant resistance to pathogens. Genome-wide massive tools have come along to complement traditional breeding based on genetic linkage maps, expressed sequence tag (EST) libraries, gene-based markers, and comparative genomics (Rodda et al. 2017). The first release of the genome assembly, CDC Redberry v1.2 (Ramsay et al. 2016), was a significant breakthrough for lentil's genomics-aided breeding. It consisted of 7 pseudochromosomes and approximately 2.7 Gb of assembled sequence. Although a big leap from previous lentil pre-genomic era, this first assembly covers barely two thirds of the predicted size and displays high levels of fragmentation. A new improved draft, v2.0, is available upon request at https://knowpulse.usask.ca/ (Ramsay et al. 2019). The assembly has over 3.7 Gb, close to the expected lentil genome size of about 4 Gb. Currently, the use of next-generation sequencing (NGS) technology in lentil breeding programs is not widespread compared to other crops (Kumar and Gupta 2020). As improved assemblies are coming to light, researches will be able to tackle genome-wide approaches.

5.9.1 Transcriptome Analyses

Until a reference-quality genome sequence becomes available, de novo transcriptome assemblies are strategic in marker discovery and transcript profiling (Kaur et al. 2011; Verma et al. 2013; Sudheesh et al. 2016b; Gutierrez-Gonzalez and Garvin 2017). They have also proven to be an effective tool to unravel plant-pathogen interactions. Using RNA-seq, Khorramdelazad et al. (2018) compared AB resistant and susceptible lentil genotypes at 2, 6, and 24 h post-inoculation, with a focus on studying the physiology of the interaction between lentil and *A. lentis*. They found genotype- and time-dependent differential expression and identified genes with putative roles in primary, secondary and tertiary defense responses. Among these, there were genes coding for transcription factors (TFs), fungal elicitors' recognition, early signaling, structural and biochemical responses, hypersensitive reaction, and cell death and systemic acquired resistance.

Recently, Garcia-Garcia et al. (2020) were able to highlight the pathways that are most affected following *A. lentis* infection by using massive analysis of cDNA ends (MACE). The precise plant-pathogen recognition mechanism is not well understood for *A. lentis*. Nevertheless, some common patterns that are frequently seen after infection may give researchers a hint. For instance, authors demonstrated that the JA and lignin biosynthesis pathways were up-regulated in the resistant lines compared to the susceptible genotype. Conversely, the response to chitin, the SA pathway and the auxin response were activated in the resistant genotype. A majority of disease resistance genes in plants encode nucleotide-binding site leucine-rich repeat (NLR) as part of the R-protein mediated recognition of fungal effectors. Garcia-Garcia et al. (2020) found 42 tags that were assigned to the NLR gene family, although most of them did not show significant changes after the infection. Other transcriptomics research has been carried out by Cao et al. (2020) on resistance to BS and already described in Sect. 7.2, and by Sari et al. (2018) who found that lentil cultivars CDC Robin and 964a-46 activated cell surface receptors tentatively associated with pathogen-associated molecular patterns (PAMP) recognition and NLR upon *A. lentis* infection, and differedin their activation of SA and JA signal transduction pathways.

Anthracnose of lentil is another devastating fungal disease in some parts of the world. It is caused by pathogens of the hemibiotrophic species *Colletotrichum lentis*, where the transition from biotrophy to necrotrophy is critical for a successful infection. To shed light into the mechanisms regulating this transition, Bhadauria et al. (2013) assembled expressed tags into unique genes (unigenes). Among the assembled transcriptome, 387 unigenes were predicted to have stress and defense related roles. There were also membrane and transport associated sequences (101) and unigenes implicated in signal transduction (159), some of them thought to be part of the inducible plant response. The molecular mechanisms triggering the symptomatic phase of infection have also been investigated (Bhadauria et al. 2017b). Authors identified a total of 22 putative effectors, and 26 resistance genes implicated in the recognition of fungal effectors, signaling of pathogen perception, phytohormone level changes, and TFs. These resistance genes included both positive and negative regulators of plant immunity in an intricate molecular interplay between disease resistant proteins and effectors, in which, during a compatible interaction, the pathogen appears to exploit the defense responses mounted by the host.

5.9.2 Genomic Selection

Genomic selection (GS) is a promising approach in breeding programs as it provides opportunities to increase genetic gain of complex traits per unit time and cost (Bhat et al. 2016). It uses all marker data as predictors of performance to deliver more accurate predictions, but in turn requires the availability of genome-wide, high-throughput and cost-effective markers. A well-fitted statistical model is also required for the training population, which is phenotyped and genotyped. This model will be later applied to the breeding population that has been genotyped but not phenotyped.

Some SNP genotyping platforms, especially the GBS and SNP chips, as well as a polished genome assembly draft, have opened GS to lentil breeding programs. Haile et al. (2020) have tested several statistical prediction models specifically for lentil breeding. They suggested that GS can be implemented to make predictions within populations and across environments, as moderate to high accuracies were obtained. Across-population predictions were much lower, and thus, their use is discouraged when the population size is small. It is expected that GS will gain importance in the coming years.

5.9.3 Novel Genomic Tools in Other Plant Species

Genome-wide approaches successfully used to understand the responses to biotic stresses in other species could also be applied to lentils. Recently, Laflamme et al. (2020) designed a pangenome based analysis to unravel the complex interrelationship between pathogens and plants, supplying invaluable information about gene families involved in the resistance. The work was carried out on *Arabidopsis thaliana*, which was infected with one of the most common plant pathogens, the bacteria *Pseudomonas syringae*. Authors generated a *P. syringae* Type III Effector Compendium (PsyTEC) from 494 strains and identified the genes responsible for effector-triggered immunity in *Arabidopsis*. This pangenome analysis revealed that relatively few *A. thaliana* genes are responsible for recognizing the majority of *P. syringae* effectors. Furthermore, they identified new *Arabidopsis* immune NLR receptors able to recognize effectors expressed by most of the strains. These results provide insight into why most pathogenic microbes only infect specific plant species.

Multi-genome assemblies have also allowed identifying genetic differences between wheat lines that are important for breeding (Walkowiak et al. 2020). The research team was able to track the unique DNA signatures of genetic material incorporated into modern cultivars from several of wheat's undomesticated relatives. These wheat relatives have been used by breeders to improve disease resistance and stress resistance of wheat. For instance, a DNA segment from one of these relatives contains disease-resistant genes and provides protection against a number of fungal diseases. This segment can improve yields by as much as 10 per cent. The pangenome was also used to isolate an insect-resistant gene (*Sm1*) that enables wheat plants to withstand the orange wheat blossom midge, a pest which can cause millions in annual losses to producers. As more pangenomes are being announced this information could be validated and extrapolated to other plant species. Kumar and Gupta (2020) have highlighted the new opportunities of pangenome analysis in lentil breeding.

NGS was also used for large-scale pathogen diagnoses in soybean (Díaz-Cruz et al. 2019). Several bacteria, fungi, and viruses known to infect soybean were detected, as well as pathogens not previously identified. For some microorganisms, this technique was able to disentangle the different pathovars present and/or assemble their genome sequence. Since NGS generated data on the whole spectrum of flora

and fauna that thrive in leaves, it was possible to identify residual pathogens (i.e., pathogens of crops other than soybean) and multiple species of arthropod pests. Finally, the assembled NGS data allowed for the development of polymerase chain reaction-based diagnostics for some pathogens.

5.10 Recent Concepts and Strategies

The application of traditional breeding techniques to lentils, such as the development of molecular markers, QTL identification, and MAS, has led to important achievements. However, approaches that rely on the use of transgenic plants and plant tissue techniques are currently lagging behind. Lentils are long known to be recalcitrant to plant tissue culture, whole plant regeneration, and micropropagation (Polanco and Ruiz 1997; Fratini and Ruiz 2003; Khatib et al. 2011).

5.10.1 Research on Other Plant Species

Recent studies using model plant species have emphasized the complexity of the plant-pathogen response and have suggested novel and complementary pathways for resistance in crop species. For instance, in a genome-wide association mapping study in *Arabidopsis*, Aoun et al. (2020) dug into the genetic basis of the resistance to *Ralstonia solanacearum* under heat stress. They discovered multiple QTLs and the identity of the candidate genes underlying the 14 major QTLs. The nature of those genes is highly diverse, not matching the typical resistance genes encoding NLRs. Interestingly; the QTLs they found at 27 °C were different from those at 30 °C, indicating distinct genetic architectures for the responseto *R. solanacearum* at changing temperatures. Among non-classical defense-related candidate genes there is *SDS*, which encodes a meiotic cyclin-like protein related to cyclins previously described as being required for DNA repair. Its functional validation as a gene for susceptibility represents the first demonstration of the involvement of *SDS* in the plant response to a bacterial pathogen under heat stress. According to the authors, SDS acts together with other proteins to suppress unscheduled cell wall synthesis. Other candidate genes encode for proteins involved in cell wall and lignin polymerization. We think this genome survey reflects the complexity of the response pathways to biotic stresses, and, that despite of the progress made in the last years, *omic* approaches will have to provide further knowledge for us to fully understand the responses of crops to biotic and abiotic stresses.

Another example of this complexity is provided by Ngaki et al. (2021).They proved how a single gene (*Glycine max disease resistance 1; GmDR1; Glyma.10g094800*) can confer resistance to various pathogens and pestsi n soybean. Overexpression of its encoded plasma membrane protein led to enhanced resistance not only against the fungal pathogen *Fusarium virguliforme*, but also against

spider mites (*Tetranychus urticae*), soybean aphids (*Aphis glycines*) and soybean cyst nematode (*Heterodera glycines*). Authors also investigated if chitin, a PAMP, can significantly enhance defense pathways in *GmDR1*-overexpressed transgenic soybean lines. They concluded that chitin-induced SA- and JA-pathways could be involved in broad-spectrum resistance against pathogens and spider mites, for which no known resistance genes have been identified in soybean and in most crop species. It is likely that some of these results on *GmDR1* could be extrapolated to lentils, due to their taxonomic proximity.

Plant stomata play important roles in the response to stresses in plants. The perception of some biotic and abiotic stresses leads to stomatal closure. The flow of calcium ions (Ca^{2+}) across the plasma membrane is key in this response, but the calcium channel involved was not known. Thor el al. (2020) found that the *Arabidopsis thaliana* Ca^{2+}-permeable channel OSCA1.3 controls stomatal closure during defense response. In fact, OSCA1.3 is rapidly phosphorylated upon sensing PAMPs. Genetic and electrophysiological data revealed that OSCA1.3 is permeable to Ca^{2+}, and that BIK1-mediated phosphorylation increases this channel activity. Thus, OSCA1.3 and its phosphorylation by BIK1 are critical for stomatal closure during defense. Notably, OSCA1.3 does not appear to regulate stomatal closure upon sensing abscisic acid, a plant hormone associated with abiotic stresses. Their research suggests that there is specificity in the Ca^{2+} influx mechanisms in response to different stresses, opening new targets for pathogen resistance in crop plants.

The advent of NGS technologies has allowed the cataloging of genes, gene products and gene interactions within the biological context. TF-driven gene regulation underlies most aspects of organisms' biology, including the response to biotic stresses. High-throughput gene expression profiling is dramatically changing our views on how gene regulation networks are coordinated: from single-gene activities to gene interactions (Ko and Brandizzi 2020). Data gathered on interacting networks are valuable to integrate molecular communications and derive models to describe biological systems. Behind this is the idea of leveraging the interactions between genes and TFs over function of components alone (Ko and Brandizzi 2020). Thus, to understand the complex response of plants to pathogens and pests we will have to look at them as a whole.

Because they accumulate more recombination events, multi-parental segregating populations can offer better resolution than traditional biparental populations for the mapping of complex traits. They also have more genetic diversity and minimal population structure. Several multi-parent populations have been constructed in legumes, including peanuts, soybean, cowpea, and faba bean. Their utility ranges from being a tool for mapping quantitative trait loci to a means of providing germplasm for breeding programs (Scott et al. 2020).

Improved in situ hybridization (ISH) techniques have come out. One of them is RNAscope® (Wang et al. 2012), an ISH assay for detection of target RNA within intact cells through a novel signal amplification and background suppression. This method is capable of simultaneous detection of multiple target RNAs down to the single molecule level in individual cells, allowing researchers to study spatiotemporal patterns of gene expression. By applying confocal laser microscopy, Solanki

et al. (2020), designed an optimized method for RNAscope® detection to determine the spatial expression and semi-quantification of target RNAs. The generalization of RNAscope® method to lentils and other legumes will assist in gene expression studies, as researchers not only know the genes that are expressed, but also when and in which cells.

RNA transport and localization *in planta* represent important post-transcriptional regulation mechanisms. Plants have the capacity to transport mRNA molecules beyond the cell boundaries through plasmodesmata and over long distance by phloem. Peña et al. (2021) have described in plants an in vivo method for RNA-labelling which allows monitoring cell-to-cell transport of mRNA. Technical advances like these offer new and complementary alternatives for fine analysis of gene expression in various situations, including stress response.

5.10.2 Gene Editing

Precision gene editing by the CRISPR/Cas9 reagent is a powerful technique for the genetic manipulation of crop genomes and can be carried out by either targeted mutagenesis or gene targeting (Scheben et al. 2017). During the last years gene-editing methods have been established for some crop and model legumes species such as chickpea, cowpea, soybean, *Lotus japonicus* and *Medicago truncatula*, as reviewed by Bhowmik et al. (2021). However, the recalcitrance of other legumes to in vitro gene transfer and regeneration has posed a serious challenge to application of gene editing. Targeted mutagenesis, or gene knock-out, is the easier technique due to lower host plant transformation efficiency requirements. Gene targeting, or gene knock-in, is a more advanced technique that uses a donor template containing the desired DNA changes to be incorporated into the targeted region and requires a greater transformation efficiency to recover successfully edited plants.

Currently, the ability to manipulate DNA using CRISPR/Cas9 (Anzalone et al. 2019) exceeds the transformation technologies required to deliver reagents into the plant. Not surprisingly, improvements to the delivery of reagents has become a hot area of research which is attempting to address problems such as inefficient in vitro shoot regeneration, Agrobacterium-mediated T-DNA delivery, shoot regeneration from protoplast tissue and optimization of transgenic selection. Recent research has demonstrated the capability of morphogenic regulators to effectively generate transformed plants and this technology shows great promise for improvements to legume transformation and gene editing (Anand et al. 2018; Hoerster et al. 2020; Maher et al. 2020). As it is typical for many grain legumes, the lentil has a long and frustrating history of tissue culture and in vitro regeneration. In comparison with model plant species and many other crop species, lentil is a relatively recalcitrant species in relation to plant tissue culture, whole plant regeneration and micropropagation, hindering further biotechnological modifications (Pratap et al. 2018). Encouragingly, lentil plant transformation has been reported in several genotypes to date including Laird, Sultan and L-4076 at a transformation efficiency ranging between 0.9–3.1% (Gulati and McHughen, 2003; Akcay et al. 2009, 2015; Chopra et al. 2011). Improvements

to these procedures and/or the implementation of morphogenic regulators, combined with cultural practices such as micrografting transgenic shoots to non-transformed rootstocks to establish transgenic plants will likely improve transformation efficiencies and widen the range genotypes that can be transformed (Khatib et al. 2011). Genome editing technologies have been also reviewed by Gupta et al. (2020).

5.11 Role of Bioinformatics as a Tool

Most of the published lentil sequences are found in the National Center for Biotechnology Information (NCBI) and the European Bioinformatics Institute (EBI) databases, (in this chapter the information has been searched and referenced in the NCBI database). In the NCBI there are 33,503 entries of Nucleotides using "Lentil" as searching word. The vast majority of the sequences comes from the cultivated species, (29,240 entries), although numerous sequences from wild species can also be found: *L. orientalis*, 1,606; *L. ervoides*, 893; *L. nigricans*, 479; *L. odemensis*, 254; *L. tomentosus* 161; and *L. lamottei*, 86.

The most numerous entries related to a pathogen in the database refer to*Colletotrichum truncatum.* Data were obtained in a series of works analyzing the interactions between lentil and the pathogen (Bhadauria et al. 2011, 2013, 2017b).

Members of the Division of Crop Improvement of Indian Institute of Pulses Research from Kanpur analyzed the lentil genomic resources available in the public databases in a recent review (Kumar et al. 2020). Sequence-based markers are available from the NCBI databases. Among the first works used to obtain maps and markers is that by Kaur et al. (2011). They obtained 15,298 small-sized TSA (Transcriptome Shotgun Assembly) sequences from 6 lentil genotypes (BioProject PRJNA65667, 14-Apr-2011, Table 5.3). Sharpe et al. (2013) compared 11 genotypes (including two of *L. ervoides*). The raw data obtained with 454 GS FLX Titanium are found in the BioProject PRJNA192531 (6-Mar-2013). Yilmaz Temel et al. (2015) obtained 97,528 contigs of cDNAs from two genotypes (PRJNA210522, 7-Jan-2014). The entry of these BioProjects is shown in Table 5.3.

Without doubt the most important specialized database on pulse crops is KnowPulse (knowpulse.usask.ca) developed by the University of Saskatchewan (Sanderson et al. 2019). In it, numerous markers based on sequences obtained by the Sanger's technique and by NGS-based 454 and Illumina procedures are collected. These markers are located on the draft v1.2 of the *Lens culinaris* genome whose sequences come from the CDC Redberry variety. On that page it is possible to perform BLAST searches and browse the lentil genome with the JBrowse tool and perform other queries. The genes have been detected by comparing the genome with different lentil transcriptomes and the putative lentil orthologous genes to *Medicago* 4.0, *Arabidopsis* 10, chickpea 1.0 and soybean 2.75 genomes have also been located. Access to the data of this genome is limited and for a more complete use it is necessary to contact Dr. Kirstin E. Bett.

The v1.2 of the lentil genome consists of 2,748 Mb (38,998 genes) assembled in 7 large pseudomolecules corresponding to the 7 chromosomes of the species with 339,

317, 199, 246, 263, 210 and 247 Mb, respectively, in addition to another 128,639 small fragments or contigs containing the rest of the approximately 927 Mb. The raw data of sequence reads used in lentil genome construction is available from the NCBI in BioProject PRJNA343689 (21-Sep-2016, Hiseq 2000). The project includes 22 SRA experiments, with 1,087 Gb in raw data that are assembled in a total of 2,748 Mb of the 4,063 Mb of the haploid lentil genome (Table 5.3). Many of the annotated genes come from or have been verified with data from bioprojects focused on cDNAS (PRJNA434239, uploaded to the NCBI in February 2018). Both the raw data of the genome and the cDNAs used for their annotation were submitted by the research group at the University of Saskatchewan.

Other lentil cDNA sequences can be found in two BioProjects: PRJNA218843 is the oldest (11-Sep-2013, 4 Gb, submitted by India NIPGR) in which only one sample was analyzed; and PRJNA352096 (2-Nov-2016, 160 Gb) in which Sudheesh et al. (2016b) compare the transcripts of seven different tissues of the Cassab variety. The project with greatest sequencing effort, PRJNA497358 (18-Oct-2018, 207 Gb) of the Shadong Center of Crop Germplasm Resources (unpublished), includes six biological samples and 18 sequencing experiments. This project representsa new and significant contribution of new lentil transcripts, although it does not fully specify the data.

In addition to the nuclear genome, the NCBI database contains the lentil chloroplast genome sequence. The complete sequence can be found assembled in the BioProject PRJNA285561 submitted by the University of British Columbia (2-Jun-2015), although not much data of the technique used to obtain it is provided.

It is also possible to identify genome sequences from both prokaryotes and fungi that are part of the microbiota of the lentil root. Fungi are explored in the BioProject PRJNA470968 by analyzing the ITS1 spacer of ribosomal genes, the University of Saskatchewan is again participating in the project (10-May-2018). Prokaryotes were also studied by researchers at Assam University from 10 different samples. The analysis was based on the sequences of a fragment of the coding gene for ribosomal RNA that includes the variable regions V3 and V4 (PRNJNA622390, submitted at 8-Apr-2020). A new whole metagenomic analysis of two-samples has recently been performed by researchers at Bidhan Chandra Agricultural University (PRJNA639655, Jun-16–2020). Also, there are complete genomes of two of the most important lentil pathogens, *Colletotrichum lentis* (PRJNA407672, 14-Aug-2018, Bhadauria et al. 2019) and *Ascochyta lentis* (PRJNA506513, 22-Nov-2018, Curtin University) available from NCBI.

Numerous sequencing projects have focused their objectives on exploring the diversity of lentil at the genomic level. Among the firsts of them there is the study of 83 samples genotyped by sequencing (GBS) carried out by Wong et al. (2015), whose raw data can be obtained from the BioProject PRJNA261418(18-Sep-2014, 44 Gb). Two other GBS studies, based on genomic data, are included in the BioProjects PRJNA528610 (22-Mar-2019, 121 Gb) and PRJEB38912 (1-Oct-2020, 55 Gb). In the first one, Pavan et al. (2019) compared 349 lentil accessions, mostly landraces, while in the second, Liber et al. (2021) chose 190 genotypes of both cultivated and wild species to study the history of lentil domestication and spread. Ogutcen et al.

(2018) developed an exome capture array for lentil using 16 wild lentils and 22 cultivars accessions (PRJNA433205, 6-Feb-2018). The greatest effort in sequencing made to know on the diversity of lentil has been carried out by Dissanayake et al. (2020), although instead of GBS they studied RNAs from 467 accessions, including wild species (*L. culinaris* 304; *L. orientalis*, 57; *L. ervoides*, 57; *L. nigricans*, 24; *L. odemensis*, 22; *L. lamottei*, 1; two unidentified *Lens* accessions and no samples of *L. tomentosus*). The BioProject that collects the data from Dissanayake et al. (2020) is PRJNA625627 (16-Apr-2020, 1598 Gb).

The rest of the bioprojects devoted to lentil analyze the differential expression at the messenger level of lentil samples subjected to some type of stress, either abiotic or biotic. Although abiotic stress is not the main objective of this chapter, we must mention the two RNAseq studies in which the response to drought is analyzed, BioProjects PRJNA308969 (16-Jan-2016, 94 Gb) and PRJNA474098 (1-Jan-2018, 120 Gb) by Singh et al. (2017b) and Morgil et al. (2019) respectively, and the two studies dedicated to temperature, the one by Barrios et al. (2017) studies the effect of cold and uses the superSAGE technique (PRJEB14947, 9-Dec-2016, 1 Gb) and Shing et al. (2019) that analyses exposure to high temperatures (PRJNA423129, 20-Dec-2017, 63 Gb). Three other projects submitted by Lorestan University collect data on abiotic stresses, although the indications in the NCBI database are not too clear, they analyze the effect of temperature, drought and salinity (PRJNA378872, 12-Mar-2017, 43 Gb; PRJNA379217, 15 -Mar-2017, 60 Gb; and PRJNA379218, 15-Mar-2017, 52, Gb).

Several experiments analyze gene expression in relation to pathogens, affording messenger sequences to databases. They are all related to the infection of the fungus *Ascochyta lentis*. The first data come from the study by Khorramdelazad et al. (2018), in which three replicates were analyzed by treatment of the ILL7537 (resistant) and ILL6002 (susceptible) accessions at three times (2, 6 and 24 h after inoculation - hpi) with spores of the fungus or mock setting (PRJNA321618, 15-May-2016, 79 Gb). In the analysis by Garcia-Garcia et al. (2019) only the 3' terminal ends of the messengers were analyzed with the MACE technique 24 hpi with the fungus or mock setting, the genotypes chosen in the study are the susceptible cultivar 'Lupa', the moderately resistant 'ILL558' and the resistant wild accession of *L. orientalis* 'BG 16,880' (PRJNA356810, 9-Dec-2016, 1 Gb). Another study of RNAseq is that of Sari et al. (2018) that used the CDC Robin and 964a-46 lines as resistant and the Eston cultivar as sensitive. Samples were taken at eight different times after inoculation ranging between 0 and 60 h, the raw data were collected in the 24 SRA of the BioProject PRJNA422815 (18-Dec-2017, 40 Gb).

Finally, in the study by Polanco et al. (2019), the messengers of 78 RIL lines from the cross of the sensitive cultivar ALPO and the resistant ILWL235 accession of the wild species *L. odemensis,* the parents used for the cross, were analyzed 24 h after having been inoculated with spores of the *Ascochyta* isolate AL-84. Six replicates of each parent inoculated with spores or mock setting were analyzed from the parents to serve as a control, obtaining total of 6,306 polymorphic markers from the parents were used to obtain a high-density map. The raw data are found in the BioProject PRJNA523792 (22-Feb-2019, 416 Gb).

Table 5.3 Registered BioProjects in the NCBI Database with lentil nucleotide sequence public data

Bioproject	Registration date	# of SRAs	Biosamples (in NCBI)	Gbases	Technology	Strategy/Aims (Stress)	Publication
PRJNA65667	14-Apr-2011				454 GS	cDNA SSR markers	Kaur et al. (2011)
PRJNA192531	6-Mar-2013	11	1	2	454 GS	cDNA markers 6 lines	Sharpe et al. (2013)
PRJNA218843	11-Sep-2013	1	1	4	Illumina	RNAseq	India NIPGR
PRJNA210522	7-Jan-2014	2	2	11	Illumina	cDNA markers	Yilmaz Temel et al. (2014)
PRJNA261418	18-Sep-2014	166	83	44	HiSeq 2500	GBS	Wong et al. (2015)
PRJNA285561	2-Jun-2015					Chloroplast Genome	University of British Columbia
PRJNA308969	16-Jan-2016	5	1	94	HiSeq 2500	RNAseq/Drought	Singh et al. (2017a, b, c)
PRJNA321618	15-May-2016	36	2	79	Ion Torrent	RNAseq/Ascochyta	Khorramdelazad et al. (2018)
PRJNA343689	21-Sep-2016	1	22	1087	HiSeq 2000	Genome WGS	University of Saskatchewan
PRJNA352096	2-Nov-2016	7	7 tissues	160	HiSeq 2000	RNAseq	Sudheesh et al. (2016a, b)
PRJNA352318	3-Nov-2016	2	1	1	Illumina	cDNA/Various stresses	University of León
PRJNA356810	9-Dec-2016	6	3	1	HiSeq 2000	MACE/Ascochyta	García et al. (2019)
PRJEB14947	23-Dec-2016	2	1	1	454 GS	SuperSAGE/Cold	Barrios et al. (2017)
PRJNA378872	12-Mar-2017			43		RNAseq/Temperature	Lorestan University
PRJNA379217	15-Mar-2017		1	60		RNAseq/Drought and heat	Lorestan University

(continued)

Table 5.3 (continued)

Bioproject	Registration date	# of SRAs	Biosamples (in NCBI)	Gbases	Technology	Strategy/Aims (Stress)	Publication
PRJNA379218	15-Mar-2017		1	58		RNAseq/Salt	Lorestan University
PRJNA422815	18-Dec-2017	24	3	40	HiSeq 2500	RNAseq/Ascochyta-time	Sari et al. (2018)
PRJNA423129	20-Dec-2017	12	1	63	HiSeq 2000	RNAseq/Heat	Singh et al (2019)
PRJNA474098	1-Jun-2018	18	18	120	HiSeq 4000	RNAseq/Drought	Morgil et al. (2019)
PRJNA433205	6-Feb-2018	38	38	343	HiSeq 2500	cDNA Diversity	Ogutcen et al. (2018)
PRJNA434239	15-Feb-2018	17	17	261	Miseq	cDNA annotation	University of Saskatchewan
PRJNA470968	10-May-2018	112	112	4	MiSeq	ITS1 Fungi Microbiome	University of Saskatchewan
PRJNA407672	14-Aug-2018	1	1		HiSeq 2000; MiSeq	*Colletotrichum lentis* genome	Bhadauria et al. (2019)
PRJNA497358	18-Oct-2018	18	6	207	HiSeq 4000	cDNA	Shandong Center of Crop Germp. Resour
PRJNA506513	22-Nov-2018	6	6	14	NextSeq 500	*Ascochyta lentis* genome	Curtin University
PRJNA523792	22-Feb-2019	102	80	416	HiSeq 2500	cDNA Map/Ascochyta	Polanco et al. (2019)
PRJNA528610	22-Mar-2019	1	349	121	HiSeq 2500	GBS Diversity	Pavan et al. (2019)
PRJNA623690	8-Apr-2020	10	10	1	MiSeq	16S (V3-V4) Root Microbiome	Assam University
PRJNA625627	16-Apr-2020	467	467	1598	HiSeq 3000	cDNA Diversity	Dissanayake et al. (2020)
PRJNA639655	16-Jun-2020	2	2	14	HiSeq 2500	Metagenomic	Bidhan Chandra Agricultural University

(continued)

Table 5.3 (continued)

Bioproject	Registration date	# of SRAs	Biosamples (in NCBI)	Gbases	Technology	Strategy/Aims (Stress)	Publication
PRJEB38912	1-Oct-2020	190	190	55	NextSeq 550	GBS Diversity	University of Algarve

454 GS = GS-FLX Titanium shotgun; SRA = Sequence Read Archive; MACE = Massive Analysis of cDNA Ends; SuperSAGE = Supertag sequences; GBS Genotyping by sequencing

5.12 Future Perspectives

In a recent review on the status and prospects of biotechnological interventions for plant breeding the authors (Singh et al. 2020b) pointed out the following set of actions: (1) Deployment of genomic resources for trait discoveryand crop improvement by whole-genome sequencing, resequencing and pangenome analysis together with the development and deployment of molecular markers for breeding; (2) identification of QTLs associated with agronomic traits; (3) genomics-assisted breeding for trait improvement including marker-assisted backcrossing and recurrent selection, and genomic selection and speed breeding; (4) biotechnological interventions for crop improvement including the expression and overexpression of candidate genes for desired phenotype, RNA interference for in vivo knockdown of target genes, and gene and genome editing. While some significant advances have been achieved in the development of genomic resources, development of molecular markers and QTL identification and their use in lentil "molecular breeding", and many more are being and will be developed in the near future, most of the biotechnological interventions depend on the use of transgenic plants and plant tissue techniques, which represents a bottleneck in the application of the biotechnological interventions in current lentil breeding. Lentil is a relatively recalcitrant species in relation to plant tissue culture hindering further biotechnological modifications (See Sect. 5.12).

Third-generation single-molecule sequencing technologies reduce the cost of sequencing and can be used for sequencing the long DNA fragments expediting the assembling and scaffolding of complex genome. Hence use of these technologies can overcome problems associated with the large genome size of lentil and in coming years, use of NGS will boostgenetic gain in lentil (Kumar and Gupta 2020).

Recent publications on model species have again emphasized the enormous complexity of the response to pathogens in plants and suggest complementary or new pathways in the search for resistance in crop species.

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Chapter 6
Development of Biotic-Stress Resistant Pigeonpea

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Abstract Pigeonpea is the second most important pulse legume crop of the tropics and subtropics. Pigeon pea infested by a plethora of insect-pests and diseases resulting in lower production and productivity. Among the various biotic stresses, Fusarium wilt, Sterility mosaic disease, Phytophthora blight, leaf spot, pod borer, pod fly and *Maruca vitrata* are the major constraints which affect the productivity of pigeonpea. Synthetic pesticides were used extensively to control biotic stress factors. However, host plant resistance provides a cost-effective and long-term pest management solution. As a result, imparting resistance to these pests and diseases is critical for achieving global food security. The genetic variability for insect and disease resistance is available in wild species of pigeonpea, which can be used for interspecific gene transfer through conventional hybridization. In addition to conventional breeding approaches, modern genomics tools such as molecular markers linked with biotic stresses offer a great opportunity for development of resistant cultivars. Particularly, marker-assisted selection (MAS) or marker-assisted backcrossing (MABC) has played a great role in resistance breeding, as it helps in easy assessment of the phenotypes and transfer of large number of resistance genes. In light of this, this chapter deliberates on the major diseases and pests of pigeonpea, as well as their diagnostics, epidemiological factors, breeding approaches for improved productivity, and the use

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of markers for developing resistant/tolerant genetic resources against major diseases and pests.

Keywords *Cajanus cajan* L. · Biotic stress · Molecular markers · *Fusarium udum* · *Helicoverpa armigera* · Marker assisted breeding

6.1 Introduction

Pigeonpea, (*Cajanus cajan* (L.) Millspaugh), also known as red gram, is the sixth most important grain legume crop grown in the semi-arid tropics of Asia, Africa, Latin America and the Caribbean region (Mula and Saxena 2010). Pigeonpea belongs to the subtribe Cajaninae of the agriculturally most important tribe Phaseoleae under subfamily Papilionoideae of the family Fabaceae (Leguminosae). The true origin of pigeonpea is still disputable, however, various studies have indicated that pigeonpea originated from its closest wild relative *C. cajanifolius* (van der Maesen 1980; Panigrahi et al. 2007) most probably in India and later it spread to the continents of Africa and Australia where some of its wild relatives still exist. It is a hardy, widely adapted and drought tolerant crop with large temporal variation for maturity (90–300 days). The crop is most suitable for intercropping as it is slow growing and does not compete with short duration annual crop. Green pigeonpea seeds are highly nutritious, contains high levels of proteins and significant amount of essential amino acids like lysine, methionine and tryptophan. Dry pigeonpea seeds contain protein (20–22%), carbohydrate (57.3%), fat (1.5%) and ash (8.1%). Its protein has two globulins, cajanin and concajanin accounting for 58% and 8%, respectively. The dried stalks are used as fuel, for making baskets and thatching material. Besides the ability of plant to fix atmospheric nitrogen makes pigeonpea an important component of sustainable cropping system.

Although India has been the world's largest producer and consumer of pigeonpea, the productivity has stagnated in recent years (Varshney et al. 2012) due to a range of vagaries under the climate change scenario. The large number of insect pests and diseases which attack pigeonpea in India is perhaps the main constraint to increased production. Hence, the major challenge for pigeonpea improvement is not only increasing productivity but also stress mitigation. More than 200 species of insects have been found feeding on pigeonpea, although only a few of these cause significant and consistent damage to the crop. Besides, pigeonpea is affected by various diseases which cause a reduction in its production and productivity. Nene et al. (1996) reported over 100 pathogens attacking pigeonpea comprising fungi, bacteria, viruses, nematodes and mycoplasma-like organisms. Pigeonpea diseases of economic importance are Fusarium wilt (FW) caused by *Fusarium udum* Butler, Phytophthora blight caused by *Phytophthora drechsleri* Tucker f. sp. *cajani*, Macrophomina root rot caused by *Macrophomina phaseolina* (Tassi) Goid and Sterility mosaic disease (SMD). However, the disease of minor importance includes *Phoma cajani* (Rangel) and Alternaria blight caused by *Alternaria* spp. etc.

The management of biotic factors mainly depends on the use of fungicides, antibiotics and insecticides, which leads to pesticide residue in food products, pesticide resistance offered by pathogen (fungicidal and antibiotic resistance) and environmental pollution (Hawkins et al. 2019). The best alternative method of disease management is use of pest and disease resistant/tolerant varieties. Resistant varieties have ability to prevent the pathogen infection and multiplication in host plant. Hence resistance breeding offers a cheap and eco-friendly management practice against wide range of insects and diseases (Sharma and Ortiz 2002). To develop cultivars tolerant to biotic stresses, plant breeders have been using various classical breeding approaches viz., single plant selection based on its field performance, wide hybridization with wild relatives, gene pyramiding for multiple resistance and backcross breeding to transfer resistant genes.

6.2 Biotic Stresses in Pigeonpea

6.2.1 Fungal Diseases of Pigeonpea

6.2.1.1 Fusarium Wilt

Fusarium wilt is a major disease of pigeonpea caused by *Fusarium udum* Butler, a soil and seed borne fungus. It spreads through wind, water and soil and can survive up to 3 years on infected plant debris (Shinde et al. 2014). The incidence and yield losses differ from place to place owing to the existence of variability in pathogen isolates, so for in India five variants (I, II, III, IV and V) of *F. udum* have been identified and documented (Mishra 2004; Tiwari and Dhar 2011). Fusarium wilt accounts for yield loss ranging from 30 to 100% (Pawar and Mayee 1986).

The causal organism: The wilt of pigeonpea was recorded for the first time in India by Butler (1906). The causal organism described initially as *Fusarium udum* by Butler during 1910, was subsequently described as *F. butleri*, *F. uncinatum, F. lateritium* var. *uncinatum*, *F. oxysporum* f. sp. *udum*, *F. lateritium* f. sp. *cajani* and *F. udum* f. sp. *cajani* (Dhar et al. 2005). However, the name *F. udum* was accepted as an imperfect state (Booth 1971) because the macro-conidia having well distinguished prominent hook (Rai and Upadhayay 1982). *Fusarium udum* is highly host specific and its damage limited to pigeonpea (Padwick 1940; Subramanian 1963; Booth 1971).

Dispersal of the fungus: The pathogen is host specific and infection documented only on pigeonpea and its wild relative, *Atylosia* spp. (Kannaiyan et al. 1984). It is a soil borne, facultative parasite that enters through roots and later becomes systemic (Nene et al. 1979). It is dispersed through irrigation, rain water and displacement of host debris by termites that feed frequently on dead wilted plants. It has also been found to be of a seed borne nature (Upadhyay and Rai 1982).

Symptoms: The infected plants may show symptoms of water shortage from seedling to maturing stage even through there may be plenty of moisture in soil. The wilting is characterized by gradual, sometimes sudden yellowing, withering and drying of leaves followed by drying of the entire plant or its branches. Generally, wilt symptoms appear 4–6 weeks after sowing, but are most common in flowering and podding stage/reproductive stage. Patches of diseased plants are scattered through the field. The initial visible symptoms are loss of turgidity in leaves and slight interveinal clearing. In advance stage of disease branch dry from tip to downwards and show blackening internal xylem vessels (Reddy et al. 1990). The xylem gradually develops black streak and brown or dark purple bands appear in the stem of wilted plants expending upwards from the base of affected plants (Reddy et al.1993; Pandey et al. 2013). When the bark of such bands is peeled off, browning of the wood beneath can be seen. Lateral root infection results in partial wilting, whereas tap root infection resulted in complete wilting and also showed dry rot symptoms.

Variants of *F. udum*: Based on multilocational testing of pigeonpea genotypes, prevalence of five variants of the pathogen has been reported in different parts of India. Of these, race 2 is considered to be the most virulent and widely distributed in India (Patil et al. 2014).

Genetics of resistance: Resistance to FW governed by major R-genes (Singh et al. 2016a, b) (monogenic dominant gene). Resistance to FW in pigeonpea is governed by single dominant or a recessive gene (Jain and Reddy 1995) or more than one gene (Joshi 1957) i.e., two complementary genes (Okiror 2002), duplicate recessive (Patil et al. 2014) and even multiple factors in different crosses. Therefore, molecular tagging of resistance genes or quantitative trait loci (QTLs) for different races of wilt pathogen is required to facilitate molecular marker assisted resistance breeding in pigeonpea. Limited studies have reported identification of molecular markers linked to FW resistance in pigeonpea i.e., random amplified polymorphic DNA (RAPD, Kotresh et al. 2006), simple sequence repeat (SSR, Singh et al. 2013), amplified fragment length polymorphism (AFLP, Ajay et al. 2015), and single nucleotide polymorphism (SNP, Singh et al. 2016a, b). Six SSR markers namely, ASSR-1, ASSR-23, ASSR-148, ASSR-229, ASSR-363 and ASSR-366 reported to be associated with FW resistance were tested and it was concluded that the markers ASSR-1, ASSR-23 and ASSR-148 may be used for screening of parental genotypes in pigeonpea FW resistance breeding programs (Singh et al. 2016a, b).

Management of Fusarium wilt disease:

Resistant cultivars: A number of resistance sources for Fusarium wilt and sterility mosaic diseases are available within the primary gene pool. The first wilt resistant variety of pigeonpea in India ICP 8863 (Maruti) was released during 1986 and even today it is cultivated for wilt resistance in India. Another wilt resistant variety ICPL 87119 (Asha) was popular among the farmers. ICRISAT screened several genotypes of pigeonpea and found ICPL 20109, ICPL 20096, ICPL 20115, ICPL 20116, ICPL 20102 and ICPL 20094 as resistant genotypes (Sharma et al. 2015). Phenotyping of lines in sick plots across locations led to identification of two RAPD markers (Kotresh et al. 2006), four sequence characterized amplified region (SCAR) markers

(Prashanthi et al. 2009) and six SSR markers (Singh et al. 2013; Pazhamala et al. 2015) for Fusarium wilt resistance. The wilt resistant cultivars are available for short duration, extra short duration and long duration groups. Dhar et al. (2005) listed following cultivars/lines resistant to *Fusarium* wilt of pigeonpea: ICP 8863, AWR 74/2, Banda Palera, DA 11. Varieties C-11, C-28, C-36, F-18, NP-15, NP-38, NP-41, and T-17 had been considered resistant to wilt.

Biocontrol: *Bacillus subtilis* showed antagonistic property against *F. udum* (Vasudeva et al. 1963) and *Pseudomonas florescence* controlled pigeonpea wilt and has significant effect on grain yield.

6.2.1.2 Phytophthora Blight (PB)

Phytophthora blight is a seedling disease of pigeonpea, causing heavy mortality in low lying areas of the field. The disease predominantly affects the stem and occasionally the leaves. The pathogen greatly affected by the weather parameters.

Causal agent: Phytophthora blight of pigeonpea caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* (Mahendra Pal, Grewal and Sarbhoy) Kannaiyan, Ribeiro, Erwin and Nene (Pal et al. 1970). Kannaiyan et al. (1984) first recorded the diseases at Indian Agricultural Research Institute, New Delhi, India and subsequently its endemic occurrence was observed in different parts of India. Mycelium of *Phytophthora* is coenocytic, aseptate, hyaline, and profusely branching mainly of monopodial branches. The septa are formed at the time of reproduction. Chlamydospore is thick-walled long-term survival spores, as they are produced through asexual means of reproduction. Whereas oospores are sexual spores, these are produced from fertilization of the oogonium by an antheridium.

Symptoms: The symptoms of the PB on pigeonpea have been described as stem rot (Pal et al. 1970), stem blight (Williams et al. 1975; Amin et al. 1978), stem canker and root rot (Kaiser and Melendez 1978). The symptoms of PB in different growth stage as follows.

Plant part/growth stage	Symptoms
Seedling (up to 30 days)	Young seedlings are killed within 3–10 days
Foliage (30–45 days)	Water soaked lesions on young leaves, and whole foliage gives desiccated appearance
Stem (45 to >75 days)	Brown to black sunken lesions on their stems and petiole, distinctly different from the healthy green portions. Lesions enlarge rapidly and girdle the stem. The infected stems easily break at the lesion site. A dark brown to purple streak band that extends upward from the soil level and is usually only visible on one side of the stem. Phloem is smoky gray coloured and the xylem remains clear. It is also common to find stems and branches that are swollen at the base or have transformed into cankerous hypertrophied structures

Epidemiology: The fungus is soil borne; which survives as chlamydospores, oospores, and dormant mycelium in the soil and on infected plant debris. Moist cloudy weather with drizzling rain for about 6–8 h (RH = 90–95%) with temperatures of 25–28 °C favour the development of disease. Zoospores of the pathogen are the primary source of inoculum and wind acts as dispersing agent over short distances. PB incidence was reported to be higher, when maximum temperature (28–40 °C), minimum temperature (12–24 °C) relative humidity 75–96% is coupled with 300 mm rainfall within a week (Sharma et al. 2006a, b; Pande and Sharma 2010). Pal and Grewal (1975) reported correlation between disease incidence and soil nutrition indicated that in the high doses of nitrogen (N) and absence of potassium (K), increased PB incidence. Stage of the crop is very important for PB disease incidence. 100% susceptibility to disease was noticed in the 15-day-old seedlings and 25% on 4-month-old plants (Mishra and Shukla 1986).

Management of blight

Cultural control: The best option for management of *Phytophthora* blight is to avoid pigeonpea cultivation in fields prone to water logging. Ridge method of sowing was superior to flat method with regard to reduction in disease and higher yield. Intercropping of pigeonpea with soybean, cowpea, groundnut, mungbean and urdbean also helps in reduction of the disease. Agrawal and Tripathi (2003) reported pigeonpea: sorghum (2:2) intercropping resulted lowest seedling mortality.

Chemical control: Seed treatment with Metalaxyl 4 g kg^{-1} and foliar spraying Metalaxyl + Mancozeb @ 0.3% is necessary to manage the disease. Integration of resistant cultivar (KPBR 80-2-1), ridge sowing and intercropping with mungbean in NEPZ (Chauhan et al. 2005) and combination of ridge sowing + soybean as cover crop and Metalaxyl seed treatment @ 4 g kg^{-1} seed in central zone proved most effective for management of Phytophthora blight of pigeonpea.

Resistant cultivars: Resistance sources have been identified for stem blight of pigeonpea i.e. *C. platycarpus, C. scarabaeoides* and *C. sericeus* are found resistant to Phytophthora blight. Resistance to the P3 race of stem blight is available only in a wild species (*C. platycarpus*) from tertiary gene pool. Since this disease is now taking a form of an epidemic in the low-lying and high rainfall areas (Pande et al. 2011), its genetic solution through inter-specific crop breeding involving *C. platycarpus* as a donor is essential. Some of the inbred lines derived from crosses involving *C. cajan* and two wild species *C. acutifolious* and *C. platycarpus* were found to have moderate to high levels of resistance to P3 race of phytophthora blight (Mallikarjuna et al. 2006; Sharma 2017). It was also reported that the resistance to phytophthora blight in *C. platycarpus* (ICPW 61) was under the control of a single recessive gene pair (Mallikarjuna et al. 2006).

Recently, ICRISAT identified the Phytophthora resistant lines viz., ICP 11376-5, ICP 12730, ICP12751, ICP 12755, ICPL 20093, ICPL 20100, ICPL 20101, ICPL20104, ICPL 20105, ICPL 20109 (Pande et al. 2012). Gupta et al. (1997) discovered the monogenic dominant nature of resistance and the involvement of minor genes in the resistance against *Phytophthora.*

Chemical and biological control: The fungicides such as apron (metalaxyl), ridomil MZ (metalaxyl + mancozeb), capton (captaf), difolatan (captafol), thiram and bavistin (carbendazim) @ 0.3 and 0.6% were compatible with *P. fluorescens* while *B. subtilis* was compatible only with apron at 0.3% (Singh and Dubey 2010).

6.2.1.3 Dry Root Rot of Pigeonpea

Rhizoctonia bataticola (Taub.) Butler [*Macrophomina phaseolina* (Tassi) Goid] emerged as serious problem in late sown or summer and in perennial or ratoon pigeonpea (Kaur et al. 2012). Pathogen infects more than 500 host plants including cultivated and wild plant species belonging to 100 families around the world (Mihail and Taylor 1995; Pande et al. 2004). The pathogen causes severe damage especially when an off-season summer crop is taken particularly in black soil (Nene et al.1979). Under favorable condition, disease will infect quickly and cause huge economic losses ranging from 10 to 100% (Smitha et al. 2015).

Resistant sources: The genotypes GRG-820 and GRG-811 were found to be least susceptible, while eight genotypes ICP-14832, BDN-2008-8, AGL1666, AGL-1919, AGL-2013, ICP-8793, AGL-1603 and GRG-177 were resistant to dry root rot. Maruti et al. (2019) reported 11 genotypes viz., GRG-177, GRG-811, TS-3R, ICP-14832, BDN-2008-8, GRG-820, AGL-1666, AGL-1919, AGL-2013, ICP-8793 and AGL-1603 resistant to *R. bataticola* of pigeonpea.

Chemical and biological control: The fungicide Ziram @ 0.1, 0.2 and 0.3% was significantly superior and systemic fungicides tested, tebuconazole @ 0.05, 0.10 and 0.15, and propiconazole @ 0.10 and 0.15% concentration showed complete inhibition of *R. bataticola* (Maruti et al. 2017). Whereas, *Trichoderma viride* (Tv-B) was found more effective in inhibiting the mycelial growth (77.20%) of *R. bataticola* followed by *Trichoderma virens* (Tvn-B) (75.76%) (Maruti et al. 2017).

6.2.1.4 Powdery Mildew

Powdery mildew (*Oidiopsis taurica* SALM.) though a disease of minor importance in pigeonpea cultivation, it occurs regularly and in severe forms in certain areas. Powdery mildew is wide spread in the semi-arid areas of India and eastern Africa (Nene et al. 1996). Sterility mosaic is known to predispose pigeonpea to powdery mildew attack (Prameela et al. 1990). There was a significant reduction in nodule number, nodule weight, shoot weight and root weight due to infection. *Sterility mosaic virus* had a greater adverse effect than mildew and when both the pathogens infects at a same time, had much greater effect on yield. Although often present on the older leaves it is generally not regarded as a cause of crop loss and management is not considered necessary. However, the disease was frequently encountered in Tanzania during the survey conducted by Kannaiyan et al. (1984) who regarded the

disease of economic importance. Powdery mildew was also moderately severe in parts of Kenya but less so in Malawi.

Etiology: Powdery mildew is caused by *Leveillula taurica* (Lev) Arnaud (*Oidiopsis taurica*) on a wide range of crops, although isolates from one host do not always cross inoculate onto other hosts. The primary inoculum is probably the conidia. Conidia germinate on the leaf surface under a wide range of humidity. The germ-tube penetrates through the stomata and much of the subsequent mycelial development takes place within the mesophyll. Cleistothecia are formed only under cool climatic conditions and are short-lived in dry climates. The symptoms are seen on the leaf as white patches of spore-bearing mycelia. The pathogen is able to survive due to the wide host range amongst crops and weed species.

Control: No serious attempts have been made to control powdery mildew in Africa. Reddy and Sheila (1994) reported a high degree of resistance to powdery mildew in some Kenyan germplasm lines (ICPs 9150, 13107, 13156, and 13232).

6.2.1.5 Cercospora Leaf Spot

Cercospora leaf spot is found in most countries where pigeonpea is grown. The disease is reported to cause substantial losses where pigeonpea is grown under humid conditions with yield losses as high as 85% (Rubaihayo and Onim 1975; Onim 1980).

Etiology: Leaf spot is caused by *Cercospora cajani* Hennings (perfect stage: *Mycovellosiella cajani* (Henn.) Rangelex. Trotter). The pathogen probably survives in crop residues and perennial pigeonpea. Spores are splash-dispersed, to infect the leaves of nearby pigeonpea plants during wet weather, causing small brown spots that increase in size and coalesce. Often, only the older leaves are affected but disease development is favoured by prolonged high humidity and rapid spread is facilitated by wet conditions. Under these circumstances, younger leaves can be affected, leading to premature defoliation.

Control: Crop rotation may be useful in reducing the sources of primary inoculum. Fungicides such as benomyl and Mancozeb have been shown to be effective in reducing disease severity and increasing yield (Onim 1980). Onim and Rubaihayo (1976) reported a number of sources from Kenya having a high degree of resistance to Cercospora leaf spot (UCs 796/1, 2113/1, 2515/2, and 2568/1). Recently, several sources of resistance have been identified in genotypes belonging to different maturity groups in Kenya: KCCs 50/3, 60/8, 119/6, and 423/13 (early maturing), KCCs 81/3/1, 576/3, 657/1, 777 and ICPL 13081 (medium maturing), and KCCs 66, 605, 666, and ALPL 6-2 (late maturing) (Songa et al. 1991).

6.2.2 Viral Diseases of Pigeonpea

Fifteen viruses are known to naturally infect pigeonpea. Of these, diseases caused by the *Pigeonpea sterility mosaic virus* (PPSMV) and new whitefly-transmitted bipartite begomoviruses have been shown to be economically significant (Kumar et al. 2003).

6.2.2.1 Pigeonpea Sterility Mosaic Virus

Sterility mosaic disease (SMD) is a serious constraint for pigeonpea cultivation in India, Bangladesh, Nepal, Thailand, Myanmar, and Sri Lanka with an estimated annual loss of over US$ 300 million in India alone (Kumar et al. 2003). SMD caused by *Pigeonpea sterility mosaic virus* (PPSMV) containing multipartite, negatively oriented single-stranded RNA genomes and double membrane-bound virus-like particles of 80–200 nm in diameter of the virus classified under the newly created genus, Emaravirus (Kumar et al. 2003; Mielke-Ehret and Muhlbach 2012). The yield losses caused by SMD vary depending on when the crop is infected; early infection (45 days) can result in a 95–100% yield loss, whereas late infections can result in 26–97% yield losses. Aside from reducing yield, SMD infection predisposes plants to subsequent infection by fungal diseases and spider mites (Kumar et al. 2003).

PPSMV is transmitted by the eriophyid mite *Aceria cajani* in a semi-persistent manner. Recently, an isolate of PPSMV from ICRISAT-Patancheru (India) was fully sequenced and was shown to contain five segments of RNA (Elbeaino et al. 2014). DAS-ELISA, DIBA and PCR have been developed to detect PPSMV (Kumar et al. 2003).

Symptomatology: SMD symptoms depend on the genotype and usually there are of three types of symptoms were seen (Reddy et al. 1998) i.e. (i) Complete sterility: Severe mosaic on leaflets, plants without flowers and pods, this happens when infection takes place at the early stage (before 45 days). (ii) Partial sterility: Mild mosaic on leaflets in a few branches that do not bear flowers or pods. This is seen when the infection is in its later stages i.e. beyond 45 days. (iii) Ring spot: This was distinguished by leaves with green islands surrounded by a chlorotic halo. As the plants mature, such symptoms fade. This type of reaction is seen only in certain genotypes like ICP-2376.

Genome organization: Based on genomic organization and sequence characterization, PPSMV-1 and PPSMV-2, comprises economically important plant viruses with negative-sense segmented RNA genomes, consisting of 4–8 single stranded RNA segments depending on the species. The first sequence of PPSMV published and renamed as PPSMV-1, was reported to contain five genomic RNA segments of 7022 nt, 2223 nt, 1442 nt, 1563 nt and 1689 nt coding for an RNA-dependent RNA polymerase (RdRp), a glycoprotein (GP), a nucleocapsid protein (NP), a movement protein (MP) and p5, respectively (Elbeaino et al. 2014, 2015). Subsequently, another *Emaravirus* species, PPSMV-2, was reported to be associated with SMD of pigeonpea (Elbeaino et al. 2015).

Natural transmission: In nature, the PPSMV is transmitted by the vector, eriophyid mite-*Aceria cajani* (Acari: Eriophyidae). It mainly distributed South Asian countries such as Bangladesh, India, Myanmar (Burma), Nepal, and Sri Lanka. It is uniformly distributed on the leaves with major concentration in the younger leaves. More than 90% population of *A. cajani* occur on the lower surface of leaves in the diseased plants. These mites are very small but can be seen easily under a stereo binocular (40x) microscope. This mite is highly host-specific and is largely confined to pigeonpea and its wild relative's viz., *Cajanus scarabaeoids* and *C. cajanifolius* commonly found on wastelands and field bunds. *A. cajani* has short chellceral stylet mainly feeds on the lower surface of the leaf. Because of the short stylets length, a mite feeding cause no obvious damage to pigeonpea but allows only penetration of epidermal cells. The expression of disease involves the interaction between virus, vector, cultivars and environmental conditions. A single mite is sufficient to transmit the virus successfully but requires 10 viruliferous mites for 100% infection. Nymphs and adult's mites are equally effective in transmission of the virus (Janarthanan et al. 1973).

Molecular markers for disease resistance: Varietal identification is critical for genetic resource documentation. Traditional methods such as morphometric trait observation and biochemical techniques based on protein and isozyme polymorphism were used. However, fingerprinting of crop varieties using DNA markers is very useful for differentiation and characterization of varieties at the molecular level, and it has been found to be more reliable than traditional markers. Microsatellite markers, also known as simple sequence repeat markers, are short tandem repetitive DNA sequences with repeat lengths ranging from 1 to 5 base pairs. Microsatellite markers are increasingly being used to assess plant genetic diversity and population structure (Li et al. 2002). The high variability of repeat numbers among individuals has led to the use of microsatellite markers for the development of genome specific DNA fingerprints. Burns et al. (2001) described a set of ten SSR markers in pigeonpea. A total of 12 different pigeonpea accessions were screened for polymorphic SSRs using 20 primer pairs. Of the 20 primer pairs, 10 loci exhibited polymorphism when applied to the set of 12 diverse pigeonpea accessions. Odeny et al. (2007) discovered 19 polymorphic SSR primers among 15 cultivated and nine wild pigeonpea accessions, indicating cross-species transferability within the genus *Cajanus*. Diversity array technology (DArT) markers revealed low level of genetic diversity in cultivated pigeonpea as compared to wild relatives (Yang et al. 2006). Ganapathy et al. (2009) identified SSR and AFLP markers associated with the sterility mosaic disease in the F2 population of the cross TTB 7 (susceptible) and BRG 3 (resistance).

Genetics of resistance: The digenic recessive, complementary gene controlling resistance against sterility mosaic disease (Bisht et al. 2019). In case of SMD four independent loci, two duplicate dominant genes (Sv1 and Sv2) and two duplicate recessive genes (sv3 and sv4) are mainly responsible for the of resistance for sterility mosaic disease (Saxena 2008). The introgression of genomic segments related to disease resistance through genomics-assisted breeding (GAB) is an important strategy for the development of disease-resistant varieties. The inheritance pattern of resistance in pigeonpea for the diseases is governed by monogenic dominant.

Disease Management strategies:

The better ways to reduce pigeonpea production loss by preventing mite vector multiplication in the field through adopting prophylactic spray of acaricides and growing of resistant/tolerant varieties.

Cultural method: The various crop management methods to reduce mite infestation include: the destruction of mite-infested plants at an early stage of the crop growth, sowing new pigeonpea crops away from perennial pigeonpea, the rotation of pigeonpea with other crops to reduce vector populations, and the removal of perennial and volunteer plants of pigeonpea (Raychaudhary and Nariani 1977). However, Singh and Rathi (1996) found no significant difference in the disease incidence in various dates of sowing. However it depends on the variety chosen and the geographical location. Inter-cropping with sorghum, and pearl millets or both, and border and inter-cropping with jowar and sunhemp (Singh and Rathi 1995) had no effect on incidence of SMD.

Resistant donors and cultivars: Resistance to the mite vector is the most practical and cost efficient disease control measure. Moreover, use of resistant cultivars would enhance the efficacy of other disease control measures in an integrated disease management strategy. The screening of germplasm for SMD resistance, three methods are being used for experimental transmission of PPSMV viz., the 'leaf stapling technique' (Nene and Reddy 1976), infector-hedge technique' (Nene et al. 1981) and 'spreader row' inoculation technique (Nene et al. 1981). In all these methods mites transmits the virus from source leaf to the healthy plant. However, the presence of distinct strains/isolates of PPSMV in different locations makes broad-spectrum resistance difficult to incorporate. Resistance to various isolates of PPSMV has been reported in a few cultivars, including ICP7035, which has been approved for cultivation. Wild *Cajanus* species were shown to have resistance to multiple isolates of PPSMV. Screening for resistance to three PPSMV isolates from South India was conducted for 115 wild Cajanus accessions belonging to six species, *C. albicans, C. platycarpus, C. cajanifolius, C. lineatus, C. scarabaeoides,* and *C. sericeus.* Accessions, ICP 15614, 15615, 15626, 15684, 15688, 15700, 15701, 15725, 15734, 15736, 15737, 15740, 15924, 15925, and 15926 showed resistance to all the three isolates (Kumar et al. 2005). Singh et al. (2014) evaluated pigeonpea germplasms against various diseases and found that Sehore 367, DPPA 84-61-3, DPPA 84-8-3, ICP 786, ICP 8327, DA12, DA13, DA51, DA11, MA 97, Rampur, Bahar, Bageshwari, Pant A3, Pant A104, Pant A8505 were resistant to sterility mosaic disease. These diverse accessions that are resistant to FW or SMD will be useful to the pigeonpea resistance breeding program.

Chemical Control: Fenazaquin (0.1%) spray reduced the mite (*Aceria cajani*) population by 81.9% followed by winter green oil 30 EC (2%) with the population reduction of 72.3%. Among the plant products tested, winter green oil 30 EC (2%) was effective by recording 19.0% SMD incidence with 58.7% disease reduction (Rajeswari et al. 2016). Manjunatha et al. (2012) also reported that Fenazaquin @ 0.25% reduced the mite population by 60.4%. Therefore, Fenazaquin could be the best chemical for the management of SMD in pigeonpea under field conditions.

Seed treatment with systemic acaricides will prevent early mite infestations. Foliar sprays to check the secondary spread in the field and the cultivation of sterility mosaic disease (SMD)-resistant pigeonpea cultivars can limit the perpetuation of *A. cajani* in the field. Spraying of propargite 0.1% at 25 DAS and 40 DAS recorded significantly lowest sterility mosaic disease incidence of 7.72% with highest yield of 875 kg ha^{-1} (Maurya et al. 2017).

No specific early warning systems are available to indicate outbreaks of *A. cajani*. However, the summer rain (in March to April) contributes to a high mite infestation and an increased incidence of SMD on new pigeonpea crops. This is because the summer rain supports the survival of leftover SMD-infected pigeonpea plants in the fields. Such plants harbour *A. cajani* and allow a high multiplication of the vector during the off-season. The plants act as a source of *A. cajani* which spreads and contribute to an increased mite infestation on the newly sown crop.

6.2.2.2 Yellow Mosaic Virus Disease (YMD)

Pigeonpea YMD is caused by whitefly-transmitted begomoviruses and occurs in Sri Lanka, India, Jamaica, Nepal, and Puerto Rico. Although the incidence of YMD in pigeonpea is low, late-sown pigeonpea can have a higher incidence, resulting in a yield loss of up to 40%. Various begomovirus species that include MYMV, *Rhyncosia mosaic virus* and Tomato leaf curl New Delhi virus have shown to be associated with YMD (Biswas and Varma 2011).

6.2.3 Pigenonpea Nematodes

Pigeonpea crop is significant damaged by plant parasitic nematodes (PPN) and may directly affect the physiological functions of the plants to decrease the yield. Kaiser (1981) reported many species of plant parasitic nematodes associated with pigeonpea *Criconemoides* spp., *Hoplolaimus galeatus, Helicotylenchus dihystera, M. javanica, Meloidogyne arenaria, P. schribneri*, *Pratylenchus brachyurus, Rotylenchulus reniformis, Tylenchorhynchus claytoni, Trichodorus christiei* generally producing root rot. Of these, Nene et al. (1996), *Heterodera cajani*, *Meloidogyne* spp. and *Rotylenchulus reniformis* are known to be important (Sharma et al. 1992). *Heterodera cajani*, *M. incognita*, *M. javanica* and *R. reniformis* are serious pests of pigeonpea in India (Syed Abuzar and Akhtar 2009). Annual yield loss to pigeonpea by PPN has been estimated at 13.2%, worldwide (Abd-Elgawad and Askary 2018). High soil moisture, warm regions and high temperature, crop stands in the field for long duration favours the nematode infestation in pulses crops. Most widely distributed nematodes of pigeonpea are cyst nematode (*H. cajani*), root-knot nematode and reniform nematode is the most widely distributed cyst nematode of pigeonpea in India (Varaprasad et al. 1997). *M. incognita* and *M. javanica* have been reported to attack

pigeonpea in Australia, India, Pakistan; *M. javanica* in Brazil, India, Pakistan. Nematodes also affect the severity of soil-borne pathogens and reduced the efficiency of beneficial microbes like *Rhizobium* in nodule formation.

6.2.3.1 Pigeonpea Cyst Nematode (*Heterodera cajani*)

At least 80 species has been reported in Genus *Heterodera*, which caused a serious crops yield reduction (Subbotin et al. 2010). Swarup et al. (1964) were the first to recorded cyst nematode on pigeonpea. Lemon-shaped females possessing a short neck and terminal cone. Cysts are pale yellow which later becomes hard-walled and turn brown then black in colour. This protective cyst enables the nematode to withstand desiccation and greatly enhances their survival and dispersal (Waeyenberge et al. 2009). Mature females are quite distinct having a large egg sac which is double the size of the cyst. Damage to the root is done by J2s by feeding intracellularly on the elongation region of the growing root. Formation of syncytial cells by the female nematode around cephalic region blocked xylem and phloem vessels and reduced root efficiency (Aboul-Eid and Ghorab 1974; Sharma et al. 1992). This condition becomes even more crucial if there is association of these nematodes with the wilt pathogen, *Fusarium* spp. The disease reaction to both wilt-resistant and wilt-susceptible pigeonpea cultivars to Fusarium wilt is increased by the presence of nematodes in the soil (Hillocks et al. 2000). The association of Fusarium wilt and root-knot nematodes (Hillocks and Songa 1993; Marley and Hillocks 1996), cyst nematode, *Heterodera cajani* (Sharma and Nene 1989) and reniform nematode, *Rotylenchulus reniformis* (Jain and Sharma 1996) have been well-established.

The nematode caused a disease called pearly root in pigeon pea. Sharma and Nene (1989) reported a significant reduction in plant growth parameters at the level of 500 and 1000 J2/500 cc soil. Chlorosis, stunting, reduction in development of leaves, flowers, ultimately leading to reduced pods and yield (Gaur and Inderjit 1977). Nitrogen content and phosphorous increase in roots but decrease in the shoot. Potassium decreases both in roots as well as of the shoots of pigeon pea.

6.2.3.2 Root Knot Nematode (*Meloidogyne* spp.)

The most important symptoms of root knot nematode infestation are identified by formation of galls on the roots. The shape and size of galls vary, depending upon the nematode species and population density. The above ground symptom includes stunted growth, smaller size of leaves and pods, yellowing of foliage, wilting was observed in patches. Pods may ripen and dry prematurely and remain partially filled and undersized. Formation of galls reduction in number, size and weight of root nodules, consequently affecting nitrogen fixation. Besides, its direct affect, mixed population of root-knot nematodes (*Meloidogyne javanica* and *M. incognita*) form a complex with *Fusarium udum* in the root of pigeon pea and showing more susceptibility to Fusarium wilt (Marley and Hillocks 1996).

6.2.3.3 Reniform Nematode (*Rotylenchulus reniformis*)

Reniform nematode being sedentary semi endoparasitic in nature causes tremendous loss in pigeonpea when cultivated in light soil. The symptoms are observed in patches and number of such patches increases under water stress condition. Infected plants shows chlorosis on new leaves followed by dieback of twigs and main stem. The premature death of plants has also observed in some cases. The most characteristic below ground symptoms are the presence of soil covered with egg masses and necrotic, dark-coloured regions on the root portion. Hence the disease caused by this nematode is often known as dirty root disease.

Management of pigeonpea nematodes:

Resistant sources: Resistant sources against pigeonpea nematodes is not available in current cultivated germplasms. The promising pigeonpea lines such as ICPL 83024, ICPL 85045, ICPL 8357, 85068, 85073, 89050, 89051, and 90097 have been identified as tolerant to the reniform nematode (Sharma 1997). Sharma et al. (2015) also reported tolerance lines bearing accession numbers ICP 16329, ICP 16330, ICP 16331, ICP 16332, and ICP 16333 in genebank of pigeonpea at ICRISAT against reniform nematode. They reported that sources of tolerance to the nematode lies in the pigeonpea landraces.

Seed and soil treatment: Soaking of pigeon pea seeds in solution of Aldicarb or carbofuran for 30 and 60 min. Soil solarisation with irrigation reduces the population of *H. cajani* cysts, eggs and larvae (Sharma and Nene 1990).

Bio-management of plant-parasitic nematodes in pigeon pea field

Management of nutrient and a combination of nutrient and botanical extract like neem showed a remarkable effect on the population of pigeon pea nematodes. It was reported that 220 kg N/ha urea effectively suppressed the nematode populations and enhanced the performance of plant. A combination of urea and neem product showed an additive effect on the growth of the plant and effective in suppressing nematode populations.

6.3 Insect Pests of Pigeonpea

Nearly 200 insect species have been reported to feed on pigeon pea (Reed and Lateef 1990), however few of them know to inflict significant damage to the crop. On an average, 30–80% losses occur due to insect pests (Asthana et al. 1997).

Taxonomy, races, isolates and biotypes of insects: In pigeon pea, the insect belongs to order Lepidoptera, Hemiptera, Diptera and Coleoptera poses major problems. These insects belongs to different food habit, i.e. oligophagous to polyphagous and exploit the resources in different manner i.e. surface feeder, concealed feeder, chewing and biting, piercing and sucking type (Shanower et al. 1999).

6.3.1 Gram Pod Borer, Helicoverpa armigera *(Hubner) (Lepidoptera: Noctuidae)*

6.3.1.1 Geographical Distribution

Geographical distribution widely distributed in countries of Asia, Europe, Africa, Australia, South and Central America.

6.3.1.2 Host Plants

The larvae known to feed on more than 187 host plants, however major pest on Cotton, Tomato, Pigeon pea, Chickpea, Sunflower, Maize and Sorghum.

6.3.1.3 Biology

The life cycle of *H. armigera* is depends upon the geographical location and also on the crop on which the larvae grown. The adult moth has a stout body with a typical noctuid appearance. The female adult has yellowish to orange coloured fore-wing, whereas greenish grey in males with distinct kidney shaped marking in anterior margin of forewing. Hind wings are pale grey in colour with darker marginal band. The female lays the eggs individually on all the parts of the plant, however the young shoot and florets were preferred for oviposition. The incubation period lasts for 3 days (at optimum temperature of 27–28 °C). The caterpillars are greenish with darker broken lines along the side of the body, however coloration can vary considerably (yellow, brown, red or cream) due to diet content (larvae exhibit body colour polymorphism) (Yamasaki et al. 2009). The larval and pupal period in normal conditions lasts for 17–23 and 9–11 days, respectively.

6.3.1.4 Damage Symptoms

The young larvae feed on the tender foliage, may result in pinhole damage. Older larvae bore into the pods and completely feed on the seeds. While feeding it thrusts its head inside the pod leaving the rest of its body outside. They eat away completely the seed and feeding results in characteristic round holes on pods. The older larvae can destroy pods. They can cut hole on one to another locule and feed 20–25 pods in its lifetime.

6.3.2 *Redgram Plume Moth,* Exelastis atomosa *(Walsingham) (Lepidoptera: Pterophoridae)*

6.3.2.1 Geographical Distribution

India, Sri Lanka, Indonesia and Madagascar.

6.3.2.2 Host Plants

Redgram, Lablab, Niger, Horse gram, Cowpea, Mung beans and Soy beans.

6.3.2.3 Biology

The adults are small, slender and light brown in colour. The wings are deeply fissured. The forewings are elongated and divide into 2–4 divisions, whereas the hindwings have 3 divisions. The adults survived for 6–7 days, and lay nearly 60 green colored eggs individually on buds and developing pods (Sharma et al. 2010). The egg hatched in 2–3 days and bore into the pod feed on tender seeds. The larvae complete its development in five instar. The pupa is active and pupation noticed on the surface of the pod. The pupa is covered with short hairs and in close resemblance to larvae. Total larval and pupal duration vary between 15–18 and 6–9 days, respectively.

6.3.2.4 Damage Symptoms

The activity of moth noticed during pre-flowering and flowering stage of the crop. Immediately after hatching the larvae first scrapes the surface of pods, enters into the pod by cutting a small hole and feeds on seeds. Attacked seed is completely eaten away by the caterpillars, unlike pod fly. The larva also feeds on unopened flower buds consuming the anthers and causing flower drop.

6.3.3 *Redgram Pod Fly,* Malanagromyza obtusa *(Malloch) (Diptera: Agromyzidae)*

6.3.3.1 Geographical Distribution

The pigeon pea pod fly native to tropical Asia (Shanower et al. 1998), being present in south Asian countries like India, Sri Lanka, Bangladesh, Nepal, Pakistan and south east Asian countries like Myanmar, Philippines, Thailand, Vietnam, Taiwan and also

recorded in Central America and Caribbean (Dominican Republic, Guadeloupe, Haiti and Puerto Rico).

6.3.3.2 Host Plants

Chickpea, Phaseolus (bean), Mung bean, Cowpea, Rhyncosia, Tephrosia, Dunbaria, Flemingia.

6.3.3.3 Biology

The adult of both sexes are 2–3 mm long, and appear as black in color to the naked eye, but the closer observation shows distinct coloration in thorax and abdomen i.e. green metallic in colour. The adult female insert egg into shell of the green tender pod. The incubation period lasts for about 3 days. The newly hatched maggot bore into the epidermis without rupturing the seed coat and the late instar maggot feed on the cotyledon. Most of the cases one seed is sufficient for a maggot to complete its development. Before entering to pupation the final instar maggot makes an exit hole on the pod and cover it with translucent epidermal cells. The total larval and pupal duration vary between 6–11 and 9–23 days, respectively. After completing pupal duration, the adult fly come out through the exit hole made by the maggot. The adult is short lived (less than 12 days), during its adult duration the female lays about 80–100 eggs.

6.3.3.4 Damage Symptoms

All the immature stages found inside the pod, hence monitoring the pest without damaging pod is very difficult. The maggot feeds on the starchy portion of the seed and also the embryo. The damaged seeds will not germinate and also unfit for consumption, hence has no value.

6.3.4 Spotted Pod Borer, Maruca vitrata *(Testulalis) (Geyer) (Lepidoptera: Pyralidae)*

6.3.4.1 Geographical Distribution

The insect is a pantropical insect pest of leguminous crops, reported to be present over 100 countries of Asia, Africa, South America and Australia.

6.3.4.2 Host Range

It has wide host range however limited to legume crops like Pigeon pea, *Cajanus cajan*, Jack bean, *Canavalia ensiformis*, *Crotalaria*, Jewelvine, *Derris*, Soyabean, *Glycine max*, Hyacinth bean, Lablab purpureus, Lima bean, *Phaseolus lunatus*, Common bean, *Phaseolus vulgaris*, Tropical kudzu, *Pueraria phaseoloides*, Hoary-pea, *Tephrosia*, Cowpea, *Vigna unguiculata.*

6.3.4.3 Biology

The adult moths have dark brown forewings with three white spots and a brown border on greyish white hind wings. The eggs are laid on buds or petals or sepals of flower and sometimes on the young pods regardless of the host plant. Each female is capable of laying about 400 eggs either individually or in overlapping group of 2–14. The eggs were white in colour and dorso-ventrally flattened. The incubation period varies between 2–5 days. The larvae are cream white in color with dark brown head and prothorax. The later instar larvae (II–V) have characteristic six rows of black spots. The larval and pupal duration vary between 8–14 and 6–9 days, respectively. The pupation may occur within the larval web or in the soil within a pupal cell made by the final instar larvae and covered it with debris. The total life cycle of *M. vitrata* is typically 18–25 days, sometimes can be as long as 57 days.

6.3.4.4 Damage Symptoms

The larvae webs together the foliage including the flowers and pods and feeds on them. The larvae feed inside a webbed mass of leaves, flowers, pods and rendering them unmarketable. At the entrance of larval furrow larval webbing, mass excreta can be seen. The larvae cause extensive damage to floral buds and flowers, resulting in discoloration and dropping of flower. The concealed nature of larvae (the damaging stage) and pupal stage complicates management by chemicals or other conventional means.

6.3.5 Pod Sucking Bugs

Clavigralla gibbosa Spinola, *C. scutellaris* (West wood), *C. tomentosicollis* Stal. *Riptortus* sp. *Anoplocnemis* sp. (Hemiptera: Coreidae) *Nezara viridula* (L.) (Hemiptera: Pentatomidae).

6.3.5.1 Geographical Distribution

Three *Clavigralla* species are major and causing economic damage to pigeon pea viz., *Clavigralla tomentosicollis* is widespread in sub-Saharan Africa, the *Clavigralla scutellaris* is found from Kenya, Yemen, Oman, Pakistan, and India. The third species, *Clavigralla gibbosa*, is limited to India and Sri Lanka (Sharma et al. 2010). *Riptortus pedestris* (Fabricius) occurs in Korea, Japan, China, India and South Asian countries.

Nezara viridula is a cosmopolitan insect pest widely distributed in tropical and subtropical regions of Americas, Africa, Asia, Australasia and Europe.

6.3.5.2 Biology of *Clavigralla gibbosa*

The adults are stout (about 10 mm long), wedge shaped, dusky brown in colour and having a pair of elongated spines projecting interiorly on pro-thorax. A single female may lay about 250 eggs, occasionally as many as 430 eggs in clusters of varying size. The eggs are pinkish to orange in colour.

6.3.5.3 Damage Symptoms

The nymphs and adults of all the aforesaid bug species suck the sap from the developing pigeon pea pods and seeds. As a result of desapping the pods shriveled and the shoot fades. The seeds become dark and dried up, and they are difficult to differentiate from the seeds damaged due to drought stress. The damaged seeds do not germinate and are unmarketable as they are not acceptable as food. The damage due to *C. gibbosa* and *C. scutellaris* vary across geographical location and occasionally results in 50% loss in pod yield due to bugs' damage.

6.3.6 *Redgram Sterility Mite:* Aceria cajani *(Acarina: Eriophyidae)*

6.3.6.1 Geographic Distribution

The mite limited to South Asia and South East Asia. The mite is identified to present in India, Bangladesh, China, Myanmar, Nepal, Sri Lanka and Thailand.

6.3.6.2 Host Range

The *A. cajani* is highly host-specific and is largely confined to pigeon pea, *C. cajan* and its wild relatives viz., *C. cajanifolius, C. platycarpus, C. scarabaeoides, C. sericeus* growing as wild plants in the fields.

6.3.6.3 Biology

It is hard to see the mites with the naked eye. The adults are 0.2 mm long, light pink in color and spindle shaped. The pigeon pea mite completes its life cycle in three developmental stages viz., egg (30 × 40 μm), nymphs and adults (200–250 μm).

6.3.6.4 Damage Symptoms

A. cajani feeding causes no direct damage to the host plant, as a result no distinctive symptoms attributable to it. However damage severe due to its ability to transmit Pigeon pea sterility mosaic virus (PPSMV), which cause sterility mosaic disease (SMD). The mites inhabit the under surface of the leaf and usually found in the symptomatic leaves of PPSMV infected plants. The plants infected by virus appears bushy and pale green in color, stunting and excessive vegetative growth however severe reduction in leaf size is noticed. The diseased plants show complete or partial cessation of flowering i.e. it produce sterile flower. The mites have passive mode of dispersal mainly assisted by wind currents.

6.3.7 Pulse Beetle

Callosobruchus chinensis. C. analis, C. maculatus (Bruchidae: Coleoptera).

6.3.7.1 Geographic Distribution

C. chinensis has been reported from African countries like Egypt, Kenya, Nigeria; and Asian countries like China, Japan, Indonesia, Burma and India.

6.3.7.2 Host Range

All whole pulses, beans and grams like Mung bean, Cowpea, Chickpea, Peas, Lentil. Beside pulses, the beetles also known to feeds on cotton seed, maize and sorghum.

6.3.7.3 Biology

The adult beetles are chocolate or reddish brown in color and oval in shape with characteristic dark stripes on dorsal elytra. The elytra don't cover the abdomen exposing the pygidium. The fecundity of the insect varies between 30–130/female. The eggs are small and oval to spindle shaped, individual eggs are glued over the surface of the grain. The egg hatches within 4–5 days of laying. The grub after hatching bore onto the grain, thereafter feed inside the grain and complete its larval and pupal stage. The average larval and pupal duration vary between 10–38 and 4–28 days, respectively. It takes 117–168 days for the hibernating larvae to complete their growth.

6.3.7.4 Damage Symptoms

The infestation starts from the main field before harvesting the mature pods (Field infestation or cross infestation). However the pest causes no or least damage in the main field condition, however they do serious damage in storage. Both grubs and adults of pulse beetle feeds on whole content of the grain, leaving only the shell behind. The damage due to pest is severe that every grain in a lot or bag is infested. The infested grain is easily identified by exit holes and white eggs on the seed surface. The infested grains are unfit for human consumption and also unfit for sowing. The damaged grain is often converted into flour.

6.3.8 Blue Butterfly, Lampides boeticus *(Linnaeus) (Lepidoptera: Lycaenidae)*

6.3.8.1 Geographic Distribution

The butterfly is known to be present in Europe, Africa, South and Southeast Asia, and Australia. In India, the insect is active throughout the year.

6.3.8.2 Host Range

Pigeon pea, *Cajanus cajan*, chickpea, *Cicer arietinum*, sunn hemp, *Crotalaria juncea*, soybean, *Glycine max*, hyacinth bean, *Lablab purpureus*, Lucerne, *Medicago sativa*, lima bean, *Phaseolus lunatus*, common bean, *Phaseolus vulgaris*, pea, *Pisum sativum*, tropical kudzu, *Pueraria phaseoloides*, faba bean, *Vicia faba*, cowpea, *Vigna sinensis*, black gram, *Vigna mungo*, mung bean, *Vigna radiata.*

6.3.8.3 Biology

The male forewings with a blue tinge and dark grey in females. The adults have characteristic tail like prolongation from the apical margin of the hind wing and also have prominent black spot at the posterior end. The eggs are greenish blue in colour and turn pale before hatching. The eggs are turban shaped, flat on top and at bottom. The incubation period lasts for 2–3 days. The larvae are apodus and moulted four times and complete its larval duration in 8–12 days. The pupal duration lasts for 5–8 days and pupates in the soil. Total life of the insect completed in 20–25 days (Palem et al. 2015).

6.3.8.4 Damage Symptoms

The female prefer to lay the eggs on the growing shoots, flower buds, green pods and leaves. Upon hatching the legless caterpillar enters unopened flower and feed inside. Later the larva also feed on the developing grain.

6.4 Management of Insect Pest

6.4.1 Cultural Methods of Control

Cultural methods are the most practicable, simple and low-cost pest management practiced since ages by the farmers (Morales 2002). Deep summer ploughing exposes all resting stages of insects, such as larva and pupa to abiotic and biotic factors. It helps in removing quiescent pupae of *H. armigera*, *Lampides boeticus* and *M. vitrata* from the field. Host plant resistance acts as a first line of defense against the both insect pests and diseases. Selection of suitable resistant or tolerant varieties against endemic pest or diseases helps in maintaining pest population, besides assuring good yield (Bosque-Perez and Buddenhagen 1992). It adds no cost towards cultivation, however save the crop from the insect and diseases. Adoption of following varieties like ICPH 2740 which is resistant to wilt and sterility mosaic diseases and ICPL 332 WR (TDRG 4) which is resistant to wilt and tolerant to *Helicoverpa* damage (Anonymous 2015).

Selecting companion crops has also been explored as a means of reducing pest damage by increasing environmental diversity. Sowing of one row of sunflower as intercrop, over nine row of pigeon pea is helpful to control the pod borer population. Mixing of sorghum/maize seed @ 250 g/ha to function as live bird perches, shelter several natural enemies and also helpful in reducing wild diseases (Gopali et al. 2009; Mula et al. 2015). Clean sanitation is an important cultural strategy for protection of crops from arthropod pests like insect and mite. Collection and destruction of wild pigeonpea, ratoon crop and volunteer pigeonpea plants which acts as sources

of inoculum for SMD. Regular removal and cutting of SMD plants/plant parts helps in reduce the subsequent spread to healthy plants. The farmers can adjust date of sowing or harvesting of pigeonpea to avoid damage from specific pest viz., early sowing of redgram escapes the pod-fly (*M. obtuse*) damage. Early planting by mid-June in North West Plain Zone avoids *H. armigera* and Timely planting in North East Plain Zone escapes *H. armigera* (Sharma et al. 2010).

6.4.2 Chemical Methods of Control

Considering the economic importance of pigeon pea, as well as the seriousness of the insect damage and related yield loss, farmers apply insecticide to control the insect pest. Several reports quoted that plant have the ability to compensate 50% loss caused due to defoliation, even if occur at the podding stage (Sithanantham 1987). However, the insects which damage the crop during flowering and pod formation stage inflict severe damage to the crop, which necessitates chemical application. The chemical pesticides are recommended only when the pest crosses Economic Threshold Level (ETL) and when epidemic situation arises. Selection of insecticides is also important, as the pods are harvested for consumption, beside it should have least effect on non-target organisms. Spraying of effective molecule like Indoxacarb 14.5 SC % @ 300 ml/ha or Emamectine benzoate 5% SG @ 11 a.i. gm/ha or Spinosad 45 SC @ 56–73 a.i. gm/ha is effective against pod borer complex viz., *H. armigera*, *M. vitrata* and Jassids can become a serious threat if the seedling stage is heavily infested. Under these conditions, application of any contact or systemic insecticide is adequate restraint.

6.4.3 Biocontrol Methods with Natural Products and Biotic Agents

Biological insect pest control is the best alternative tool for replacing chemical pesticides and their effect on the non-target organism and environment (Rani et al. 2021). A large number of natural enemies have been identified and reported from pigeonpea ecosystems that have the potential to restrict the population to non-damaging levels (Lingappa and Hegde 2001). In order to make the biocontrol program successful farmers need to preserve existing natural enemies and augment potential natural enemies within the ecosystem. Several natural enemies were found significant in reducing the pop borer population viz., *Trichogramma chlionis, Copidosoma floridanum*, *Bracon hebetor*, *Cotesia* spp. *Campoletis chlorideae, Eucelatoria* spp. *Apanteles taragama, Carcelia illota*, *Goniopthalmus halli, Palexorista laxae, Chrysoperla carnea*, *Mantid* spp. and *Cheilomenes sexmaculatus* (Romeis and Shanower 1996). A large number of predatory birds are reported to feed on pod borer larvae in the pigeon

pea ecosystem. Among them, black drongo, *Dicrurus adsimilus*, house sparrows, *Passer domesticus*, blue jays, *Coracias bengalensis*, cattle egret, *Bulbulcus ibis*, rosy pastor, *Sturnus roseus* and mynah, *Acridotheres ginginianus* have been commonly documented to be predators on larvae of *H. armigera* and lepidopteran insects on chickpea and pigeonpea crops (Beri et al. 1972; Ghode et al. 1988; Lingappa and Gopali 1994). For the management of borer complex farmers were advised to release *T. chlionis* in field @ 1.5 lakh/ha/week for 3–4 times. Application of HearNPV @ 250 LE/ha with teepol 0.1% and Jaggery 0.5% for 2–3 times at fortnight interval commencing from flowering stage, is helpful to control *H. armigera* (Santharam et al. 1981). As the pigeonpea pods were consumed at green pod (as vegetable) farmers must use safe agents like *Bacillus thuringiensis* serovar *kurstaki* (3a, 3b, 3c) 5% WP1000–1250 g/ha or 2% neem oil or Azadirachtin 0.03% WSP 2000–3000 ml/ha against pod borer. Even farmers also prepare their own neem formulation (NSKE 5%) and sprayed during pod formation stage controls multiple pests.

6.4.4 IPM

Commercial agriculture depends heavily on chemical pesticides for the management of insect pest and diseases, which is responsible for wide spread environmental problems. There is a growing awareness world over on the need for promoting environmentally sustainable agriculture practices (Sagar 1991). Integrated pest management (IPM) is a globally accepted strategy for promoting sustainable agriculture and human livelihood. IPM utilizes all the available management techniques in a compatible manner to reduce the pest populations below the injury levels through an ecologically sound, economically practicable and socially acceptable manner (Ha 2014). Although it is possible to exterminate the pest population with the use of chemical pesticides, it is not without unfavourable effects. Application of chemicals results in serious problems like insecticide resistance, pest resurgence and pesticide residues. Such outbreak and reoccurrence of pests have induced farmers to apply more frequent of application of insecticides at higher doses. This sort of approach in pest management is due to the ignorance of the role played by the natural enemies in controlling the pest populations. Besides developments of resistance, toxic hazards associated with residues of chemicals, has stimulated the interest in evolving approaches that limit the application of pesticides and used as a last resort in managing pest population in compatible with other pest control methods. Various cultural, physical, mechanical and biological methods are integrated to bring down the pest populations to economically lower levels with little disturbance to ecosystem particularly to natural enemy populations. The various field operations like deep summer ploughing expose the resting stages of insects to predatory birds and sunlight, use of pest free and resistant planting material offers protection against particular pests, sowing in right time as early or late sowing escapes insect infestation, proper removal of diseased plants, alternate host plants and weeds, applying balanced fertilizers and irrigation at such

a level that pests are not encouraged, choosing bio pesticides like NPV, *Bt* formulations and neem based formulations not only control the pests but also encourage the activity of natural enemies and pollinators. Applications of green or blue labelled insecticides must be a last resort in managing pest populations and applied only when the pest population reaches injury level.

6.5 Traditional Breeding Approaches

6.5.1 Genetic Resources

To achieve the global food security in twenty-first century, research must focus on curbing the menace of biotic stress, which affects both quality and quantity of the crop. Among the various biotic stresses in pigeonpea, Fusarium wilt (FW), *Sterility mosaic disease* (SMD), Phytophthora blight (PB), leaf spot, pod borer, pod fly and *Maruca vitrata* are the major constraints which affect the productivity of pigeonpea. The host plant resistance (HPR) is the most effective and economic way to manage these biotic stresses (Keerthi et al. 2021). The use of crop wild relatives in pigeonpea resistance breeding program has been successful in providing cultivars which are resistant to various biotic stresses. Many of the wild species has shown resistance against multiple pest and diseases of pigeonpea (Table 6.1). Most of the pigeonpea genetic resources are in ex situ gene banks, however the species diversity gap analysis found that India and northern Australia are the hotspots for pigeonpea crop wild relatives (Khoury et al. 2015). The largest pigeon pea germplasm was conserved at gene banks such as ICRISAT with over 14,500 accessions and NBPGR with 11,221 accessions, which include trait specific germplasm for Fusarium wilt, sterility mosaic disease, Phytopthpra blight, pod fly, pod wasp and pod borer resistance. The collections mainly include landraces, wild relatives and breeding materials. In this context ICRISAT and NBPGR has made considerable efforts in identification of wild species, introgression lines and landraces which are resistant to major diseases and insects in pigeonpea. These germplasm materials are utilized in development of biotic stress resistance varieties which can be successfully cultivated across different regions of the world.

6.5.2 Breeding for Disease Resistance

About 210 pathogens have been found to infect pigeonpea plants including 83 fungi, 4 bacteria, 19 viruses and mycoplasma and 104 nematodes (Nene et al. 1996). Among which, the Fusarium wilt (*Fusarium udum*), SMD, Phytophthora blight (*Phytophthora drechsleri* f. sp. *cajani*), and Alternaria blight are the major biotic stresses which decrease the productivity of pigeonpea globally. To develop cultivars resistant to

Table 6.1 Sources for disease and pest resistance in wilt relatives of Pigeonpea

Genus	Species	Gene pool	Traits of importance	References
Cajanus	*sericeus* (Benth. Ex Bak.) van der Maesen	2	Resistance to SMD, phytophthora, and alternaria blight; pod borer, pod fly	Sharma (2006), Upadhyaya (2006)
Rhynchosia	*bracteata* Baker	4	Tolerant to pod borer, pod fly and pod wasp	Sharma (2006)
Cajanus	*albicans* (W. & A.) van der Maesen	2	Resistance to SMD, alternaria blight	Pundir and Singh (1985)
Cajanus	*cajanifolius* (Haines) van der Maesen	2	tolerant to pod borer, pod fly and pod wasp	Singh (1990), Pundir and Singh (1985), Sharma et al. (2006a, b)
Cajanus	*lineatus* (W. & A). vander Maesen	2	Resistance to SMD and alternaria blight	Pundir and Singh (1985), Reddy et al. (2000)
Cajanus	*platycarpus* (Benth.) van der Maesen	3	Resistance to phytophthora and alternaria blight	Reddy et al. (2000), Srivastava et al. (2006)
Cajanus	*reticulates* (Dryander)F.v. Muell	2	Hardy, fire-tolerant, resistant to pod borer	Reddy et al. (2000)
Cajanus	*scarabaeoides* (L.) Thouars	2	Resistance to wilt, SMD, PB, (both for P2 and P3 isolates), alternaria blight, pod borer, pod fly, pod wasp, cyst nematode, and possesses combined resistance to diseases and insects	Pundir and Singh (1985), Reddy et al. (2000)
	sericeus (Benth. ExBak.) van der Maesen	2	Resistance to SMD, Phytophthora, and alternaria blight; pod borer, pod fly, pod wasp	Sharma (2006), Upadhyaya (2006)
Rhynchosia	*bracteata* Baker	4	Tolerant to pod borer, pod fly and pod wasp	Sharma (2006)

(continued)

Table 6.1 (continued)

Genus	Species	Gene pool	Traits of importance	References
Cajanus lineatus	ICPW 40, ICPW 41	–	Resistance to Phytophthora	Pande et al. (2011)
Flemingia stricta	ICPW 202	–	Resistance to Phytophthora	Pande et al. (2011)
Cajanus lineatus, Cajanus sericeius	–	–	Resistance to Phytophthora	Pande et al. (2011)
C. platycarpus			Resistant to the P3 race of Phytophthora blight disease	Kassa et al. (2012)

biotic stresses, plant breeders have been using various classical breeding approaches viz., single plant selection based on its field performance, wide hybridization with wild relatives, gene pyramiding for multiple resistance and backcross breeding to transfer resistant genes. Breeding for resistance to Fusarium wilt, Phytophthora blight and sterility mosaic disease has been attempted and considerable success also been obtained in developing resistant cultivars in pigeon pea (Sharma et al. 2012).

The information on genetic nature of resistance can be used to introgress the disease resistance to highly adapted cultivars (susceptible genotypes) through conventional as well as marker assisted backcross breeding. Inheritance of resistant genes in pigeonpea against wilt disease is governed by multiple factors. Earlier Jain and Reddy (1995) reported that a single recessive gene governs resistance to Fusarium wilt. Wilt resistance is dominant over susceptibility, which is controlled by two genes (Saxena et al. 2012) and Bhanu et al. (2018) also confirmed the presence of two complementary genes for wilt resistance.

Studies on inheritance of sterility mosaic disease indicated that the resistance is controlled by two independent non-allelic genes with complementary gene interaction (Nagaraj et al. 2003). But Patil and Kumar (2015) and Singh et al. (2003) confirmed the presence recessive genes in governing resistance to sterility mosaic disease. For Phytophthora disease, very limited work has been done on inheritance of resistance. Studies on inheritance of resistance for P2 race confirmed the presence two homozygous recessive genes for resistance (Singh et al. 2003). However, for P3 race it is reported that resistance is controlled by single dominant gene and some minor genes (Gupta et al. 1997). Sharma et al. (1982) reported the presence of single dominant gene in governing resistance to Phytophthora blight. Similar to Phytophthora blight, very limited information is available on genetics of inheritance of Alternaria blight resistance. A single recessive gene controls the resistance for Alternaria blight in pigeonpea (Ojha et al. 1993). Sharma et al. (1987) also confirmed the presence of single recessive single recessive gene (abr_1) for resistance. FW resistance has been governed by one dominant gene in BDN-2004-1 and BDN-2001-9, two duplicate

dominant genes in BWR-133 and two dominant complimentary genes in resistance source IPA-234 in F_2 segregation pattern (Singh et al. 2016a, b).

Fusarium wilt is the most destructive vascular wilt disease of pigeon pea, which causes yield loss up to 50–70% during pod filling stage. The selection of breeding method for developing resistant varieties is dependent on genetic nature of resistant sources. For biotic stresses the resistance sources were available in wild relatives and land races. Hence the resistant landraces were effectively utilized in resistant breeding through pedigree selection. One such example was a wilt-resistant variety, ICP 8863 (Maruti), which is a selection from the landrace ICP 7626. This variety has been the best performer in the farmer's field to date.

The higher incidence of SMD at early stages of plant severely affects the pod number, seed size and seed yield per plant and it causes yield loss of 90–100%. Many researches documented that the wild *Cajanus* species are resistant or tolerant to SMD (Kulkarni et al. 2003; Rao et al. 2003). But those wild relatives belong to different gene pools hence pose gene transfer difficulties. It is difficult to transfer resistance genes directly from wild species to cultivated variety. Thus the development of introgression lines by crossing wild *Cajanus* species with an appropriate variety is an ideal approach for the efficient use of wild relative in the pigeonpea resistance breeding. Recently, Interspecific population was developed through advanced backcross breeding by crossing *C. acutifolius* and *C. cajanifolius* with pigeonpea variety ICPL 87119, which is resistant to SMD and Fusarium wilt (Sharma et al. 2019).

Wild *Cajanus* species such as *C. sericus* and *C. scarabaeoides* are reported as resistant to Phytophthora blight, which can be effectively utilized as a source of resistance to pigeonpea breeding program (Pande et al. 2006). Because of frequent evolution of new pathotypes and lack of durable genetic resistance for Phytophthora blight, there is very limited progress on the resistance breeding program for Phytophthora blight. The novel resistance genes which are resistant to multiple races of Phytophthora blight are essential for development of resistant cultivars. In this regard, gene pyramiding using molecular tools holds promise to develop a cultivar resistant to multiple races of this pathogen. Similarly, Alternaria blight of pigeonpea is the major disease noticed in the post rainy season.

6.5.3 Breeding for Insect Resistance

Among the insects damaging pigeonpea, pod borer, *H. armigera*, pod fly, *M. obtusa* and spotted borer, *M. vitrata* are the major pests which cause significant economic loss. Because of blanket application of pesticides by farmers, the insects developed a resistance to a large group of insecticides. Besides, it also resulted in several environmental problems such as residues etc. It also increases the cost of production. In such cases host plant resistance (HPR) is a suitable approach to combat the insect damage. The genetic variability for insect resistance is available in wild species of pigeonpea but transfer of resistant genes through conventional backcrossing is not a successful

approach in this crop. However, the wild relatives such as *C. scarabaeoides* and *C. acutifolius* are cross compatible with cultivated pigeonpea, which can be used for interspecific gene transfer through conventional hybridization (Mallikarjuna and Saxena 2002; Upadhyaya 2006).

The presence of dominance of resistance over susceptibility for pod borer resistance in wild relatives was confirmed through cross between *C. cajan* and *C. scarabaeoides* (Aruna et al. 2005). The inheritance studies in segregating generations of a cross between the susceptible cultivar Pant A-3 and *C. scarabaeoides* confirmed that resistance to pod fly and pod borer is governed by two recessive genes and one dominant gene, respectively (Verulker et al. 1997). The studies on mode of gene action for resistance to plume moth revealed the presence of single dominant allele that is designated as PPM_1 (Mishra et al. 2015). The resistance to insects is not available in primary gene pool but available in the secondary and tertiary gene pools of wild relatives. For example, *C. scarabaeoides* shows higher resistance to pod borer and pod fly, hence genetic control of resistance to these pests are studied in interspecific crosses including wild relatives.

Selection of resistant plants among the available germplasm is the cheapest and quickest method of development of resistant variety for biotic stress. One such example is the variety Abhaya, which is resistant to pod borer. It was the selection from germplasm line ICP1903. However, attempts have also been done to develop pod borer resistant cultivars through wide hybridization between *C. platycarpus* and *C. cajan* (Mallikarjuna and Moss 1995). But it was not successful because of post zygotic barriers leading to completely sterile F_1 hybrids. Most of the wild species which are possessing resistance to insects belong to secondary and tertiary genepool. It led the difficulty in introgression of resistant genes to cultivated genepool. So the modern biotechnological tool such as embryo rescue technique plays a great role in obtaining fertile hybrid. In-ovule embryo culture is also used for direct culturing of the hybrid embryos on medium. So there is significant scope for transfer of pod borer resistant genes through interspecific hybridization using compatible species (Mallikarjuna et al. 2007).

Another important pest, pod fly, *M. obtuse* also causes remarkable damage to yield loss in pigeonpea, but little effort has been made on development of resistant cultivars. The resistant germplasm lines for pod fly have been identified. These lines are having small seeds and are not preferred by pod fly for egg laying. These lines can be used as potential genetic sources for pod fly resistance breeding. The genotypes ICP 8266, ICP 8102, ICP 8595-E1-EB, ICP 12510-1, ICP 12759, ICPL 20120, ICP 8087 and ICPL 332 WR have been identified as potential source of resistance to pod borer, pod fly and pod bug. Sharma et al. (2003) reported that the germplasm accessions belonging to *Cajanus scarabaeoides, C. sericeus, Rhynchosia bracteata, C. acutifolius*, *C. lineatus*, and *C. albicans* showed resistance to pod fly. But some of the accessions of *C. scarabaeoides*, *R. bracteata, C. albicans,* and *F. stricta* showed resistance to both pod fly and pod wasp damage. These germplasms can be effectively utilized for development of resistant cultivars for pod fly.

Maruca vitrata (Geyer) is also serious insect pest of pigeonpea. It causes yield loss up to 100% if left uncontrolled. The promising accessions which are resistant

to *Maruca* have been identified through selection from germplasm in pigeonpea (Saxena et al. 2002). In general, the pigeon pea plants with determinate growth habit are more susceptible to *Maruca* damage in comparison to indeterminate plant type (Saxena et al. 1996). The breeding programs aimed at development of *Maruca* resistant variety should consider this trait as prior importance. So identification of resistant germplasm and development of resistant cultivar is the best way control of insect damage.

The efforts have also been made on development of genomic resources for insect resistance in pigeonpea. The molecular markers coupled with bulk segregant analysis have been used in pigeonpea for tagging insect resistance gene in pigeonpea. For the first time Mishra et al. (2015) developed a SCAR marker which is linked to resistance to plume moth with the use of an interspecific mapping population. Among the wild relatives, *C. scarabaeoides* had higher level of resistance to insects affecting pigeonpea and it is extensively used in development of interspecific population for genetic analysis. The genetic linkage map for traits contributing to resistance for pod borer, plume moth and blue butterfly also developed based on interspecific population developed through cross between *C. cajan* and *C. scarabaeoides* (Sahu et al. 2015).

Gene pyramiding with two different insecticidal genes and tissue-specific expression to reduce the risk of developing insect resistance is another attractive option to combat this *H. armigera* for durable resistance. Expression of a chimeric *cry1AcF* (encoding cry1Ac and cry1F domains) gene in transgenic pigeonpea has been demonstrated towards resistance to *H. armigera* (Ramu et al. 2012).

6.6 Use of Morphological and Molecular Markers

Along with conventional breeding, modern genomics tools such as candidate genes and molecular markers linked with biotic stresses offers a great opportunity for development of resistant cultivars. Particularly, marker-assisted selection (MAS) or marker-assisted backcrossing (MABC) has played a great role in resistance breeding, as it helps in easy assessment of the phenotype and transfer of large number of resistance genes. Molecular markers closely linked to resistance to disease and insects help in assessment of resistant and susceptible plants at seedling stage and it also it eliminates the repeated phenotyping of segregating population and progenies. Very early, the mapping efforts have been made with the help of RAPD markers (Kotresh et al. 2006) and AFLP markers (Ajay et al. 2015) for Fusarium wilt disease resistance.

Six SSR markers namely, ASSR-1, ASSR-23, ASSR-148, ASSR 229, ASSR-363 and ASSR-366 are associated with FW resistance and this could be used to introgress FW resistance into susceptible but highly adopted cultivars through MABC and in conventional breeding programs (Singh et al. 2016a, b). Saxena et al. (2017a, b) identified three QTLs *qFW11.1*, *qFW11.2* and *qFW11.3* for FW resistance by using a genotyping-by-sequencing approach from three populations (PRIL B, PRIL C, and F_2 Population), respectively. Two RAPD markers (Kotresh et al. 2006), four SCAR

markers (Prasanthi et al. 2009) and six SSR markers (Pazhamala et al. 2015) were detected to be linked for FW resistance.

As a result of pigeonpea genome initiative the much of genome resources have been developed. Use of advanced molecular tools has facilitated the trait discovery through linkage and association mapping. A number of mapping population has been developed in pigeonpea for various biotic and abiotic stresses. Moreover, the molecular markers, genetic map, map based cloning of resistant genes will facilitate the molecular breeding for biotic stress resistance in pigeonpea. The availability of genome sequence information in pigeonpea revolutionized the mapping efforts with the help of sequence based markers. The high density genetic map also been developed for Fusarium wilt resistance by using genotyping by sequencing approach (Saxena et al. 2017a, b).

The molecular markers have also been extensively employed in pigeonpea for differentiation of genotypes based on response to diseases. The SSR marker also has proven its potential in differentiating the contrast genotypes for SMD infection in pigeonpea (Naik et al. 2012). These genotypes can be utilized in development of mapping population and identification of RIL combined with stable yield and resistance to SMD. The identification of QTLs associated with disease resistance aids the easy transfer of resistance genes through molecular breeding approach. Among various molecular markers, SSR markers have proven to be highly reliable, environment insensitive and reproducible as compared to other markers. A large set of SSR markers have been developed for identification of genes associated with resistance to SMD (Gnanesh et al. 2011), and Fusarium wilt (Khalekar et al. 2014). Four independent loci, two duplicate dominant genes (*Sv1* and *Sv2*) and two duplicate recessive genes (*sv3* and *sv4*) are responsible for the inheritance sterility mosaic disease resistance and SMD is expressed only when one dominant allele at locus 1 or 2 and homozygous recessive genes at locus 3 or 4 are present. The GBS approach was used for simultaneous identification and genotyping of SNPs, and the candidate genomic region identified on CcLG11 was the promising QTL for molecular breeding in developing superior lines with enhanced resistance to SMD (Saxena et al. 2017a, b). Six QTLs explaining phenotypic variation were identified on LG7 and LG9 after extensive phenotyping for SMD resistance (Gnanesh et al. 2011).

The developed markers help in molecular tagging and mapping of genes for biotic stress resistance in pigeonpea. Furthermore, which can be successfully used in resistance screening of germplasm and marker assisted introgression of resistance to biotic stress in susceptible cultivars. Another type of functional marker such as expressed sequence tags (EST) developed in pigeonpea can be successfully employed in identifying the resistant genes expressing differentially in different genotypes. Since pigeonpea is prone to high pests and diseases, the pyramiding of resistant alleles for multiple biotic stresses to single cultivar is also an effective approach. Currently genomic assisted breeding is emerging as novel approach to develop FW and sterility mosaic disease resistant varieties (Saxena et al. 2020).

6.7 Transgenic Pigeonpea for Resistance to Pod Borer

The resistant source for insects particularly for pod borer is not available in primary genepool. Thus the transferring of resistant genes from wild to cultivated genepool poses a difficult challenge. The effective strategy to control pod borer is transgenic expression of cry genes which encode for *Bacillus thuringiensis* insecticidal crystal proteins (ICPs). The introgression of Cry1 series of insecticidal crystal proteins and Cry2Aa has already proved its efficacy against pod borer (Singh et al. 2018). The *Agrobacterium tumefaciens* mediated transfer of cry genes of *B. thuringiensis* has been identified as efficient method for transferring gene of resistance to pigeon pea (Sharma et al. 2006a, b). It is not only an additional strategy to get resistant plants but also an effective way to prevent economic yield loss in pigeon pea. As the insects develop resistance to particular toxin, gene pyramiding is the better alternative strategy to overcome failure of transgenics. Here transgenics play a greater role in developing plants with absolute resistance (Table 6.2).

Table 6.2 Crop wild relatives for major disease and pest tolerance in pigeon pea

Biotic stress	Wild relatives	References
Fusarium wilt	*C. scarabaeoides, C. platycarpus*	Nagaraja et al. (2016), Mallikarjuna et al. (2011)
Sterility mosaic disease	*C. acutifolius, C. albicans, C. crassus, C. lineatus, C. platycarpus, C. scarabaeoides, C. sericeus*	Mallikarjuna et al. (2011), Saxena (2005)
Phytophthora blight	*C. platycarpus, C. sericeus, C. platycarpus*	Saxena (2005), Mallikarjuna et al. (2005)
Alternaria blight	*C. albicans, C. lineatus, C. scarabaeoides, C. sericeus, C. cajanifolia, C. volubilis*	Sharma et al. (1987)
Pod borer	*C. acutifolius, C. albicans, C. platycarpus, C. scarabaeoides, C. sericeus, Rhynchosia aurea,* R. bracteata, *Flemingia bracteata*	Mallikarjuna et al. (2007, 2011), Saxena et al. (2005)
Pod fly	*C. acutifolius, C. albicans, C. lineatus, C. scarabaeoides, C. sericeus, Rhynchosia bracteata*	Sharma et al. (2003)
Pod wasp	*C. albicans, C. scarabaeoides, Flemingia stricta, R. bracteata*	Sharma et al. (2003), Choudhary et al. (2013)

6.8 Marker Assisted Breeding for Resistance to Fusarium Wilt and Sterility Mosaic Diseases

For the development of FW and SMD-resistant pigeonpea lines, we need to identify the genomic regions/QTLs or the genes which confer disease resistance to the plants. Once that gene is identified, we can use MAS for introgressing disease resistance in the susceptible genotypes. Availability of mapping populations and genetic maps is the basic requirement for identifying the marker-trait associations. The markers thus identified are subsequently used for practicing MAS for crop improvement. Marker assisted breeding (MAB) may be strategically useful to diminish the effect of complex and unpredictable environmental interactions and in identification of race-specific resistant genes and gene pyramiding.

Various studies to explore the genomic architecture of FW and SMD resistance have revealed that there are significant associations between marker and trait which can be used for pigeonpea breeding programs. However, these DNA markers have not been used extensively in genomics-assisted breeding for developing FW and SMD resistant varieties primarily due to genetic variability in pathogens, effect of different locations, lesser phenotypic variance explained by the reported QTL and cost-inefficiency of the genotyping assays. To enable genomics-assisted breeding in pigeonpea, the Pigeonpea Genomics Initiative (PGI) was established in 2006. It was funded by Indian Council of Agricultural Research under the umbrella of Indo-US Agricultural Knowledge Initiative, which was further aided with financial support from the US National Science Foundation's Plant Genome Research Program and the Generation Challenge Program (Varshney et al. 2010).

Ganapathy et al. (2009) employed BSA to identify SSR and AFLP markers associated with SMD resistance in the F_2 population of TTB 7 (susceptible) X BRG 3 (resistant) parents, where they identified four polymorphic markers potentially useful for MAS. Prasanthi et al. (2009) screened 88 pigeonpea lines and identified resistant sources with specific amplification for resistance to Fusarium wilt at 920 bp with OPGO8 primer, namely TRG-32, TRG35 WRG-65, TRG-40, TRG-36, TRG-38, ICPL 8863 and ICPL-87119. Gnanesh et al. (2011) described the existence of five different SMD isolates in India, of which three had been characterized: Patancheru, Bangalore and Coimbatore. Composite interval mapping (CIM) based QTL analysis by using genetic mapping and phenotyping data provided evidence for four QTLs for Patancheru SMD isolate and for two QTLs for Bangalore SMD isolate. Sharma et al. (2015) performed multi-environment field testing, and identified four genotypes (ICPLs 20094, 20106, 20098 and 20115) as the most stable and resistant to SMD. Daspute and Fakrudin (2015) identified 32 out of 300 short decamer random DNA markers that showed polymorphism between Gullyal white (SMD susceptible) and BSMR 736 parents (SMD resistant).

To investigate the inheritance of resistance to FW disease in pigeonpea, Singh et al. (2016a, b) found that it was governed by one dominant gene in BDN-2004-1 and BDN-2001-9, two duplicate dominant genes in BWR-133 and two dominant complimentary genes in resistance source IPA-234. Singh et al. (2016a, b) used

sequencing-based bulk segregant analysis (Seq-BSA) to map the FW and SMD resistance genes in pigeonpea. The effort of Seq-BSA uncovered seven candidate SNPs, which included a total of four candidate nsSNPs (non-synonymous single nucleotide polymorphisms) in four genes associated to FW resistance and four candidate nsSNPs in three genes related to SMD resistance. Saxena et al. (2017b) worked on using genomics-assisted breeding for QTL mapping for Fusarium wilt resistance in pigeonpea. They developed high-density genetic maps using two RILs: ICPB 2049 × ICPL 99050 designated as PRIL_A and ICPL 20096 × ICPL 332 designated as PRIL_B and one F_2 (ICPL 85063 × ICPL 87119).

Candidate genes from *C. cajan 03203* (for FW resistance) and *C. cajan* 01839 (for SMD resistance) turned out to be most promising from the results of in silico protein analyses and expression profiling. Saxena et al. (2017a) took five parental lines: ICPL 20096, ICPL 332, ICPL 20097, ICP 8863 and ICPL 87119 with contrasting SMD resistance for mapping populations. Out of these lines, ICPL 20096, ICPL 20097 and ICPL 87119 were resistant to SMD while ICPL 332 and ICP 8863 were susceptible to SMD. Three mapping populations were created, namely, ICPL 20096 × ICPL 332 (PRIL_B), ICPL 20097 × ICP 8863 (PRIL_C) and ICP 8863 × ICPL 87119 (F_2). They used GBS approach for the simultaneous identification and genotyping of SNPs on these populations. They identified the genomic region CcLG11 as promising for molecular breeding for developing better lines. Very recently, Saxena et al. (2020) developed a diagnostic kit for identification of suitable FW and SMD resistant lines. Two genes *of* C. *cajan_03691* and *C. cajan_18888*) for FW resistance and four genes (*C. cajan_07858, C. cajan_20995, C. cajan_21801* and *C. cajan_17341*) for SMD resistance had been identified. In conjunction with a cost-effective Competitive allele-specific PCR genotyping assay, they identified 9 robust markers for FW resistance and 10 robust markers for SMD resistance in pigeonpea.

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Chapter 7
Application of Genetic, Genomic Strategies to Address the Biotic Stresses in Faba Bean

Kedar Nath Adhikari, Lynn Abou Khater, and Fouad Maalouf

Abstract Faba bean (*Vicia faba* L.) is a widely grown cool season grain legume crop for human consumption and animal feed. It is one of the oldest domesticated crops dating back to the Neolithic era around 9,000–10,000 B.C. Although area under the crop has decreased significantly over the years, its productivity has increased making it the third largest cool season legume after chickpea and lentil. One of the reasons for decreasing area under faba bean is its unstable yield owing to the effect of biotic stresses. Now there is considerable knowledge accumulated on identification, genetic variation, genetic control and strategies to overcome the effects of diseases, weeds and insect pests. With the availability of next-generation sequencing and high-throughput genotyping methods, high-density genetic maps are being developed which will facilitate marker assisted selection based on gene based molecular markers. Resistance to major diseases, Ascochyta blight, chocolate spot and rust are available and successfully integrated into the breeding programs. However, resistance breeding for chocolate spot, Cercospora, gall disease and root rot diseases are not so advanced due to poor understanding of the diseases and lack of strong resistance in the germplasm. Availability of improved genotypes tolerant to parasitic weeds in combination with the low rate of herbicide application is allowing successful cultivation of faba bean in the affected areas. Herbicide tolerant genotypes particularly to imidazolinone and metribuzin will provide effective measures of in-crop broad leaf weed. All these measures will enhance the breeding for biotic stress tolerance and successful cultivation of protein rich faba bean crop in the future.

Keywords Disease resistance · Herbicide tolerance · Parasitic weeds · Genetic enhancement · Genomic research · Resistance breeding · Molecular markers

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7.1 Introduction

Faba bean (*Vicia faba* L.) is the third most important cool season food legume crop grown in many parts worldwide. It was domesticated at the beginning of agriculture in the Fertile Crescent of the Near East following the Neolithic era around 9,000–10,000 BC. Subsequently, its cultivation has spread around the world through different routes from the Near East to Europe and to other parts of the world (Cubero 1974). Currently, faba bean is cultivated under rainfed and irrigated conditions in more than 55 countries and it is grown under different cropping systems for dry grains, green pods for vegetable, animal feed and green manuring. Faba bean contributes to the sustainability of cropping systems through its ability in fixing nitrogen that can be used for succeeding crops. The area under faba bean was reduced from 5.4 million ha in 1961 to 2.2 in 1995 and its production was reduced from 5.5 million tons to 3.6 million tons in the same period due to several biotic and abiotic stresses. Since then, its area increased slightly to 2.6 million ha in 2019, but the production has increased to 5.5 million tons (FAOSTAT 2021). This equates to nearly 2% yield increase/year. The increase in productivity can be attributed to effective breeding programs and agronomic research (Fig. 7.1).

In addition to its economic value, faba bean has an important nutritional value as it is rich in protein (25–36%) and many essential minerals and vitamins (Crepon et al. 2010). Faba bean is also a valuable source of essential amino acids, such as arginine, lysine, and leucine, containing up to 67 g kg^{-1} dry matter (Koivunen et al.

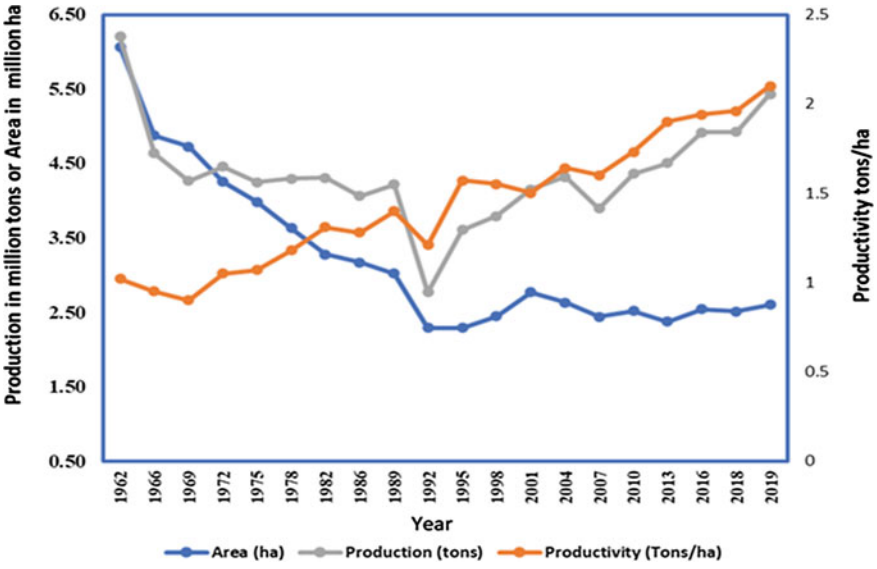

Fig. 7.1 Trend of the world harvested area and production of faba bean since 1961–2018 (FAOSTAT 2021)

2016). Faba bean also has a wide range of variation for iron (22–78 mg/kg) and zinc (45–61 mg/kg) contents in evaluated faba bean accessions (Maalouf et al. 2021). The crop is used as human food and animal feed mainly for pigs, horses, poultry and pigeons in many countries all over the world (Singh and Bhatt 2012). Due to its high protein content (25–36%) faba bean is used as a main source of protein to vegetarians and people who cannot offer animal protein through meat. Faba bean is consumed in various forms—falafel, stew or ful medames and roasted beans. The faba bean grains is also eaten roasted in India, where it is used as a coffee extender (Yitayih and Azmeraw 2017).

Finally, faba bean crop has an important role in improving the soil structure through its ability to fix nitrogen. Faba beans can fix up to 219 kg nitrogen/ha in favorable environments and 63 kg/ha under rainfed conditions (Neugschwandtner et al. 2015). Its nitrogen fixing ability is highest among the cool season grain legumes and it can fix nitrogen even in the presence of already available nitrogen in the soil (Herridge et al. 2008). However, the ability to fix nitrogen depends on environmental conditions, the variety of the crop, the physical and chemical properties of soil and *Rhizobia* present in the soil (Argaw and Mnalku 2017).

Despite having high level of protein, faba bean contains the alkaloid glycoside vicine and convicine which can trigger acute anemia in people who lack glucose 6-phosphate dehydrogenase enzyme in their system. This can be a fatal condition if immediate medical attention is not available. However, low vicine-convicine faba bean lines have been identified and the trait has been incorporated into some modern cultivars. Faba bean breeding programs around the world are in the process of eliminating these glycosides through breeding. Recently a molecular marker has been identified (Khazaei et al. 2019, 2020) that is being used by breeding programs to minimize the level of vicine-convicine in faba bean.

Being an important crop in many production regions, especially in North and East Africa and West Asia, the faba bean program has formed as part of the Consultative Group for International Agricultural Research (CGIAR) agenda at the Arid Land Agriculture Development since 1972 to fulfil the needs of the West Asia and North Africa in cooperation with the Agricultural Research Institute of Lebanon. Consequently, the International Center for Agricultural Research in the Dry Areas (ICARDA) was established in 1977 with faba bean as one of its mandate crops. Since then, the international faba bean breeding program has been working to supply the global communities with improved germplasm and genetic stocks mainly for biotic stresses. In this chapter we will address yield losses due to diverse biotic stresses, the major achievements on improving diseases and Orobanche resistance as well as achievement on genetic mapping and genomic research associated with resistance to major biotic stresses.

7.2 Major Biotic Stresses in Faba Bean

Several biotic stresses affect faba bean and reduce its production in many production areas. The dominant biotic stresses are foliar diseases, viruses, insect pests and parasitic weeds (Table 7.1). The major biotic stresses that affect faba bean are described as below.

7.2.1 Foliar Diseases

The major diseases are chocolate spot (*Botrytis fabae* Sard.), rust (*Uromyces viciae-fabae* (Pers.) Schroet.), Ascochyta blight (*Ascochyta fabae* Speg), faba bean gall (*Physioderma fabae*) and powdery mildew (*Erysiphe pisi*). Faba bean rust can cause moderate to high yield losses in North Africa, Ethiopia and Australia (Emeran et al. 2011; Shifa et al. 2011; Adhikari et al. 2016). Faba bean gall is a newdisease that has caused major production constraint in Ethiopia (Hailu et al. 2014; Teferi et al. 2018).

Ascochyta blight is a devastating disease in many countries, causing up to 90% yield losses as well as quality losses on susceptible faba bean cultivars (Hanounik 1980). It can affect all above ground parts of faba bean including pods. It not only reduces grain yield but also the quality of the grain due to staining. Genetic resistance to the disease has been reported and accession 29H has been classified as the most resistant line (Atienza et al. 2016). Several quantitative trait loci (QTLs) have been reported from studies in controlled conditions (Díaz-Ruiz et al. 2009; Kaur et al. 2014; Atienza et al. 2016), but they need to be validated in the field conditions. Two pathotypes of the fungus have been detected in Australia and varieties PBA Amberley, PBA Bendoc, PBA Samira and Nura are resistant to moderately resistant to both pathotypes.

Chocolate spot generally appears around flowering time in Australia when the humidity is more than 70–80% over extended period. If the conditions are favorable for disease development, substantial yield losses are expected as was observed in 2016 in Australia. It can cause damage even at the seedling stage if the conditions are conducive. Major yield reduction due to chocolate spot has also been reported in Egypt, Maghreb countries and Ethiopia (Tivoli et al. 2006; El-Sayed et al. 2011). A high level of resistance to the disease has not been found and the resistance is believed to be controlled by multiple genes (Beyene et al. 2016) making difficult to breed for high level of resistance. Some resistant lines were reported earlier (Bouhassan et al. 2004), but no information is available on their level of resistance. However, recently a variety, Amberley, has been released in Australia with the significant improvement to the disease.

Rust is another important disease in warmer area causing up to 70% yield losses (Adhikari et al. 2016). The disease is found throughout the faba bean growing areas in the world except North America. Several sources of resistance have been reported

Table 7.1 Major biotic constraints affecting faba bean production in diverse production regions

	Foliar disease				Viruses		Weeds		Insects	
Regions	Ascochyta blight	Chocolate spot	Rust	Gall	BYMV	FBNYV	Parasitic weeds	Annual weeds	Aphids	Stem and pod Borers
South Asia		X	X		X			X	X	
East Africa		X	X	X		X	X	X	X	
West Asia	X	X			X	X	X	X	X	X
North Africa	X	X	X			X	X	X	X	X
East Asia	X	X	X	X		X		X	X	
Oceania	X	X	X		X			X		X
North America	X									
South America	X	X								
Europe	X	X	X				X	X	X	X

(Rashid and Bernier 1986, 1991; Sillero et al. 2000; Avila et al. 2003; Sillero et al. 2010; Rubiales et al. 2013; Adhikari et al. 2016), but the level of resistance is only partial. Newer Australian varieties, such as PBA Warda, PBA Nasma and PBA Nanu have moderate level of resistance to the disease and require only limited fungicidal sprays. Cercospora leaf spot (*Cercospora zonata*) is another emerging disease in southern Australia and Stemphylium blight (*Stemphylium* spp) in subtropical north-west of Australia. However, no significant losses have been reported from these diseases.

7.2.2 Soil-Borne Diseases

Faba bean is also affected by several soil-borne pathogens in irrigated agriculture specially in Egypt and Sudan causing more than 40% yield losses (Bogale et al. 2009; Belete et al. 2015). *Fusarium* species are the most common pathogens including *F. oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *F. avenacearum* (Corda ex Fr.) Sacc., *F. graminearum* Schwabe and *F. culmorum* (Smith) Sacc. (Zakrzewska and Oleksiak 1993; Helsper et al. 1994), but other fungi may also be present, including *Rhizoctonia solani* Kuehn, *Pythium* spp., *Phoma* spp., *Phytophthora pisi* and *Aphanomyces euteiches* Drechs (Salt 1982; Lamari and Bernier 1985; Heyman et al. 2013). Among them, black root rot caused by *Fusarimsolani* (Mart) Appel and Wollenw is one of the most important diseases in the major faba bean growing areas in Ethiopia (Habtegebriel and Boydom 2018). *Aphanomyces* root rot, resulting in severely stunted plants due to complete rotting of primary and secondary roots, has been reported in Australia with genetic variation for tolerance in host, (van Leur et al. 2008) but it has not become a major issue in Australia and not much work is being done.

7.2.3 Parasitic Weeds

Orobanche and *Phelipanche* species (the broomrapes) are chlorophyll-lacking root parasitic plants that drastically reduced production and area of faba bean in the Mediterranean Europe, West Asia and North Africa (Gressel et al. 2004; Khalil et al. 2004; Maalouf et al. 2011). These parasites are completely dependent on the host due to the lack of chlorophyll and functional roots. Recently, these parasitic weeds are expanding their ecological range to Ethiopia and Sudan forcing farmers to abandon faba bean cultivation in many parts of the country (Abebe et al. 2013). Although several broomrape species can infect faba bean, *Orobanche crenata*Forsk. and *O. foetida* Poir. are the most damaging and widespread weeds (Pérez-de-Luque et al. 2010; Rubiales et al. 2014). The estimated average yield losses due to *Orobanche* in Morocco was 37.4% in 1998, but it can lead to total crop failure. *Orobanche* infestation in Algeria has spread over 60% of the area in the middle coast and is continuing its spread towards the west. In Tunisia, the main *Orobanche* species include *O. crenata,*

*O. foetida*and *O. ramosa*, affecting 7.1% area of total food legume-growing areas (Abu-Irmaileh and Labrada 2014), while in Egypt all traditional cropping lands are infested by *Orobanche*.

7.2.4 Insects-Pests

Several insects such as Sitona weevil (*Sitona lineatus* L.), cowpea aphid (*Aphis craccivora* Koch), black bean aphid (*A. fabae* Scopoli), broad bean beetle (*Bruchus rufimanus*) and Heliothis cause damage by direct feeding as well as by transmission of viruses (Mwanauta et al. 2015). The weevils and borers cause holes in the seed causing a reduction in seed germination as well as qualitative degradation in the market. The crop is also affected by pod borer (*Lixusalgirus* L) in the southern Europe, the Middle East and North Africa (Weigand and Bishara 1991). The larvae of this insect are the most damaging; they are borers which grow within the stems, causing bending, leaf yellowing, wilting and drying of the plant at the vegetative stage (Chakir 1992; Khan et al. 2010) and affecting crop growth and yield (Liotta 1963). The most damaging insect pests is Australia are *Heliothis armigera* and *H. punctigera.* These are polyphagous insects attacking many crops including cotton, sorghum, maize, and oilseed and legumes including faba bean. The former is a more of problem in summer crops and the latter in winter crops. *H. punctigera* is native to Australia and is the major insect pest of faba bean. Green mirids (*Creontiadesdilutus*, *C. pacificus*) can damage faba bean in warm growing regions in Australia by feeding on immature green pods. They release a chemical while feeding on the seed inside the pod by probing their mouth part which stains the seed resulting in several black dots on the seed.

7.2.5 Viruses

More than 20 viruses infect faba bean crop in many production areas (Makkouk et al. 2012; Hema et al. 2014). The most prevalent are broad bean mottle virus (BBMV), broad bean stain virus (BBSV), bean leaf roll virus (BLRV), bean yellow mosaic virus (BYMV), alfalfa mosaic virus (AMV), pea enation mosaic virus (PEMV), faba bean necrotic yellows virus (FBNYV) and true broad bean mosaic virus (TBBMV) (Saxena 1991; Bond et al. 1994; van Leur et al. 2006). Morphologically viruses infected faba beans are classified into two categories based on their symptoms (1) yellowing/stunting/necrosis and (2) mosaic/mottling symptoms. However, it is quite difficult to distinguish these symptoms without a proper lab test. Some of the viruses are transmitted through infected seeds leading to spread of infection of new crop and yield losses. Most of viruses that infect faba bean crop are not host specific and they can happily feed and multiply on a range of food and pasture legumes as well as numerous weeds. The other means of virus survival is seed transmission, which is

almost absent in faba bean viruses except pea seed borne mosaic virus (PsbMV) (van Leur et al. 2006). Once a plant is infected by virus, it cannot be cured. Because of the uncertainty of virus epidemics and the lack of effective control options, growers can perceive viruses as a higher risk than fungal diseases. In addition, viruses such as faba bean necrotic yellow virus can cause up to 90% yield losses as reported in Egypt by Kumari and Makkouk (2007). In Australia, BYMV, BLRV and PsbMV are the most prevalent viral diseases causing sporadic losses. In 2020, however, BYMV losses up to 70% were reported in northern New South Wales area and some growers did not harvest the crop as it was uneconomical.

7.3 Agronomic and Cultural Practices

Several agronomic and cultural practices have been developed to combat these biotic stresses in faba bean. Below is a short description of cultural practices applied to control foliar diseases, *Orobanche crenata* and viral diseases.

7.3.1 Foliar Diseases

Ascochyta blight: The key cultural control methods for the major foliar diseases such as Ascochyta blight, chocolate spot and rust involve crop rotation, use of resistant varieties, control of volunteer plants before planting and uses of certified seeds. Burning of straw in countries where it is not used as animal feed can be recommended to reduce initial inoculum and reduce possibility of sexual reproduction. Efficient methods of controlling Ascochyta blight are seed treatment with Thiabendazole and benomyl and foliar spray with Chlorothalonil, Mancozeb, and Azoxystrobin (Davidson and Kimber 2007; Ahmed et al. 2016). Integrated Ascochyta blight management involves crop rotation, use of partially resistant cultivars with fungicide applications (seed treatment and foliar sprays), adjusting sowing dates and use of pathogen free seeds (Davidson and Kimber 2007; Stoddard et al. 2010). For example, early planting coupled with fungicide spays controlled Ascochyta blight and increased seed yield under Mediterranean condition (Ahmed et al. 2016).

Chocolate spot: The major source of chocolate spot inoculum is infected seed, stubbles and spores coming from infected field which can travel long distances. The life cycle can be completed in less than a week and rapid epidemic can start quickly in warm and humid conditions (Fig. 7.2a). In case of chocolate spot, the major cultural control practices are adjusting sowing dates, optimized plant population, crop mixtures, use of pathogen free seeds, weed management, crop rotation, intercropping and mixed cropping (Boudreau 2013). Chocolate spot severity can be significantly reduced by intercropping faba bean with cereals (barley, triticale, oat and wheat) but intercropping with other food legumes did not reduce disease development (Agegnehu et al. 2008; Sahile et al. 2008). Crop rotation with nonhost crops

Fig. 7.2 Development of chocolate spot (*Botrytis fabae*) (left) and rust (*Uromyces viciae-fabae*) (right) in faba bean plants in the field

will reduce primary inoculum from infected debris. Grazing of stubbles can help to minimize inoculum in countries where straw is key animal feed. Destroying volunteer faba bean plants, weeds and burning of stubbles from previous season, ploughing to bury infected straw and seeds can minimize primary inoculum, but stubble burning, and ploughing should be discouraged to promote conservation tillage. Spraying with fungicides like mancozeb and carbendazim is recommended to control the disease with repeated sprays. Carbendazim is more effective than mancozeb against chocolate spot but will not control rust. Some studies showed application of trace elements such as boron, calcium and molybdenum can decrease chocolate spot (Ali et al. 2014). Integration of cultural practices, fungicide sprays and uses of resistant cultivars is recommended to manage chocolate spot epidemics and increase yield (Davidson et al. 2007; Boudreau 2013).

Rust: Management of rust is done through fungicide sprays and use of resistant varieties. The major ones are Mancozeb, dichlofluanid, difenoconazol, epoxiconazol, chlorothalonil, tebuconazol, carbendazim, flusilazole and tebuconazole (Marcellos et al. 1995; Emeran et al. 2011). Although most of the released cultivars of faba bean in many countries in recent years have some resistance to the disease, none have been reported to be immune (Adhikari et al. 2016; Stoddard et al. 2010). These varieties can be infected by virulent pathogen populations during favourable environment for epidemic development (Fig. 7.2b). Therefore, a combination of host resistance, adjusting planting dates and fungicide sprays are recommended to increase crop productivity (Stoddard et al. 2010).

Faba bean gall (FBG)*:* This is a newly emerged disease reported in Japan, China (Li-Juan et al. 1993) and in Ethiopia (Hailu et al. 2014). In Ethiopia, the disease was reported in 2012 and devastated the crop in the cooler highlands of the country. The epidemiology of the disease is yet to be properly known and limited information is available on key management practices. The major cultural practices recommended to reduce the impact of FBG are crop rotation with cereals, nonhost legume crops like potato, intercropping of faba bean with cereals, improve drainage system (using raised beds), and sanitary measures (Alemu and Tadele 2017). Weed control can also play a role to reduce the microenvironment in the crop canopy that favors FBG development and avoid primary inoculum if some of the dicot weeds are infected by the pathogen. Although it is not established that the pathogen is seed borne, use of seeds produced in FBG free areas are encouraged. Burning of diseased crop debris left on the field can minimize primary inoculum coming to the surface of the soil. Ploughing can also play a role to reduce primary inoculum. Fungicide seed treatment (Carbendazim, thiram and Triadimefon) and foliar sprays (Triadimefon, Metalaxyl-M and mixture of Thiamethoxam, Mefenoxam and Difenoconazole) are recommended to manage FBG. In Ethiopia, 2–3 applications of Ridomil Gold WP (2.5 kg/ha) and Bayleton 25 WP (0.3 kg/ha) were found effective in managing FBG (Abebe et al. 2018).

7.3.2 Root Rot Diseases

Root rot diseases of faba bean are key diseases in high rain fall area of Ethiopia and irrigated fields in Sudan and Egypt where there is waterlogging condition. The major management practices are crop rotation with nonhost crops, adjusting plant population, adjusting planting dates and planting faba bean using ridges and beds to remove excessive water. In Ethiopia, rotation of faba bean with edible oil seed crops (*Brassica*, *Guzotia* and *Linum* spp.) and liming of acid soils are recommended to reduce root rots on faba bean (Negussie et al. 2008). Seed treatment is the most effective way of managing root rot complex diseases. Most recommended fungicides as seed treatments are Apron Star (Thiamethoxam, Mefenoxam and Difenoconazole) and Vitaflo 280 (carbathiin + thiram) (Chang et al. 2014). The integration of cultural practices (crop rotation, managing soil moisture, adjusting planting dates, acid soil management, methods of planting), fungicide and biological treatment of seeds and resistant cultivars can reduce the impacts of wilt/root rot. In Ethiopia, planting of moderately resistant faba bean cultivars on raised beds reduced the intensity of black root rot and increased seed yield (Habtegebriel and Boydom 2016).

7.3.3 Viruses

Viral diseases are spread through some vectors and aphids are the most important vectors. Therefore, the aim should be to avoid aphids coming to the faba bean field. But aphids are polyphagous insects feeding on several plant species and their avoidance is difficult if there are growing plants/weeds in surrounding areas. Different methods are used to manage virus and their vectors. Isolation distance over 1000 m from sources of inoculum is recommended to manage viruse transmitted by vectors as nonpersistent manner (Kumari and Makkouk 2007). Other cultural control measures include adjusting planting date, seeding rate and row spacing, planting borders with nonhosts are reported to help in reducing virus epidemics (Thresh 2003). Aphids like bare patches, so maintaining proper plant densities and not leaving any open bare spots in the field is crucial. The cultural methods involve, removing of infected faba bean plants to reduce secondary transmission of the virus, removing of volunteer plants, adoption of mixed cropping with nonhosts and minimum tillage. Application of insecticides targeting vectors is recommended where virus is transmitted through persistent feeding in case of luteo viruses. However, for some viruses like bean yellow mosaic, one or two probes of aphids will be enough to transmit virus. Prophylactic repeated insecticidal sprays in the beginning of the season and rouging of infected plants will be needed to avoid/minimize aphid activities for the control of bean yellow mosaic virus. Integrating control measures should include host plant resistance with 1–2 well timed insecticide sprays, optimal planting time, maintaining optimum plant densities and rouging of infected plants (Makkouk et al. 2003).

7.3.4 Parasitic Weed Management

Several reports have been published in this aspect by many authors (Abang et al. 2007; Rubiales et al. 2006, 2018; Fernández-Aparicio et al. 2016). The parasitic weed species attacking faba bean are *O. crenata, O. foetida, O. minor* and *Phelipanche aegyptiaca.* Different approachesare used to manage parasitic weeds in faba bean. The first one is cultural practices that include preventing parasitic weed seeds movement from field to field through irrigation water, farm implements and animals and removal of parasitic plants to reduce the seed bank in the soil. Soil solarization is recommended in summer under clear polyethylene cover for 4–8 weeks to kill seeds of Orobanche (Sauerborn et al. 1989a). However, its practical application on large scale is limited and has not been practiced. No till farming practices (López-Bellido et al. 2009) and low rates of herbicide application such as glyphosate, imidazolinonesor sulfonylureas (Joel et al. 2007) have been recommended. One to two foliar applications of glyphosate in low rate is used by farmers at flowering stages in many countries (Sauerborn et al. 1989b). The integration of partially resistant cultivars, herbicide application and other cultural practices such as delay planting should be followed to manage these parasitic weeds.

7.3.5 Annual Weed Management

Weeds compete with crops for soil nutrients, moisture, space and light causing significant reduction in yield. Faba bean has better ability to compete weeds compared to chickpea and lentil because of its early vigor and larger canopy. Frenda et al. (2013) examined the critical period of weed control for 5% yield loss and found that it occurred later in faba bean than in chickpea (428 and 261 growing degree days after emergence, respectively).However, weeds should be controlled within a couple of weeks, after sowing to minimize yield losses. Chemicals have come to play a key role in weed management and without them it is unlikely that faba bean would have been able to reach their current production levels in extensive agriculture. Effective control can be achieved by pre-emergence herbicides as post emergence herbicides are available only for grassy weed species, not for broad leaves.

Weed control is now becoming more difficult due to widespread evolution of herbicide-resistant weed biotypes. Therefore, continuous spraying with the same group of herbicides for a long time should be avoided by adopting crop rotations using different herbicides. Faba bean is sensitive to phenoxy herbicides containing endogenous plant hormones, such as MCPA, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Chemical drift from such herbicidal sprays can travel long distances and need to be vigilant with neighbor's spraying. Integrated weed management involving crop rotations, rotation of herbicide groups, and combination of both chemical and nonchemical methods should be adopted to control weeds (GRDC 2017).

In Australia, extensive research is carried out to develop faba bean varieties with resistance to certain group of herbicides. As a result, recently a faba bean variety, PBA Bendoc, has been released with tolerance to imidazolinone (Group B) herbicide and several lines have been identified with tolerance to metribuzin (Group C). With the availability of such varieties, more options will be available for managing broad leaf weeds in faba bean. Similarly, ICARDA recently identified faba bean sources with tolerance to metribuzin and imazethapyr (Abou Khater et al. 2021). Once such varieties are available it will make easier to control incrop weeds in faba bean.

7.4 Genetic Resources of Resistance

The wild relative of faba bean is either extinct or yet to be discovered. Caracuta et al. (2016) have identified seeds of a potential ancestor of faba bean adjacent to Mount Carmel, Israel where the remains were C-dated to 14,000 years BP (before present). Moreover, Caracuta et al. (2015) have determined that faba bean was already domesticated about 10,200 years BP in the Lower Galilee, Israel. In any case, faba bean can be considered one of the earliest domesticated crops in light of numerous archeological findings in Eurasia and Africa dating back to the early Neolithic era (Duc et al. 2015).

Table 7.2 Gene bank with more than 500 faba bean accessions (Maalouf et al. 2013 modified)

Country/city	Organization	No. of accessions
Lebanon	ICARDA/Lebanon	9,938
Bulgaria	IIPGR/Sadovo	692
France	INRA/Dijon	1,900
Germany	Genebank IPK/Gatersleben	1,920
Italy	Genebank/Bari	1,876
The Netherlands	DLO/Wageningen	726
Poland	PBAI/Radzikow	856
	IOPG-PAS/Poznan	1,258
Portugal	INRB-IP/Oeiras	788
Russia	VIR/St Petersburg	1,881
Spain	CNR/Madrid	1,622
	IFAPA/Cordoba	1,091
China	CAAS/Beijing	5,200
Australia	DPI/Victoria	2,445
Morocco	INRA/Rabat	1,715
Ethiopia	PGRC/Addis Ababa	1,118
USA	USDA/Pullman	750

The global faba bean collection is above 38,000 accessions conserved in 37 Genebanks around the world (Table 7.2). The largest and most important one is held by ICARDA accounting nearly 10,000 accessions (ECPGR website consulted on February 2021). This global collection conserves materials from 71 countries with a high percentage of unique accessions.

Several sources for biotic stresses were identified from the global collection. The first and major sources for orobanche resistance were described by Nassib et al. (1982). The first identified sources of resistance to chocolate spot (ILB 438 and ILB 938) originated from Columbia, outside the center of origin (Hanounik 1982; Khalil et al. 1984; Robertson 1984). Additional accessions with resistance to chocolate spot were identified from Ecuador (Hanounik and Roberston 1988). Rhaïem et al. (2002) identified four landraces resistant to chocolate spot that originated from Tunisia. Sources of resistance to Ascochyta blight have been reported from numerous countries. The resistance was found in England by Bond and Pope (1980) and Lockwood et al. (1985) and in the Genebank collection at ICARDA (Hanounik and Roberston 1989; Rashid et al. 1991 and Bayaa et al. 2004) and by the national research partners (Kharrat et al. 2006; Ondrej and Hunady 2007). Between 2005 and 2012, more than 2,000 faba bean landraces and breeding lines were identified with resistance to Ascochyta blight, chocolate spot, and rust at ICARDA, of which more than 400 accessions were resistant to either Ascochyta blight or chocolate spot (Maalouf et al. 2016). ICARDA also evaluated more than 1,000 accessions over two decades for

resistance to faba bean necrotic yellow virus and identified 27 of them as resistant (Kumari et al. 2018). Australia has identified several sources of rust resistance from accessions originated in Europe, Ecuador and ICARDA sources and at least two single genes have been identified (Adhikari et al. 2016) and the quest for new genes continues. Research is ongoing to identify sources for resistance to gall diseases in faba bean.

7.5 Genetics and Breeding for Resistance

Breeding for biotic stress tolerance is regarded as the most cost efficient and environmentally friendly method. However, in contrast to other crops including legumes, limited research has been done on genetics and genomics of faba bean. One of the greatest obstacles in genomic research is its genome size which is over 13 Gb that is almost three times bigger than that of field pea and lentil genome and 16 times bigger than chickpea (Khazai et al. 2021) despite being a diploid species with 2n = 12 chromosomes. Its complex genome and lack of adequate funding discouraged researchers to work on genomics. However, map-based cloning and the development of high density genetic maps from various sources have enriched the understanding on molecular aspect of the faba bean genome (Webb et al. 2016; Carrillo-Perdomo et al. 2020).

7.5.1 Orobanche Resistance

There is no clear evidence for the existence of races of *O. crenata* (Radwan et al. 1988; Cubero et al. 1994). Joel (2000), however, described a virulent population of *O. crenata* that successfully infested previously resistant vetch plants in Israel. This race seems to have evolved from the existing population. Breeding for resistance against broomrape is difficult to achieve due to its complex nature and of low heritability. However, the first significant resistance was detected in the Egyptian line F402 derived from 3 year cycle of individual plant selection in an F_7 genotype from the cross Rebaya 40 × F216 (Nassib et al. 1982; Cubero et al. 1994). Efforts to breed faba bean resistant to *Orobanche* have resulted in the release of cultivars with useful levels of incomplete resistance with varying degree of tolerance (Khalil and Erskine 1999; Kharrat et al. 2010; Maalouf et al. 2011; Rubiales et al. 2018). The resulting resistance, which might be based on a combination of resistance mechanisms, is more likely to last longer lasting than resistance based on a single gene (Rubiales et al. 2006; Pérez-de-Luque et al. 2010; Rubiales and Fernadez 2012). ICARDA further developed *Orobanche* resistant elite lines with large seed types adapted to North African conditions in collaboration with National Agricultural Research System (NARS) partners which were released as Giza843, Misr3 and Hashbenge for Ethiopia (Maalouf et al. 2018)

7.5.2 Root Rot Complex

It is often difficult to screen against root diseases in the field conditions as there can be many types of pathogens in the soil. It is therefore important to first identify the major agents in each area and to screen for resistance against it. Different methods of inoculation can be applied, such as foliar spraying onto plants, injection of plants and soil inoculation (Infantino et al. 2006). Resistance to foot and root rot has been reported for the accessions Burshtyn 56 and German KK13 (Bond et al. 1994) but with no specification on the causal agent. Stem rot caused by *Sclerotinia trifoliorum* Eriks. is a serious problem in many important forage legumes including faba bean and the resistance against this disease has been identified as a single dominant gene (Lithourgidis et al. 2005).

7.5.3 Foliar Diseases

Development of resistant cultivars against foliar diseases in faba bean has been successful in Ascochyta and rust as major genes have been identified, but the process is slow for chocolate spot which is governed by polygenes and not a strong resistance has been detected in the germplasm.

The first effective Ascochyta blight (*Ascochyta fabae*), chocolate spot (*Botrytis fabae)* and rust (*Uromyces viciae-fabae)* resistance sources were identified at ICARDA faba bean breeding program in the 1980s (Hanounik and Robertson 1988, 1989). These lines were later utilized by various faba bean breeding programs in many countries to develop locally adapted disease resistant and high yielding varieties. As a result, superior varieties were released in Australia, Canada, China, Egypt, Ethiopia, and Spain (Sillero et al. 2010; Temesgen et al. 2015; Adhikari et al. 2016). New sources of resistance to major diseases were identified later at ICARDA (Maalouf et al. 2016, 2018) and are being distributed to other faba bean programs worldwide.

Based on limited pathogenic survey, nine pathotypes of *Uromyces viciae-fabae* were detectedin Australia (Ijaz et al. 2020) indicating a wide variation in the pathogen. One of those pathotypes (pathotype 63–63) was virulent on all tested genotypes causing concern to the breeders. However, there are several genotypes which have been identified as resistance to the prevalent pathotypes in New South Wales and they are being evaluated for the presence of new genes that might be effective against the pathotype 63–63. Most of the resistance reactions described so far in faba bean are incomplete requiring one or two fungicidal sprays (Adhikari et al. 2016; Sudheesh et al. 2019) while complete monogenic resistance exists in common bean or soybean making the marker assisted selection (MAS) efficient (Miklas et al. 2006; Garcia et al. 2008). Adhikari et al. (2016) found two single dominant genes for rust resistance which were independent to each other. Molecular markers for these independent genes have been developed which will facilitate pyramiding them into a single genotype (Ijaz 2018; Ijaz et al. 2021) Many varieties, such as Doza, PBA Warda, PBA

Nasma and PBA Nanu developed in the last decade in Australia are moderately resistance to rust (Adhikari et al. 2021).

Likewise, closely linked molecular markers to Ascochyta blight have been identified which have potential to be used in the breeding programs to enhance the resistance (Díaz-Ruiz et al. 2009; Kaur et al. 2014; Sudheesh et al. 2019). Several cultivars were released by NARS partners using ICARDA sources for chocolate spot resistance. The major faba bean varieties released in Ethiopia with partial resistance to chocolate spot are Moti, Gebelch, Obsie and Walki developed by crossing ICARDA lines ILB 4432, ILB 4726, ILB 4427 and ILB 4615 with Kuse-2–27-33, Tesfa, Obsie, CS20DK and Bulga 70 respectively. The variety 'Walki' was developed for waterlogged areas and is gaining popularity in the central highlands of Ethiopia, and more recently the varieties Gora (ILB2717-1 × R878-1) and Didea, which have chocolate spot resistance and large seeds, have been released in Ethiopia. Recent research has identified sources for resistance to new Gall disease in Ethiopia. Among 14 cultivars tested, 'Degaga' and 'Nc 58' were identified as moderately resistant to gall disease (Yitayih and Azmeraw 2017).

7.5.4 Breeding for Insect Resistance

Limited knowledge is available on resistance to insect pests and therefore emphasis should be placed on integrated pest management options as described by Redden et al. (2018). Stem borer weevil (*Lixus algirus* L.) is a widespread pest causing serious damage in faba bean in North Africa. Recently, new sources for resistance to this insect were identified and needs to be utilized to develop resistant cultivars (Aittaadaouit et al. 2018). *Heliothis* sp. is a major insect of faba bean in Australia and the host resistance is not yet known. The only control method available currently for *Heliothis* is insecticidal spray, but it is becoming difficult to control as the species has developed resistance to a wide range of insecticides.

7.6 Genetics and Genomic Research on Resistance

7.6.1 Genetic Linkage Maps

Khazaei et al. (2020) recently reviewed the list of genetic linkage maps developed in faba bean using different types of populations and molecular markers. In summary, genetic mapping studies were initiated in early 1990s using morphological markers, isozymes, seed protein genes, and random amplified polymorphic DNA (RAPD) markers; then later with the development of expressed sequence tags (ESTs), microsatellites or single sequence repeats (SSRs), EST-SSRs, and single nucleotide polymorphism (SNP) markers helped to enrich faba bean genetic studies

and breeding. The first DNA-based linkage map in faba bean was constructed with only 17 markers, of which 10 were restriction fragment length polymorphisms (RFLPs) (Van de Ven et al. 1991). The first set of SSR markers were developed by Požárková et al. (2002) and then mapped by Román et al. (2002, 2004). Some QTLs associated with rust, Ascochyta blight and orobanche were identified (Table 7.3) using these markers.

Table 7.3 Major markers identified for different disease resistance (extracted from Torres et al. 2010 and updated)

Loci/QTL	Chromosome	Mapping populations	Linked markers	References
Rust resistance				
Uvf1	Unknown	2N52 × Vf176 (F_2)	*OPI20*$_{900}$*/OPL18*$_{1032}$	Avila et al. (2003)
Uvf2	3	Doza#12,034 × Fiord (RILs)	*KASP_Vf_0703*	Ijaz (2018)
Uvf3	5	Ac1655 × Fiord (RILs)	*KASP_Ac* × *F165*	Ijaz (2018)
Broomrape resistance				
Oc1	1	Vf6 × Vf136 (F_2)	*OPJ13*$_{686}$*/OPAC02*$_{730}$	Román et al. (2002)
Oc2	6		*OPAC06*$_{342}$*/OPN07*$_{849}$	
Oc3	2		*OPW15*$_{533}$*/OPAA07*$_{807}$	
Oc2	6	Vf6 × Vf136 (RILs)	*OPAI13*$_{1018}$*/OPAC06*$_{396}$	Diaz et al. (2004, 2005)
Oc3	2		*OPM15*$_{794}$*/PisGEN 4_3_1*	
Oc4	1		*OPAB01*$_{438}$*/OPM18*$_{1192}$	
Oc5	1		*OPM18*$_{620}$*/OPA17*$_{524}$	
Ascochyta blight resistance				
Af1	3	Vf6 × Vf136 (F2)	*OPA11*$_{1045}$*/OPAB07*$_{1026}$	Román et al. (2003)
Af2	2		*OPE17*$_{1272}$*/OPJ18*$_{626}$	
Af3	3	29H × Vf136 (F2)	OPD16$_{1732}$/OPG04$_{1131}$	Avila et al. (2004)
Af4	Unknown		*OPJ18*$_{655}$*/OPG11*$_{1118}$	
Af1	3	Vf6 × Vf136 (RILs)	*OPAC06*$_{1023}$	
Af2	2		*OPAG05*$_{737}$*/Mer04*$_{790}$	Diaz et al. (2005)

7.6.2 Genomic Research

Genomic research in faba bean lags behind other grain legumes, such as chickpea and lentil. A composite gene-based map, anchored with orthologous markers mapped in *Medicago truncatula* Gaertn., was developed by Elwood et al. (2008). Kaur et al. (2014) reported the first exclusively SNP-based generic map of faba bean. Satovic et al. (2013) reported the first reference consensus genetic map, which covered 4,062 cM (centiMorgan) in six main linkage groups, corresponding to the six chromosomes of faba bean. An international effort resulted in the first consensus map for six mapping populations, based on SNP markers derived from *M. truncatula* (Webb et al. 2016). It contained 687 SNP markers on six linkage groups, each presumed to correspond to one of the faba bean chromosomes. Carrillo-Perdomo et al. (2020) recently reported the most saturated consensus genetic map to date: it was constructed using three mapping populations and encompassed 1,728 SNP markers distributed in six linkage groups. Solid proof of macro-synteny was also observed between this map and the most closely related legume species that have been sequenced.

Recently, a database of ESTs, EST-SSRs, mtSSRs (mitochondrial-simple sequence repeats), and microRNA-target markers in faba bean has been launched (Mokhtar et al. 2020). Now that most pulse genomes are available, it is important to implement comparative genomic approaches, which will ultimately assist in the identification of candidate genes, QTL mapping, and in assembly of the genome in faba bean. The first faba bean QTL mapping study was reported by Ramsay et al. (1995), who detected several loci for morphological and biochemical traits including vicine convicine. QTL mapping in faba bean for biotic stresses, such as resistance to pathogenic fungi and parasitic plants has been attempted. Regarding biotic stresses, two of the major constraints in the Mediterranean climates, namely Ascochyta blight and broomrape have been widely subjected to QTL studies using F_2 and recombinant inbred line (RIL) populations. The QTLs accounting for significant proportions of Ascochyta blight resistance have been validated in multi-environment trials (Atienza et al. 2016). Ascochyta blight has two pathotypes in Australia and the resistance to both types are available. All modern varieties have moderate level of resistance to the disease. Flanking SNP markers and QTLs have been identified recently to facilitate gene pyramiding and marker assisted selection (Kaur et al. 2014; Sudheesh et al. 2019). Significant progress has been made for rust resistance breeding in Australia and all modern varieties released in rust prone area have moderate level of resistance (Adhikari et al. 2016; Ijaz 2018). Recently, two mapping populations (Fiord × Doza#12,034 and Fiord × Ac1655) have been developed at the University of Sydney, in which Kompetitive Allele Specific PCR (markers (KASP) for rust resistance genes *Uvf-2* and *Uvf-3* have been identified (Ijaz 2018; Ijaz et al. 2021).

Beyene et al. (2016)reported high variability in chocolate spot resistance with additive gene effects and identified ILB-4726, ILB-938, BPL-710 and Gebelcho as resistant to the disease. However, until now, there has been no attempt to map QTLs or genes governing chocolate spot resistance, in spite of the importance and widespread nature of this disease globally. A few RIL populations suitable for chocolate spot

genetic studies have been developed using ILB 938, BPL710—both accessions with proven resistance to chocolate spot (reviewed by Khazaei et al. 2018). ICARDA has developed RILs populations BPL710 × ILB4357 and MAGIC population combining eight parents with sources for chocolate spot resistance that can be used in QTL mapping (Maalouf et al. 2019).

7.7 Genetic Engineering

7.7.1 Mutagenesis

The first mutagenesis efforts as a tool for breeding in faba bean was carried out by Sjödin (1971) who reviewed not only observations made in the extensive mutagenesis program run by Swedish breeders Svalov-Weibull in the "Primus" genetic background, but also a series of spontaneous mutants reported by a host of previous researchers. After 23 years, Duc (1995) reported isolation of five nodulation mutants by screening 20,000 M_2 population derived from ethyl methane sulfonate (EMS) mutagenized cv. "Ascot".

In the last decade, mutagenesis research has become very crucial to create variability for the most devastating parasitic weed, *Orobanche crenata*, referred to in an earlier section. This parasite could be effectively controlled with a range of other troublesome broad-leaved weeds, if faba bean varieties with target-site mutations can become insensitive to a target herbicide (Gressel 2009). As examples Ser653 and Ala205 mutations in the AcetoLactate Synthase target of imidazolinone and amidosulfuron families of herbicide, which do not occur in nature have been documented to occur at low frequency under strong selection pressure in the field such as selection for metribuzin and imazethapyr tolerance (Abou-Khater et al. 2021). A number of imazapyr resistance mutations have been identified by Mao et al. (2014). Mutation work for herbicide tolerance in South Australia through EMS saw the release of first mutant variety, PBA Bendoc, in 2018. This variety is tolerant to imidazolinone herbicide. Now there are several lines developed for tolerance to metribuzin and imidazolinone in Australia through conventional mutation program. This development will provide effective control measures for broad leaf weeds. Recently Adhikari et al. (2021) have reported a comprehensive section on faba bean mutation including herbicide resistance.

7.7.2 Genetic Transformation

Genetic modification represents research tools permitting testing of hypotheses on gene function by overexpression, misexpression or knockdown/knockout studies

and an outlet for genetic research in generation of targeted phenotypic modifications based on knowledge of gene function. Stable germline transformation of faba bean using in vitro regeneration of *Agrobacterium*-infiltrated (nonmeristematic) internode stem segments was first reported by Böttinger et al. (2001). Adopting a somewhat different strategy, Hanafy et al. (2005) infiltrated excised (meristematic) embryo axes with *Agrobacterium* and successfully recovered stable transgenic lines. Both methods, however, reported low primary transformation efficiencies and relied on micro-grafting of putative transgenic shoot material onto nontransgenic roots, a slow and highly time-consumingprocess. Hanafy et al. (2013) later reported salt and drought tolerant fertile transgenic faba bean plants from *Agrobacterium tumefaciens*-mediated transgenic lines over expressing potato PR10a gene using their previous methods. This remains to our knowledge the sole successful demonstration to date the feasibility of a biotechnological approach on transformation. These findings were confirmed recently by the same group of authors (Desouky et al. 2021).

The prospects afforded by new insights into the phenotypic effects of allelic variation and the more refined biotechnological possibilities afforded by rapidly maturing genome editing technologies (Gaj et al. 2013) could potentially stimulate renewed interest in genetic transformation. An example of a game changing product which could readily be generated using even a medium efficiency transformation system would be herbicide resistance obtained by directed mutagenesis of endogenous herbicide target genes e.g., introduction of heterologous glyphosate resistance of bacterial origin.

In the absence of a robust and efficient transformation method, some attention has been devoted to the task of decreasing generation time using tissue-culture based embryo rescue (Mobini et al. 2015). Doubled haploidy technique has become highly effective tool in reaching homozygosity in crop breeding. However, grain legumes including faba bean seem to be recalcitrant to this technique. Nevertheless, Croser et al. (2018) has developed single seed descent method for achieving the same outcome through tissue culture techniques completing 7–8 generations in a year. Similarly, (Mobini et al. 2020) have developed method for shortening the generation time by 22 days in each cycle with the application of cytokinin and cold treatment.

7.8 Conclusions

Faba bean is relatively a minor crop and limited funding and resources are available compared to other legumes. However, significant improvements have been made to the crop as new knowledge and understanding on genetics, molecular biology and agronomy are available. Its large genome ~13 GB, the largest among diploid crops, was the most deterrent factors for researchers in the beginning, but this has been slowly defeated with the availability of next-generation sequencing and high-throughput genotyping methods. This has contributed to development of high-density genetic maps which will lead to gene based molecular markers for targeting marker

assisted selection. This will enhance the breeding for biotic stress tolerance. Resistance to major diseases, such as Ascochyta blight and rust are available and successfully integrated into the breeding programs. Resistance breeding for chocolate spot, Cercospora, gall disease and root diseases are not progressing due to poor understanding of the diseases and lack of strong resistance in the germplasm. Several improved genotypes tolerant to parasitic weeds and availability of control measures such as low rate of herbicide application is encouraging faba bean growers in the affected areas. Availability of herbicide tolerant genotypes particularly to imidazolinone and metribuzin will provide effective measures of in-crop broad leaf weed control.

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Chapter 8
Genomic Designing Towards Biotic Stress Resistance in Mungbean and Urdbean

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Abstract Mungbean (*Vigna radiata*) and urdbean (*V. mungo*) are considered two important crops amongst the Asiatic *Vigna* species, which are primarily farmed for food, fodder and manure, and have emerged as a suitable alternative to other grain legumes. Despite their agronomic relevance, these two species have a limited crop production, yielding just one-third to one-fourth of their full potential. The key factor behind low yield can be attributed to various biotic stresses (pathogenic infection and insect infestation) that occur at all phases of plant growth as well as post-harvest period. The major contributors in this regard are the Yellow mosaic virus, Macrophomina blight, powdery mildew, anthracnose, Cercospora leaf spot, bacterial leaf spot, root-knot nematodes, and post-harvest pests, such as bruchids. Development of new cultivars through conventional breeding could be an alternative, but the narrow genetic base of these two crops has hindered the progress. Additionally the time-consuming and labor-intensive nature of the traditional breeding techniques has aggravated the problem further. To accelerate the breeding process scientists have turned towards genomic tools, particularly QTL mapping and genomics-assisted breeding which provide potential ways for development of elite cultivar for biotic stress resistance. Despite sincere efforts, both mungbean and urdbean are slow runner in genomics research, although mungbean was one of the pioneer legumes targeted for genome analysis at the dawn of the plant genomics era. Completion of the mungbean genome sequence in 2014 and the recent de novo sequencing of the urdbean genome in 2020 has empowered the researchers to develop genomic resources and identification and mapping of potential gene(s) associated with biotic stress resistance. The present chapter therefore covers the various biotic constrains in the production of mungbean and urdbean and cumulates the previous, current and future endeavors on

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molecular and genetic improvements aiming at biotic stress tolerance in these two crops.

Keywords Biotic stress · Molecular mapping · Genomics · Resistance · QTL

8.1 Introduction

Grain legumes belonging to the family Fabaceae is considered as the second most nutritionally and economically important crops worldwide. It has been predicted that consumption of legumes commenced some 10,000 years back and since then they are playing a fundamental role in contributing global food and nutritional security. Apart from these, legumes play additional roles in restoring soil nutrients through biological nitrogen fixation.

Over 100 species of *Vigna* have been identified from the warm, temperate and tropical regions all around the world (Schrire 2005). Nine crop plants are part of the Asiatic *Vigna* genus: cowpea [*V. unguiculata* (L.) Walp.], mungbean[*V. radiata* (L.) R. Wilczek], bambara groundnut [*V. subterranea* (L.) Verdcourt], azuki bean [*V. angularis* (Willd.) Ohwi & Ohashi], urdbean [*V. mungo* (L.) Hepper], rice bean [*V. umbellata* (Thunb.) Ohwi & Ohashi], tuber cowpea [*V. vexillata* (L.) A. Rich], creole bean (*V. reflexo-pilosa* Hayata) and moth bean [*V. aconitifolia* (Jacq.) Maréchal].

Amongst the Asiatic *Vigna* species, mungbean is the most important grain legume both in terms of distribution and cultivation (Kim et al. 2015). There are reports of early trades from India to other countries of the Middle East, Latin America, East Africa, Australia and South America resulting in spreading of mungbean germplasm since ancient times (Poehlman 1991). Presently, the crop is under widespread cultivation throughout the South and South-East Asian countries, such as India, Bangladesh, Pakistan, Myanmar, Malaysia, Indonesia, Vietnam, Sri Lanka, Thailand, Philippines, Cambodia and Taiwan. Cultivation of urdbean or blackgram is mostly confined to South, East and Southeast Asia particularly in the Indian subcontinent. Outside Asia urdbean is cultivated in African countries like Kenya, Uganda, Gabon, Congo, South Africa, Malawi, Tanzania, Mauritius and Madagascar, while in Australia and the United States they are mostly cultivated as a fodder crop. Both the species is assumed to have originated in the Indian subcontinent and was probably domesticated in the Indo-Burma region (Chandel et al. 1984) and are cultivated as a short duration (90–120 days) rainfed crop (Purseglove 1974). Discovery of urdbean seeds from archaeological sites of Uttar Pradesh, India and reports in Kautilya's Arthashastra and several Vedic and post Vedic texts substantiate their Indian origin.

Taking advantage of its rapid growth and early maturity, these crops can be grown under various rice based cropping systems and agro-ecological practices both in the Rabi and Kharif season (Singh 1997; Pratap et al. 2013). India is by far the largest producer and consumer of both mungbean and urdbean which is grown over 4.50 million ha and with a gross productivity of 0.5–1.5 t/ha and 2.93 t/ha respectively, thus contributing to World's 70% production. What attracts geneticists and plant breeders

towards *Vigna* is its vast genetic diversity available across the wild species. This diversity results in agronomically favored genotypes which are naturally occurring such as tolerance to high-salinity, acid or alkaline soil; drought; flooding; and pests and diseases (Chankaew et al. 2014; Tomooka et al. 2014; Yoshida et al. 2016). What makes it really interesting is the fact that suitable susceptible species are also found in the wild, some of these are cross-compatible. As a result, conventional forward genetic studies have been performed to identify causative genes (Chankaew et al. 2014; Tomooka et al. 2014). Thus, *Vigna* offers a suitable model system for exploring abiotic and biotic stressors.

8.1.1 Economic Importance

Both these legume species are important members in the world agricultural scenario and impart sizeable economic impact in many developing countries. The dry grains of both mungbean and urdbean are key dietary staples for millions of people in the South, East and Southeast Asian countries. It is mainly consumed in the form of stew made from dried, dehusked or decorticated, split or entire seeds and popularly known as 'dal' or pulse. Additionally the dried seeds may also be cooked with rice or eaten as a snack by roasting and grilling. The whole grains, specially which have glossy, lustrous seeds are more preferred in the Asiatic countries. In West Bengal and Bangladesh, consumers prefer a particular mungbean variety having small, yellow grains with a pleasant aroma popularly known as 'sona mung'.

The sprouted seeds are commonly used in continental and oriental dishes. They are eaten with or without salt, fried, in salads, as a component in soups, for garnishing noodles, and sometimes stir-fried with vegetables and eggs. During the sprouting process vitamin, mineral and protein content increase substantially with corresponding decrease in carbohydrate and calories. The green pods and immature green seeds are also eaten as vegetables.

In Chinese cuisine powdered seeds are used to produce a transparent noodle (popularly known as glass noodle). Mungbean flour, also known as 'besan' is sometimes mixed with rice or wheat flour and is used as a food in some countries. Mungbean flour is also an essential component in South Indian delicacies like dosa, idli, vada etc. The paste obtained from the seeds is often mixed with butter and sugar and used as an ingredient for preparation of desserts such as sweets and pan cakes.

Both mungbean and urdbean are used in Chinese and Indian system of medicine for their use in the treatment of liver and stomach disorders, fever and general weakness. Mungbean seeds contain high level of phenolic and flavonoid compounds that substantiate its use as a therapeutic agent for human health (Kim et al. 2012). Several workers have reported the medicinal use of the *Vigna* genus towards the treatment of asthma, abscesses, hepatitis, cholera, neuritis and other neurological disorders (Pandey 2019). In China, health drinks are prepared from mungbean seed coats containing flavonoids, vitexin, and isovitexin to reduce body heat. Use of urdbean is also recommended for the treatment of sexual dysfunction, nervous, hepatic and

stomach disorders and is also a blood purifier. Both the crops are also used as livestock feed. Pods and foliage are used to supplement cattle feed or used as forage. In some countries, plant residues are used as green manure and cover crop (Purseglove 1974).

8.1.2 Constrains in Productivity

Even with the best efforts in the field, production of mungbean and urdbean has not improved significantly over the past few decades. Primary reason behind this is the low productivity of the cultivated varieties. They are mostly grown in rotation with high input crops like paddy and wheat with little or no modern yield enhancing inputs. Cultivation of these legumes on marginal lands further aggravates the problem. Concomitant with this is the threat of various biotic and abiotic stresses resulting in yield destabilization.

Among the various biotic concerns, bacterial, fungal and viral diseases pose significant threat to productivity in the South and South East Asian countries. Mungbean yellow mosaic disease caused by the Mungbean Yellow Mosaic India Virus is the most destructive viral disease causing severe economic impact. The major fungal diseases of mungbean and urdbean include Cercospora leaf spot (caused by *Cercospora canescens*), powdery mildew (caused by*Erysiphe polygoni*), anthracnose (caused by *Colletotrichum truncatum*), Macrophomina blight (caused by *Macrophomina phaseolina*) and causes substantial yield penalty. Amongst the bacterial diseases, bacterial leaf spot (caused by *Xanthomonas campestris* pv. *phaseoli*) is the most destructive one, followed by halo blight (caused by *Pseudomonas syringae* pv. *phaseolicola*) and tan spot (caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*).

Abiotic stresses affect plant growth and crop yield both in mungbean and urdbean and causes extensive losses in crop yield. Various types of abiotic stresses contribute to the process including extreme environmental perturbations such as heat, cold, or frost; drought and flood; UV radiation; salinity, nutrient deficiency and heavy metal pollution. Salinity has been the key problem in growth and productivity of mungbean and urdbean especially under irrigated conditions in arid and semi-arid environments. It mostly affect the overall crop morphology and reduces germination of seeds, affecting root and shoot architecture, reduced nodule formation and other yield attributes (Ahmed 2009). Since both the cropsare partially tolerant to drought and grown on marginal lands, but still responds to low soil moisture by reducing its growth and thereby productivity. Extreme dry conditions may result in decrease in seed yield and weight, reduction in flowering, number of pods per plant, and overall loss in biomass per plant. Temperature stresses such as high (heat stress) and low (cold stress) are damaging at all stages of growth and development, resulting in loss in crop yield. Both mungbean and urdbean are sensitive to heat and cold stress, particularly during the seedling germination, vegetative, flowering, and pod filling phase (Sharma et al. 2016). Another important abiotic constraint is waterlogging which negatively affects seed germination and plant growth. Heavy rains particularly

during the reproductive phase and pod ripening may result in yield reduction and formation of low quality seeds. Since both the crops are cultivated mainly in low land rice-fallow system, flooding remains a perpetuating cultivation problem.

8.1.3 Limitations of Traditional Breeding and Rationale of Adopting Functional Genomic Approach

Traditional breeding techniques to develop resistance against biotic and abiotic stresses necessitate following features to be taken under consideration: genetic diversity of the cultivated species, genetic distance between the source and cultivars, screening methodology and inheritance pattern of the resistance traits. Both in mungbean and urdbean the genetic base is narrow and hence the breeding efforts remain restricted only to a few cultivated lines. This indicates the need for broadening the base of genetic diversity of these crops urgently employing techniques, such as, gene pyramiding, choosing resistance source of either parent, genetic distances between breeding partners should also be taken into consideration. However, the greater the genetic distances, more chances of segregation of beneficial traits are expected; which can be reversed through repeated backcrossing with the parental lines. Although this is a time consuming and labor intensive process. Occasionally such resistance to these biotic or abiotic stresses are governed by multigenic factors which are more difficult to handle compared to resistance governed by a single gene. Additionally co-inheritance of undesirable trait/s due to genetic linkage may result in the inferior seed quality, poor seed germination and affects other yield related attributes. Another vital aspect hampering classical breeding is the generation of biotypic variations of the concerned pathogen and pests. Cultivated lines that are resistant to one pathogenic strain may remain susceptible to the other race or isolate of the same pathogen. This becomes apparent while analyzing the MYMV-*Vigna* interaction. It was observed that two separate strains of the same virus exist which are location specific and infect both mungbean and urdbean causing the yellow mosaic disease. Urdbean lines that are resistant to one pathotype, viz, MYMV showed susceptibility to the other pathotype, MYMIV; which is more prevalent at a different location. All these factors impedes the efficacy of traditional breeding approaches and necessitates the adoption of molecular techniques, which are now becoming more and more convenient in figuring out the resistance or tolerance mechanisms, and will assist in altering the genomes of mungbean and urdbean to incorporate stress tolerance.

8.2 Major Biotic Stresses of Mungbean and Urdbean

In nature, crop plants encounter various biotic agents, including bacteria, viruses, fungi, insects and nematodes. While some of these agents exist symbiotically or

synergistically, others act as pathogens or pests that impose a serious threat to its productivity. Besides quantitative reduction in yield, these biotic agents also affect the physical quality of seeds, making them unusable, unfit for human consumption. In the farmers' field, mungbean and urdbeanare susceptible to about 26 pests and pathogens and some of the major biotic stress have been listed below.

8.2.1 Yellow Mosaic Disease

Amongst the various viral pathogens, the Yellow mosaic disease (YMD) caused by the Mungbean yellow mosaic virus (MYMV; Nariani 1960) and the Mungbean yellow mosaic India virus (MYMIV; Mayo 2005) are the most destructive pathogens of mungbean and urdbean (Singh 1981). YMD is a serious threat to *Vigna* production owing to its widespread distribution throughout the South and South East Asian countries and incurs significant yield loss. The pathogen, however, is not seed, sap, soil or mechanically transmitted but spreads through its vector *Bemisia tabaci* (whitefly) in a circulative, non-propagative manner (Ahmad and Harwood 1973). The virus is a member of the family Geminiviridae, comprising twinned geminate particles, encapsidating two, circular, single stranded DNA genomes (Hull 2004). It not only infects mungbean and urdbean but also other members of the legume family such as soybean, pigeon pea, etc. About 75 weed species have been identified as collateral hosts that harbor the virus and acts as a source of inoculation. In India, YMD, is a serious hazard as it causes significant reduction in yield under conducive environments (26–32 °C, 60–99% humidity) that may reach up to 100%depending on severity of infection, specifically if the infection takes place at the juvenile stage (Basak et al. 2005).

8.2.1.1 Symptoms

The most prominent symptom of YMD appears on the leaves and sometimes on pods in the form of scattered yellow chloretic spots. These spots expand, coalesce, producing conspicuous bright yellow patches and eventually the entire foliage turns yellow. Sometimes a necrotic mottle appears in infected leaves as a result of the host's hypersensitive response against the virus. An early infection results either in seedling mortality or stunting of growth (Basak et al. 2005). Maturity is delayed in infected plants, bearing reduced number of flowers and pods. Both qualitative and quantitative deterioration of pods and seeds (deformation of pods with shriveled and undersized seeds) have been reported in case of severe infection. However, severity of disease symptoms and yield penalty depends upon the viral load, cultivar, growth stage and environmental conditions (Kundu and Pal 2012).

8.2.1.2 Biocontrol and Disease Management

It is practically impossible to reduce YMD incidence adopting a single control measure but can be efficiently managed by integrating different approaches such as avoiding sources of infection, cultivation of resistant varieties, vector control and modification of cultural practices (Hull 2004). Currently the most widely adopted strategy is the cultivation of resistant varieties as is considered as the cheapest and the most effective one. Mungbean varieties such as IW 3390, EC 398,897, TM-11-07, TM-11-34, PDM-139, IPM-02-03, IPM-02-14, ML 267, ML 337, Pusa-0672, Pusa-0871, CO-7 and MH-521 showed promising resistance against the virus (Reddy and Singh 1995; Mohan et al. 2014). Whereas urdbean lines such as PU 07-7, PU 08-1, PU 08-45, PU 06-16, Pant U31, Pant U40 showed resistance against the YMD pathogen (Azeem et al. 2019).

Intercropping with non-host crops such as rice, maize, sorghum, may reduce YMD incidence. Additionally, manipulation in the sowing date also minimizes vector populations. Secondary infection can be effectively reduced by the removal of infected plants from the field. Foliar application of systemic insecticides can also reduce vector population such as aldicarb and disyston (1 kg/ha) phorate, ekatox (0.02%), disulphoton granules (2 kg/ha), metasystox, malathion, ambithion (0.1%) is effective against whitefly populations (Sastry and Singh 1973).

Plant extracts that have been explored for their effectiveness against YMD include 4% leaf extracts of *Clerodendrumfragrans* and root extracts of *Boerhaavia diffusa.* Disease incidence was considerably reduced by the application of leaf extracts of *C. fragrans* while the later delayed the appearance of symptoms (Verma et al. 1985).

8.2.2 Macrophomina Blight

Macrophomina phaseolina (Tassi) Goid. is a devastating fungal pathogen inciting disease in broad range of cultivated crops including legumes (Srivastava et al. 2001). The pathogen infects almost all plant parts such as roots, stem, branches, leaves, pods and seeds and can cause an array of symptoms including seedling blight, collar rot, leaf blight, stem and root rot (commonly known as charcoal rot), dry root rot, tuber decay, dry rot, pod rot and pre and post emergence damping off (Kumari et al. 2012). The pathogen is a necrotroph and is prevalent in regions particularly with warm (25–40 °C), dry weather (15–20% moisture) under water stressed conditions during the growing season. The pathogen mainly propagates through black colored sclerotia and pycnidia (Wheeler 1975). The temperature resistant sclerotia can survive in the soil as long as 2–15 years or in plant debris and is the key source of primary infection. While the pycnidiospores are produced on the infected aerial plant parts (stem and leaf) to facilitate secondary infection. Since the pathogen produces scattered charcoal like symptoms in the infected tissues hence the disease is commonly known as charcoal rot disease and the fungus as charcoal rot fungus (Dhingra and Sinclair 1978). Yield loss upto 60% have been reported both in case of mungbean and urdbean.

8.2.2.1 Symptoms

M. phaseolina attacks plants on almost all growth stages and may cause death of young seedlings. The pathogen initially attacks the stem at the base (or ground level) and form dark irregular lesions. The affected part weakens and gets easily shredded (Abawi et al. 1990). In adult plants, pathogen invasion blocks xylem and causes host death due to seedling blight; stem and pod rot (Beas-Fernández et al. 2006). Sclerotia are produced on the surface of stem and roots and also below the epidermis. During pathogenesis, the fungus produces several cell wall-degrading enzymes (Javaid and Saddique 2011), hydrolytic enzymes (Kaur et al. 2012), and phytotoxins such as phaseolinone and botryodiplodin (Bressano et al. 2010).

8.2.2.2 Biocontrol and Disease Management

Since the disease is soil and seed-borne and hence management is difficult and is not suitable for small holding farmers. Seed treatment using fungicides vitavax, benlate (0.25%) and ceresan (0.1%) has been found effective against MB. Quintozene, Thiram and Captan also manage the disease avoiding seed borne infection. Application of systemic fungicides such as carbendazim and benomyl as foliar sprays at an interval of 15 days is effective during the leaf blight stage. Soil treatment can be done using zinc sulfate @ 25 kg/ha and neem cake @ 150 kg/ha. Biological control of soil and seeds using *Trichoderma viridae*, *Pseudomonas fluorescens* may help in prevention to some extent. Mungbean varieties such as ML 4, LM 162, LM 220 and urdbean lines BR 68, T 29 showed varietal resistance to the pathogen.

8.2.3 Powdery Mildew

Among the various fungal diseases encountered, powdery mildew, caused by *Erysiphe polygoni* DC. is the most destructive one that mostly affects the crop at the later stages of the life cycle (Kasettranan et al. 2010; Anjum et al. 2010). The pathogenic fungus is an obligate pathogen belonging to the family Erysiphaceae of the class Ascomycetes and infects almost all the crop legumes. The infected parts show a powdery appearance as if it has been dusted with flour and hence the name. Powdery mildew is well prevalent in India and other South East Asian countries and imposes a serious threat to the rice-based cropping system (Abbaiah 1993). The pathogen is an obligate ecto-parasite that ramifies on the host surface and thereby causes serious yield loss. Powdery mildew mostly interferes with the host physiological activities and imposes heavy damage if the disease occurs before the onset of reproductive stage. It may inflict a 20–40% reduction in the yield (Reddy et al. 1994) affecting traits like total biomass, pods per plant, number of seeds per pod and also the quality of seeds.

8.2.3.1 Symptoms

The disease is characterized by superficial, white, floury, dense mycelia and conidia that develops mainly over the stems, leaves and also on all other aerial parts of the plant. Symptoms generally initiates on the lower leaves that rapidly spreads to the upper ones under favorable environments of cool, dry season (Jayasekhar and Ebenezar 2016). While growing superficially, it sends penetrating haustoria within the epidermal cells for absorption of nutrients. Conidiophores bearing conidia are borne on the leaf in short chains. Conidia are single celled, white; thin walled; more or less elliptical in shape. Cleistothecium are minute, globose structures, brownish to black in colour, with profuse appendages. Each cleistothecium produces four to eight asci, containing four to eight elliptical, hyaline, single celled ascospores. The white powdery patches gradually become circular, increase in size, and ultimately covers the entire leaf surface. In severe infections, the foliage may shrivel, becomes distorted and the entire leaf turns yellow, resulting in premature defoliation. Powdery mildew also prompts maturity of infected plants, thereby resulting in heavy crop losses (Chaitieng et al. 2002).

8.2.3.2 Biocontrol and Disease Management

The disease incidence may be reduced by collection and destruction of the infected plants and debris. Additionally, a delayed sowing and planting them with wider spacings have been effective in minimizing disease severity (Sivaprakasam 1981). Application of chemical fungicides like thiovit and bavistin has been found most effective in treatment of powdery mildew when sprayed (0.05%) at 15 days interval. Additionally, elosal, calixin has also been found effective. Hexaconazole, benlate and copper oxychloride are some other fungicides used in the treatment of powdery mildew. Plant extract such as neem oil 20 ml/L and Eucalyptus leaf extract (10%) considerably reduced spread of infection. Cultivation of resistant urdbean lines such as Pant U 19, AKU 15, UG 301, LBG 17, PLU 63 showed resistance to *E. polygoni*. In mungbean, LM 223, LM 294, ML 131, ML 322, ML 337, TARM 1, LGG-460, Vaibhav, BPMR-145, TARM-18, Phule M-2003-3, Phule M-2002-13, Phule M-2002-17, Phule M-2001-3 and Phule M-2001-5 showed promising resistance against the fungus (Mandhare and Suryawanshi 2008).

8.2.4 Cercospora Leaf Spot

Cercospora leaf spot (CLS) is a foliar disease caused by the biotrophic fungus *Cercospora canescens* Ellis and Martin, and *Pseudocercospora cruenta* (Sacc.) Deighton (formerly *Cercospora cruenta)* (Allen and Lenne 1998), which is a potentially devastating disease, causing considerable yield losses in the genus *Vigna*. The disease is widespread throughout the *Vigna* producing areas and infects almost all the

species, in particular mungbean, cowpea, and urdbean. Both the pathogens persist in the unfavorable season on infected plant debris and seeds that spreads rapidly in the warm-humid climate particularly in the growing season. In susceptible mungbean plants, infection may cause reduction in yield of up to 50% while in susceptible cowpea loss varies between 36–42% (Schneider et al. 1976; Fery et al. 1977).

8.2.4.1 Symptoms

CLS symptoms are not visible until the crop reaches the flowering stage but the destruction is utmost at the pod-filling stage. Initially symptoms on the infected plants appear as water-soaked spots on the leaves. The spots increase in number, gradually enlarge in size, may coalesce together, producing enlarged necrotic areas on the infected leaves. Severe infections in the susceptible host may lead to malformation or wrinkling of leaves resulting in premature defoliation. Maturity is delayed in the infected plants resulting in reduced pod formation with less number and deformed seeds (Grewal et al. 1980).

8.2.4.2 Biocontrol and Disease Management

Since the pathogen perpetuates in the seed, procurement of seeds from disease free plants helps to minimize disease incidence. Destruction of infected crop debris, crop rotation with non-legume crops, elimination of collateral hosts and proper field sanitization have profound effect in reducing disease inoculum. Additionally, delayed sowing of both mungbean and urdbean has been effective against the disease. Seed treatment with bavistin (1 g/kg seed), thiram (2.5 g/kg seed) has also been found effective against the disease. Other fungicides such as benzimidazoles, dithiocarbamates and copper fungicides also showed good results. Leaf extracts of *Amaranthus spinosus*, *Leucaena leucocephala* successfully reduced disease development. Mungbean varieties such as LGG-460, GM-02-08, GM-02-13, GM-03-03, NM-98, NM-1, NM-2, BRM-188, CO-3, Basanti, PDM-11, BARI Mung-2 and VC3960-88 (Haque et al. 1997) and urdbean varieties such as Naveen, Jawahar, Barkha etc. showed resistance against the fungus (Sharma et al. 2011).

8.2.5 Root-Knot Nematodes

In addition to the above-mentioned pathogens, the root-knot nematodes belonging to the genus *Meloidogyne* is another damaging pathogen that causes considerable yield losses (Trudgill and Blok 2001). The commonest of them infecting mungbean and urdbeanare *Meloidogyne incognita* and *M. javanica.* The pathogen is an obligate, polyphagous, sedentary root endoparasites, primarily causing knots and galls on the roots of infected plants. During parasitism, eggs of the nematodes hatch out, and

the juveniles invade the roots. They move inter-cellularly thereby establishing their feeding sites and develop into adults. Concurrent enlargement of the root cells leads to development of galls that damage the vascular system of the roots. This alters the host physiology hampering the normal uptake of water, minerals and upward translocation through the roots resulting in weak and low-yielding plants (Chakrabarti et al. 2001). In Indian scenario, the root knot nematodes incur around about 18 -65% loss in crop yield in mungbean.

8.2.5.1 Symptoms

The above ground symptom includes severe nutrient deficiency, characterized by stunted growth, yellowing of the foliage and subsequent wilting that may lead to death of the plant. Root knot nematode infection unfavorably affects nodulation process in legumes thereby affecting symbiotic nitrogen fixation and yield. In association, the impaired root vascular system may also enhance the susceptibility of the host to various pathogenic bacteria and fungi (Abad et al. 2003). There are reports that disease complex involving nematodes and fungal, bacterial, mycoplasma and viral pathogens can lead to significant more crop losses than it does independently (Sikora et al. 2005).

8.2.5.2 Biocontrol and Disease Management

Seed treatment with carbosulfan (3% w/w) followed by spraying with triazophos (0.1%) was found to be effective against *Meloidogyne*. Additionally treatment with Carbofuran belonging to the carbamate group was also found to be effective. Mungbean lines PM-10-12, AKM-8802, NVL-641, IGKM-05-26-3, IPM-410-3 and AKM-4 were found resistant against the nematode (Singh et al. 2020). Additionally, the efficacy of various bio-agents (*Trichoderma* spp., *Pseudomonas fluorescens, Bacillus megaterium, Pochonia chlamydosporia* and *Purpureocillium lilacinum*)and biofertilizers (*Azotobacter* sp. *Rhizobium* sp.) were tested effective against RKN infection (Bharali et al. 2019). Leaf extracts of *Ageratum conyzoides* against RKN infection at various concentrations (2–10 ppm) was also found to be effective after 40 days treatment (Pavaraj et al. 2012). Seed extracts of *Nerium indicum* also alleviated the harmful effects of RKN infection in urdbean (Vijay et al. 2009).

8.2.6 Bacterial Leaf Spot

Bacterial leaf spot (BLS) is incited by the gram negative bacterium *Xanthomonas phaseoli* (Smith) Dowson. The rod shaped bacterium propagates within the seeds and in the infected plant debris during the unfavorable season that becomes virulent in the warm and humid climate with an optimum temperature of 30–33 °C. The

pathogen not only infects the *Vignas* but also other crop legumes such as *Phaseolas*, *Lens*, *Dolichos lablab* etc. (Borah et al. 2000).

8.2.6.1 Symptoms

The BLS pathogen causes disease symptoms on all aerial plant parts including leaves, stems, pods and seeds. Symptom appears as circular or irregular, small water soaked spots on the leaves within 4–10 days post infection. The spots gradually enlarge and infected areas appear and become raised. The center of the lesion becomes necrotic and brown and gets surrounded by a narrow strip of translucent yellow tissue. Symptoms in the stem also initiates with water-soaked areas that becomes brownish with time (Thind 2012). The diseased plants were not killed immediately, but a severe infection causes necrosis reducing the photosynthetic ability thereby weakening the plants affecting crop production.

8.2.6.2 Biocontrol and Disease Management

Disease can be controlled by using pathogen free, healthy seeds. Seed treatment using hot water was proven effective in controlling BLS. Since BLS pathogen is mainly seed borne, treatment of seeds using Streptomycin sulphate (500 ppm) or bleaching powder (0.025%) has been proven effective against the bacteria (Sahni et al. 2016). Additionally Vitavax (0.2%), Captan (0.3%) or three protective sprays of Streptocycline (100 ppm), Zineb (0.3%), Blitox-50 (0.25%), Agrimycin-100 (250 ppm) reduced disease incidence considerably. Mungbean lines such as Jalgaon 781, ML 8, ML 10 showed high degree of resistance against bacterial leaf spot (Yadav et al. 1981).

8.2.7 Anthracnose

Anthracnose is caused by the fungus *Colletotricum truncatum* (Schw.) Andrus and Moore (or *C. lindemuthianum* or *C. gloeosporioides*) is one of the most devastating fungal diseases of mungbean and urdbean. The fungus is seed-borne and is the major source of primary infection but the pathogen also perennates in the infected plant debris. Primary infection produces conidia on the infected plant that causes the secondary infection (Sharma et al. 1971). Disease development is more severe in cool (17–24 °C) and humid (100%) weather conditions with intermittent rains. In mungbean, anthracnose affects both quality and quantity of seeds in varying intensity depending on the disease severity and incurs a yield reduction of about 24–67% (Deeksha and Tripathi 2002).

8.2.7.1 Symptoms

Anthracnose is basically a foliar diseases but infected plant shows disease symptoms on all above ground parts. The disease is characterized by circular black spots with bright red–orange margins on leaves that ultimately withers off resulting in the 'shot hole' symptoms. In severe infections, the whole seedling may get blighted. Anthracnose directly affects the pods and damages the quality and quantity of the seeds.

8.2.7.2 Disease Management

Seed treatment using hot water at 58 °C for 15 min has been effective in controlling seed borne infection. Chemical treatment of seeds using fungicides like Carbendazim (0.10%), Thiram (2 g/L), Benomyl (50%), Captan (2.5 g/L) has also been proven effective. Foliar spray of Zineb (0.2%), or Ziram (2 g/L) at 15 days interval has successfully reduced the spread of symptoms in the field (Rathaiah and Sharma 2004).

8.2.8 Web Blight

The Web blight (WB) disease, caused by *Rhizoctonia solani* Kuhn [Teleomorph: *Thanatephorous cucumeris* (Frank) Donk] is one of the major fungal constraints in the production of mungbean and urdbean. *R. solani* is also known to infect other legumes such as pigeonpea, cowpea, soybean, groundnut and ricebean. It is a soil-borne pathogen that primarily survives as sclerotia or thick walled hyphae on infected plant tissues and is the major source of primary infection. The secondary infection is caused by dissemination of the pathogen by irrigated or rain water, infected or contaminated seeds or by mechanical transmission. An optimum temperature of 26–28 °C and a high relative humidity (90–100%) favours development of the disease chronically. The disease is mainly prevalent in the warm, humid tropical regions of the world including India, Pakistan, Myanmar, Sri Lanka, Philippines, West Indies, Argentina, Brazil, North and South America and Mexico. In mungbean and urdbean, it is responsible for crop losses of up to 40% depending upon the prevailing conditions (Dubey and Patel 2001; Shailbala and Tripathi 2007).

8.2.8.1 Symptoms

The first symptom appears on the leaves as small, irregular, water soaked spots. The spots enlarge, gradually turns dark brown, often with concentric bands and the leaflets shrivel and dry up (Singh 2006). The disease spreads rapidly covering the entire lamina and stem, starting from the lower leaf extending upwards. A white

cottony mycelial growth with microsclerotia develop on the affected plant parts. The mycelial growth on the infected leaves appear as spider web and hence the name web blight disease. Symptoms in the pods appears as light irregular specks, that becomes dark brown with maturity. The grains in the infected pods also gets affected and becomes small in size, shrivel and becomes pale in color (Sharma and Tripathi 2001).

8.2.8.2 Biocontrol and Disease Management

Since *R. solani* is a soil borne pathogen, management of the disease is a challenging task and no single method is available for effective management of web blight. Cultural practices include alteration in the sowing date avoiding the rainy season, plantation of crops with a wider row spacing of 50 cm or more, removal of collateral hosts and adoption of an appropriate crop rotation regime is helpful in management of the disease. Biological control includes treatment with the fungal bioagents, *Trichoderma harzianum* and *T. viride* and the bacterial bioagent, *Pseudomonas fluorescens* provides the best additive effect for better management of the disease (Dubey 2007). Chemical fungicides such as bavistin or benlate (2.5 g/kg seed) were found effective in eliminating the seed-borne infection. Additionally good results were also obtained for Captafol (3.5 kg/ha), indofil M-45 (0.25%), Propiconazole (0.10%) or oxycarboxin (0.16 kg a.i./ha) as foliar spray. In urdbean, lines such as HPBU 67, KU 304, NDU 7-24, COBG 653, IPU-2-43, KPU-1-10, KU-1106 showed high degree of resistance against the web blight fungus (Kumar et al. 2018).

8.2.9 Bruchids

Bruchids or seed weevils (*Callosobruchus chinensis* and *C. maculatus*) are the most destructive storage pests ofmungbean and urdbean that feed on the dry seeds and cause extensive post-harvest damage (Somta 2007). Bruchid infested seeds not only decrease the nutritional content and commercial value but also renders the grain unfit for human consumption. Apart from these two species, they attack almost all crop legumes including pigeon pea, cowpea, chickpea, and lentil etc. and are cosmopolitan in distribution (Rees 2004). Both the bruchid species are able to attack mungbean, however, urdbean is susceptible only to *C. maculatus* but resistant to *C. chinensis* (Srinives et al. 2007).

8.2.9.1 Mode of Infection

Bruchid infestation starts while plants are growing in the field, where a female adult lay eggs on pods; the emerged larvae penetrate the pods and grow into adults. The severe bruchid infestation starts when the adult bruchids start laying eggs directly

on the seeds after harvest. Such secondary infestation is more detrimental as it can cause a total loss of seed yield within a span of 3–4 months. In urdbean, *C. maculatus* can cause a yield loss up to 90% (Soundararajan et al. 2012), while in mungbean, both *C. maculatus* and *C. chinensis* cause 7–73% losses in yield, depending upon the genotype and the morphological and biochemical attributes of the seeds (Somta et al. 2008).

8.2.9.2 Biocontrol and Disease Management

Cultural control to eradicate the conducive environments for pest multiplication including physical elimination of bruchid eggs and larvae, elimination of bruchid infested grains before storage, periodical fumigation and disinfestation of the storage room, spraying with insect repellents is effective against the pest. Presently 10% DDT and/or 5% benzene hexachloride dust is used for disinfestation (Mishra et al. 2018). Phytochemicals that have inhibitory actions against bruchids include groundnut or coconut oil that have a toxic effect on eggs of *C. maculatus*, 5% neem extract with 0.1% soap solution. Tobacco powder, leaf or flower powder of the drumstick tree (*Moringa oleifera*) is also used as an herbal insecticide. Chemical pesticides, belonging to pyrethroids, carbamates, organochlorines, and organophosphates are used to control bruchid infestation (Mishra et al. 2018). Mungbean lines namely V1128, V2709, V2802, TC1966, ACC41, VC1973A and Jangan Mung showed high resistance against the two buchid species, while in urdbeanVM 2164, VM2011, VM3529 similar resistance (Mishra et al. 2018).

8.3 Molecular Mapping of Genes and QTLs for Resistance Breeding

In order to develop varieties resistant to pathogens and pests, wild relatives of the crops are considered vital sources of such traits. Until now, genomic assisted breeding in legumes lags far behind cereals as there is limited genomic information available in the wild species. With the recent development of genomic resources, more and more information are being available publicly providing a better understanding of the crop diversity. Therefore a combinatorial effort of genomics coupled with traditional breeding can broaden the narrow genetic bases and is imperative in legume breeding.

Although functional genomics resources are available for some legumes such as soybean, cowpea, chickpea, pigeonpea, etc., meager information are available in mungbean and urdbean. In order to assist marker-assisted breeding for MYMIV-resistance, Basak et al. (2005) screened a large segregating F_2 population and F_3 families developed from MYMIV-susceptible T9 cultivar and a mutant resistant line VM4 of urdbean and observed a monogenic recessive inheritance pattern of the resistance trait. The group screened several resistance gene analog (RGA) primers and

observed polymorphism for a single primer pair in the resistant parent. Subsequently, a 445 bp polymorphic marker VMYR1was developed that was found to be linked with MYMIV-resistanceas it co-segregates with the MYMIV-resistant progenies of F_2 population and F_3 families. In another attempt, the recombinant inbred lines (RILs) were analyzed for MYMIV resistance generated from a cross between *V. mungo* (TU94-2) and *V. mungo* var. *sylvestris* (Souframanien and Gopalakrishna 2006). They developed a sequence characterized amplified region (SCAR) marker from a polymorphic inter-simple sequence repeat (ISSR) marker linked with MYMIV-resistance. Maiti et al. (2011) screened different genotypes ofurdbean for MYMIV-resistance using degenerate primers designed from the nucleotide binding site (NBS) domain of MYMIV-resistance genes and developed two markers YR4 and CYR1. These two MYMIV-resistance-tagged SCAR markers, YR4 and CYR1 were successfully used for genotyping of various urdbean and mungbean cultivars/lines (Maiti et al. 2011). Subsequently, Kundu and Pal (2012) validated introgression of MYMIV-resistance in 35 F_2-derived RILs using these two markers and observed superior agronomic qualities of a RIL, referred as VMR 84 over others under the YMD-affected background. In another study, Gupta et al. (2013) analyzed the inheritance pattern of the MYMIV resistance gene in the F_1, F_2 and $F_{2:3}$ individuals derived from cross between DPU 88-31 (MYMIV-resistant) and AKU 9904 (MYMIV-susceptible) and found that MYMIV resistance is governed by a single gene in the resistant urdbean genotype. In the F_2 population, out of 361simple sequence repeat (SSR) markers, 31 was found polymorphic between the parents; amongst which CEDG 180 was found to be linked with the resistance gene and was located at a map distance of 12.9 cM apart. This marker has also been validated in 9 resistant and 7 susceptible lines and recommended its use in marker assisted breeding to develop MYMIV resistant genotypes in urdbean. In another study, utilizing 35 polymorphic SSR markers out of 525 tested primer pairs, Vadivel et al. (2021) screened 112 $F_{2:3}$ RILs of the cross between MDU 1 × Mash 1008 and identified two major quantitative trait loci (QTLs) for MYMV resistance in LG2 (*qmymv2_60*) and LG10 (*qmymv10_60*) showing 20.9 and 24.9% phenotypic variability (Fig. 8.1a). Subsequent validation of the QTLs in other mapping population revealed greater phenotypic variability of *qmymv10_60* highlighting its potential in MYMV resistance breeding in urdbean.

Kitsanachandee et al. (2013) in an attempt to identify QTL associated with MYMIV resistance using SSR markers reported three QTLs (*qYMIV1*, *qYMIV2* and *qYMIV3*) and two QTLs (*qYMIV4* and *qYMIV5*) for Indian and Pakistan mungbean populations, respectively. Although identified distinctly, *qYMIV1* and *qYMIV4* appeared to be located in the same locus conferring MYMIV resistance (Fig. 8.1b). Alam et al. (2014) crossed MYMIV-susceptible (BM1) and resistant (BM6) mungbean genotypes and employed F_2 and BC_1F_1 populations to identify the QTLs associated with MYMIV-resistance. Resistance to the virus was evaluated using 1,165 SSR markers from other legumes to detect polymorphism between the parents of which 61 showed polymorphism. Composite interval mapping identified two major QTLs, *qMYMIV2* and*qMYMIV7* governing resistance amongst the progenies of mungbean populations. Likewise, Lekhi et al. (2018) crossed MYMV-susceptible SML668 with-resistant Mash114 to generate F_2 lines and observed that resistance is controlled

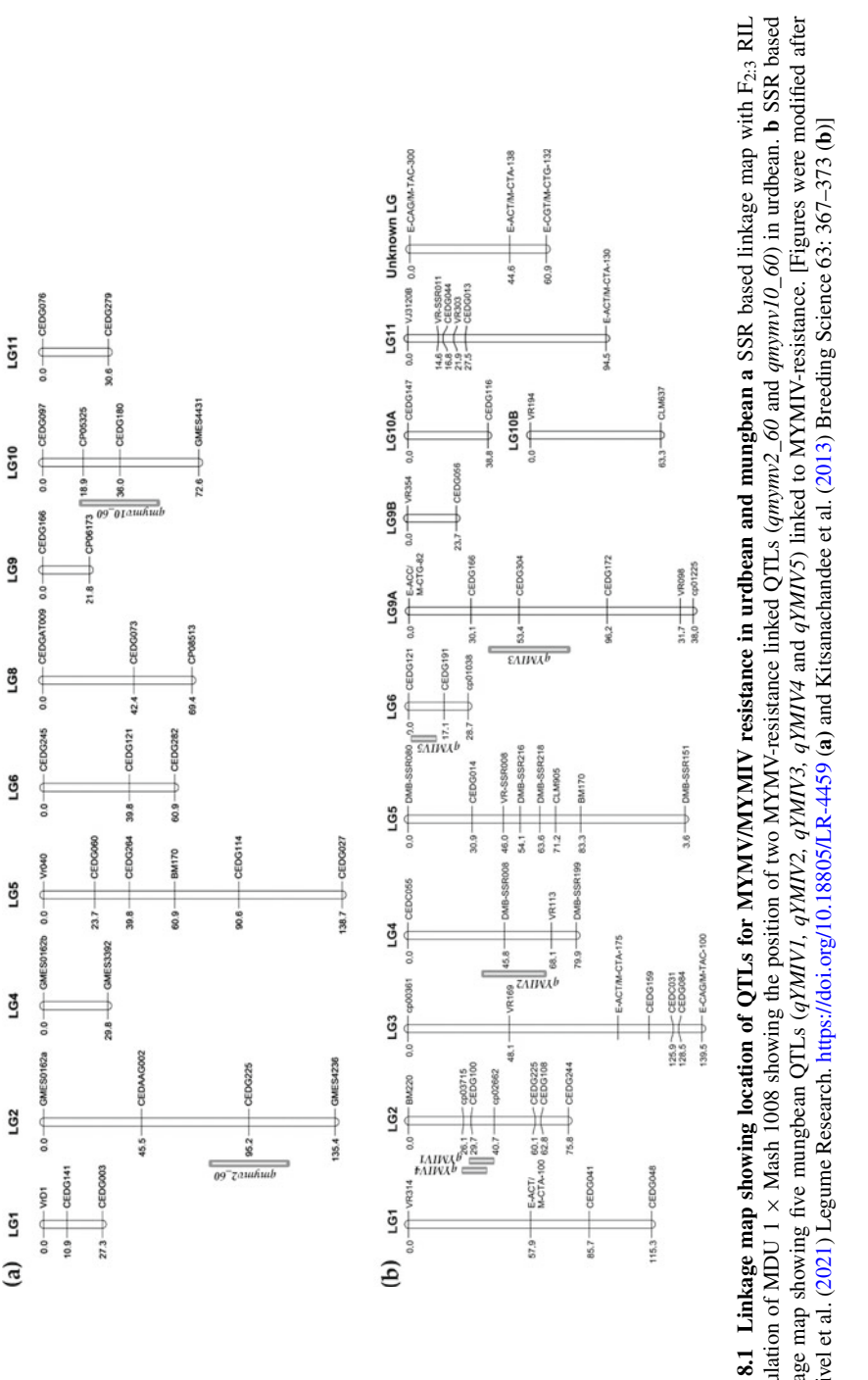

Fig. 8.1 Linkage map showing location of QTLs for MYMV/MYMIV resistance in urdbean and mungbean a SSR based linkage map with $F_{2:3}$ RIL population of MDU 1 × Mash 1008 showing the position of two MYMV-resistance linked QTLs (*qmymv2_60* and *qmymv10_60)* in urdbean. **b** SSR based linkage map showing five mungbean QTLs (*qYMIV1, qYMIV2, qYMIV3, qYMIV4* and *qYMIV5*) linked to MYMIV-resistance. [Figures were modified after Vadivel et al. (2021) Legume Research. https://doi.org/10.18805/LR-4459 (**a**) and Kitsanachandee et al. (2013) Breeding Science 63: 367–373 (**b**)]

by a single dominant gene. Bulked segregant analysis (BSA) with 67 SSR markers identified 46 polymorphic bands between the parental lines of which the SSR marker MBM 0378 was found to be associated with MYMV-resistance. Singh et al. (2020) identified 15 markers associated with MYMIV-resistance by screening 256 genome-wide microsatellite markers used in the association studies. The specific regions close to CEDG293, DMB-SSR008 and DMB-SSR059 located on linkage group 2, 4 and 9 were detected to be associated with MYMIV-resistance and suggested to be useful in marker-assisted breeding for MYMIV-resistance.

Young et al. (1993) identified three QTLs associated with the PM-resistance in mungbean. In another attempt, Chaitieng et al. (2002) attempted to map the PM-resistance gene in mungbean using restriction fragment length polymorphism (RFLP) probes but remain unsuccessful to identify any association linked to resistance. Subsequently, amplified fragment length polymorphism (AFLP) analysis and BSA identified four bands linked with powdery mildew-resistance, and five RFLP markers generated. Later, Humphry et al. (2003) observed a single QTL controlling powdery mildew-resistance in RIL population derived from resistant line ATF3680 and the susceptible cultivar, Berken. Zhang et al. (2008) developed RFLP marker (VrCS65) linked with PM-resistance. The marker was subsequently used to screen mungbean bacterial artificial chromosome (BAC) library and the positive clones were employed to develop SSR and sequence tagged site (STS) markers linked to PM-resistance. Anjum et al. (2010) mapped the PM-resistant population using RBU 38 as a source of resistant and DPU 88–31 as the susceptible genotype and assessed polymorphism using 363 SSR and 24 resistant gene homologue markers. In another study, Kasettranan et al. (2010) crossed the PM-susceptible cultivar, Kamphaeng Saen 1 and resistant line, VC6468-11-1A to generate a RIL population of 190 F_7 lines and found 15 SSR loci on three linkage groups associated with PM-resistance. Composite interval mapping identified two QTLs *qPMR*-1 and *qPMR2*, conferring PM-resistance and subsequently SSR markers were developed from primers flanking and closely linked to these QTLs, *qPMR*-1 (CEDG282 and CEDG191) and *qPMR*-2 (MB-SSR238 and CEDG166). Poolsawat et al. (2017) crossed PM-susceptible CN72 and -resistant V4718 and found that resistance is governed by a single major gene. Amplification by ISSR primers combined with RGA primers amplified 52 polymorphic loci, of which 11 were putatively associated with PM-resistance. Additionally, the major QTL, *qPMC72V18*-1 was identified which was flanked by I42PL229 and I85420 markers with a distance of 4 and 9 cM, respectively.

Marker assisted introgression of bruchid resistance is one of the first approach adopted of its kind in mungbean and for that the wild relative of mungbean, *V. radiata* var. *sublobata* TC1966 was utilized (Fujii et al. 1989). Young et al. (1992) studied the F_2 progenies from a cross derived from resistant TC1966 with a susceptible cultivar and developed 153 RFLP markers and mapped one of these at 3.6 cM distant from the resistance gene. Resistance to bruchids was also mapped in another member ACC41, the gene was tagged with arandom amplified polymorphic DNA (RAPD) marker. This marker was further modified to a SCAR marker. Later two STS markers, namely, STSbr1 and STSbr2 were developed in ACC4132 for bruchid-resistance. Of these two, STSbr1showed polymorphism among wild accession of *V. radiata* var.

Sublobata and other 12 mungbean cultivars. Analysis of113 segregating F_6lines of a cross between Sonamug, Cv. B1 with the wild accession showed 100% co-segregation of resistant locus with the *STSbr2* (Sarkar et al. 2011).

Mapping of the bruchid-resistance gene in cultivated varieties is yet to be developed. Chen et al. (2007) developed 200 RILs and used them to carry out BSA and obtained 10 RAPD markers associated with bruchid-resistance. These markers were converted to SCAR and cleaved amplified polymorphism (CAP) markers, of which the CAP marker was found to be tightly linked with the bruchid-resistance gene. More recently, Schafleitner et al. (2016) generated more than 6,000 single nucleotide polymorphism (SNP) markers through genotyping by sequencing (GBS) and these were used to map the bruchid-resistance genes. One important QTL was found to be associated with bruchid- resistance that was mapped on chromosome 5. However, co-segregation of all the markers indicated that there is single major QTL responsible for bruchid resistance.

QTL mapping has also been done for CLS-resistance in mungbean. Chankaew et al. (2011) crossed CLS-resistant V4718 and susceptible KPS1 and raised F_2 (KPS1 × V4718) and BC_1F_1 lines [(KPS1 × V4718) × KPS1]. The progeny populations were screened using 752 SSR markers, of which and 69 polymorphic markers were found in the population. Segregation analysis showed that CLS-resistance is controlled by a single dominant gene. A major QTL was identified for CLS- resistance located on linkage group 3 between the markers CEDG117 and VR393.

8.4 Genomic and Proteomic Approach with Reference to Disease Reaction

Since the omics tools offer large data sets, more and more researchers are nowadays shifting towards functional genomics including transcriptomic and proteomic approaches to elucidate molecular mechanisms underlying abiotic and biotic stress tolerance (Kundu et al. 2013a, b). However, researches on both mungbean and urdbean are slow-paced in terms of genomics studies. Although the whole genome sequencing of mungbean was planned about two decades ago, however, the first mungbean draft genome became publicly available when Kang et al. (2014) published their findings. More recently, a chromosome-scale assembly towards the urdbean genome sequencing was reported by Pootakham et al. (2020). These two reference genomes will definitely provide essential information in mapping biotic stress resistance mechanisms in both the pulse crops.

For a better understanding of the mechanisms behind stress resistance, expression profiling is considered a well-established technique for procuring information on host's transcriptomic responses against the pathogen and analyzing differential expression of genes. Kundu et al. (2015) studied the transcriptomic responses of urdbean during MYMIV infestation and identified 345 differentially expressed genes (DEGs) through suppression subtractive hybridization (SSH). Out of these

205 unigenes were derived from the resistant VMR84 library and 140 from the susceptible T9 library and predicted their plausible biological and cellular functions. Key pathways that are upregulated following MYMIV-inoculation include induction of calcium and MAP kinase signaling, expression of transcripts involved in phenylpropanoid and ubiquitin-proteasomal pathways and elicitation of the salicylic acid (SA) pathway. Later on, Chakraborty and Basak (2018) have worked upon the identification of two hypothetical models of compatible and incompatible interactions between MYMIV and urdbean using an elaborate library preparation and expressed sequence tag (EST) sequencing protocol. Initially they generated SSH libraries where they identified 145 and 109 differentially expressed transcripts in resistant and susceptible plants, respectively.Of these, the key genes were later validated by qRT-PCR. Based on the obtained differential expression data they have predicted the possible protein–protein interactions of the upregulated transcripts which enabled them to construct the hypothetical models for genomic designing of plant pathogen interaction.

Transcriptome profiling and analysis has also been explored in urdbean in response to MYMIV reactions using next generation sequencing (NGS) based technologies that utilizes assembly, annotation and pathway analysis following sequencing using Illumina Hiseq platform (Ganguli et al. 2016; Kundu et al. 2019). The key findings of the work was to generate a comparative RNA-Seq transcriptomes of resistant (VM84) and -susceptible (T9) plants in order to identify genes potentially involved in urdbean resistance against MYMIV. Distinct gene expression landscapes were observed in VM84 and T9 with DEGs. Functional analysis of the altered 2158 and 1679 DEGs in resistant and susceptible plants respectively identified multiple regulatory pathways in MYMIV-resistance.

Transcriptomic responses in the resistant background (VM84) reflected a cascaded immune reaction indicating involvement of an efficient pathogen response mechanism leading to activation of basal and induced immune responses. Functional analysis of the altered DEGs were carried out using MAPMAN that further identified multiple regulatory pathways to be activated or repressed over time. qRT-PCR validations of the responsive genes including the *NB-LRR*, *WRKY33*, *ankyrin, argonaute* and NAC transcription factors showed a propensity of their accumulation in the resistant background suggesting their potential roles in MYMIV-resistance. Other candidate genes including *phenylalanine ammonia lyase* and non-expresser of *pathogenesis related gene 1* (*NPR1*) are the core components of SA mediated signaling; these were found to be highly induced in the resistant background after pathogen inoculation (Kundu et al. 2019). *NPR1* acts as a cytosolic receptor of SA interacting with TGA transcription factors and activates pathogenesis related (*PR*) genes providing antiviral response eliciting the canonical systemic acquired resistance (SAR). Activation of these members indicates a post-inoculation burst of SA biosynthesis in urdbean that primarily occurs through the phenylalanine dependent pathway (Kundu et al. 2019). This transcriptomic intervention also revealed a strong anti-oxidative defense mechanism that is operative in response to MYMIV-reaction and a rapid detoxification of free radicals was found to be critical for the survival of

host plants under high virus load. Most of the DEGs detected in the resistant background are known to involve in defense response and are operative in recognized pathways including biotic stresses, biosynthesis of secondary metabolites, defense signaling, antioxidant activity and transcriptional regulation. The RNA-Seq results were validated using the qRT-PCR which further demonstrated a tendency of defence related gene expression in the resistant background.

Paul et al. (2014) identified urdbean microRNAs from leaf small RNA transcriptome through deep sequencing and analyzed their expression using qRT-PCR under salinity, drought and cold stress. This study was followed by a high-throughput Illumina sequencing-based investigation where the group identified the responsive microRNAs (miRNAs) involved in the MYMIV-*Vigna* interaction (Kundu et al. 2017). Expression of several conserved miRNAs such as miR156, miR159, miR160, miR166, miR398, miR1511, miR1514, miR2118 and some novel members like vmu-miRn7, vmu-miRn8, vmu-miRn13 and vmu-miRn14 were found to be modulated after challenging with the virus. The obtained results were also validated through qRT-PCR analyses, and the findings correlated with the expression pattern like that of the Illumina reads.

Earlier Kundu et al. (2013a, b) carried out the comparative proteome analyses of two contrasting urdbean genotypes using 2D gel electrophoresis together with MALDI-TOF identifying 109 differentially expressed proteins at 3, 7 and 14 days post-inoculation. The proteomics data mostly corroborates with the transcriptomics data revealing the validity of both the documentation. A severe alteration in the photosynthesis related proteins were noted in the susceptible background resulting in total disruption of the photosynthetic related processes under MYMIV-stress. Eventually the MYMIV-susceptible plants either perished or became less productive (Kundu et al. 2013a, b).

Lin et al. (2016) studied the mechanism of bruchid resistance in mungbean seeds using transcriptomic and proteomic approach. They have identified 399 DEGs from the isogenic lines VC1973A (bruchid-susceptible) and VC6089A (-resistant) of which 251 displayed high expression and 148 showed low expression. They also recognized 45 differentially expressed proteins in the two contrasting genotypes using the iTRAQ technology based proteomic analysis. Twenty one of them showed higher accumulation in VC6089A, and 24 showed high level expression in VC1973A. Comparing both the transcriptome and proteome data, three genes including resistant-specific g39185, gag/pol polyprotein g34458, and aspartic proteinase g5551 were recognized amongst which the g39185 is implicated in conferring bruchid-resistance. Baruah et al. (2017) employed SSH to identify transcripts in urdbean seeds infested with bruchid eggs. A total of 277 ESTs were obtained and 134 unigenes were annotated. Amongst these, 20 defense related transcripts were subjected to qRT-PCR analyses, of which 12 showed up-regulation that includes defensin, PR- related protein and lipoxygenases in the oviposited population.

8.5 Bioinformatic Analyses and Genomic Research in *Vigna*

More than 80 species of the genus *Vigna* are quite widespread throughout the tropics. Both the species dealt in this chapter, viz. *V. radiata* and *V. mungo* belong to the Papilionoid subfamily and subgenus *Ceratotropis* having the same chromosome number $2n = 2x = 22$. Mungbean have a genome size of 579 Mb (Kang et al. 2014), whereas the genome size of urdbean is 560 Mb (Arumuganathan and Earle 1991). If we explore it from an evolutionary standpoint then we would be able to identify phylogenetic proximity of *Vigna* with agriculturally important genera such as *Phaselous, Cajanus* and *Glycine* (Gepts et al. 2005). Thus, it is important to generate information on the genome, transcriptome and metabolome of the different species of *Vigna*, which would be beneficial not only for conventional plant breeding programs but also enable the formulation of suitable transgenic strategies for the amelioration of biotic and abiotic stress factors, which directly influence crop productivity. Apart from that comprehensive genome and metabolome information could also provide important insights on probable lead compounds which might be useful for therapeutic purposes.

8.5.1 Next Generation Sequencing Based Genome Information

One of the first reports on the application of modern genomics technologies in *Vigna* species can be attributed to Naito et al. (2013). They created a hybrid assembly of the organelle genome sequences of *V. angularis* using NGS data generated by Roche GS Titanium and Illumina Hiseq 2000. It was clear from the obtained data that both the organellar genomes were highly similar in size as well as gene content to those of mungbean. A closer look into the organellar genome structure revealed the evidences of a number of recombination events within the mitochondrial DNA (mtDNA) sequence; while the chloroplast DNA (cpDNA) exhibited higher percentage of similarity. The complete sequences of cpDNA (AP012598) and mtDNA (AP012599) are publicly available at DDBJ (DNA data bank of Japan, http://www.ddbj.nig.ac.jp/updt-form-j.html). The analysis of raw sequencing reads including assembly was performed using the CLC Genomics workbench using default parameters with the help of de bruijn graphs, integrated BLAST and other contig extension programs.

The first de novo genome sequence of *Vigna* species was reported by Kang et al. (2014). They sequenced three different *Vigna* cultivars, namely, domesticated diploid *V. radiata* var. *radiata* ($2n = 2x = 22$), its polyploid relative *V. reflexo-pilosa* var. *glabra* ($2n = 4x = 44$), and its wild relative *V. radiata* var. *sublobata* ($2n = 2x = 22$). Pure line VC1973A was chosen for genome sequencing in case of *V. radiata* var. *radiata*. A high-quality draft genome sequence of the diploid *V. radiata* var. *radiata* VC1973A with an estimated genome size of 579 Mb (1.2 pg per 2C) was constructed. Five libraries were generated and sequencing was performed using Illumina HiSeq 2000 including 180 and 500 bp paired-end libraries and 5, 10 and 40 kb mate-pair

libraries. In addition, GS FLX + sequencing produced long reads that provided fivefold genome coverage. The short reads were assembled using ALLPATHS-LG software, producing 2,800 scaffolds where N50 length was 1,507 kb. The long reads generated by GS FLX + were assembled into 180,372 contigs using Newbler 2.5.3 software. In total, 144,213 of the GS FLX contigs were consistent with the scaffolds from ALLPATHS-LG. The non-matched GS FLX + contigs were divided into 5 kb pseudo-mate-pair reads and assembled using ALLPATHS-LG software to improve the quality of the assembly, resulting in 2,748 scaffolds with an N50 length of 1.52 Mb. The total length of the produced scaffolds was about 431 Mb, representing 80% of the genome size of 543 Mb estimated from 25-base kmer frequency distribution.

Recently Souframanien et al. (2020) have constructed a draft genome sequence of urdbean, by employing hybrid genome assembly with Illumina reads and third generation Oxford Nanopore sequencing technology. The final *denovo* whole genome of urdbean was reported to be ~475 Mb (82% of the genome) and has maximum scaffold length of 6.3 Mb with scaffold of 1.42 Mb. Genome analysis identified 18,655 genes with mean coding sequence length of 970 bp. Around 96.7% of predicted genes were annotated. It was noted that genome comprised of large proportion of repetitive elements with 47.3% of retrotransposon elements. A total of 166,014 SSRs were reported from the genomic DNA. Out of the 18,665 in silico translated proteins, 678 proteins exhibited domains designated for R-genes, of which majority (372) belongs to the KIN class and RLK and N are 79 each. While exploring the chromosome scale assembly of urdbean genome, Pootakham et al. (2020) have employed the 10X genomics linked-read technology to obtain a de novo whole genome assembly of *V. mungo* cultivated variety Chai Nat 80 using Illumina Hi Seq technique. The preliminary assembly was reported to contain 12,228 contigs and had an N50 length of 5.2 Mb. Subsequent scaffolding using the long-range Chicago and HiC techniques yielded the first high-quality, chromosome level assembly of 499 Mb comprising 11 pseudomolecules.

Raizada and Souframanien (2019) have reported the identification of SSRs in *V. mungo* var. *silvestris* using Illumina Miseq technology. They predicted 38,753 coding sequences (CDS) from 40,178 transcripts that were assembled and successful annotation of 28,984 CDS was done using BLASTX. These were then mapped to GO and KEGG databases revealing their associations with 140 unique pathways. For the segment of identification of SSRs, tri-nucleotides were found to be most abundant (39.9%) followed by di-nucleotide (30.2%). About 60.3 and 37.6% of SSR motifs were present in the coding sequences (CDS) and untranslated regions (UTRs), respectively. SNPs were genotyped using a High Resolution Melting (HRM) Assay and a validation rate of 78.87% was achieved.

Employing 454 Sequencing technology Tangphatsornruang et al. (2009) has identified 1493 SSR motifs having potential for generating genomic markers in mungbean. These microsatellite primers have the capacity of cross-species transferability amongst other pulse species. Maximum compatibility was noted in *V. angularis* (91.6%). On the contrary, the transferability of these markers was quite low in *Phaseolus vulgaris* and *Glycine max*.

8.5.2 *Other* Vigna *Resources*

Over the years various data repositories have incorporated different aspects of *Vigna* genome and associated information. Table 8.1 summarizes the important resources

Table 8.1 Web resources containing genome and accessory information of *Vigna* cultivars

Serial number	Name of the resource	Brief description and URL	References
1	VigSatDB	Genome-wide microsatellite DNA marker database of three species of *Vigna* for germplasm characterization and improvement http://webtom.cabgrid.res.in/vigna_ssr/	Jasrotia et al. (2019)
2	The *Vigna* genome Server, VigGS	A Genomic Knowledge Base of the Genus *Vigna* Based on High-Quality, Annotated Genome Sequence of the Azuki Bean, *Vigna angularis* (Willd.) http://viggs.dna.affrc.go.jp	Sakai et al. (2016)
3	VmTDB	*Vigna mungo* transcriptome database: http://webtom.cabgrid.res.in/vmtdb/	Jasrotia et al. (2017)
4	Virus—host DB	Information regarding virus and host: https://www.genome.jp/virushostdb/3915	Mihara et al. (2016)
5	Ensembl plants	General information about Vigna genomes that have been sequenced so far. Also provides information regarding protein coding genes and their corresponding sequences https://plants.ensembl.org/Vigna_radiata/Info/Index	Howe et al. (2020)
6	Legume information system	General Information regarding various aspects of Vigna cultivars including genomic and other important information https://legumeinfo.org/organism/Vigna/radiata	Dash et al. (2016)
7	Eppo global database	https://gd.eppo.int/taxon/PHSMU	EPPO (2021) EPPO Global Database (available online)

which can be explored by any research enthusiasts willing to work ongenomic designing of Vignas.

8.5.3 *Comparative Genomic Sequencing* Vigna *Species*

Pootakham et al. (2020) had explored the computational pipelines such as OrthoFinder (Emms and Kelly 2019) to compare sequence orthologues of urdbean. They compared the sequences obtained through de novo transcriptomics against nine different legume members such as *Glycine max, V. unguiculata, V. angularis, V. radiata, V. reflexo-pilosa, P. vulgaris, Arachis duranensis, Cicer arietinum and Medicago truncatula.* Apart from the legumes they had also included information of two cucurbit species (*Cucumis sativus* and *C. melo*). These were included in the phylogenetic analyses as their divergence time was known, in addition two rosid species (*Prunus persica* and *Arabidopsis thaliana*) were also included due to the availability of their complete genome information and one monocot (*Oryza sativa*) included as an outgroup member. The phylogenetic tree of single copy orthologous group was generated using standard tools, such as, MUSCLE and RAXML. Analysis have revealed that a large percentage (21.62%) of genes areurdbean specific, while only 1.23% of the genes are found to be shared among other 14 legumes surveyed. Thus, this data paves way for future studies on Pan genomes of the related *Vigna* species and the core genomes of the individual members that may provide insights into the events of acquisition or rearrangement events as a result of transposon activity (Pootakham et al. 2020). It was estimated that the majority of LTR retrotransposon elements were integrated intothe genomes of mungbean and urdbean within the last 5 million years ago (Mya). Based on the comparative genomics analyses it was revealed that mungbean and urdbean diverged approximately 2.7 Mya (Pootakham et al. 2020).

The genome assembly and the information gathered on the genomic variations among different germplasm provide important resources for generating useful varieties through marker-assisted breeding and genomic designing for crop improvement in general including stress tolerance.

8.5.4 *Metabolomic Information Regarding* Vigna *Cultivars*

Recent studies have explored the numerous bioactivities of *Vigna* metabolites, since data suggests that these compounds can have numerous therapeutic implications. In one such study mungbean protein was observed to prevent non-alcoholic fatty liver disease in high-fat fed mice (Watanabe et al. 2017). Apart from that, mungbean coat extract has been reported to exhibit hypoglycaemic activity, which has been attributed to the inositol and phenolic contents (Tang et al. 2014; Yeap et al. 2012; Mushtaq et al. 2014). Tang et al. (2014) have opined that due tosignificant changes in the

metabolites contents during mungbean germination the nutritional and medicinal qualities are enhanced significantly. With the above premise Wang et al. (2020) embarked upon an integrated study in mungbean using a combined transcriptomics guided metabolomics approach, where the pathway predictions obtained from the transcriptome data was used as a parameter for setting up the gas chromatography tandem mass spectrometry (GC–MS)-based metabolomics approach. They were able to identify 160 different metabolite signals of which 57 were validated using their respective standards. It was found that out of the 57 compounds identified majority of them were byproducts of the primary metabolism cascade. In a similar study a few years back Goufo et al. (2017) had analyzed the importance of the metabolites towards osmo-protection using *V. unguiculata* as the model species. In their analysis they used the Gas Chromatography instrument with the DB-35MS column and then passed on the column elutes to the Pegasus HT time-of-flight mass spectrometer. Chromatograms and mass spectra thus obtained were further processed using the deconvolution algorithm of ChromaTOF and Tag Finder. Multivariate analysis of the 88 metabolites identified in this study, proline, galactinol, and a quercetin derivative, were found to be associated with drought stress response and significant correlation was observed with the beneficial sets.

Recently Wu et al. (2020) have explored the metabolome of mungbean during seed germination. The objective of the study was to identify the different metabolomic composition associated with seed germination. Apart from the products of primary metabolism, this study reports for the first time the presence of shikimate pathway-mediated secondary metabolites. Since the detection pipeline was mostly NMR based and it would be prudent to use NMR spectroscopy to identify metabolome profile of*Vigna* cultivars for comparison in future.

8.6 Future Perspectives

8.6.1 Potential for Expansion of Productivity

Productivity of mungbean and urdbean has been seriously affected in the past few decades as a number of biotic stressors jeopardized the production of both the crops. As per the Asian scenario, both of them has been projected as crucial members to be included for vertical and horizontal expansion to achieve self-sufficiency in pulses. Therefore, it was felt that an imminent thrust in basic and advanced research is needed to improve their quality and quantity to make them a contributory element in the future pulse revolution.

Biotic stresses are the foremost constraints responsible for the loss in yield potential of the pulse crops. Therefore, a sustainable increase in the production of plant proteins is necessitated to fulfill the increasing demand of the growing world population. Majority of the yield loss suffered by mungbean and urdbean is due to yellow mosaic, powdery mildew, CLS, anthracnose diseases and the post-harvest menace by

stored grain pest *Callosobruchus*. A number of disease resistant varieties have been developed through conventional breeding in order to counteract the loss incurred by these biotic stresses however, only a few sources of resistance have been identified for other fungal and bacterial diseases, making the task harder. Development of molecular markers using genomic information against these pests and pathogen is demanded to be utilized in marker assisted breeding to develop disease resistant lines. Additionally genomic markers can minimize the time and labor required for phenotyping in field-trials during evaluation of diseases incidence. Such markers can also aid in transferring the resistance traits from other legumes to mungbean and urdbean. However, there are chances of introgression of undesired traits from the resistant sources to the cultivars. Therefore a synergistic effort involving conventional breeding along with molecular techniques can eliminate the problem. Another important aspect is to combine multiple pathogen/pests resistant genes into the same cultivar (gene pyramiding), as this could help to achieve resistance/tolerance simultaneously against various pathogens and insect-pests and can nullify the development of pathogenic strains or biotypes. Additionally, identification of resistance linked genes/QTLs may provide the foundation in improving the biotic stress tolerance in mungbean and urdbean by non-conventional experimental designing.

8.6.2 Potential for Expansion of Non-traditional Techniques

Genomic techniques are gaining more and more popularity in dissecting the mechanisms of resistance and susceptibility that will help in altering the genome of crops plants to counter biotic stresses. Time course expression profiling following pathogen inoculation using techniques such as cDNA-AFLP and SSH has already provided substantial information in elucidating the pathogen responsive pathways. With the advent of RNA-Seq technologies, a real-time information at the global transcriptome level can be gathered that will bridge the gap between the genotypic information and expression of plant phenotypes. NGS technologies have further offered GBS which is considered a promising technique to identify SNPs and help in generation of high-density genetic maps. Genome wide identification of the responsive miRNAs under pathogenic stress can help us to formulate appropriate RNAi technology that can be used to enhance biotic stress tolerance inmungbean and urdbean. Additionally, genome editing tools such as CRISPR/Cas can also be deployed to alter the plant genome and confer resistance against biotic stresses. In the recent past this system has been successfully used to impart resistance to Beet Severe Curly Top Virus in *Nicotiana* and *Arabidopsis*. All these information gathered at the molecular level will assist in predicting pathogenic ingression and can contribute in preventive and control measures thereby minimizing disease incidence. Thus apart from conventional breeding approaches, a comprehensive effort towards exploring the molecular and biochemical mechanisms involved in the biotic stress tolerance through identification of genes, proteins and metabolites may help in imparting resistance and thereby aid in the development of improved cultivars by genomic interventions.

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Chapter 9
Genomic Designing for Biotic Stress Resistance in Grasspea

Rishu Sharma, Krishnendu Paramanik, Joydeep Banerjee, Arpita Das, and Kanchan Bhan

Abstract Grasspea (*Lathyrus sativus* L.) is a cool-season legume crop with a broad range of genetic diversity prevalent across the continents. Grasspea is an underutilized source of calories and protein for populations residing in areas with frequent droughts and marginal areas of Asia, Africa and in few pockets of Australia. It is a viable crop option for agro-ecosystems, where successful cultivation of major crop species is difficult especially under the changing scenario of climate change. The major constraint in grasspea production is a neurotoxin known as β-*N*-oxalyl-l-α,β-diaminopropionic acid known as (β-ODAP) causing neurolathyrism, a neurotoxic disease in humans, thus making it unfit for the human consumption. The strategic reduction of ODAP through genetic manipulation is the sole option to obtain the benefits of this "orphan crop". *Lathyrus* genetic resources in large ex situ collections have been done in various gene banks of the world by undertaking collection, conservation, evaluation, characterization and utilization. It has found that no significant efforts have been made for alien gene transfer in grasspea, in spite of a large number of wild relatives with useful traits. The grasspea is well-adapted to a number of biotic stresses but yet incur considerable yield losses of approx. 15–25%. Till date, negligible genetic resources have been exploited to develop grasspea genotype

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resistant against biotic stresses viz. diseases and pests. Foliar diseases are predominantly responsible for the substantial yield loss. This chapter reviews the present status of genomic resources of grasspea and their use in developing biotic stress resistant genotypes.

Keywords Grasspea · *Lathyrus* · Biotic stress · ODAP · Pathogens · Genomics

9.1 Introduction

Lathyrus is popularly known as Grasspea across the globe. It is a Neolithic cultivated crop with a long history of domestication (Budge 1928) with cultivation spread over three continents. The genus *Lathyrus* L. includes nearly 187 species (Allkin et al. 1983, 1986). These species are allotted throughout the Northern Hemisphere and extending upto tropical regions of East Africa and Southern USA. It is a vigorous and most pioneer resilient crop species to climate change, low input requirement (Kumari 2001; Urga et al. 2005) and has emerged as promising legume crop during drought and famines. Additionally, it has a capacity to grow in various soil types, including marginal ones due to its hardy penetrating root system. Grasspea is an atmospheric nitrogen fixer, thus can fulfill its nitrogen requirements and is beneficial to the following crops. However, overconsumption of the grasspea seeds can cause a crippling neurological disorder, termed as neurolathyrism. In a balanced diet plan and sociological set up in which grasspea is part of a balanced diet, neurolathyrism is virtually non-existent. The overemphasis on the toxic properties have degraded the exceptionally viable agronomic properties and dietary benefits of Grasspea. The cause of neurolathyrism and the deficiency in methionine in the diet has been ignored. In order to combat the effects of global climate change, this orphan crop deserves more emphasis to meet the nutritional requirement. Grasspea could be a "wonder crop" if we could detach and diminish the stigma labeled on it of a "toxic" crop. Also, current studies have deciphered the prospects of this legume as a nutraceutical for health welfare. Breeding and developing grasspea varieties with improved essential amino acids might come handy for enhancing the nutritional value without threatening the multiple stress tolerance of this promising crop.

9.1.1 Origin and Cultivation

Grasspea is a pulse and fodder crop widely cultivated (Campbell 1997); GrassPea has been reviewed as one of the most ancient crops of the world. The archaeological studies in Turkey and Iraq have found and collected the seeds of *Lathyrus* species. Similarly, seeds of grasspea have been identified from excavations done in India 2500 BC and from Balkan in 8000 BC (Budge 1928; Kislev 1989). According to another school of thought, the queen from Sheba in the tenth century BC brought the grasspea

to Ethiopia (Budge 1928). The name *Lathyrus* is derived from a word in Greek "la thyros" which points towards thrilling and few aphrodisiacal qualities in this legume (Loudon et al. 1855). The word "lathyrism" was given by Cantani in 1873 from Naples to identify a disease consuming seeds of grasspea in excess (Barrow et al. 1974). Egyptian pyramids have shown presence of different legume seeds as offerings counting grasspea. Then, items prepared from grasspea were served as a delicacy to kings unlike its status today where its consumption has been labeled "survival food" for the poor. The civilizations which flourished in the Middle East incorporated grain legumes in their diets with optimum amounts of amino acids (Essential). The cuisines cooked from seeds of grasspea are highly relished by communities. Since time immemorial, they are famous in few European nations (e.g., Poland, Italy, France, Portugal, Spain) African countries (Ethiopia) and Asian countries (e.g., Nepal, India and Bangladesh). In Ethiopia, the consumption of boiled whole seeds of grasspea (*nifro*), conventional sauce (*kik wott and shiro wott*), whole seeds roasted (*kollo*), traditional drink (called as *areke*), and green seeds for snacks as greens (*eshet*) (Fikre et al. 2011). Also, many Europeans have given local names to grasspea (Gry et al. 1998) *titos* ("running for grasspeas"). This game revolves around trespassing the neighbors' fields with grasspea to eat the unripe green seeds without being caught by the landlords. While, the status of grasspea in same nations today is almost of a bygone crop, it is sown in some small plots to fulfill the requirements during the religious activities (Caminero and Grajal 2009).

9.1.2 Grasspea Cultivation: A Boon or Bane?

Grasspea is an annual grain legume grown for its grain, fodder and green manure (Rahman et al. 1995). Grasspea usually exhibits self-pollination but cross-pollination might be there up to 30%, thus genetic integrity of grasspea varieties during must be maintained during the regeneration. Grasspea is considered a nutritionally complete diet as it has high percentage of proteins (28.7–34.2%), micronutrients and optimum qualities of essential amino acids (Sammour et al. 2007). Despite many advantages, till date very little effort has been made in existing Lathyrus conventional breeding programs on producing grasspea cultivars with low β-ODAP content. At present, many breeding lines or released varieties of *L. sativus* or *L. cicero* containing β-ODAP approx. 1% of the original wild have been developed across several laboratories all over the world (high 0.5–1.5%, lower 0.01%) (Roy et al. 1993; Kaul et al. 1986; Santha and Mehta 2001; Pandey et al. 1997). This is anticipated that these varieties could lead grasspea in achieving its full potential as a resilient crop with high protein content through sustainable agriculture with the change in climate and providing food and nutritional security.

9.1.3 Grasspea A "Climate Resilient Crop"

Grasspea is one among the priority crops in the project "Adapting Agriculture to Climate Change" by Kew's Millennium Seed Bank and the Global Crop Diversity Trust. Its main objective is on conserving and preserving the genetic diversity of important food crops plants containing traits that could be exploited for adaptation to new climatic conditions (Lambein et al. 2019). The various factors for increasing agricultural output are numerous viz. water scarcity, decrease in soil fertility, climate change etc. The efficient exploitation of genetic resources and adoption of sustainable soil and water resource management will be pivotal in gaining the optimum productivity (Cobb et al. 2013). The diversity in genetic resources is the foundation of the improvement in plants (Patto and Rubiales 2014). The present-day emphasis on climate resilient crops has shifted the limelight on grasspea. Genomic reservoir which has a low ODAP lines have been developed, and a sudden rise of this crop is anticipated to provide us with combination of protein source and nutritional security in the times of changing climate and sustainable. In the era of global climate change, grasspea considered an orphan crop deserves more attention being very adaptable and nutritious. Grasspea could be a "wonder crop" if workers could mitigate its status as of a toxic plant and poor man's food. And, in latter-day research, the hidden potential of grasspea as a nutraceutical for human health has been unveiled. The development of grasspea varieties with an optimum presence of essential amino acids and thus enhancing the diet by uplifting its nutritional value in addition its ability to tolerate multiple stress. Among the pathogens attacking plants, fungi are the most taxing to manage because it exhibits genetic plasticity and versatility, which capacitate various fungi to deal and adapt rapid to their altering vicinities and environments (Perez-Nadales et al. 2014). Large-scale genomic studies have equipped us with different plant disease-resistance mechanisms against the fungal pathogens viz. biotrophic, hemi-biotrophic and necrotrophic. Substantial studies have been carried out to decipher the disease interaction in legumes at molecular level using molecular markers. Fusarium wilt is one among the most devastating disease in legumes that is as a result of host-specific *Fusarium oxysporum* strains. Many of *Fusarium oxysporum* f. sp. *Ciceri* were marked around the world with cultivating areas under chickpea. Chickpea interactions with *Foc*1 have been used to studied using various methods viz. cDNA-AFLP (amplified fragment length polymorphism), cDNA-based microarrays, and cDNA RAPD (random amplified polymorphic DNA) (Ashraf et al. 2009; Gupta et al. 2009, 2010; Nimbalkar et al. 2006; Gurjar et al. 2012). A study by Xue et al. (2015), marked 122 resistance associated gene fragments which are scattered across the genome and observed that this spread of genes could serve as molecular markers for tagging in breeding programs. RNA seq evaluation of Soybean attacked by each pathogenic and non-pathogenic *F. oxysporum* strains showed activation of defense-associated genes same as responsible for causing necrosis in resistant plants (Lanubile et al. 2015). The differences in defense responses in *Glycine max* at a molecular level, between the different species towards pathogen *F. oxysporum* was also done using RNA seq (Chang et al. 2019). *Phakospora pachyrhizi* causes Asian

soybean rust (ASR), is a destructive disease which is identified among the uppermost biotic challenges to agricultural practices (Pennisi 2010). Earlier studies have been done to identify and characterize the crucial players in resistance to ASR. Initial studies in identifying vital components in *R*-gene controlled reaction of ASR utilized complementary DNA method (Soria-Guerra et al. 2010; Choi et al. 2008). Diverse antimicrobial peptides which includes thionin, defensins and genes in soybean and cowpea attacked by *P. pachyrhizi* causing rust could also be diagnosed the usage of SuperSAGE technique (Kido et al. 2010).

9.1.4 Limitations of Traditional Breeding and Rationale of Genomic Designing

Traditional breeding relies solely on germplasm diversity. In the present day, the genetic and genomic resources are feeble and there is absence of non-standardized methods for germplasm curation for the enhancement of grasspea. This obstructs significantly the utilization of these resources for the improvement of grasspea by breeding whereas molecular breeding in grasspea is the need of the hour by avoiding the external environmental factors. The grasspea data with genomic information is vital for breeding and study of new transmissible variation in the already existing genotypes (Hao et al. 2017). There are very less handy cases with reference to the expansion of genomic resources in grasspea, which might be because of its huge genome length (8.2 Gbp) and the confined characterization. The changing climate and growing population is a challenge to world's food supply, innovative teams of scientists are turning to advanced genomics tools to build solution. Grasspea got more attention as a hardy pulse crop in the past. It's ability to resist against different pathogens and pests. (Palmer et al. 1989; Campbell et al. 1994). But, it harbors high neurotoxin (β-N-oxalylamino alanine) percentage in grasspea leaf and seed (Grela et al. 2001) which requires instant effort to decrease its percentage so as to increase its nutritional benefits. The presence of this toxin β-ODAP has been the major constraint for promotion of this crop for commercial cultivation (Parihar et al. 2015). Therefore cultivation of low-ODAP toxin (<0.1%)/ODAP free grasspea varieties or cultivars with higher yields having desirable attributes like disease and pest resistance along with matching production technologies are highly desirable. And there are no suitable and well adapted high yielding varieties of grasspea with low or free toxin available.

9.2 Stresses in Grasspea Crop

Grasspea is largely grown in areas with dry or rainfed conditions on lands like marginal and submarginal who usually have normally poor soil fertility. In comparison to rest of the legumes, grasspea is more resistant to plant pathogens when

compared with other legumes (Tiwari and Campbell 1996). However, grasspea crop has few production constraints viz. a broad range of biotic stresses ex. powdery mildew, downy mildew, rust, thrips and few abiotic causes like high moisture and waterlogging, which in totality causes a reduction of yield potential by 15–25% (Campbell 1997). Few other causes that can be detrimental to grasspea production include (i) insufficient seed supply of improved and high yielding cultivars with desirable attributes like disease and pest resistance (ii) lower productivity of rice fallows (iii) lack of large scale adoption of novel crop production technologies (iv) unbalanced use of fertilizers (v) untimely sowing and low seed rates, and (vi) weed infestation. Using micronutrients in deficient soils can be followed to boom the grasspea production however farmers are reluctant to adopt these practices, because of their low economic reputation (Baghel et al. 1995; Mehta 1997). Inadequate channel for the transfer of applicable technologies remain another constraint.

9.2.1 Diseases in Grasspea

Downy mildew caused by *Peronospora lathyri-palustris,* a destructive grasspea disease within South Asia (Campbell 1997). Although, absolute resistance is not found in grasspea, and those landraces which exhibited reduction in the level of infection, could be because of mechanisms like tolerance or escape (Campbell 1997). Resistance was found in accessions of *L. sativus* against Ascochyta blight (*Mycosphaerella pinodes*) (Gurung et al. 2002; Pang et al. 2000; Weimer 1947; Skiba et al. 2004a), the species viz. *Lathyrus ochrus*, *Lathyrus clymenum* (Gurung et al. 2002). Ascochyta blight is predominantly found in grasspea causing production losses, but complete resistance has not been found in this species against the pathogen (Skiba et al. 2004b). Distinct partial gradations in resistance and hypersensitive response was pronounced in accessions of *Lathyrus sativus* and *Lathyrus ciceraare* against rust causing pathogens viz. *Uromyces pisi, Uromyces viciae sativae; U. cicierisarietini* (Vaz Patto et al. 2004). Among various diseases, rusts in grasspea is predominant inside the Northwestern regions of Ethiopia (Campbell 1997). The genes responsible for resistance in grasspea may be used in chick pea, local pea as well. *Lathyrus sativus* and *Lathyrus ciceraare* are discovered in broom-rape without showing any resistance (Linke et al. 1993; Sillero et al. 2005). However, excessive degrees of resistance to *O. crenata* through few Lathyrus species, viz. *L. ochrus* and *L. clymenum* has been observed (Sillero et al. 2005; Linke et al. 1993). The principle mechanism of resistance in these species is believed to create the primary line of defense against broomrape, which turned into observed leading to very few broomrape tubercles under the sphere situations (Sillero et al. 2005). A moderate level of resistance was recorded in Slovakian grasspea germplasm against *Fusarium oxysporum* (Benková and Záková 2001).

9.2.1.1 Resistance to Rusts

Infection in genus *Lathyrus* by the rust species has been pronounced to be due to *U. ciceris-arietini* and *U. viciae-fabae* ex. *V. sativa.* The genetic breeding for resistance is the main excellent strategy to govern rusts in Lathyrus thinking about that it's miles most inexpensive and green manage technique (Rubiales et al. 2011). But, any change in virulence of pathogenic population could cause resistance breakdown (McDonald and Linde 2002). The underlying process of plant protection are operative at distinct levels of the contamination system initiation by spore deposition to haustoria formation. *Lathyrus sativus* and *Lathyrus ciceraare* which are the germplasms from Iberia are observed to be highly resistant towards *Uromyces viciaefabae e.g. Uromyces sativa*, *Uromuces ciceris arietini* (showed most effective in *Lathyrus sativus*) showing robust hypersensitive reaction, whilst a well suited reaction (susceptible) or determine its first-class or quantity by way of scoring to envision its resistance towards *Uromyces pisi* infection (Vaz Patto et al. 2009; Vaz Patto and Rubiales 2009). A study by Vaz Patto and Rubiales (2014), level of disease resistance was determined using the disease severity (DS) which showed differential reactions among various accessions. To conclude, accessions from *Lathyrus sativus* are higher resistant (lower DS) to *Uromyces pisi* in comparison to *Lathyrus cicera* accessions, in the open and controlled environment. The disease occurrence was observed to be lower in despite a compatible reaction (high IT), could be due to frequent partial resistance in *L. sativus* (Patto and Rubiales 2009), and was found to a lesser extent in *Lathyrus cicera* (Patto et al. 2009). Disease score was found to be positively correlated in experiments under the field and controlled conditions. The appearance of high resistance or immunity to *Uromyces ciceris-arietini* and *Uromyces viciaefabae* was found in maximum accessions from *Lathyrus cicera* and *Lathyrus sativus* accessions which were examined can be attributed due to non-host resistance. Also, prehaustorial resistance is rare in non-hosts, but does play a vital role in host resistance is partial in nature (Rubiales and Niks 1995) similar was observed by Sillero and Rubiales (2002). It is mostly found in *Uromyces ciceris-arietini*, some accessions of *Lathyrus cicera* were found to be moderately susceptible *U. viciae-fabae.* This shows incongruity to the already mentioned most often shows susceptibility to *Uromyces pisi.* The resistance against rust is found in *Uromyces. pisi* has been described largely in cool-season legumes (Rubiales et al. 2011; Sillero et al. 2006). The maximum observed interactions exhibited incomplete resistance, though in few cases complete resistance was found to be associated with necrosis in plant cells also known as hypersensitivity, was found on *Lathyrus cicera* accessions but not in *Lathyrus sativus* accessions. Some of the *L. cicera* accessions showed mixed disease reactions in accessions expressing hypersensitive (Patto et al. 2009).

9.2.1.2 Resistance to Powdery Mildews

The data on availability of resistance against powdery mildew in the genus *Lathyrus* and the mechanisms responsible is very sparse till date. *Lathyrus sativus* lines

from India and Syria exhibited mild resistance to powdery mould (Asthana and Dixit 1998). Also, Campbell et al. (1994) and Robertson and El-Moneim (1996) observed the similar results however the disease reactions were now not analyzed deeply. In latter day, germplasm of showing resistance to mould have been characterized in info underneath the field and controlled conditions (Patto et al. 2006a, b, 2007). In the growth chamber, *Lathyrus sativus* and *Lathyrus cicera* both confirmed a compatibility of high IT without a external signs of hypersensitive reaction but, DS numerous fantastically in *Lathyrus sativus* accessions than in *Lathyrus cicera* with frequent low DS values (Patto et al. 2006a, b, 2007). The accessions with reduced DS and high-IT showed partial resistance against powdery mildew were identified under the growth chamber as well as field conditions (Patto et al. 2006a, b, 2007); some of the *Lathyrus. cicera* accessions showed resistance only in the adult stage of plant growth was observed by Vaz Patto et al. (2007). *Lathyrus belinensis,* its hybrids with *Lathyrus odoratus showed* quality resistance to *Erysiphe pisi*, sporelings collapsed immediately after the germination was observed (Poulter et al. 2003). The hybrid plants of *Lathyrus odoratus* × *Lathyrus belinensis* and others which are developed by using *Lathyrus odoratus* for back crossing were found to be resistant to *Erysiphe pisi* by Poulter et al. (2003). Fondevilla et al. (2007) found that continued backcrossing leads to introgression in closely resembling plants the of *Lathyrus odoratus* parent and showed absolute resistance or susceptibility against *Erysiphe pisi* after segregation, with depicting 2.5:1, indicating towards the occurrence of a single resistance gene for resistance. In few cases, *P. sativum* and wild relatives showed partial resistance to *Erysiphe pisi.* It was observed that the moderate resistance ranges are managed by unmarried recessive gene known as *er1* studied by Fondevilla and Rubiales (2012). The gene *er1* also give comprehensive resistance because of its prolonged durability in some locations (Fondevilla et al. 2006). In this, a latest observation confirming resistance shown by *er1* is because of the deterioration in the feature of PdMLO1, a gene MLO coding for mold Resistance Locus O was given by Hamphry et al. (2011). Some single genes even independently are also responsible for providing resistance in pea against *E. pisi.* In powdery mildew inoculation, mixed disease reactions have been a whole lot extra frequent on *Lathyrus sativus* than on *Lathyrus cicera* (Patto et al. 2006a, b, 2007) when pollinated, crossed with susceptible accessions (Patto et al. 2006a, b, 2009) also by Patto and Rubiales (2009). The next generation of such breeding gives authentic information on inheritance of this resistance.

9.2.2 Common Pests in Grasspea

Thrips are most extreme pest of grasspea, and there is very meager data on resistance to thrips except in *Lathyrus aphaca* (Pandey et al. 1995). The Indian accessions JRL6and JLR41 have suggested tolerance to thrips (Asthana 1995). In some instances root knot and cyst nematodes are additional reason to create losses in this legume crop (Cocks et al. 2000) instead, fortunately, resistance was observed in *Lathyrus sativus* by Campbell (1997). Also, Robertson and Abd El-Moneim (1995) mentioned

maximum *Lathyrus cicera* accessions are immune to low temperature, while accessions of *Lathyrus ochrus* and *Lathyrus sativus* are commonly discovered to be very at risk of bloodless. *Lathyrus ochrus*, a Portuguese accession has been observed to be tolerant to cold (Abd El-Moneim and Cocks 1993).

9.2.3 Conventional Methods of Disease Control

Grasspea is attacked by various diseases and pests causing substantial yield losses. We can manage these pathogens with the application of integrated disease management methods to mitigate the field and post-harvest losses. These methods include physical, cultural, and biological methods which could be practicing rotation in crops, varieties with resistance and pesticides in isolation or as an integrated approach. However, indiscriminate and continuous application of pesticides deteriorates the biological, chemical and physical and properties of the soil. It also shows its implications on the non-target organisms and leads to development of resistance in pathogens in opposition to these chemicals (Sharma et al. 2016). For example, *Sclerotium rolfsii* is a soil pathogen causing foot and root trot in numerous crops spices of tropical and subtropical areas of the globe. Foot rot (*F. oxysporum* and *S. rolfsii*) is a very several so destructive disease of pulses in all the legume-growing countries. It results in seedling dying at the very early plant growth stage that leads to very poor plant stand also decrease in yield. Although, this disease is managed by applying chemical pesticides but it aggravates the environmental pollution and creates health hazards too and comes with high economical cost too making is non feasible for the farmers. Hence, the application of biological control agents (BCA's) viz. Arbuscular mycorrhizal fungi (AMF) and Rhizobium can be vital for sustainable agriculture. AMF creates symbiosis with the roots of higher vegetation, and feature a potential to enhance the nutritional popularity of their host plant and to defend them in opposition to various soil-borne plant pathogens (Harrison 1999; Bi et al. 2007). AMF are the major rhizospheric component in many plants and play a vital role in decreasing plant disease incidence as a biocontrol agent. Similarly, use of Rhizobium as a biofertilizer improves the crop productivity and soil fertility as an alternative approach to chemical fertilizers making it economically feasible and environmentally sustainable. In a study by Rahman et al. (2017), it was observed that it improved the nodulation substantially and fixes the nitrogen also under adverse soil conditions. Also, they concluded that AMF species and AMF combinations with rhizobial inoculums have been significant in effective Arbuscular Mycorrhizal symbiosis and lowers the occurrence of foot and root rot disease in grasspea. This study suggested that a dual mixture of AMF p and rhizobium became determined to be simplest in managing the foot and root rot disease of grasspea better to conventional approaches of sole application of the biocontrol agent and hinting toward the established order of sustainable agricultural systems (Barea et al. 1997). Another powerful alternative to the use of standard fertilizers which can contribute to crop disease reduction become shown by means of three lines of *Enterococcus species*, AAUGPR-53, 91 and 92, exhibited

a maximum collection identification (99%) to Enterococcus species and had been used as microbial inoculants for trials under the managed and area conditions. The use of Enterococcus species could be used as a basis for future research leading to figuring out potentially beneficial biocontrol strain found in the rhizosphere of grasspea (Mussa et al. 2018).

9.3 Resources of Resistance Genes

Genetic resistance is considered as the most effective, economical and eco-friendly methodology for managing any plant disease. The resistance breeding in grasspea has many constraints viz. losses, causative agents, and varietal response studies have not often been significantly reviewed (Campbell 1997). The screening for resistance is highly dependent on standardization of methods, as not enough descriptive literature has been found on the resistance sources. The process of germplasm identification and characterization of any crop is the basis of any breeding program was studied by Yunus and Jackson (1991). In-depth information of grasspea's close relatives and their initiation are vital steps in this breeding process (Schaefer et al. 2012). An idea to develop a stable crop classification of plants and their relatives based on cross ability and ease of gene transfer was given by Harlan and de Wet (1971). The improvement of *L. sativus* is done by the exploitation of the germplasm resources using conventional means (Yunus and Jackson 1991). There is a huge potential in landrace material for a higher betterment due to high variability found in the primary gene pool in *Lathyrus sativus* accessions. Yunus and Jackson (1991) were pioneers in identifying the *Lathyrus* gene pools. *sativus* along with *Lathyrus amphicarpos* while *Lathyrus cicera* was positioned to a confined gene pool (Secondary) and few Lathyrus species had been positioned in a gene pool (tertiary extended). Later, Heywood et al. 2007), elongated the secondary *Lathyrus sativus* gene pool with *Lathyrus chrysanthus, Lathyrus gorgoni, Lathyrus. marmoratus* and *L. pseudocicera* (Table 9.1). The rest of the species comprises the gene pool (tertiary). A huge inquisitiveness

Table 9.1 Gene pools of *Lathyrus sativus*

Gene pool (primary)	Gene pool (secondary)	Gene pool (tertiary)
Cultivated and wild races of *L. sativus* races	*L. amphicarpus* *L. blepharicarpus* *L. cicera* *L. choranthus* *L. chrysanthus* *L. gorgoni* *L. hierosolymitanus* *L. hirsutus* *L. marmoratus* *L. pseudocicera*	Other *Lathyrus* spp.

Source Heywood et al. (2007)

is there in exploring secondary gene reservoir of *Lathyrus odoratus* for getting new colors and essence. *Lathyrus odoratus* was bred with *Lathyrus hirsutus*, *Lathyrus chlorantus* by Khawaja (1988) and *Lathyrus belinensis* by Hammet et al. (1994).

9.4 Glimpses on Classical Genetics and Traditional Breeding

9.4.1 Classical Breeding Achievements

Till date, a meager effort has been made in the direction of grasspea improvement despite its numerous benefits. Also, because of recurrent presence of lathyrism in humans, the conventional breeding programs in *Lathyrus* have emphasized on developing varieties with low percentage of β-ODAP in the seed. *L. cicera* or *L. sativus* breeding lines having β-ODAP content approx. 1% from original wild (Kaul et al. 1986; Lal et al. 1986; Roy et al. 1993; Santha and Mehta 2001). *L. sativus* undergoes pollination on its own, but expresses a high percentage of outcrossing also carried out by bees observed by Hanbury et al. (1999). Chowdhury and Slinkard (1997) observed 2% of outcrossing in each generation, but Kaul et al. (1986), found a higher variability ranging from 4–16%. In a study by Hanbury et al. (1999) concluded that outcrossing percentage in *Lathyrus cicera* is almost similar to those of *Lathyrus sativus* due to their similar biology. Because of this outcrossing percentage, grasspea breeding programs are performed in the greenhouse with controlled conditions. The breeding programs in grasspea across the world (India, Canada, Nepal, Bangladesh, ICARDA and Ethiopia) had been doing hybridization with selected lines followed by evaluating the further generations to transmit low ODAP percentage to popular adaptable, better yielding lines with better phenotypic qualities (Campbell 1997). Improved yield is a predominant basis for the selection of the crop development programs. But, few parameters that alters yield include double pods or higher number of seeds in each pod. The biomass yield of *Lathyrus sativus* has started to get hold of interest best since last some years only (Campbell 1997; Abd El Moneim et al. 2001). It has emerged as a completely vital area of study because of the huge capacity of this crop for giving fodder and straw within the Northern areas of Africa and regions of South Asia (Campbell 1997). The expedited efforts on decreasing the β-ODAP content lead to lots of different regions of crop evaluation and improvement which comprises neglecting the resistance to both biotic and abiotic stresses. But, the varieties with low β-ODAP traces, showed an improvement in resistance against standard pests and pathogens has been strengthened. Grasspea is typically cultivated by needy farmers under bad management practices, where it is tough to use chemicals for controlling diseases caused by pathogens and pests. Thus, the improved varieties resistant to conventional disease causing biotic factors viz. pests and pathogens is the call of the day to develop a sturdy pulse crop by incorporating strenuous efforts. Variation noticed within the Lathyrus germplasm gives enormous opportunity to

develop grasspea cultivars by breeding of closely related legume species. *Lathyrus sativus* has shown resistance to powdery mildew viz. *Lathyrus ochrus* and *Lathyrus clymenum* (Gurung et al. 2002), *Lathyrus aphaca* (Pandey et al. 1995) hybrid sweet peas (*L. odoratus* × *L. belinensis*) by Poulter et al. (2003). Researchers in India are working towards the transfer of powdery mildew resistance genes to high-yielding newly added lines (Campbell 1997). Quantitative The resistance to *Erysiphe pisi* is quantitative and occurs because of resistance created by epidermal cells of the host and not by the necrosis of the host cell, was defined in accessions from *Lathyrus sativus* and *Lathyrus cicera* (Patto et al. 2004) and segregating populations are being generated to study its genetic control (Patto et al. 2006a, b). The Slovakian grasspea germplasm showed resistance against *Fusarium oxysporum* (Benková and Záková 2001). *Lathyrus* improvement programs are focusing on these breeding priorities for resistance to broomrape, several fungal diseases (Campbell et al. 1994).

9.5 Diversity Analysis

Genetic diversity is an essential requisite for continuation of plant progeny and crop development. diversity in genetic sources offer possibility to plant breeders for developing new and advanced cultivars containing suitable traits which might be farmer (high yield, bold type seed etc.) and friendly for breeders (pathogen resistance and photosensitivity etc.). Crop evolution relies on the available genetic diversity in the test population through natural or human choice. Diversity is the gradation of variability among or within species. The occurrence of variations due to intra-specific and inter-specific variations is the basis of all crop improvement programs (Bhandari et al. 2017). Because the initiation of step wise plant breeding, study of variability and degree of divergence in crop plants have been marked to improve the crop species. Genetic diversity in plants is affected by various factors. Natural powers viz. selection, migration, mutation along with genetic forces the ongoing changes in the frequency of alleles within a population creates the genetic diversity. The prominent centres of diversity in *Lathyrus* L. extends from Mediterranean to Irano-Turanian regions (Kupicha 1983). International conservation policy recognizes biodiversity at three levels, ecosystem, species and genetic, and that management should aim to retain all three (Convention on Biological Diversity 2007). Genetic diversity in the *Lathyrus* genus can be detected by means of diverse molecular markers, including, among others, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and sequence related amplified polymorphism (SRAP) (Chtourou-Ghorbel et al. 2001; Tavoletti and Iommarini 2007; Nosrati et al. 2012; Marghali et al. 2016). The study of genetic diversity between *Lathyrus sativus* L. and its relative species may yield fundamental insights into evolutionary history and provide options to meet the challenge of climate changes. Thirty simple sequence repeat (SSR) loci were employed to assess the genetic diversity and population structure of 283 individuals from wild and domesticated populations of *Lathyrus sativus* L. from Asia, Africa, Europe, and

Table 9.2 *Lathyrus* diversity in biotic stress resistance

Stress	Germplasm	References
Melaidogyne halpa (root knot nematode)	*L. latifolius, L. sylvestris, L. hirsutus*	Rumbaugh and Griffin (1992)
Fusarium oxysporum	*L. sativus* (Slovakia)	Benková and Záková (2001)
Mycosphaerella pinodes	*L. sativus* (worldwide)	Gurung et al. (2002)
Erysiphe pisi (powdery mildew)	*L. sativus, L. cicero* (India, Syria, Iberian Peninsula)	Campbell et al. (1994), Robertson and Abd El Moneim (1996)
Uromyces pisi (rust)	*L. sativus, L. cicera* (Iberian Peninsula)	Asthana and Dixit (1998), Vaz Patto et al. (2006a, 2007)
Orobanche crenata (broomrape)	*L. sativus, L. cicero,* eight other *Lathryus sp.* (worldwide)	Sillero et al. (2005)

ICARDA. The number of alleles per loci varied from 3 to 14. The average gene diversity index was observed to be 0.5340 and average polymorphism information content (PIC) was 0.4817 (Wang et al. 2015). It provided a basis for understanding genetic diversity of *Lathyrus sativus* and its relatives at molecular level. Many evaluation studies related to diversity in biotic stress resistance of Lathyrus germplasm has been carried out by various scientists (Table 9.2) with variable outcomes and slight discrepancy in pathogens linked to diseases. For example, powdery mildew infecting the Lathyrus is mainly *Erysiphe pisi*, but it is also a possibility that various other species can infect *Lathyrus* sp., as in pea it has been observed recently (Fondevilla et al. 2013). The existence of specialized forms and races continues to be ambiguous, however a unique ability to infect distinctive plant species has been documented. It is observed that a strain of *E. pisi* accumulated on *L. odoratus* can infect faba bean but not pea, while every other strain accumulated from *L. latifolius* become able to infect pea and faba bean (Cook and Fox 1992). Further, *Lathyrus* sp. infected with the aid of rust is determined to be because of *Uromyces pisi* and *U. viciae-fabae both* (Barilli et al. 2011, 2012).

9.5.1 Phenotype-Based Diversity Analysis

Morphological variation studies by Jackson and Yunus (1984) on Lathyrus accessions from across the globe revealed the largely differentiate able into numerous distinct forms, based on color of the flower, seed size and leaf sizes in *L. sativus*. Thus, a discreet distinction between the blue coloured flowers from Indian subcontinent, South-west Asia and Ethiopia and the white-blue-coloured flowers with white seeds that are spread in the west. This variation is possibly due to the geographical separation and selection by man. This grouping of white-seeded with large seeds

Table 9.3 Losses in *Lathyrus sativus* due to biotic stresses

Broomrape (*Orobanche* sp.) Downy mildew (*Peronospora* sp.) Powdery mildew (*Erysiphe* sp.)	Major diseases	Campbell et al. (1994), Linke et al. (1993), Pandey et al. (1995)
Ascochyta blight (*Ascochyta pisi* and *Mychospaerella pinodes*) Grey mold (*Botrytis* sp.) Rust (*Uromyces* sp.)	Diseases causing damage to some extent	Cocks et al. (2000), Pandey et al. (1995)
Aphids (e.g. *Aphis craccivora*) Thrips (*Caliothrips indicus*) Pod borer (*Etiala jhinkinella*)	Serious pest in India, Bangladesh, Ethiopia and Nepal	Pandey et al. (1995)

originating from Europe and North Africa while the colored-seeded with relatively small seeds originating from Asia and Ethiopia was in congruence to studies by Przybylska et al. (1998, 2000) on the basis of quality based on quality analysis and based on agronomic testing by Hanbury et al. (1999). Those lines which have originated from Mediterranean or European were high yielding with large seed sizes and later phenology (Hanbury et al. 1999). They also showed a lower ODAP content (Abd El Moneim et al. 2001). These areas have a preference for larger seed varieties as well as to other grain legumes such as lentil (*Lens culinaris*), chickpea (*Cicerarietinum*) and faba bean (*Vicia faba*) (Chowdhury and Slinkard 2000). Analogous studies on the assessment of grasspea landraces under the field conditions demonstrated high variation in morphological and agronomical traits. Such studies were also carried out in Chilean, Ethiopian, Italian, Indian, Spanish and Slovak germplasm (Benková and Záková, 2001; Kumari 2001; Tay et al. 2000; Tadesse and Tavoletti et al. 2005; De la Rosa and Martín 2001). Diversity among and within populations exhibited a high breeding potential (Table 9.3).

9.5.2 Molecular Markers Assisted Assessment

Molecular markers are important in plant genome studies to envision the diversity in genetic sources, assessing the plants genotype, genetic maps construction, as a probe for screening of traits, tagging of traits and genome mapping, to estimate the hybrid overall performance and identification and marker assisted selection (Shanmuga et al. 2011). They have the capacity to transfer genes from different organisms which has an impact on genomic research in plant breeding. These methodologies offer new era for upgrading prominent agronomic traits in Lathyrus and diverting the gene transfer barriers to related legume species. Molecular markers are attached to *E. pisi* and *U. pisi,* genes involved in conferring resistance or quantitative trait loci (QTLs) are available in pea and due to the phylogenetic relation between *P. sativum* and *L. sativus* or *L. cicera* (Wojciechowski et al. 2004) could be used for marker

assisted selection (MAS) in *Lathyrus*. Although, molecular marker based studies have been quite successful among these three species (Almeida et al. 2013a, b) but none of the SSR markers linked with pea resistance such as that conferred by *er1* for *E. pisi* (Ek et al. 2005) or given by the QTL *Qruf* for *U. fabae* resistance (Rai et al. 2011) was cross amplifiable to *L. sativus* or *L. cicera* (Almeida et al. 2013a, b). Likewise, if the genes conferring resistance in *Lathyrus* will be identified, they could also be used for resistance improvement in the *Lens* and *Vicia* sp. The present array of resistance mechanisms against rust and powdery mildew could be increased by phylogenetically related *Vavilovia* and *Pisum* (Schaefer et al. 2012). The SSR markers are still in wide use, are co-dominant, high polymorphism and ubiquitous in many eukaryotic species, highly repeatable and are user friendly. SSR is a strong marker for evaluating germplasm and modern breeding. Various research groups use these markers in genetic diversity, DNA fingerprinting, genetic linkage map, QTL mapping and mining of alleles. However, very few SSR markers are useable for this orphan crop in comparison to other crops (Almeida et al. 2014). Hao et al. (2017) implemented RNA Seq using two different accessions of grasspea, and RNAs isolated from root, stem and leaf tissues were sequenced and 5916 SSR markers from the resulting sequence data were identified, designed primer pairs were designed and 284 of these markers were validated. Their studies revealed that 87 (30.6%) SSRs markers were polymorphic and 88 (31.0%) were found to be monomorphic. The rest of the marked SSRs had no specific target bands or were found to be very complex to be identified. Kompetitive allele specific PCR (KASP) markers are strong tool for testing single nucleotide polymorphism (SNP). Though, there are many methods for testing SNP's viz. as Taqman assay, allele-specific PCR, DNA microarray-based SNP genotyping but it is costly (Singh and Singh 2015). A recent technique of SNPs is KASP which relies on PCR using specific alleles using forward primers (two) and a reverse primer (one) (Graves et al. 2016; Lister et al. 2013). This technique is highly precise but cost of application is high (Khera et al. 2013). Also, in a research by Hao et al. 2017 in grasspea, they concluded that more than 80% SNP loci were turned into KASP markers. The dendrograms developed using SSR and KASP markers proved that the different markers are not absolutely consistent.

9.6 Mapping of Resistance Genes and QTLs

Mapping of quantitative trait loci (QTLs) leads to detection of the genes with combined effect and the identification of the molecular markers which could be used for stacking of genes and breeding for developing stable rust resistance (Soriano and Royo 2015). Backcross population based first linkage map was developed in *Lathyrus sativus* and the QTLs associated with resistance to ascochyta blight was also investigated (Skiba et al. 2004b). QTL detection for Ascochyta blight resistance was done by Skiba et al. (2004a, b), Three markers (Cf-9, B04_1100, M16_500) have been recognized as being related to ascochyta blight resistance, at an LRS threshold

of 9.22 by single-factor analysis. These markers have been placed in a single, continuous location on linkage group 1. farther two markers, located collectively on linkage group 3, P10_1200 and B07_1400, fell quick of the LRS threshold; but, the *P*-values had been <0.05, suggesting that those markers may be extensively related to a QTL. First RNA sequencing-derived markers based genetic linkage map was developed in *Lathyrus cicero* using a RIL population. In this study, transcriptome of *Lathyrus cicero* was analysed in reaction to rust (*Uromyces pisi*) infection with the objective of identification of the candidate resistance gene/genes conferring resistance against rust. The map contained 307 markers, which covered around 724.2 cm also organised in seven major and two minor linkage groups, with an average mapping distance of 2.4 cm. Powdery mildews are considered as one of the most important fungal diseases for a wide range of crop species. The loss of function mutation specifically at *Mildew Locus O* (*MLO*) has long been discovered to be associated with plant resistance to powdery mildew disease. Recently the location of the Lathyrus *MLO1* gene have been mapped on the linkage map developed using a *Lathyrus sativus* RIL population.

9.7 Molecular and Genomics-Assisted Breeding

With the advancement of new sequencing technologies and SNP genotyping with high throughput methodologies facilitated the construction of genetic maps with high density in wheat (Maccaferri et al. 2015; Wang et al. 2014; Winfield et al. 2016), augmenting our potential to extract the economically important traits such as disease resistance (Kthiri et al. 2019). In a study by Hao et al. (2017), KASP method proven 50 SNP primers amongst 43 grasspea genotypes. The outcomes confirmed polymorphism in the array of the 40 SNPs where SNPs came out to be monomorphic. Although, SNPs are crucial and used on a larger scale due to their stability and high-throughput outcome. The ability of SNPs for exceptionally efficient and accurate for gene detection makes them superior from other markers (Klepadlo et al. 2017). This primary gene expression data of the *Lathyrus sativus* against ascochyta infection delivered a precious candidate resistance genes which could be for precision breeding in future by Hao et al. (2017). DeepSuperSAGE investigation was used to obtain a genome-wide overview of the response of the transcriptome of a resistant *L. sativus* genotype to *A. lathyri* infection in comparison to a non-inoculated control. DeepSuperSAGE analysis was done on a genotype resistant to ascochyta blight by using inoculated plants and control, generated 14.387 UniTags. Recently a reference grasspea and rust interaction transcriptome assembly mapped gave a 95.7% match to this analysis (Almeida et al. 2014). Out of total mapped UniTags, 738 were expressed differentially among the control and inoculated, 625 of which were annotated in public domain of plant databases. Genomic tools have enabled a global view of transcriptome changes during the these interactions between plant and pathogens, from which several key players in both the resistant and susceptible interactions have been identified (Kankanala et al. 2019). Till date, the functional studies in Lathyrus using biotechnological tools have not been explored well. The expression analysis

studies of *Mycosphaerella pinodes* was done on *Lathyrus sativus* by inoculating with 29 expressed sequence tags (ESTs) with genes representing the codes for actively involved enzymes and proteins in the defence (Skiba et al. 2005). A few EST libraries in Lathyrus have been found to be helpful in the construction of genetic linkage maps with high-density for identifying the molecular markers, marking and assessing the function of putative genes which are involved in resistance in *Lathyrus sativus* and *Lathyrus cicera* against rust (Almeida et al. 2012). The *Lathyrus sativus–Ascochyta* sp. interface has been studied using NGS through SuperSAGE analysis for quantitative gene expression (Almeida et al. 2013a, b). Gurung and Pang (2011) constructed EST libraries in Lathyrus using seeds and pods to achieve a mark in the reproductive tissues. Gurung and Pang (2011), concluded that present populations with mutants are not suitable to identify the effects of gene deletions or silencing by applying targeting-induced local lesions in genome (TILLING). Mutation breeding has been applied hired on numerous occasions to for creating higher variability in numerous traits e.g. plant growth, ODAP, methionine or lysine content (Patto et al. 2011). Tissue culture studies have also revealed high variation in different morphological traits, and thus have been explored at large in breeding programs in Lathyrus (Kumar et al. 2011).

9.8 Social, Political and Regulatory Issues

Grasspea has been considered toxic causing a neurotoxic disease "neurolathyrism" due overconsumption under certain circumstances (Lambein and Kuo 2009). The long intake of grasspea has lead to disabling effects due to the presence of the neurotoxin b-N-oxalyl-L-a,b-diaminopropionic acid (ODAP). Thus, it was recommended to be avoided as food and selling of seeds were also prohibited in few countries (Enneking 2011). However, in the present scenario of increased demand for resilient food crops, national and international research centers are focusing on prioritizing the improvement of grasspea (Patto and Rubiales 2014). Many cultivars with low ODAP are being released in the last 50 years exploiting the grasspea breeding (Kumar et al. 2011). Also, it has been observed that ODAP content does not cause any disability as a sole component because grasspea if consumed within the limits of balanced diet does not cause any harm to humans and animals (Getahun et al. 2002, 2003, 2005) and similar was found by Lambein and Kuo (2009). The detoxing of seeds in grasspea may be executed by methods including fermentation, pre-soaking in alkaline solutions or with the aid of cooking (Kumar et al. 2011; Kuo et al. 2000). Llorens et al. (2011), have given a hypothesis recently reporting that nitriles cause neurolathyrism instead of ODAP. Also, potential pharmacological benefits of ODAP should not be overlooked (Lan et al. 2013). Recently, the Department of Biotechnology, Ministry of Science and Technology, Government of India felt its importance in national economy and nutritional security, and supported the activity for popularizing grasspea cultivation of low ODAP varieties (Ratan and Prateek) in Bihar under Biotech-KISAN hub at Bihar Agricultural University, Sabour with the title"

Revival of GrassPea Cultivation in Bihar. India is the largest producer and consumer of pulses, thus it is imperative that it becomes self-sufficient in the pulse production and ensuring nutritional security of common masses towards: Atmanirbhar bharat. Grasspea once being an economically feasible crop in India but lost its popularity due presence of high ODAP content, thus a ban was imposed but in 2016, it was lifted by the Government considering the role of GP in feed, fodder and protein fulfilling crop. In 2018, to popularize the varieties Ratan (Bio L 212) and Prateek with low ODAP, having <0.1% ODAP content in their seeds which needs demonstration in farmer's field. Many severe legislative measures have been laid by many countries, the area underneath grasspea is continuously decreasing extensively since last many years. Though, many varieties containing lower amounts of toxin had been developed by the pioneer institutes like ICAR and other agricultural institutes and but farmers are still taking up the crops with higher value. Therefore, a steep reduction has been observed in the area and productivity of grasspea across India. The countrywide area of grasspea has decreased from 1.67 to 0.58 mha during the last four decades. A comparable pattern is observed in its production, which has reduced drastically from 0.84 million to 0.43 million tons during the same period of time (http://agricoop.nic.in/) (Dixit et al. 2016).

9.9 Future Perspectives

Grasspea (*Lathyrus sativus* L.) is an 'orphan crop' which has high potential due to its capacity to survive in adverse situations of biotic stresses. However, grasspea is less studied due to its less available genomic information and slow breeding process. Despite being a crop which is considered "minor", grasspea is an indispensable crop in the arid regions or sometimes in semi-arid conditions (Dixit et al. 2016; Patto et al. 2006a, b; Yan et al. 2006). Many researchers have emphasized recently on Lathyrus and its relatives from wild (*Lathyrus cicera*) due to their high inherent resistance to biotic stresses (Wang et al. 2015). Although, it is difficult to cultivate grasspea at a larger scale for agricultural output worldwide due to its large size of genome (8.2 Gb), outcrossing percentage (2–30%) (Chowdury and Slinkard 1997; Rahman et al. 1995) and the occurrence of β-ODAP (Hillocks and Maruthi 2012). The application of Next Generation Sequencing for the genes with complex traits is simpler to elucidate using RNA-Seq, RAD-Seq and GBS technologies (Singh and Singh 2015).

9.9.1 Potential for Expansion of Productivity

Lathyrus, a legume crop if grown after rice can improve the soil fertility and soil health. The areas practicing monocropping in rice permits the additional crop cultivation with a potential of using soil moisture to convert areas with monocropping into

areas with double-cropping leading to an increase in the productivity and sustainability. Also, a large chunk of small landholdings are the sole option for with poor farmers and that holds the key to subsistence farming by incurring income with limited options could be exploited for growing this legume crop. However, these kind of expansions could be done using the varieties with low toxin contents, well suited to mechanized farming and relay cropping (Dixit et al. 2016). Some of the parameters which could increase the productivity by extensive research and field trials are extensive germplasm from unexplored regions by collection, conservation, and evaluation, development of genotypes with lower ODAP, breeding focus should be targeted on increased forage and fodder production, study of mechanisms involved in tolerance against droughts, recognition of resistance sources to biotic stresses, identification and alteration of enzymes involved in ODAP production at genetic level, upgrading the ODAP detoxification method with higher accuracy, and development of saturated linkage maps. Genes conferring disease resistance genes in cultivars can bust in the field due to the high selection pressure during co-evolution of the pathogens. Thus, crop protection against pathogens is a continuous defense. The attack by many pathogens on legumes makes it very susceptible. In the modern-day genomic era, high-throughput which are low cost and powerful genomic tools are rampant, has changed our mindset about the interactions among legumes and pathogens (Kankanala et al. 2019). A thorough study of resistance mechanism in addition to breeding for resistance will assist in gaining high productiveness in grasspea particularly in dry or drought-inclined zones because of worldwide weather change within the coming times.

9.9.2 Potential for Expansion into Nontraditional Areas

In a study by Rao (2011), nitric oxide biosynthesis could occur alternately by a component "homoarginine" found in *L. sativus*. Nitric oxide plays vital role in cardiovascular system and overall health, thus a regular intake of homoarginine in small quantities of *L. sativus* can be beneficial and requires apt attention. ODAP is also responsible for can also initiate the spark the protein kinase C (PKC) which has opened new avenues in research for its use in therapy in the sphere of neurons and memory related diseases such as Alzheimer's disease (Rao and Northup 2011). Genetic variation has been observed in the germplasm of *L. sativus* with homoarginine content (Piergiovanni and Damascelli 2011). It can be use as functional food due to their higher antioxidant activity in its polyphenols besides other legumes e.g. soybean (*Glycine max*) chickpea, lupin (*Lupinus* sp.) (Pastor-Cavada et al. 2009). Additionally, Paneda et al. (2001) also concluded that *L. sativus* seeds could be used in ameliorating diabetic symptoms by changes in insulin-mimetic process.

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