Chittaranjan Kole *Editor*

Genomic Designing for Biotic Stress Resistant Vegetable Crops

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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 and gratitude for his generous appreciation 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 the emergence of new diseases and insects together with newer biotypes, and physiological races of the causal agents on 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 the world population by 2050 is about 9.3 billion which would necessitate an increase in crop production by about 70%. On the other hand, the additional losses due to climate change and global warming somewhere in the range of 10 to 15% should be minimized. Therefore, an increase in the crop yield as well as minimization of its loss should be practiced simultaneously focusing both on 'adaptation' and 'mitigation'.

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

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

Knowledge of 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 the 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 the possibility of multiple utilities. For this purpose of 'precise' breeding, employment of the genetic and genomic techniques individually or in combination as and when required will play a crucial role.

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

There are obviously a number of reviews and books on the individual aspects of plant molecular breeding, genetic engineering and genomics-aided breeding on crops or on agro-economic traits which include the 100-plus books edited by me. However, there are no comprehensive reviews or books available that have 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 Vegetable Crops" includes nine chapters focused on Tomato, Potato, Pepper, Eggplant, Vegetable Brassicas, Cucurbits, Onion and Garlic, Vegetable Amaranths and Carrot contributed by 49 scientists from 9 countries including Canada, Egypt, India, Italy, Norway, Republic of Korea, Spain, Uruguay 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 ambiance to pursue my efforts for science and society pleasantly and peacefully.

New Delhi, India Chittaranjan Kole

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Abbreviations

6-MM	6-Methoxymellein
ABA	Abscisic acid
AFLP	Amplified fragment length polymorphism
AG	Anastomosis group
AH	Alpha-helical
AMF	Arbuscular Mycorrhizal Fungi
AmLMV	*Amaranthus leaf mottle virus*
AMoV	*Amaranthus mosaic virus*
AMP	Antimicrobial Peptides
ARS	Agricultural Research Service
AUDPC	Area under the disease progress curve
AVRDC	Asian Vegetable Research and Development Centre
AYSYN	Aster yellows synthetic
BAC	Bacterial artificial chromosome
BC	Backcross
BLAST	Basic local alignment search tool
BLTVA	Beet leafhopper transmitted virescence agent
BMT	Biochemical and molecular technique
BS	Bacterial spot
BSA	Bulked segregant analysis
CaCV	*Capsicum chlorosis virus*
CAPS	Cleaved amplified polymorphic sequence
CarVY	*Carrot virus Y*
Cas9	CRISPR-associated protein 9
CAT	Catalase
CDS	Coding sequence
Ce	Cercospora leaf spot
CFU	Colony-forming unit
CGIAR	Consultative Group on International Agricultural Research
ChiLCV	*Chili leaf curl virus*
ChiVMV	*Chili veinal mottle virus*

CIFP	Centro de Investigaciones Fitoecogenéticas de Pairumani
CIP	International Potato Centre
cM	CentiMorgan
CMS	Cytoplasmic male sterility
CMV	*Cucumber mosaic virus*
CMVY	*Cucumber mosaic cucumovirus Y*
CNT	Multi-walled carbon nanotube
CPC	Commonwealth potato collection
CR	Clubroot
CRISPR	Clustered regularly interspaced short palindromic repeats
CVYV	*Cucumber vein yellowing virus*
CWE	Compost water extract
CWR	Crop wild relative
DArT	Diversity array technology
DAS-ELISA	Double antibody sandwich ELISA
DBD	DNA binding domain
DBM	Diamondback moth
DDBJ	DNA Data Bank in Japan
DDI	Domain–domain interaction
DH	Doubled haploid
DM	Doubled monoploid
DREB	Dehydration responsive element binding
DSB	Double strand break
DUS	Distinctness, uniformity and stability
EB	Early blight
EBN	Endosperm balance number
ECPD	European Cultivated Potato Database
Eh	*Erysiphe heraceli*
ELISAs	Enzyme-linked immunosorbent assays
EMBL	European Molecular Biology Laboratory
EMS	Ethylmethane-sulfonate
EST	Expressed sequence tag
ET	Ethylene
ETI	Effector triggered immunity
FAO	Food and Agriculture Organization
FAOSTAT	FAO Corporate Statistical Database
FBR	Fusarium basal rot
FCPri	Fruit calyx prickliness
FCRR	Fusarium crown and root rot
FISH	Fluorescence *in situ* hybridization
Foc	*Fusarium oxysporum* f. sp.*conglutinans*
FOC	*Fusarium oxysporum* f. sp. *Cepae*
Fol	*Fusarium oxysporum* f. sp. *lycopersici*
FS	Fruit shape
FW	Fruit weight

FW	Fusarium wilt
G×E	Genotype–environment interaction
GBNV	Groundnut bud necrosis virus
GBS	Genotyping by sequencing
GD	Genomic designing
GE	Genome editing
GGE	Genotypes and genotype by environment
GM	Genetically modified
GMO	Genetically modified organism
GMS	Genetic male sterility
GO	Gene ontology
gRNA	Guide RNA
GRSV	*Groundnut ringspot virus*
GS	Genomic selection
GS	Gene silencing
GWA	Genome-wide association
GWAS	Genome-wide association study/studies
Hab	Plant growth habit
HdR	Homology-directed repair
HDR	Homology-dependent repair
H_E	Expected heterozygosity
HEN	Homing endonuclease
HR	Hypersensitive response
HTG	High-throughput genotyping
HTP	High-throughput phenotyping
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
IIHR	Indian Institute of Horticultural Research
IIVR	Indian Institute of Vegetable Research
IMP	Integrated pest management
InDel	Insertion/deletion
INIAP	Instituto de Investigaciones Agropecuarias
INIFAP	Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias
IPM	Integrated pest management
IRCS	Inducer resistance chemicals
ISSR	Inter-simple sequence repeat
iTAG	International Tomato Annotation Group
ITR	Internal transcribed region
ITS	Internal transcribed spacer
IYSV	Iris yellow spot virus
JA	Jasmonic acid
LB	Late blight
LD	Linkage disequilibrium
LD	Long day

LePri	Leaf prickliness
LG	Linkage group
LOD	Logarithm of odds
LRR	Leucine-rich repeat
MAB	Marker-assisted backcrossing
MAGIC	Multi-parent advanced generation intercross
MAPK	Mitogen-activated protein kinases
MAS	Marker-assisted selection
Mbp	Million base pair
MeBr	Methyl bromide
Mh	*Meloidogyne hapla*
miRNA	MicroRNA
Mj	*Meloidogyne javanica*
mRNA	Messenger RNA
MSN	Mesoporous silica nanoparticle
MTA	Marker trait association
NAM	Nested association mapping
NBPGR	National Bureau of Plant Genetic Resources
NBS	Nucleotide binding site
NCBI	National Center of Biological Information
NCRPIS	North Central Regional Plant Introduction Station
NGS	Next generation sequencing
NHEJ	Non-homologous end joining
NIAB	National Institute of Agricultural Botany
NILs	Near-isogenic lines
NLR	Nucleotide binding domain and leucine-rich repeat
NMR	Nuclear magnetic resonance microscopy
NM	Nanomaterial
NP	Nanoparticle
NPGS	National Plant Germplasm System
NTSR	Non-target site resistance
ODC	Ornithine decarboxylase
PA	Polyamine
PAL	Phenylalanine ammonia lyase
PAM	Protospacer adjacent motif
PAMP	Pathogen-associated molecular pattern
PCD	Programmed cell death
PCN	Potato cyst nematode
PCR	Polymerase chain reaction
PepGMV	*Pepper golden mosaic virus*
PepLCV	*Pepper leaf curl virus*
PepSMoV	*Pepper severe mottle virus*
PeYV	*Pepper yellow virus*
PGI	Potato Genome Identification
PGSC	Potato Genome Sequencing Consortium

PHYVV	*Pepper Huasteco yellow vein virus*
PIC	Polymorphism information content
PLRV	*Potato leaf roll virus*
PM	Powdery mildew
PMMoV/PepMoV	*Pepper mild mottle virus*
POX	Peroxidase
PPO	Polyphenol oxidase
PPR	Plant recognition receptor
PPV& FR	Protection of Plant Varieties and Farmers' Rights
PR	Pathogenesis-related
PSTVd	Potato spindle tuber viroid
PTC	Purple Turkey Carrot
PTGS	Post-transcriptional gene silencing
PTI	PAMP triggered immunity
PTIR	Predicted tomato interactome resource
PVM	*Potato virus M*
PVMV	*Pepper veinal mottle virus*
PVS	*Potato virus S*
PVX	*Potato virus X*
PVY	*Potato virus Y*
PYFV	Parsnip yellow fleck virus
qRT-PCR	Quantitative reverse transcription PCR
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
R gene	Resistant gene
RAPD	Random amplified polymorphic DNA
RDR	RNA-dependent RNA polymerase
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RILs	Recombinant inbred lines
RISC	RNA-induced silencing complex
RKN	Root-knot nematode
RLK	Receptor-like kinase
RLP	Receptor-like protein
RNAi	RNA-interference
RNA-seq	RNA-sequencing
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
SCAR	Sequence characterized amplified region
SCoT	Start codon targeted
SD	Short day
siRNA	Small interfering RNA
SIX	Secreted in xylem
SLon	Seed locule

SLS	Septoria leaf spot
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SOL	International Solanaceae Genome Project
SSCP	Single-stranded conformation polymorphism
SSN	Sequence-specific nuclease
SSR	Simple sequence repeat
STS	Sequence-tagged site
STTM	Short tandem target mimic
TAC	Transformation-competent artificial chromosome
TALE	Transcription activator-like effector
TALEN	Transcription activator-like effector nuclease
TCSV	*Tomato chlorotic spot virus*
TeMV	*Telfairia mosaic virus*
TF	Transcription factor
TGMV	*Tomato golden mosaic virus*
TGS	Transcriptional gene silencing
TILLING	Targeting-induced local lesions in genomes
TMV	*Tobacco mosaic virus*
TMX	Thiamethoxam
TNAU	Tamil Nadu Agricultural University
ToBRFV	*Tomato brown rugose fruit virus*
ToLCB	Tomato leaf curl betasatellite
ToLCNDV	*Tomato leaf curl New Delhi virus*
TP	Training population
TRV	*Tobacco rattle virus*
TSP	Trisodium phosphate
TSWV	*Tomato spotted wilt virus*
TuMV	*Turnip mosaic virus*
TYLCV	*Tomato yellow leaf curl virus*
UNAP	Universidad Nacional del Altiplano
UNSAAC	Universidad Nacional de San Antonio Abad del Cusco
UPOV	International Union for the Protection of (New) Plant Varieties
USDA	United States Department of Agriculture
UTRs	Untranslated region
VIGS	Virus-induced gene silencing
VRS	Vegetable Research Station
VW	Verticillium wilt
WBP	Wisconsin Carrot Breeding Program
WCR	Wisconsin carrot inbred
WES	Whole-exome sequencing
WFP	World Food Programme
WGRS	Whole-genome re-sequencing
WGS	Whole-genome sequencing
WVC	World Vegetable Center

Xcc	*Xanthomonas campestris*
YAC	Yeast artificial chromosome
ZFN	Zinc-finger nuclease
ZYMV	*Zucchini yellow mosaic virus*

Chapter 1
Genomic Tools for Improving Tomato to Biotic Stress Resistance

Ciro Gianmaria Amoroso, Dilip R. Panthee, Giuseppe Andolfo, Felipe Palau Ramìrez, and Maria Raffaella Ercolano

Abstract Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops. It also represents a model plant for studying genetic traits related to disease and pest resistance and molecular processes underlying plant-pathogen interactions mechanisms. Tomato crop can be endangered by stressful conditions, which can cause intensively yield lost in temperate areas. In the next years, it has been forecast that rising temperature and CO_2 levels, will affect agricultural production globally. The sequencing of tomato reference genome (*S. lycopersicum* Heinz 1706) allowed to improve our knowledge on important agronomic traits. In this species, important breeding achievements have been obtained thanks to extensive molecular mapping and molecular assisted selection (MAS) efforts. The advent of genomic-based technologies facilitated the identification of genes involved in tomato biotic stress and the design of more tailored varieties. Databases collected on tomato large-scale data were developed and are available to support the identification of genetic resources, markers, key genes, proteins and biochemical processes involved in biotic stress resistance. Different plant genetic engineering approaches were applied to promote more precise genome modification processes. Stable or transient plant transformations can be used to develop new resistant tomato lines able to adapt to the rapid climate

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changes and new diseases spreading. To date, laws about genetic modified (GM) tomatoes are quite stringent in many countries, but researchers made great progress using alternative biotechnological methodologies, based on DNA repair mechanisms such as genome editing technology, able to generate short insertion/deletion (InDel) in specific genomic locations leading to highly selective mutation. The current legal system on plant variety rights should be updated according to new biotechnological advances. The increasing knowledge on tomato overall response to biotic stress, including genome signature, gene identification, proteins and metabolite function combined to emerging biotechnological methodologies will unfold the full potential for accelerating tomato breeding for biotic stress resistance.

Keywords *Lycopesicon esculentum* · Disease resistance · Sequencing · Molecular markers · Database · Biotechnology · Plant-breeding rights

1.1 Introduction

1.1.1 Economic Importance of Tomato

Tomato (*Solanum lycopersicum* L.) is a species native of South America belonging to Solanaceae family that includes many other economically important vegetable crops such as potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), and eggplant (*Solanum melongena* L.). Tomato production in 2019 reached a worldwide global value of 182 million tons with a cultivated area of 4.8 million hectares. More than 60% of total production is concentrated in Asia, followed by Europe, America, and Africa with 13.5%, 13.4%, 11.8% of total production, respectively (FAOSTAT 2019). A picture of the economic importance of tomato worldwide is given by its global market value. The six major countries playing a significant role in the tomato international market are USA, Spain, Portugal, Italy, China and India (Fig. 1.1), which in 2018 produced a total revenue of $190.4 billion with an average annual rate of increase of 3% in the previous 10 years.

The economic and nutritional importance of tomato, place it among the most widely studied crop, becoming a plant model to understand molecular process related to development, fruit metabolism, and plant pathogen interaction (Liu et al. 2018; Quinet et al. 2019). Tomato genome sequence released in 2012 represents an important resource for the improvement of agronomic traits, becoming in few years an essential tool for basic and applied research (Tomato Genome Consortium 2012; Sahu and Chattopadhyay 2017).

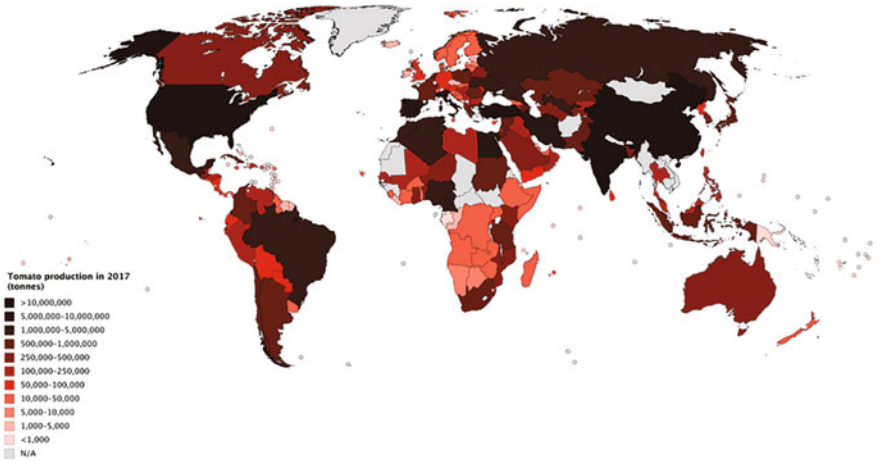

Fig. 1.1 Tomato production in tons, based on data from the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT 2017)

1.1.2 Reduction in Yield and Quality Due to Stress

Severe yield losses due to major pests and diseases can cause considerable yield and fruit quality reduction in tomato (Severin et al. 2001). Several diseases are caused by bacteria (*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *syringae*) fungi (*Alternaria porri* f. sp. *solani*, *Cladosporium fulvum*, *Phytophthora infestans*, *Verticillium dahliae* and *Fusarium oxysporum*) and virus such as *Tobacco Mosaic Virus* (TMV), *Tomato Spotted Wilt Virus* (TSWV), *Tomato Yellow Leaf Curl Virus* (TYLCV) and *Tomato Brown Rugose Fruit Virus* (ToBRFV) (Thompson and Tepfer 2010; Mândru et al. 2017). High atmospheric humidity and the presence of drops of water on the foliage can promote infection of *Phytophthora infestans, Xanthomonas campestris* pv. *Vesicatoria,* and *Pseudomonas syringae* pv. *syringae* (Costache et al. 2007; Tamir-Ariel 2007). *Cladosporium fulvum* in favorable conditions may cause premature defoliation, affecting the photosynthetic activity of affected plants and the consequent productions (Babadoost 2011). *Alternaria porri* f. sp. *solani* and other major tomato pathogens, can cause collar rot in the basal part, leaf and stem stains and rotting of fruits (Walker 1952). Sometimes biotic and abiotic stresses can act synergistically or additively causing stronger symptoms and serious damages (Cappetta et al. 2020a, b). Some studies showed that modulating the reactive oxygen species (ROS) response could be an important way to improve plant multi-stress tolerance (Sewelam et al. 2016). Depending on the plant stage and duration of the stress and interaction with other stresses yield loss can increase up to 70%. Taken together these data point out that if tomato stresses are not adequately treated it can lead to more than $133 billions of economic losses every year.

1.1.3 Impact of Climate Change

The major agricultural challenge is to provide food and nutritional security to the annually growing global population. Tomato world consumption is increasing from year to year. In 2018/2019 the estimated global consumption was 38.3 million mT (raw material equivalent) with an 8% increase against the previous year (35.5 million mT) and 4% increase compared to the average of the three previous years (Branthôme 2020).

Countries that typically showed the highest tomato consumption belong to the North American and Western European nations that to date remains the main commercial route for tomato products. However, it is important to highlight the increasing importance in the global market of emerging regions especially in the Middle East, South America, the Far East, and West Africa. Tus, the increasing tomato demand places these markets at the same level of the "classical" markets of America and Europe demand of which is in slightly decline; in total these two areas are accounted for approximately the 44% of world tomato consumption. It seems that on mentioned markets are growing fast from the beginning of the new millennium, and it is probable that in the next years they will reach a complete "maturity".

It is known that the climate is changing, average temperatures of our planet have risen about 1 grade Celsius over the last 200 years. In particular, the past 20 years have seen a rapid increase in global warming (Fig. 1.2). Every year there are new record temperatures with 2020 that has been registered as the warmest year ever.

Climate changes are in part consequential stages of our planet, but they are also driven and speed up by atmospheric greenhouse gases, land transformation and other human-made emissions into the atmosphere (Asseng et al. 2015). The "global warming" process is arousing an increasing interest in recent years, due to its high impact on human life, including the rivers and lake drying, animal species extinction and a substantial reduction of crop productivity (Wheeler and Von Braun 2013; Fahad et al. 2017). There is a real risk that climate changes that can affect the food security

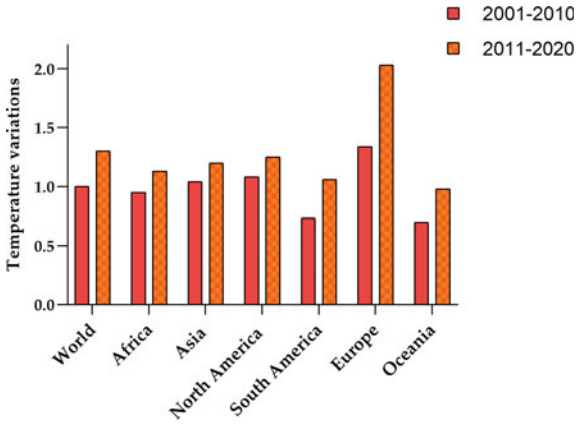

Fig. 1.2 Mean annual temperature measured globally and, in each continent in last two decades (FAOSTAT 2021)

worldwide. The global warming can reduce food availability or affect food quality. Climate change is mainly refleced in extreme weather events, and reductions in water availability, with huge impacts on agricultural productivity. For instance, in Italy, one of the major tomato producers worldwide, 2019 production season registered a reduction of tomato yield due to persistent rainfall and temperature variation from the seasonal average. Due to these climate effects, tomato plants showed a slow fruit ripening, because of winds and storms that damaged the fruits, and sudden heatwaves that reached 40 °C. Overall stressful conditions caused a 50% of total yield lost in temperate areas. Different published models show how in the next years rising temperature, and more elevated CO_2 levels will affect agricultural production all around the world (Kheir et al. 2019).

1.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

Traditional plant breeding allowed breeders to obtain improved tomato varieties through techniques based on phenotypic selection. However, several years are required to develop a new and stable variety (in terms of phenotypical and genotypical traits), which may not meet the requirements related to the fast climate changing scenarios described above. Innovative technologies potentially can address many of these challenges. The design of more tailored varieties can take advantage of a more precise and complete understanding of plant functioning. A global vision of overall tomato response to biotic stress, including genome signature, gene identification, proteins and metabolite function can be obtained by combining different genomic methodologies. Integration of computational data showed to be effective in identifying key components of stress response (Cappetta et al. 2020a). The development of molecular marker techniques and their applications drastically changed the fate of plant breeding for biotic stress in tomato (Ercolano et al. 2012). However, marker assisted selection (MAS) for quantitative trait loci (QTLs) is promising and strategies able to predict the genomic potential can be more effective. In this regard, genomic selection (GS) provides new opportunities for selection using genome-wide marker data (Cappetta et al. 2020a, b). Transcriptomic analysis of plants exposed to biotic stresses allow identifying important targets involved in disease resistance process (Padmanabhan et al. 2019; Zhao et al. 2019). To date, different engineering approaches to obtain disease resistant varieties based on genetic transformation, RNA silencing strategies, and emerging gene editing techniques were developed. Overall, established and emerging technologies such as transcription activator-like effector (TALE) and clustered regularly-interspaced short palindromic repeats (CRISPR) associated Cas protein 9 (CRISPR/Cas9)-based technologies enlarged the range of opportunities for obtaining tomato resistant varieties (Andolfo et al. 2016). Genomic editing tools allow to modify DNA sequence in a thoroughly selective manner, resulting very promising breeding tools (Malzahn et al. 2017; Waltz 2018).

1.2 Molecular Mapping for Disease Resistance

1.2.1 A Brief History of Mapping Efforts

Since restriction fragment length polymorphism (RFLP) marker was first used for genetic mapping in 1980 (Botstein et al. 1980), a variety of DNA-based molecular markers have been developed that have been used in plant breeding to select the plants of interest from segregating populations without phenotype screening (Tanksley et al. 1989; Yang and Francis 2005; Foolad 2007; Foolad and Panthee 2012). The abundance of single nucleotide polymorphisms (SNP) and the advent of next-generation sequencing (NGS) makes it more feasible to simultaneously select thousands of markers, which allows cultivar development with significantly reduced phenotypic screening, hence shortening the breeding cycle. Although, single marker cost is low, the high total cost prevents many breeders from adapting GS in their breeding practice.

Different approaches have been adopted to map and fine-map the gene(s) and QTLs in tomato. Depending upon the purpose, various mapping populations have been used for mapping QTLs in tomatoes. An F_2 population derived from crossing two inbred lines has the advantage to reduce the time to generate it. Backcross populations (BC) including BC1 and BC2 are extremely useful while doing targeted mapping. Both F_2, as well as BC populations, are early generations. Recombinant inbred line (RIL) populations get a better estimation of additive effects of QTLs and trials can be replicated. However, it takes a long time to develop them. Several tools such as Map Maker, QTL Cartographer, Join Map, iCIMapping, QTL Mapper, MapChart, SolQTL, R/QTL, and Map/QTL can be employed to perform a mapping experiment, two major reviews report details to better exploit them (Cheema and Dicks 2009; Semagn et al. 2010).

1.2.2 Molecular Genetic Maps

Tomato genetic maps has been created by using the previously mentioned software. There are several genetic maps developed using mapping populations derived from *Solanum lycopersicum* by wild relatives (*S. pimpinellifolium, S. pennellii*, or *S. habrachaites*). Those populations used for mapping are F_2, backcross, or RILs. The first molecular linkage map in tomato was developed in 1992 using RFLP molecular markers consisting of 1,030 RFLP markers (Tanksley et al. 1992). This map was updated combining cleaved amplified polymorphic sequences (CAPS), RFLP and simple sequence repeat (SSR) marker information in Tomato EXPEN2000 (Fulton et al. 2002; Frary et al. 2005). A more comprehensively map was later obtained adding a few more CAPS, SNPs, and expressed sequence tag (EST) and SSR markers which is widely called the Tomato-EXPEN2000 map (Shirasawa et al. 2010). The total length of the chromosome was 1,503.1 cM resulting from a total of 2,116 molecular

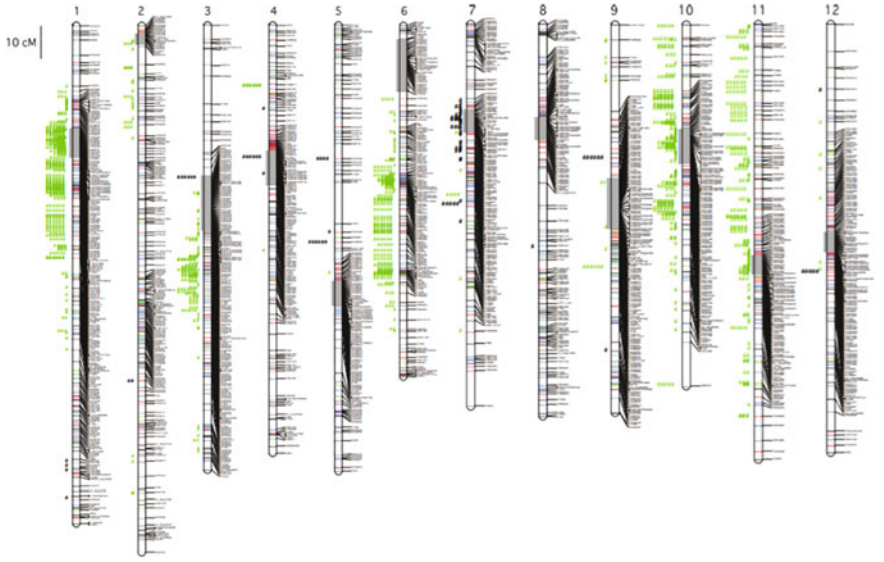

Fig. 1.3 Genetic linkage map of tomato genome derived from *S. lycopersicum* × *S. pennellii* using 2,116 molecular markers spanning 1,503.1 cM genetic distance (Shirasawa et al. 2010)

markers (Fig. 1.3; Shirasawa et al. 2010). A comprehensive list of mapping populations, markers types, number of markers, and publication information is provided by Labate et al. (2007).

1.2.3 Mapping Efforts for Identifying Resistance Traits to Major Tomato Fungal Diseases

Several bacterial, fungal, and virus diseases are common in tomatoes causing a significant yield loss throughout the world. There is a considerable research interest to investigate the genetic control of these diseases so that resistance genes or QTL can be introgressed.

Among the major diseases, late blight (LB), caused by *Phytophthora infestans* de Bary, is one of the most important diseases in the world in tomato. Three genes *Ph1, Ph2,* and *Ph3* have been identified to confer resistance to this disease. The dominant gene *Ph1* was identified in the wild relative *Solanum pimpinellifolium* and was mapped to the distal end of chromosome *7* (cited in: Foolad et al. 2008). However, this gene was not effective for a long time due to the emergence of new races of *P. infestans.* The *Ph2*, a partially dominant gene was found in the same wild relative *S. pimpinellifolium*, which was mapped to chromosome *10* (Moreau et al. 1998). The resistance conferred by this gene was also not found effective for a

long time. The *Ph3* was identified from LA3708 of *S. pimpinellifolium*, which was mapped to chromosome *9* (Chunwongse et al. 2002).

In addition, QTLs associated with late blight resistance were found on chromosome *4 7, 8* and *12* in *Solanum habrochaites* (Brouwer et al. 2004; Li et al. 2011).

Quantitative resistance to LB has also been reported from LA716 (*S. penelli*) (Smart et al. 2007). In addition, QTLs conferring resistance to LB were mapped on chromosome *5* (Haggard et al. 2013), and on chromosome *11* (Haggard et al. 2015). In order to make the resistance durable, Li et al. (2011) have suggested the pyramiding of resistance gene and/or QTLs from multiple species.

Subsequently, fine mapping of these QTLs made potential MAS for LB resistance. In another population derived from intraspecific crosses, the location of minor QTLs was found close to the R gene (Panthee et al. 2017). Such QTLs resulted consistent in all the environments tested, although the LOD score was slightly different (Fig. 1.4; Panthee et al. 2017).

Early blight (EB) resistance is a quantitative trait, which makes selection more difficult. Foolad et al. (2002) used a backcross population derived from NC84173 × PI126445 to map resistance QTLs for EB. They found ten resistance QTLs for EB in both BC_1 and BC_1S_1 populations, which were highly consistent across generations, and years explaining 8.4–25.9% of total phenotypic variation (Foolad et al. 2002). A selective genotyping approach detected seven QTLs for EB resistance, validating

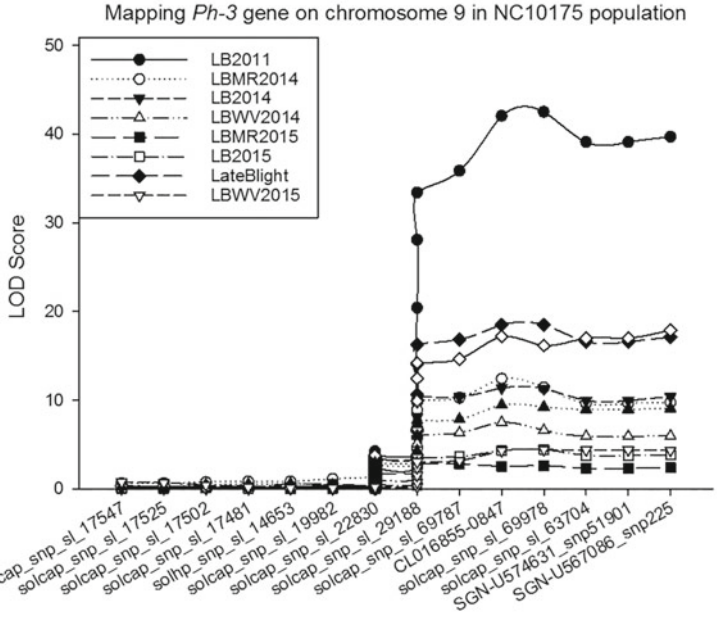

Fig. 1.4 Mapping *Ph-3* on chromosome *9* in segregating tomato population derived from an intraspecific cross (Panthee et al. 2017)

four of detected in a previous study using PI126445 of *S. habrochaites* (Zhang et al. 2003). A trait-based marker analysis for resistance to EB was performed in F_2 and F_3 populations derived from a cross between *S. lycopersicum* cv. Solentos (susceptible) and *Solanum peruvianum* LA2157 (resistant) (Chaerani et al. 2007). A total of six QTL regions were mapped to chromosomes *1, 2, 5, 6, 7*, and *9*, including three resistance QTLs to stem lesions in the field that explained 35% of the phenotypic variation. After extensive screening of 300 accessions of *S. pimpimellifolium*, an accession LA2093 with good EB resistance was selected for QTL mapping (Ashrafi and Foolad 2015a, b). Ten QTLs conferring EB resistance on chromosomes *2, 3, 4, 5, 6, 7, 9*, and *12* with individual effect of 7.6×13.4% and combined effect of 44% of total phenotypic variance were detected (Foolad et al. 2008). In another study, five major QTLs for EB resistance were identified on chromosomes *2, 5, 6*, and *9*, using RILs of the same cross (LA2093 × NCEBR-1) (Ashrafi and Foolad 2015a). QTLs on chromosomes *2* and *6* were from LA2093, whereas QTLs on chromosomes *5* and *9* were from NCEBR-1. Two stable QTLs on chromosomes *5* and *6* were used in EB resistance breeding. The detected QTLs were also co-localized with other resistant genes and candidate ESTs (Ashrafi and Foolad 2015a). A review on EB resistance including QTL mapping is provided by Adhikari et al. (2017).

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) is a devastating disease of tomato (Agrios 2005). Three races, race-1, race-2, and race-3, of *Fol* have been reported to cause this disease. Corresponding to these races, three loci *I-1, I-2,* and *I-3*, have been identified which confer resistance in tomato (Sarfatti et al. 1989, 1991). The *I-2* was mapped between the RFLP markers TG105 and TG36, 0.4 cM from TG105 on chromosome *11* (Ori et al. 1994). The *I-3* gene from wild tomato *S. pennellii* accessions LA716 and PI414773 that confers resistance to *Fol* race 3 was mapped to chromosome *7* (Hemming et al. 2004).

In contrast to the fungal diseases discussed above, there is a lack of knowledge on QTL and molecular markers for Septoria leaf spot (SLS), Verticillium wilt (VW), Powdery mildew (PM), and other fungal diseases of tomatoes.

In summary, several disease resistance genes have been mapped onto the tomato genome. It has helped to advance the MAS in tomato breeding programs throughout the world.

1.3 Marker-Assisted Breeding for Disease Resistance

1.3.1 Germplasm Characterization and DUS

Germplasm characterization is one of the foundations for launching successful plant breeding. Phenotypic characterization was the basis for the identification of suitable germplasm to be used as parents in a breeding program. With the abundance of molecular markers and their association with several disease resistance traits, this information can be utilized for the selection of germplasm in a breeding program.

After selection, variety registration is an important step to provide the plant breeders right and to regulate the seed production process. For that, a variety to be eligible to be released as a unique variety, should meet the criteria of distinctness, uniformity, and stability (DUS). Some of the traits are difficult to measure phenotypically to provide the DUS certification. In this case, molecular testing might be useful. It has been optimized and employed for the testing of some of the diseases in tomatoes as explained by Arens et al. (2010). A similar approach can be adapted for other crops as well.

1.3.2 Marker-Assisted Gene Introgression

Molecular markers associated with disease resistance genes have been optimized and used extensively (Foolad and Panthee 2012). Molecular markers can be used when plants are very young, saving the field stage. The use of molecular markers at early generation also helps to discard the unwanted materials advancing the useful materials. The use of reliable molecular markers helps to even avoid phenotypic characterization. This is useful when inoculum pressure or screening facility is an issue for some of the diseases or evaluation of some of the diseases may be extremely difficult because of their safety concern. The MAS can be more effective than phenotypic selection under certain situations, including when there is a lack of selection environment such as enough inoculum pressure, trait expression is developmentally regulated, the trait is controlled by a recessive gene(s), or multiple trait selection is desired (Foolad and Panthee 2012).

1.3.3 Gene Pyramiding

Combining multiple sets of genes in a single genotype is the goal of a plant breeder. While they have been doing it by conventional breeding for a long time, it is very time-consuming. The MAS has been instrumental to combine the multiple genes in a single genotype. Gene pyramiding has been done to combine late blight (*Ph2* and *Ph3*), root-knot nematode (*Mi-1.2* gene), and *Tomato Yellow Leaf Curl Virus* (*Ty1*, *Ty2*, and *Ty3* genes) resistance genes in tomato (Kumar et al. 2019; Kim et al. 2020; Prabhandakavi et al. 2021). It would have taken at least ten years to combine all three genes in a single genotype by a conventional method. It took a single season by the use of molecular markers.

1.3.4 Limitations and Prospects of MAS

In most of the modern tomato breeding programs, the MAS is integral component and is being used on regular basis. These will be used even more frequently with the development of SNP markers. This approach has been helpful to advance the breeding programs. With the reduction of cost per sample analysis, tomato breeders may likely integrate the molecular approach even at wider level. They may expand the use in more traits. One of the limitations is that it may be challenging to keep up with the fast-changing technologies. Also, it may be challenging to handle the ever-increasing genotypic data since sequence-based SNPs are being generated in most cases.

1.4 Genomics-Aided Breeding for Resistance Traits

1.4.1 Structural and Functional Genomic Resources

Rapid advances in genomics technologies provide new opportunities to assess the biological function of important tomato loci, which, in turn, will greatly enhance our ability to utilize these genes in breeding programs. A high-density molecular map containing >2,000 markers (Rick and Yoder 1988; Sim et al. 2012) a large collection of well-characterized mutants, wild species and near-isogenic lines (NILs) are available for tomato (Eshed and Zamir 1995). In addition, tomato has a relatively small genome size (~950 Mb), (Martin et al. 1992; Bonnema et al. 1996), and a routine *Agrobacterium*-mediated transformation system (McCormick et al. 1986). The publicly available tomato large insert libraries (YAC: yeast artificial chromosome; BAC: bacterial artificial chromosome and TAC: transformation-competent artificial chromosome) were used as valuable research tools for the isolation of several agriculturally important genes by positional cloning (e.g., Martin et al. 1993; Geethanjali et al. 2010) to be directly transformed into the plant genome via *Agrobacterium*-mediated transformation (Hamilton et al. 1996; Li et al. 2000) or to be used as templates in shotgun sequencing (Boysen et al. 1997). More recently, the development of high-throughput genomics resources is improving our understanding the entire tomato genome organization and functioning.

1.4.2 Genome Sequencing

In 2004, an international consortium of 10 countries (Korea, China, the United Kingdom, India, the Netherlands, France, Japan, Spain, Italy, and the United States), as part of a larger initiative called 'International Solanaceae Genome Project' (SOL), launched the initiative to sequence the tomato genome. The first step of SOL project

was to generate a high-quality tomato euchromatic genome sequence. An ordered BAC approach was chosen to sequence the tomato genome and the libraries were constructed from the Heinz 1706 tomato line (Barone et al. 2009). The BAC-by-BAC strategy involves the anchoring of BACs or contigs of BACs to a reference genetic map. These anchored BACs are sequenced, and the sequence information is used to further extend the contigs. A total of 837 markers were used to anchor the contigs, mainly composed of euchromatic sequences, to the tomato genetic map. The tomato physical map was validated using fluorescent in situ hybridization (FISH) on pachytene complements with entire BAC clones as probes, and by genetic mapping of anchored BACs using panels of tomato introgression line populations. The genome of the inbred tomato cultivar 'Heinz 1706' has been released over nine years ago (TGC 2012). The tomato genome was sequenced and assembled using a combination of Sanger and 'next generation' technologies. The scaffolds were linked with two BAC-based physical maps and anchored/oriented using a high-density genetic map, introgression line mapping and BAC FISH. The predicted genome size is approximately 900 megabases (Mb), of which 760 Mb were assembled in *12* tomato chromosomes (TGC, 2012) . The latest tomato genome version (SL4.0) was assembled de novo from PacBio long reads and scaffolded using Hi-C contact maps and it is available at the Solanaceae Genomics Network Current (SGN; http://sgn.cornell.edu) (Hosmani et al. 2019).

1.4.3 Gene Annotation

A high-quality automated annotation of the genome was produced by the international tomato annotation group (iTAG) to rapidly allow the use of sequenced sequences to the tomato breeders community. The iTAG performed repeats annotation, and masking of pseudomolecules, mapping of different protein sequence sets, ESTs and full length cDNAs, as well as RNA-Seq reads from Illumina, 454 and SOLiD platforms. In addition, independent ab initio predictions were performed with GENEID (https://genome.crg.es/software/geneid/), AUGUSTUS (http://bioinf.uni-greifswald.de/augustus/), and TWINSCAN (https://bio.tools/twinscan), all specifically trained for tomato. The above listed extrinsic data were integrated using the a priori informed gene prediction software. EuGene prediction, followed by manual expert curation, produced a consensus annotation of 34,727 protein encoding genes for the tomato (iTAG v2.3) nuclear genomes (TGC, 2012) . To date, the latest tomato gene annotation available at the Solanaceae Genomics Network is iTAG4.1 (SGN; http://sgn.cornell.edu) through BLAST database, Pathway database (SolCyc: https://solgenomics.net/pages/solcyc/) and Apollo (Dunn et al. 2019). About 5,000 novel genes were identified and most of the updated genes have extensions in the 5′ and 3′ UTRs (Hosmani et al. 2019). The release of the tomato genome annotation provided an excellent opportunity to steer the studies of gene characterization. Scientists and breeders around the world actively use the tomato genome sequence for breeding and research activities. Indeed, a full annotation of pathogen recognition genes was

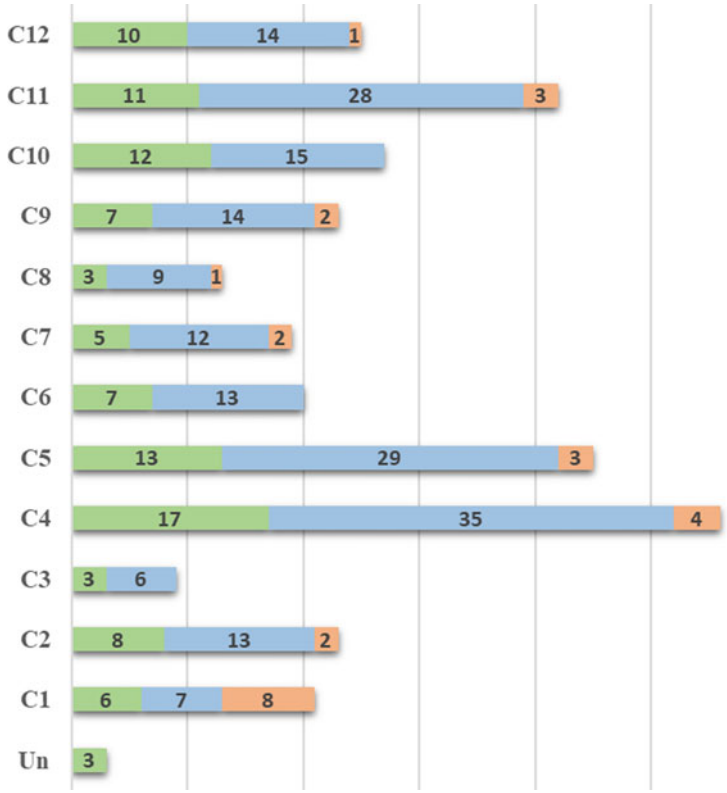

Fig. 1.5 R-gene family identified and annotated in *S. lycopersicum* Heinz 1706 v2.4 genome. The tomato defense arsenal was displayed with respect to chromosomal position (C1–C12 and unassembled region (Un)) and R protein domain structure (CNL in blue; TNL in orange and partial genes in green). The total number of CNLs, TNLs, and partial genes was shown

released immediately after the publication of tomato genome sequence (Andolfo et al. 2013). Over 770 genes, belonging to nucleotide binding domain and leucine-rich repeat (NLR), receptor-like protein (RLP) and receptor-like kinase (RLK) protein classes, were finely annotated and characterized in tomato genome (Andolfo et al. 2013) providing a useful tool, for breeders and scientists, to identify novel disease resistance traits to introduce in tomato cultivars (Andolfo et al. 2014) (Fig. 1.5).

1.4.4 Impact on Germplasm Characterization and Gene Discovery

The sequencing of tomato genome has totally revolutionized the accuracy of germplasm characterization and the pace of gene discovery (Andolfo et al. 2021). The

development of the *S. lycopersicum* Heinz 1706 reference genome made possible to study the genetic variation of tomato accessions and wild relatives. Considering the overwhelming interspecies genetic variability, tomato germplasm collections represent a gene pool with unprecedented possibilities to address new breeding demands imposed by climate change, world population increase, and consumer needs. During the domestication the tomato genome went through a genetic bottleneck, reducing its genetic diversity to less than 5% (Sim et al. 2010). Moreover, several disease resistance traits have been disregarded as a result of human selection for yield and quality related traits. Consequently, tomato cultivars have become more susceptible to various pathogens (Foolad 2007). Introgression of traits from wild-species into domesticated species is a widely used practice for increasing diversity in crop plants. Indeed, numerous disease resistance genes have been introgressed in tomatoes from wild species such as *Solanum chilense, S. peruvianum, S. habrochaites, S. pennellii*, and *S. pimpinellifolium* (Catanzariti et al. 2017; Yamaguchi et al. 2018; Andolfo et al. 2021). The selection process can be accompanied by linkage-drag, which require many rounds of backcrossing and fine-mapping to eliminate (Labate and Robertson 2012). Thus, the ability to define the borders and contents of wild-species introgressions can contribute significantly to speed up the selection process and can help to identify the putative resistance gene loci (Andolfo et al. 2021). The whole-genome sequencing approach provides detailed information on genic content and the origins of the introgressed regions through comparison of wild species genomes with genomic background of breeding lines obtained (Labate and Robertson 2012).

The increasing of accessions resequencing allowed to explore extant genetic variation in tomato, providing a major boost to identification of valuable alleles (Aflitos et al. 2014; Ercolano et al. 2014; Gupta et al. 2020). The millions of informative markers (SNPs/InDels) and structural variations identified through comparison of genome sequences of domesticated and wild tomatoes will promote investigations into the genetic and molecular basis of the disease resistance process. This will not only help identify useful SNPs from the wild accessions but also rare SNPs within domesticated varieties (Ercolano et al. 2014; Tranchida-Lombardo et al. 2018). Tomato breeders can identify gene variants in the wild species associated with desirable traits such as disease or pest resistance and introduce them into cultivars to exploit the diversity of tomato germplasm. The tomato genome sequence facilitates QTL identification, mapping and cloning of underlying genes, and provide new SNP markers for marker-assisted breeding (Arafa et al. 2017; Gonda et al. 2019). Availability of the tomato genome sequence will speed up the understanding of gene function in plant disease resistance by mapping relevant wild tomato traits. The advent of NGS and available genome sequences should make characterization of large collections of tomato accessions even more rapid and robust.

1.4.5 Application of Structural and Functional Genomics in Tomato Breeding

The growing body of tomato genomic data is accelerating the transfer of beneficial traits into new tomato varieties (Andolfo et al. 2021). The use of the reference genome for genetic analysis has become increasingly beneficial to enhance tomato breeding efforts. Genetic mapping of resistance traits speeds up breeding for plant disease resistance. Markers available for tomato have been widely used to locate and tag genes or QTLs for disease resistance (Arafa et al. 2017; Panthee et al. 2017). Indeed, mapping of resistance genes to different viruses, bacterial, nematode, and fungal diseases provided important information for tomato genomics aided breeding. The success of this strategy depends on the availability of technological platforms based on automated large-scale screening. To date, several technologies for automatic large-scale small-variants detection have been set up, increasing markers specificity levels. The completed genome sequence of *S. pennellii, S. pimpinellifolium* and *S. chilense* (Bolger et al. 2014; Stam et al. 2019; Wang et al. 2020) and several transcriptomic data for wild tomatoes are available. Therefore, the polymorphism between resistant and susceptible genotypes could be more easily explored in order to identify SNPs or InDels useful as gene markers in dissecting complex resistance traits (Pachner et al. 2015). The increasing availability of information on resistance genes deriving from the sequencing of the wild tomato genomes (Seong et al. 2020), will facilitate large-scale annotation for gene-assisted selection (Andolfo et al. 2014). Several tomato wild relatives are used to broaden the genetic diversity of tomato through the introgression of required alleles (Jablonska et al. 2007; Zang et al. 2014; Catanzariti et al. 2017). The identification and transfer of new resistance alleles assisted by genomic data provide more reliable and precise methods for tomato breeding. In many cases, one or few polymorphic amino acids are sufficient to determine resistance in the plant host (Ashikawa et al. 2012; Stirnweis et al. 2014; Giannakopoulou et al. 2015).

GS is a predictive approach that has emerged as a valuable method for improving complex traits that are controlled by many genes with small effects (Cappetta et al. 2020a, b). This promising breeding framework has already been shown to be feasible superior genotypes during breeding programs (Liabeuf et al. 2018). Genome editing technologies can improve the development of varieties with desirable wild genes/alleles (Wang et al. 2019).

1.5 Genetic Engineering for Resistance

1.5.1 Transgenic Technologies

Since 1983 with the first transgenic tobacco plant, the genetic engineering science have undergone great improvements, reaching impressing accuracy levels (Lemaux 2008). To date, plant genomes can be modified in a highly selective manner and in

near future, it is expected that engineered plants (free from the transgenic backbone and selectable marker genes) may take an important role in agricultural productions. The genetic engineers are working hard to promptly enhancing desired tomato traits by genome modification processes. In this context, transgenic approach of genetically modified (GM) tomatoes represent an important weapon. To date, laws about GM tomatoes are quite stringent, but researchers made great progress using transgenic technologies. Genes isolated in sexually compatible species (cisgenes) can be introduced through genetic engineering. Cisgenic science should be considered similar to traditional breeding, because the final result is the same of a crossing between two compatible species. Cisgenic tomato plants resistant to *Phytophthora infestans* were obtained by Faino et al. (2010). More recently cisgenic tomato lines resistant to bacterial wilt disease (*Ralstonia solanacearum*) were obtained by Morais et al. (2019) through the identification of *PPC20*, an alpha-helical (AH) peptide derived from plant protein sequences, and *SlP14a* (a pathogenesis-related protein). Cisgenic methods have been also used in other Solanaceae such as potato, introducing two R genes conferring resistance to *Phytophthora infestans*: *Rpi-sto1* and *Rpi-vnt1.1* in three potato commercial varieties, from the crossable species *Solanum stoloniferum* and *Solanum venturi;* they obtained resistant marker-free potatoes plants (Jo et al. 2014). A more efficient homologous recombination system, with a subsequently highly precise transgene insertion can be obtained with plastid DNA transformation. Foreign proteins can be expressed to extremely high levels with the absence of epigenetic effects (Oey et al. 2009). More genes can be introduced simultaneously stacking them in operon systems (Boehm and Bock 2018). Furthermore, plastid engineered does not allow the transmission of transgenic genes to the progeny. The genetic sequence of the tomato chloroplasts (plastome) has also been determined by Kahlau et al. (2006) facilitating tomato plastid experiments (transplastomic tomato).

1.5.2 Gene Silencing

In order to discover new gene functions, scientists can downregulate gene expression by several gene-silencing approaches. A method to downregulate gene expression was originally developed by Hiatt et al. (1989), using the expression of an antisense RNA strand which then caused base pairing with the sense RNA strand originally synthetized by plant, reducing the availability of targeted RNA and subsequently the protein accumulation. More efficient silencing technologies were further developed after discovering of RNA interference (RNAi) and virus-induced gene silencing (VIGS), two post-transcriptional gene silencing techniques. Through RNAi approach, a gene portion is expressed in double-strand flanking a linker DNA region. At this point a dicer protein cuts the double-stranded RNA into smaller pieces of approximately 22 nucleotides long, producing small interfering RNA (siRNA). These siRNA form the RNA-induced silencing complex (RISC) with the target gene, blocking the translation. Gene downregulation can also be achieved using

microRNA (miRNA) which binding to the 3′ untranslated regions of target mRNAs represses its expression. In the last decades these methods became quite popular among researchers worldwide (Eulalio et al. 2008; Galvez et al. 2014; Tiwari et al. 2014). VIGS involves the use of engineered viral vectors that contain a sequence of a gene of interest to silence. The recombinant virus can be introduced into plant cells through *Agrobacterium tumefaciens* infections. In many studies, it has been demonstrated that the use of gene silencing in tomato provides resistance against biotic and abiotic stress. Singh et al. (2020a, b) targeting a key polyamine (PA) biosynthesis gene *ornithine decarboxylase* (*ODC*) of the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* using RNAi, obtained transgenic lines with moderate and high resistance to *Fusarium oxysporum.* Singh et al. (2014) obtained transgenic tobacco and tomato plants, using small interfering RNA, targeting two RNAi suppressor proteins (*AC2* and *AC4*) of *Tomato Leaf Curl New Delhi Virus* (ToLCNDV); showing that after virus inoculation, most of the plants displayed no disease symptoms. Other experiments carried out in tomato, showed that silencing of *miR482b* (involved in *Phytophthora infestans* infections) using short tandem target mimic (STTM) resulted in enhancement of tomato resistance (Jiang et al. 2018). *SLNLC1* gene silencing using VIGS technology provides resistance against the pathogen *Stemphylium lycopersici* in tomato (Cui et al. 2018).

1.5.3 Gene Editing

Plant genetic editing (GE) involves technologies that could be applied to modify valuable plant traits for increasing resistance to herbicides, insects, and diseases. Gene editing technologies enable scientists to make DNA modifications, leading to changes in phenotypic traits. To date, widespread genome editing technologies allow scientists to alter, add, or remove a specific locus. Gene editing requires engineered enzymes (endonucleases) able to bind a specific DNA sequence to achieve the desired genetic changes. Once reached the nucleus, they can introduce cuts into the double-strand of DNA, leading to an non-homologous end joining (NHEJ) that subsequently results in a random mutation or in presence of a DNA donor, to an homology directed repair (HDR) useful to introduce determined DNA fragments. There are different types of nucleases: the zinc finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs) operate through the fusion of sequence-specific DNA binding domains (DBDs) and nucleases. Following the recognition of the target sequence by the DBDs, nucleases provide double-strand breaks (DSBs) leading to NHEJ and to InDel causing gene mutation and a consequently loss-of-function (Chandrasegaran and Carroll 2016). More recently, the CRISPR/Cas9 system is already being explored for a wide number of applications in agriculture fields. This technology consists of a nuclease driven to the DNA target sequence by a specifically designed guide RNA (gRNA). To date, several tomato genes involved in biotic or abiotic stress pathways have been well characterized through this technique. The CRISPR/Cas9 system it

was extensively used in the scientific community because it requires only a protospacer adjacent motif (PAM), usually NGG, and a complementary 17–22 bp guide RNA to match the target gene (Ran et al. 2013). However, the genome editing technique mentioned above requires a sequenced plant genome to selectively identify the genome targets.

1.5.4 Nanotechnology

Agricultural engineered crops are a promising solution to meet the increasing food demand worldwide also in the face of a growing population and climate changes. In the last few years, new strategies in plant genetic engineering have been developed, including the use of nanoparticles (Fig. 1.6). Nanomaterials (NMs) offer new solutions for incorporating agrochemicals and biochemical molecules into plants (Kole et al. 2013; Khan et al. 2017). To date, systems used to transfer biomolecules into plant cells such as a DNA fragment are mainly based on biological delivery systems such as *Agrobacterium*-mediated transformation. However, not all plant species can be transformed by *Agrobacterium*. Another commonly used tool for plant transformation is a biolistic particle delivery (gene gun) in which microparticles of

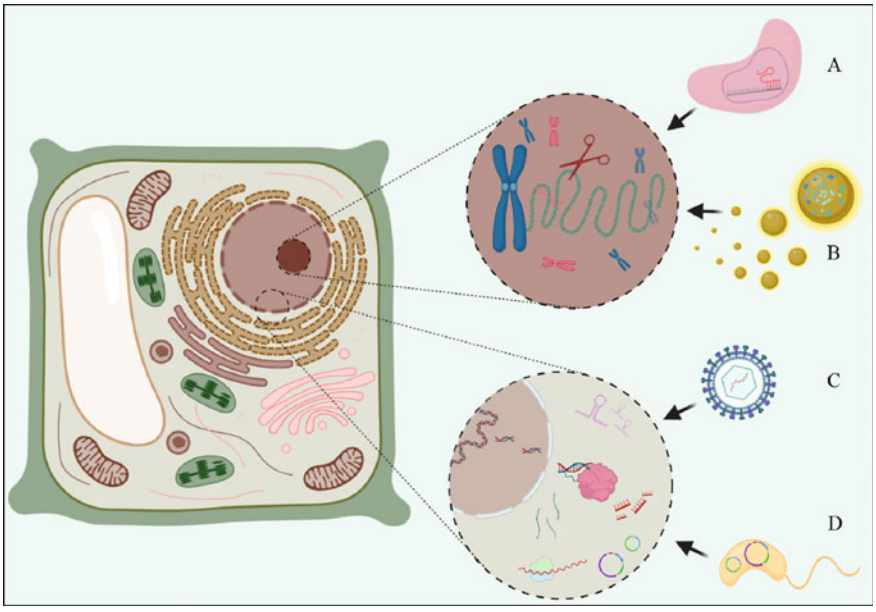

Fig. 1.6 Schematic representation of different biotechnological techniques for gene modifications: (A) Genome editing; (B) Biolistic approach; (C) Virus delivering ssRNA; (D) Agrobacterium-mediated Virus Inducing Gene Silencing. Created with BioRender.com

gold are introduced in plant tissues through a high-pressure gene gun. Recently, interesting results have been obtained with the use of nanoparticles with size of less than 100 nm able to penetrate the plant cells main barriers: (1) the hydrophilic cell walls able to exclude molecules bigger than 5–20 nm; (2) internal double-layer lipid membrane which can exclude molecules of more than 500 nm. Multi-walled carbon nanotubes (CNTs) and carbon dots allowed efficient DNA delivery into both nuclear and chloroplast genomes achieving gene silencing (Demirer et al. 2019, 2020; Kwak et al. 2019). Graphene, fullerenes, and polymeric nanoparticles (NPs) including polyethyleneimine-coated NPs have promising efficiency for DNA, RNA, or protein delivery into plant cells (Cunningham et al. 2018). Mesoporous silica nanoparticles (MSNs) were employed in Arabidopsis plants (Chang et al. 2013) and double-layered hydroxide clay nanosheets in *Nicotiana tabacum* (Mitter et al. 2017). More recently Zhang et al. (2019) using a system of DNA origami nanostructures delivered RNAi molecules in *Nicotiana benthamiana.* Nevertheless, further studies are needed to improve NMs' physic-chemical properties and to optimize nanoparticles characteristics for different cellular destinations and plant tissue or organ explant.

1.5.5 Target Traits for Biotic Stress Resistance

Genome editing techniques are generally applied in the perspective of producing genetically improved crop varieties. Target traits might be chosen to improve plant resistance to a specific biotic stress, or an established plant pathogen. Specific application, targeting multiple genes, can lead to wild species domestication. Several plant species have been genetically modified using genome editing tools, especially CRISPR/Cas9 technology and RNAi or VIGS. Tomato represents one of the most well-studied crops, probably because of its economic importance and the availability of a whole-sequenced genome. CRISPR/Cas9 mediated genome editing allowed enhancing tomato resistance to biotic stress (Nekrasov et al. 2017; Tashkandi et al. 2018). Moreover, using RNAi targeting *HyPRP1* gene (to inhibit gene translation) scientists obtained tomatoes with improved characteristics of resistance against both biotic and abiotic stresses (Li et al. 2016). In Table 1.1 are shown CRISPR/Cas9 studies related to tomato biotic stress resistance, conducted in last three years.

1.6 Bioinformatics Repositories

1.6.1 Gene and Genome Databases

In last years, a large amount of tomato genome and gene sequences was generated and stored in public repository, affecting the research approaches for carrying out

Table 1.1 List of CRISPR/Cas9 experiments conducted to improve tomato resistance to biotic stresses

Organism	CRISPR/Cas9 system	Target	Effects	References
S. Lycopersicum cultivar Moneymaker	CRISPR/Cas9 double guide RNAs	Jas domain of *SlJAZ2* gene	*Sljaz2Δjas* mutants are resistant to *Pseudomonas syringae* (PtoDC3000)	Ortigosa et al. (2019)
S. Lycopersicum cultivar Moneymaker	CRISPR/Cas9 four single-guide RNAs	*Powdery Mildew Resistance 4* (*PMR4*)	Enhanced resistance against *Oidium neolycopersici*	Martínez et al. (2020)
S. Lycopersicum *cultivar BN-86*	CRISPR/Cas9 four guide RNAs and two guide RNAs respectively	eRF1_1 domain in the *SlPelo* gene and exon 11 of the *SlMlo1* gene	complete resistance to powdery mildew fungus and reduced accumulation of TYLCV virus	Pramanik et al. (2021)
S. Lycopersicum cultivar Micro-Tom	CRISPR/Cas9 double guide RNAs	*SlPDS* and *SlMYC2* genes	reduced the plant growth and fruit resistance to *B. cinerea*	Shu et al. (2020)
S. Lycopersicum cultivar Zaofen No. 2	CRISPR/Cas9 multiplexing—three guides RNA targeting the stem-loop structure	MicroRNAs miR482b and miR482c	Mutants showed a reduced disease symptom against *Phytophthora infestants*	Hong et al. (2020)
S. Lycopersicum cultivar Ailsa Craig	CRISPR/Cas9 Not specified	*SlMAPK3*	reduced resistance to *B. cinerea* and enhanced the content of *ROS*	Zhang et al. (2018)
S. Lycopersicum cultivar Moneymaker	CRISPR/Cas9 duble guide RNAs	*SlMlo1*	Improved resistance against *Oidium neolycopersici*	Nekrasov et al. (2017)
S. Lycopersicum cultivar Moneymaker	CRISPR/Cas9 single guide RNA	TYLCV genome at coat protein (CP) site	Improved resistance against TYLC virus	Tashkandi et al. (2018)

genetic investigations and expanding the opportunity to get a response to a scientific question. The availability of an high-quality reference genome, the resequencing of hundreds of genomes (Aflitos et al. 2014; Lin et al. 2014; Ercolano et al 2014) and the release of large RNA-seq experiment data (Du et al. 2015; Yang et al. 2017; Shi and Panthee 2020) provided new insight into biological knowledge of *Solanum* species. Several databases collect tomato data and allow cross analysis of metadata coming from various entries. The Sol Genomics Network (SGN; http://solgenomics.net), a clade-oriented genomics platform for Solanaceae species, hold

several features and tools able to deal with tomato genome variation and gene family structural and functional investigation. Other large data access portals such as Ensembl Plants, PlantGDB Phytozome, and PLAZA, collect sequenced genomes, providing powerful tools to analyze annotated gene family datasets. The proper utilization of the existing large scale tomato data is challenging and many collection databases have been developed, including: KaTomicsDB, (http://www.kazusa.or.jp/tomato), TOMATOMICS (http://bioinf.mind.meiji.ac.jp/tomatomics), and Tag-SNP, an online Solanaceae genome Browser for capturing information on SNPs (Jeong et al. 2020). Tomato large scale RNA-seq data are available at the Tomato Functional Genomics Database (TFGD) (Fei et al. 2011), (TFGD, http://ted.bti.cornell.edu), TomExpress (http://gbf.toulouse.inra.fr/tomexpress/www/welcomeTomExpress.php), Kazusa Tomato Genomics Database Plant Expression Database (PLEXdb, http://www.plexdb.org/index.php).

Tomato Genetics Resource Center database (TGRC, http://tgrc.ucdavis.edu) can be interrogated for genetic resources and information on microRNA identified in expressed sequence tags (ESTs) can be obtained by miSolRNA (Bazzini et al. 2010) and in SolmiRNA (Kim et al. 2011). In addition, several mutant resources derived by Ethyl methanesulfonate (EMS), gamma-rays, fast neutron mutagenesis are publicly available and can be exploited by tools such as Mutmap, and MutChromeSeq, to accelerate the mutation breeding in tomato (Chaudhary et al. 2019).

To retrieve information related to tomato R genes and other Solanaceous species we can easily browse through the plant resistance gene database (PRGdb: http://prgdb.org/prgdb/). This web resource collects manually curated reference R-genes as well as plant putative R-genes. The PRGdb database is organized in four sections: plants, genes, pathogens, and disease. A set of pre-defined queries can be cross explored to identify putative R-proteins thanks to the distinctive structural domains of resistance genes such NB-LRR and TIR present into NB-LRR proteins and receptor kinase domains belonging to RLK and RLP proteins (Sanseverino et al. 2010). In addition, a BLAST search tool and a DRAGO pipeline allows to annotate resistance genes (Osuna-Cruz et al. 2018). A new section reporting plant-pathogen transcriptome experiments in model species, was added in the last database updated. (PRGdb 4.0). From the home page (Fig. 1.7A) is possible to select the species for visualizing data related to reference and predicted genes (Fig. 1.7B) or to explore the results of different expression studies. Differential gene expression analysis (DEG) lists to conduct further analyses are also provided (Fig. 1.7C).

1.6.2 Comparative Genome Databases

The evolution selection pressure acting on resistant loci significantly can affect species variation. The reconstruction of evolutionary trajectories that shaped tomato gene repertories can be improved using orthologs analysis. Comparison among plant species showed to be a valuable strategy to facilitate proper classification of genes and for exchanging information related to putative protein functions across species,

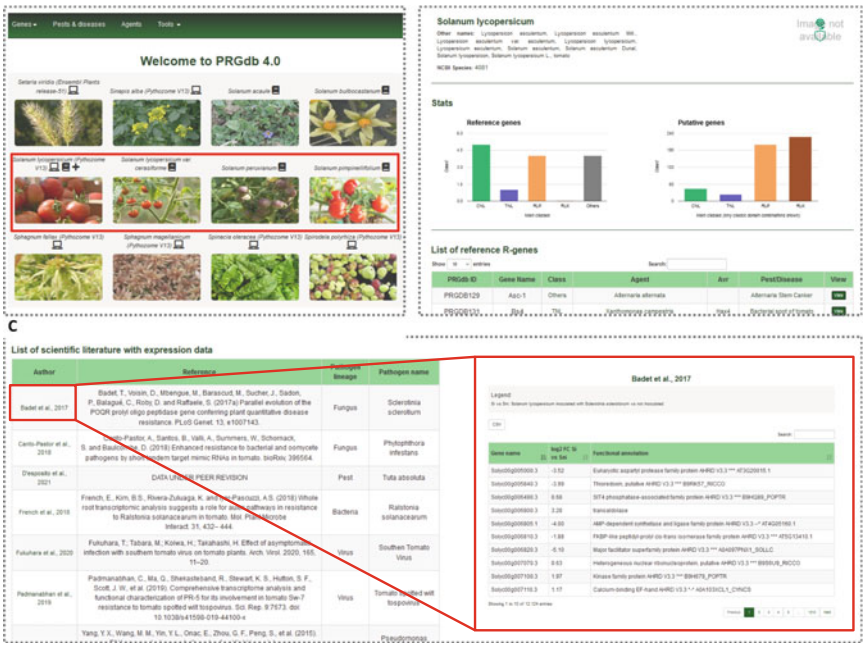

Fig. 1.7 Overview of PRGdb 4.0 main sections

raising important questions related to genome organization (Andolfo et al. 2021). Tools able to identify putative orthologous genes from different plant species are available through several websites such: Ensembl Plants, PlantGDB, Phytozome and PLAZA. A phylogenetic analysis can help to identify the likely orthologs of resistance genes for species of interest (Andolfo et al. 2013). It represents a good starting point to identify putative tomato orthologs of a given gene involved in a resistance process. Translational and/or comparative genomics methodologies can be integrated to detect homology sequences and block of synteny for trait-associated genes discovering (Di Donato et al. 2018).

1.6.3 Gene Expression Databases

Numerous tomato RNA-Seq datasets have been generated and published. Although the raw data are publicly available (e.g., via the NCBI sequence read archive, https://www.ncbi.nlm.nih.gov/sra), they are not curated and their use in direct comparisons can be tedious due to the diversity of genetic sources, pathogen treatments and sequencing methodologies. Expression browsers aim to collect and reanalyzing public datasets, normalizing parameters used to count expressed reads, and ideally

allowing retrieval of expression information in a list of genes under different conditions. Three main tomato expression browsers are currently available: The Tomato Expression Atlas that provides tissue-specific expression data based on single cell laser dissection (Fernandez-Pozo et al. 2017). The TomExpress platform developed to support tomato research community of a public RNA-Sequencing browser with integrated web tools, including data mining graphic outputs, such as expression bar plots, heatmaps of hierarchically clustered expression data and co-expressed genes networks (Zouine et al. 2017) and Co-expressed Pathways DataBase on Tomatoma platform (http://cox-path-db.kazusa.or.jp/tomato) developed by Narise et al. (2017). All these resources provide a powerful way for generating hypothesis using tomato-specific data. The web-based resources can be explored to get useful information for specific experimental aims. However, comparisons with gene expression profiles in response to various treatments could be more useful to gain new insights in specific tomato stress interactions. A dedicated platform to plant-pathogen interaction transcriptomic experiments is definitely needed.

1.6.4 Protein or Metabolome Databases

TomatoCyc (https://plantcyc.org/content/tomatocyc-5.0) is a large-scale computational prediction platform for pathways and their catalytic enzymes, compounds, and genes. Most of pathway pictures were extracted from literature. Kegg (https://www.genome.jp/kegg/pathway.html) can be widely used to check reference proteins as well as Biocyc (https://biocyc.org/web-services.shtml) that allows to retrieve pathways, reactions, compounds, genes, proteins, and RNA or transcription-unit resembling the underlying pathway tools schema. The Co-expressed Pathways DataBase for Tomato (http://cox-path-db.kazusa.or.jp/tomato) allow to predict pathways that are relevant to a query gene, which would help to infer gene functions. Predicted tomato interactome resource (PTIR) (http://bdg.hfut.edu.cn/ptir/index.html), covering approximately the 30% of the entire tomato proteome, is based on experimentally determined orthologous interactions in six model organisms, evaluated by shared gene ontology (GO) terms, co-evolution, co-expression, co-localization and available domain-domain interactions (DDIs) (Yue et al. 2016). Reconstructing protein interaction networks may be a powerful method for deciphering molecular mechanisms and potential gene function.

1.6.5 Integration of Different Genomic Data

Various web resources-based tomato omics information and bioinformatics tools have been developed. In addition, repositories collecting genetic valuable material including natural and artificial mutants are available. To enhance the efficiency of

acquiring tomato biology information coming from different sources we must integrate knowledges. Large-scale sequencing projects continue to be launched and it is important to combining them with validated data on genes function and interaction. SGN (Fernandez-Pozo et al. 2015) and TOMATOMICS (Kudo et al. 2017) provide large-scale omics information with gene structures, expression profiles and functional annotations, full-length mRNA through search functions and the genome browser. However, a more comprehensive effort for integrating genomic tools and datasets can facilitate gene characterizations. Translational strategies showed to be feasible to investigate plant defense responses. Multi-layered omics data can be combined to better explore network of interactions and biological behavior in a synthetic manner (Choi 2019). A broader vision will provide deeper insights in studied process accelerating the discovery of new traits. Knowing the location of given R-gene locus can be of great advantage for mining its nucleotide sequences using both genetic recombination analysis and protein prediction data. Once a resistance source has been phenotypically characterized, sequencing, genetic and functional analysis can be employed to link predicted sequence to gene function. Identification of syntenic regions among related genomes or collocation of a predicted gene with similar function in a related species can help to select candidate genes for the given trait. Analysis of chromosome recombination rate data and putative R-gene prediction resulted useful to select promising candidate genes (Andolfo et al. 2014).

1.7 Plant Protection and Patent Regulatory Issues

In many countries the regulation for the protection of plant varieties is based on a traditional approach set up prior the development of genetic engineering and genomics methodologies (Official Journal of the European Union n° L 227 of 01/09/1994 pp. 0001–0030) here in after "ROV". The UPOV Convention establishes a specific title for the protection of plant varieties, different from the patent, excluding from patentability, in its first drafts, all plant varieties. This prohibition is also included in article 53(b) of the ROV, relating to the community protection of plant varieties. On the other hand, Directive 98/44/EC of the European Parliament and of the Council of July 6, 1998 on the legal protection of biotechnological inventions (Official Journal of the European Union n° L 213 of 30/07/1998 pp. 0013–0031), allows the patentability of inventions consisting of plants or plant material, provided that no whether they are new plant varieties, or their application is not limited to a specific plant variety (Garcia-Vidal 2017). Effects and intensity of the protection are different from those of patent law, since they touch, in principle, the variety's reproduction material and, only when it has not been possible to exercise actions against the production and commercialization of this vegetal material, cascading actions can be exercised against the fruits and the products obtained by said fruits. Hence, despite the prohibition of the patentability of plant varieties, there have been several attempts to achieve their patentability. TOMATE II case was successful in this regard since the High Chamber of Resources of the Office European Patent, interpreting that

article 53 b) of the European Patent Convention did not exclude the patentability of plants as products (Torralba-Simon 2019). The search for the patentability of plant varieties shows the interest of the tomato industry in greater protection for their biotechnological inventions, so that they can recover and obtain greater profitability from the investment made. This forces to consider the fundamentals of the Law of plant varieties and consider whether the protection granted is currently sufficient, taking into account the development of biotechnological research. The holder of the plant variety rights has the right to exclusively carry out certain operations with the plant material, requiring any third party of their authorization for its execution (Arts. 13.1 and 2 ROV, 12 LOV and 14.1 UPOV Convention). These operations, which are exhaustively listed, are production or reproduction (multiplication), packaging for propagation, putting up for sale, sale or other commercialization, export, import and storage with a view to perform any of the above operations (Petit-Lavall 2017).

The extension of the scope of protection of the breeder's rights to the product of the harvest and to the products directly obtained from the plant material is nuanced by the cascade configuration of said protection, which already places the plant variety right at a clear disadvantage with respect to the right of patent. In this way, the harvested material is only protected if the following two conditions apply: it has been obtained through the unauthorized use of components of the protected plant variety and the owner has not had a reasonable opportunity to exercise his rights over said components of variety (Arts. 13.3 Regulation ROV, 13.1 LOV related to art. 7 ROV and art. 14.1 CUPOV). For the holder of the right to benefit from the extension of the protection on the crop product, he must have previously carried out the necessary actions to exercise said right in the multiplication or reproduction phase and, only in the case of proving these actions are not possible, he may try to exercise his rights over the harvest product.

It could well underlined a limitation of the protection of the breeder's rights to protect farmers and traditional breeders interests. It is necessary to reflect on the interests that base the plant variety right and the adequacy of the current legal system for its protection, since there is no doubt that any weakening of the breeder's rights must cause a flight to other protection systems such as know-how or patent law, as has been seen, is occurring despite the express prohibition of patentability of plant varieties, through recourse to product claims obtained by a certain procedure. The pressure on the patent system to protect plant varieties, which as has been advanced has been successful on several occasions but has been stopped by the Enlarged Board of Appeal of the European Patent Office issued Opinion G 3/19 (Pepper) on May the 14th, 2020. As with other industrial property rights (art. 59 LP and art. 38 LM, in Europe see art. 67 CPE and 9.3 RMC), the applicant for a plant variety has the right to demand reasonable compensation appropriate to the circumstances of whoever performs acts of exploitation that, granted the plant variety to be protected, would constitute acts of infringement, during the period started with the publication of the application and ended with the concession (Arts. 95 ROV; in art.18.2 LOV and art. 13 UPOV). The actions for violation of the right do not extend to this period of provisional protection, in which the protection of the owner of the rights is limited to compensation for the negative effects caused by the exploitation of the plant

variety by third parties. Obviously, whatever the criteria used to fix the amount of reasonable compensation (Espinosa-Calabuig 2016), only the negative consequences of the exploitation of the variety during the period of provisional protection would have to be take into account.

Biotechnological advances, which require an investment in plant innovation fully comparable to that made in other technology matters, together with a possible consolidation of the interpretation of the courts that is very restrictive of the scope of the protection of breeder's rights, translate into pressure on patent offices to achieve the patentability of plant varieties, by considering them products obtained through the use of microbiological procedures. Undoubtedly, the cascade protection of plant variety rights and their extension only to essentially derived varieties, and not to all derived varieties or dependent varieties, is a transcript of a traditional or "natural" conception of plant variety law that must probably outperform.

The attaching of regional and national regulation in the UPOV Convention places the international community before a huge challenge, such as the debate and reform of the Law of plant varieties, attending to all interests in presence, the public interest in food safety and the sustainability of agriculture, and the interest of farmers and rights holders, ceasing to oppose said interests and seeking a balance between them, but taking into account the current reality of the state of science, such as new publishing techniques genetics that are being developed, and the need to promote the advancement of technology.

1.8 Future Perspectives

Genomic information extracted in different stages of resistant plant design process can be used to define target genes, to select target trait to begin studies, to extract information relevant for identifying a gene or obtaining desired varieties. The genetic advance achieved through genomic scanning depends on the ability of capturing superior alleles. Modern breeding is a dynamic, and evolving research discipline for minimizing efforts. Traditional breeding has been integrated with molecular aided selection, but many traits are very complex to dissect and variation in gene expression level may cause difference in resistance response variability. In such complex situation, it is important to offer the possibility to screen for allelic differences at the expression level (Torti et al. 2021) and to discriminate superior allelic forms with high throughput and sensitive detection methods (Singh et al. 2020a, b). After generating and analyzing new data, the comparison with information stored in large-scale repositories is essential to understand and interpret the resulting data and to draw conclusions. A wide range of technologies that might be used to genetically engineering plant's genome are also available or are under development. Several countries (Argentina, Australia Japan Canada and US) acknowledge the potential of gene editing to improve plant traits without introducing foreign DNA. In other countries, the debate is still ongoing (EU, UK, Russia, India, China and South Africa). A

more comprehensive effort for making use of genomic tools and datasets can enlarge the availability of new tomato resistance traits to biotic stress in the next future.

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Chapter 2
Genomic Designing for Biotic Stress Resistance in Potato

Jagesh Kumar Tiwari, Virupaksh U. Patil, Riccardo Aversano, Domenico Carputo, G. Vanishree, Dalamu, and Manoj Kumar

Abstract Potato is a globally important food crop. In addition to various other factors, potato suffers from many biotic stresses. The important diseases are late blight, viruses, bacterial wilt, bacterial soft rot, dry rot, charcoal rot, common scab, black scurf and wart; and insect-pests are like aphid, whitefly, mite, potato tuber moth, potato cyst nematode, potato leaf hopper and white grub. Of which, late blight is the most devastating disease, whereas aphids and whiteflies are more important pests. These biotic factors limit crop growth and reduce tuber yields. The genus *Solanum* is one of richest source of genetic diversity and provides great opportunities for genetic enhancement of potato applying classical genetics, traditional breeding and modern genomics tools. With the available knowledge on potato genetic resources, genetic diversity, molecular markers, mapping, gene tagging, marker-assisted selection and high-resolution maps, there had been a considerable advancement in potato. The availability of the potato genome sequence and recently sequenced some more wild species, next-generation breeding tools like genome editing, high-throughput genotyping using single nucleotide polymorphism array and genotyping by sequencing, phenomics, genome wide association mapping, genomic selection and other omics resources further provide tremendous opportunities for next-generation breeding of potato. This chapter highlights on genomic designing for biotic stress resistance in potato.

Keywords Biotic stress · Genetic diversity · Breeding · Genomics · Potato

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2.1 Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important crop of the world after rice, wheat and maize and is a major food crop consumed by over a billion people globally (Chakrabarti et al. 2017). It is also one of the most efficient food crops. It is a good source of high-quality protein and rich in minerals, vitamins and phytochemicals. Potato can produce over twice as much dry matter and calories per unit area and time compared to wheat, rice, and maize. Besides being an efficient food producer, it has a broader flexibility in planting and harvesting time and, as such, can fit into many prevalent cropping sequences, thereby giving potato growers a wider choice of crops (Singh et al. 2020). These virtues make potato a good candidate crop for providing food and nutritional security to the developing world. Keeping this in view, FAO declared it as the "food for future" and the year 2008 was announced as the International Year of Potato by the United Nations.

Potato is affected by various diseases and pests, which causes severe yield reduction. Among them, late blight, viruses, bacterial wilt and storage rots are the major diseases, whereas aphids, whiteflies, thrips, mites and potato cyst nematodes are economically important insect-pests. Late blight is the most devastating, which can cause complete loss of crop within a week time if severe infestation is found, whereas viruses are serious from the seed potato quality point of view. Viruses transmit over the generations through clonally propagated materials like tubers and degenerate seed quality, ultimately cause yield reduction. Bacterial wilt is becoming more important in the high temperature growing regions; unfortunately, very limited resistant sources are available against this disease. Soil and tuber-borne diseases impacting tuber storage are dry rot, charcoal rot, bacterial soft rot and common scab (Singh et al. 2020).

With the rising global temperature under climate change scenario, management of these biotic stresses is inevitable for sustainable potato production to meet the world population food requirement in the future. To achieve this, traditional breeding methods have impacted a lot to improve potato varieties. However, breeding efforts suffer from many problems; among them the polyploid nature of cultivated *S. tuberosum*, hybridization barriers, poor selection efficiency, and length of breeding programs (it takes over 10 years to release a superior variety), and cumbersome trait phenotyping methods in field conditions. Hence, there is a need to strengthen potato research by applying genomics resources and modern genomics tools. After the potato genome sequencing in 2011 (Xu et al. 2011), the application of structural and functional genomics is still limited in potato. Therefore, discovering novel genes and the deployment of genomics resources is mandatory to enhance tuber production and quality. This chapter aims to update knowledge on modern breeding strategies to obtain improved varieties for sustainable crop production. Figure 2.1 depicts technologies to be used for genomic designing for biotic stress tolerance in potato.

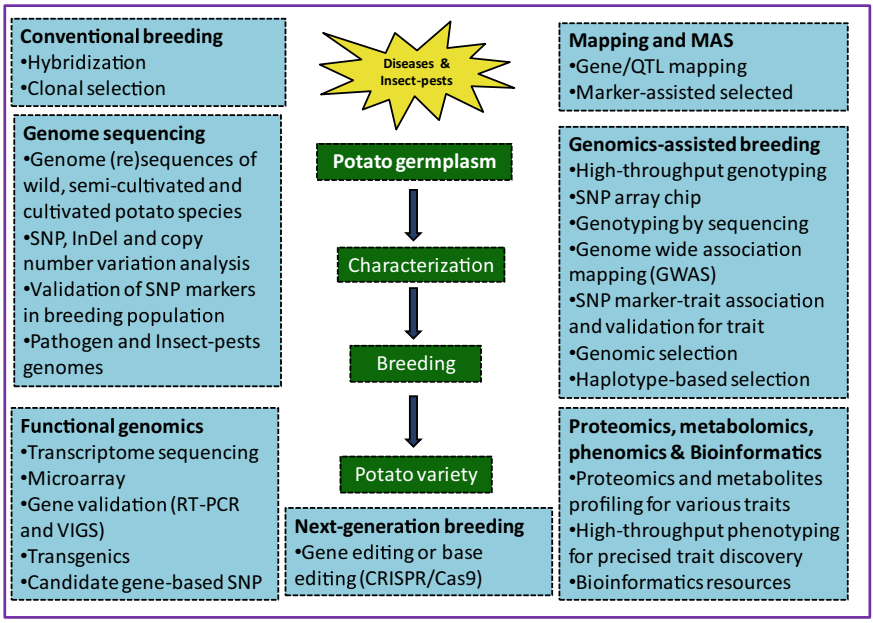

Fig. 2.1 A schematic outline of modern genomics tools used in potato improvement

2.2 Biotic Stresses in Potato

2.2.1 Late Blight

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is the most devastating disease of potato worldwide (Tiwari et al. 2013). In mid nineteenth century, late blight caused a complete wipeout of crop in the European countries, particularly in Ireland and popularly known as 'Irish Famine' (1845). Since then, it spread worldwide, causing now crop loss of up to 10–12 billion US dollars per annum world over. It affects all plant parts (leaves, stems and tubers), causing water-soaked black necrotic spots on leaves. Sporangia of the pathogen can be seen on the lower surface of leaves in the form of white cottony growth around the necrotic lesions. The favorable conditions for disease development are mild temperature (18 ± 2 °C) and high relative humidity (>90%). Due to these specific environmental needs, disease forecasting systems have been developed as complementary tools to manage this disease.

Late blight is controlled mainly through fungicides application and the use of resistant varieties. Besides, cultural practices like sanitation, crop rotation, fertilizers and crop geometry can be successfully applied. Spray of fungicides like metalaxyl and cymoxanil is the most common way to control late blight. Several resistance (R)

genes from wild or cultivated potatoes have been exploited through either conventional breeding approaches or biotechnological tools. Among donor wild species, worth to mention are *S. demissum*, *S. bulbocastanum*, *S. microdontum*, *S. pinnatisectum*, *S. cardiophyllum* and *S. verrucosum* (Tiwari et al. 2013). Biopesticides and biocontrol agents like *Trichoderma viride* and *Pseudomonas aeruginosa* have also been attempted as safer options to manage this disease. Nevertheless, there is a need for genomic designing for durable resistance to late blight.

2.2.2 Viruses

Potato is infected by more than 30 viruses, which cause yield reduction depending upon disease severity. The major potato viruses are *Potato virus X* (PVX), *Potato virus Y* (PVY), *Potato virus S* (PVS), *Potato virus M* (PVM), *Potato leaf roll virus* (PLRV), *Tomato leaf curl New Delhi virus-potato* (ToLCNDV) in India, and *Potato spindle tuber viroid* (PSTVd). In general, crop losses are higher in the case of mixed infections. PVY and PLRV are the most devastating, causing up to 80% yield losses, whereas PVX, PVS and PVM are mild, causing up to 30% yield loss (Tiwari et al. 2012). *Groundnut bud necrosis virus* (GBNV) causes stem necrosis disease in high-temperature regions of central and western India in early planted crop. The newly emerged white fly-transmitted ToLCNDV causing apical leaf curl disease is also serious in northern India. Most viruses are transmitted through contact, mechanical, infected seeds and/or vectors like aphids, whiteflies and thrips. Aphid transmit viruses in two ways (i) persistent and circulative such as PLRV, (ii) non-persistent like PVY, PVA, PVS, and PVM. These viruses cause mosaic or leaf curl and various other mixed symptoms on plants. Circulative virus like PLRV can be managed through the control of aphids.

In general, virus management methods include: prevention of viral transmission, eradication of infected sources, control and avoidance of vectors, and use of virus-free healthy seeds, resistant varieties and biotechnological tools. Conventional and molecular breeding approaches have been applied to breed resistant varieties. The Ry_{adg} gene from *S. tuberosum* Gp. Andigena has been found effective against several strains of PVY. Besides, there are many genes like Ry_{sto} from *S. stoloniferum* conferring PVY resistance and Rlr_{etb} from *S. etuberosum* providing resistance to PLRV (Tiwari et al. 2012). Resistance to virus vectors would be important to control potato viruses; particularly interesting in this context is wild *S. berthaultii,* which confers resistance to aphids. Moreover, transgenic approaches have been developed either through pathogen-derived coat protein genes or through disruption of host translation elongation factor.

2.2.3 Soil and Tuber Borne Diseases

Potato is affected by several soil and tuber-borne diseases, causing heavy losses, particularly during storage (Sagar and Sanjeev 2020). Dry rot, charcoal rot and bacterial soft rot cause losses during storage, whereas black scurf and common scab impact tuber appearance and reduce marketable value. Bacterial wilt is another serious potato disease. Dry rot, caused by a fungus *F. oxysporum* (*F. sulphureum/F. sambucinum*), is an important post-harvest disease-causing losses during transport and storage. The favorable temperature range for fungus growth is between 15 and 28 °C. Dry rot is managed by sanitation, use of disinfected and healthy quality seeds and boric acid (3%) treatment. Charcoal rot disease, caused by the fungus *Macrophomina phaseolina*, is more prevalent at high soil moisture combined with high temperature (over 28–30 °C). This disease is managed by crop rotation and rescheduling in planting dates. Bacterial soft rot, caused by *Pectobacterium atrosepticum* and *P. carotovorum* (syn. *Erwinia carotovora* subsp. *atroseptica* and *Erwinia carotovora* subsp. *carotovora*, respectively), is another devastating disease of the potato during harvest, transport and storage. The infected tubers develop wounds, holes and rots. This disease is managed by cultural practices, correct harvesting (e.g., avoid harvesting when temperature rises above 28 °C), curing, boric acid (3%), treatment of harvested tubers and cold storage. Black scurf is caused by *Rhizoctonia solani* and affects tuber quality and causes moderate yield losses. The optimum temperature for the development of stem lesions is 18 °C. This disease is managed using healthy seeds, boric acid treatment (3%), soil solarization and crop rotation. Common scab is caused by *Streptomyces scabies* and causes lesions on tuber skin. Congenial environments for disease development is pH (5.2 to >8.0), temperature (20–30 °C) and low soil moisture. The disease is managed by using healthy seeds, boric acid treatment (3%), cultural practices, and crop rotation. Bacterial wilt or brown rot is one of the most damaging diseases of potato caused by *Ralstonia solanacearum*. The favorable soil temperature for disease development is between 15 and 35 °C. Since there is no resistant source of resistance available, this disease is mainly controlled by the use of healthy seeds and crop rotation (avoidance of other Solanaceous crops). Potato wart, caused by *Synchytrium endobioticum*, is a problem of hilly regions, like Darjeeling hills in India. An average temperature less than 18 °C and rainfall nearly 700 mm favor disease development. It is managed by sanitation and crop rotation. Very few resistant varieties are available for most of these soil and tuber borne diseases (Sagar and Sanjeev 2020), and genomics assisted breeding is critical to solve this issue.

2.2.4 Insect-Pests

Potato is infested by many insect-pests such as aphids, whiteflies, thrips, white grubs, cutworms, leaf hopper, potato tuber moth and mites (Shah et al. 2020). Aphids (mainly *Myzus persicae*) are vectors of potato viruses (PVY, PVA, PLRV, PVS and

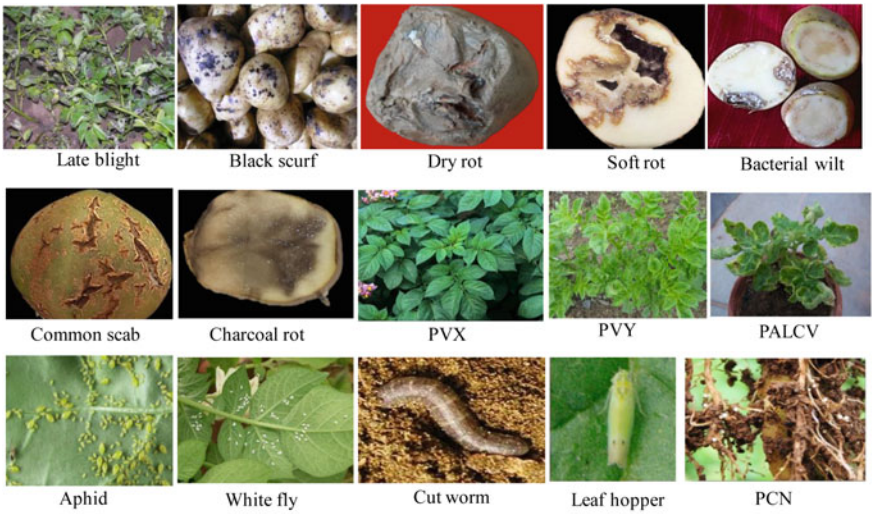

Fig. 2.2 Different diseases and insect-pests infecting potato crop

PVM); they transmit viruses from infected to healthy plants causing mosaic and leaf curl symptoms. Whitefly (*Bemisia tabaci*) is a severe emerging problem in potato under climate change scenario; it transmits ToLCNDV-potato virus and causes mosaic, chlorosis and curling of apical leaves symptoms. Potato leaf hopper (*Amrasca biguttula biguttula*) also damages the potato crop, causing hopper burn symptoms. Thrips (*Thrips palmi*) are vectors of groundnut bud necrosis virus, causing stem necrosis. They are particularly common in high-temperature regions. White grub (*Brahmina coriacea*) is destructive in hilly regions, damaging potato by large shallow and circular holes in the tubers. Cutworm (*Agrotis segetum*) is another destructive pest of potato in both hills and plains. Mature larvae damage the stem at the ground and make irregular holes in the tubers. Potato tuber moth (*Phthorimaea operculella*) is a serious pest causing damage in storage and fields. Mite (*Polyphagotarsonemus latus*) damages early planted crop when temperatures are high. Mostly, these insect-pests are managed by cultural practices and insecticides (Shah et al. 2020). Additionally, potato cyst nematodes (PCN) (*Globodera rostochiensis* and *G. pallida*) are the major problems in the world particularly temperate or hilly regions such as Nilgiri and Kufri hills in India, and European countries (Fig. 2.2).

2.3 Genetic Resources

The cultivated potato, *S. tuberosum* L. belongs to the genus *Solanum* in the family Solanaceae. According to Hawkes' classification, the genus is very large and contains over 2000 species, of which nearly 235 are tuber bearing (Hawkes 1990). Potato

species are rich sources of genes for resistance to various biotic stresses. They form a polyploid series ranging from diploid ($2n = 2x = 24$) to hexaploid ($2n = 6x = 72$), with the basic chromosome number of 12. About 73% of the tuber-bearing *Solanum* species are diploids, 4% triploids, 15% tetraploids and 8% pentaploids/hexaploids (Hawkes 1990). The potato cultivated worldwide, *S. tuberosum,* is a tetraploid ($2n = 4x = 48$). The recent classification by Spooner et al. (2007) distribute the cultivated potato species as following: (i) *Solanum tuberosum* Andigenum Group of upland Andean genotypes containing diploids ($2x$), triploids ($3x$) and tetraploids ($4x$); and *Solanum tuberosum* Chilotanum Group of tetraploids ($4x$) of lowland Chilean landraces, (ii) *S. ajanhuiri* ($2x$), (iii) *S. juzepczukii* ($3x$), and (iv) *S. curtilobum* ($5x$) (Table 2.1). *S. tuberosum* is generally divided into two subspecies, namely subsp. *tuberosum*, the universally cultivated potato, and subsp. *andigena*, a primitive taxon cultivated to a limited extent in the Andes region. An "effective ploidy" of potato species is represented by the endosperm balance number (EBN). The EBN is a number, ranging from 1 to 4, assigned to each potato species following intra/interploidy crosses (Johnston et al. 1980). It is a powerful tool to predict the success of interploidy/interspecific hybridization, in that normal endosperm development occurs only when there is a 2:1 maternal to paternal EBN ratio in the hybrid endosperm.

The primary genepool includes the cultivated potato (*S. tuberosum* spp. *tuberosum*) (4 EBN); no sexual barriers occur within genotypes of the primary genepool. The secondary genepool refers to 4 EBN wild species such as *S. demissum,* which can be crossed with cultivated *S. tuberosum,* and nearly 180 diploid 2 EBN wild species. The tertiary genepool includes wild species that are not crossable with cultivated/wild species due to differences in ploidy number and EBN; among them *S. bulbocastanum* and *S. commersonii*, both $2x$ (1 EBN). Many useful genes from wild sources cannot be transferred into the cultivated genepool through conventional techniques because of sexual incompatibilities, primarily due to differences in ploidy and EBN. A few strategies have been proposed to overcome this problem. For example, to introgress resistance to *R. solanacearum* and *P. carotovorum* possessed by 1EBN species *S. commersonii*, Carputo et al. (1997) proposed a breeding scheme based on doubling the chromosome number of *S. commersonii*, and on the production of

Table 2.1 Taxonomic classification of cultivated potato species according to Hawkes (1990) and Spooner et al. (2007)

Hawkes (1990)	Spooner et al. (2007)
Solanum ajanhuiri ($2x$)	*S. ajanhuiri*
S. curtilobum ($5x$)	*S. curtilobum*
S. juzpeczukii ($3x$)	*S. juzepczukii*
S. tuberosum subsp. *tuberosum* ($4x$) subsp. *andigena* ($4x$)	*S. tuberosum* Andigenum Group Chilotanum Group
S. chaucha ($3x$)	*S. tuberosum* (Andigenum Group)
S. phureja ($2x$)	*S. tuberosum* (Andigenum Group)
S. stenotomum ($2x$)	*S. tuberosum* (Andigenum Group)

triploid and pentabloid bridges. Additional methods to circumvent sexual barriers are manipulation of ploidy and EBN, mentor pollination and embryo rescue, hormone treatment, and reciprocal crosses (Jansky 2006). Notably, also somatic hybridization has been extensively used in potato to overcome sexual barriers and produce genetic variability at both nuclear and cytoplasmic DNA. Interspecific somatic hybridization is a multi-step process involving protoplast isolation and fusion, culture and regeneration of fusion products and, finally, the identification of somatic hybrids among regenerants. Since several important traits exhibit wide variation in the somatic hybrids produced, further breeding efforts are necessary before a genotype combining several useful characteristics is identified. A recent review by Tiwari et al. (2018b) provides progress in somatic hybridization research in potato over 40 years. For example, somatic hybrid between *S. tuberosum* dihaploid 'C-13' ($2n = 2x = 24$) and diploid ($2n = 2x = 24$) wild species *S. cardiophyllum* (Chandel et al. 2015), and *S. pinnatisectum* (Sarkar et al. 2011) for late blight resistance with wider genetic base.

Potato genetic resources are collected and preserved worldwide. The International Potato Centre (CIP) (https://cipotato.org/), Lima, Peru is a CGIAR research centre for global potato research and development. CIP is amongst the largest international potato gene bank in the world, which provides potato germplasm throughout the world. Other potato gene banks are the US Potato Genebank (NRS) USA, the CGN Potato Collection at the Centre for Genetic Resources, the Netherlands (CGN), and the Commonwealth Potato Collection (CPC) of the Vavilov Institute (VIR) etc. The European Cultivated Potato Database (ECPD) (https://www.europotato.org/) is an online database of the European cultivated potato varieties; an online potato pedigree database resource is also available (http://www.plantbreeding.wur.nl/PotatoPedigree/). UK has also developed its own potato variety database (http://varieties.ahdb.org.uk/). Worth to mention is also the PotatoPro (https://www.potatopro.com/) database, which describes potato statistics at the world level.

2.4 Classical Genetics and Breeding

The cultivated potato is tetraploid and highly heterozygous suffering from acute inbreeding depression. The clonal propagation of tubers preserves the heterozygosity in commercial cultivars. Conventional breeding is a cumbersome task because most traits show tetrasomic inheritance and also chromatid segregation. Due to the small size of potato chromosomes, it is very difficult work at cytological level. In the past, chromosome identification and mapping were carried out using a set of restriction fragment length polymorphism (RFLP) marker-anchored bacterial artificial chromosomes (BAC) as fluorescence in situ hybridization (FISH) probes (Dong et al. 2000). This led to assign genetic linkage groups to specific chromosomes and was also used for chromosome numbering. Potato breeding objectives mostly focus on high tuber yield, quality and resistance to diseases, insect pests and abiotic stresses. More than fifty desirable traits are to be combined while developing a new potato

variety. These traits include morphological features, yielding ability, tuber characters, ability to withstand various stresses, wider adaptability, quality parameters, consumer and industrial acceptability. It is perhaps a herculean task to combine all traits in a single variety because of the complex heterozygous potato nature. The genetic base of the existing and newly released potato varieties is relatively narrow compared to that available in the genepools. Only a fraction of useful genes from wild species have been successfully introgressed into potato varieties. The history of conventional potato breeding reveals that many varieties took nearly 30 or more years from hybridization and clonal selection before being released. Conventional potato breeding is often carried out at the tetraploid level, involving selection of specific tetraploid *S. tuberosum* parents, hybridization and phenotypic recurrent selection in seedling and clonal generations at targeted locations for a wide range of desirable characters. Alternatively, it is performed at the diploid level, involving hybridization between diploid species and *S. tuberosum* dihaploids ($2n = 2x = 24$). Haploid-species hybrids are selected for traits of interests and for a propensity to produce 2n gametes to be employed in sexual polyploidization crossing schemes. At both ploidy levels, however, conventional breeding strategies usually take many years and seek huge investments. In addition, they involve human resources and, compared to molecular breeding, they delay the accessibility of the targeted variety to stakeholders.

2.5 Molecular Markers to Assess Genetic Diversity

To study the diversity of plant species, various type of markers can be employed. Classical tools for such investigations are morphological markers. They are easily identifiable simply on the phenotype of an organism. Later, biochemical markers (or isozyme) were discovered; they are based on the relative mobility of enzyme isoforms. However, both markers are influenced by the environment and plant growth stage. A quantum jump towards genome mapping was made possible after introducing DNA markers, which are based on the DNA sequence variation and are least affected by the environment and growth stages. A wide range of molecular markers have been developed over the past four decades. Different types of molecular markers have been developed. The first molecular marker technique was RFLP, followed by PCR-based marker systems such as RAPD, SSR, AFLP, SSCP and CAPS. Sequencing technologies allowed the detection of single nucleotide polymorphisms (SNPs) markers. The progress of next-generation sequencing (NGS) technologies and the decreasing prices for sequence runs have led to a number of novel techniques for the detection of polymorphic markers. Some recent examples are genotyping by sequencing (GBS) and SNP array chip.

Genetic diversity analysis, marker-assisted selection and DNA fingerprinting (genotyping) are the important applications of molecular markers. Unlike morphological descriptors, profiles created by using molecular data are independent of environmental effect. Therefore, the International Union for the Protection of (New) Plant

Varieties (UPOV) has constituted a working group to critically examine the feasibility of using biochemical and molecular techniques (BMT) for variety identification. DNA fingerprints can be used to establish distinctness and check the uniformity and stability of a particular variety. A wide range of genetic diversity studies have been carried out in potato world over. Consequently, genetic relationships have been established in cultivated and wild potato species. Various molecular markers such as RAPD, ISSR, SSR, AFLP, DArT and SNP have been extensively used for diversity analysis in potato germplasm and varieties. Isozyme, RAPD and AFLP markers were used for diversity analysis and to test the genetic integrity of potato after micropropagation and long-term conservation (Aversano et al. 2011). SSR has been used to analyze genetic diversity within wild species showing noteworthy resistances to *R. solanacearum* and aphids. A new set of 24 highly informative SSR markers (two from each linkage group) named as the Potato Genome Identification (PGI) kit has been used worldwide in potato varieties (Tiwari et al. 2018a) and wild species (Tiwari et al. 2019). The role of cytoplasmic markers (T/β, W/α, W/γ and A/ε) has also been studied in potato using plastome- and chondriome-specific markers (Tiwari et al. 2014). A wide range of SNP arrays have recently been developed and applied in potato to characterize germplasm and gene discovery.

2.6 Association Mapping

Linkage mapping is the genetic association of traits with segregating alleles of molecular markers in a defined mapping population. It detects genomic regions that explain phenotypic variation in a trait of interest and subsequently identifies genes/QTLs in that region. QTL mapping in potato is mainly carried out at the diploid level due to the potato highly heterozygous nature. Many QTLs for resistance to biotic stresses like *P. infestans* and root cyst nematodes are known. On the contrary, association mapping is a method to identify genes or QTLs associated with phenotypic variation in natural populations based on historical recombination events related by descent. The method takes advantage of historical meiotic recombinations and linkage disequilibrium (Flint-Garcia et al. 2003). For association mapping, a population consisting of diverse germplasm including cultivars, breeding clones and landraces is assembled and phenotyped for the complex traits of interest. Molecular markers are then analyzed in the population and marker-trait associations between phenotypic and genetic variation are detected. Marker-trait association approaches are known to identify markers linked with the genes/QTLs. In potato, tetraploid or diploid potatoes have been utilized for association mapping for desirable agronomic traits and biotic stresses. Gebhardt et al. (2004), for the first time, used association mapping in tetraploid potato germplasm to identify markers for late blight resistance and maturity traits in 600 potato cultivars. Further, association mapping was applied based on candidate genes for resistance against *Verticillium dahliae* (Simko et al. 2004) and *Phytophthora infestans* (Pajerowska-Mukhtar et al. 2009). Genome wide association mapping (GWAS) has been deployed to identify genes/QTLs at whole

genome level in potato by D'hoop et al. (2008). GBS is effectively used for SNP discovery and trait association mapping for multiple traits including biotic stresses (Uitdewilligen et al. 2013; Sharma et al. 2018).

2.7 Molecular Mapping of Resistance Genes and QTLs

Gene mapping is foremost important for molecular breeding. RFLPs were first used to create linkage maps in potato, which showed conserved markers with tomato. Later, PCR-based molecular markers such as SSRs and AFLPs were applied for mapping of genes and QTLs. More than 10,000 AFLP markers were used to generate dense maps exploited by the "Potato Genome Sequencing Consortium". Many genes for biotic stress resistances have been mapped in potato. Particular focus has been given to simply inherited genes, such as those conferring resistance to late blight, viruses and nematodes. Disease scoring provides clear patterns of qualitative evaluation of genotypes. A number of techniques like bulked segregant analysis have been extensively used to identify markers. A recent study describes mapping of *H2* resistance effective against *Globodera pallida* pathotype *Pa1* in tetraploid potato (Strachan et al. 2019). A summary of some work on mapping and molecular markers is presented in Tables 2.2, 2.3 and 2.4.

Table 2.2 Summary of some linkage maps and molecular markers used in potato

Mapping population (number)	Parent species	Marker type (number)	Map length (cM)	References
F_1 (65)	*S. phureja* x [diploid *S. tuberosum* x *S. chacoense*]	RFLP (134)	606	Bonierbale et al. (1988)
BC_1 (67)	*S. tuberosum* (2x)	RFLP (263)	690	Gebhardt et al. (1989)
F_1 (246)	*S. phureja x* Diploid *S. tuberosum*	RAPD (170) AFLP (456) SSR (31)	773.7 (*S. tuberosum*) 987.4 (*S. phureja*)	Ghislain et al. (2004)
BC_1 (67), F_1 (91)	*S. tuberosum* (2x)	SSR (55)	879	Milbourne et al. (1998)
F_1 (136)	*S. tuberosum* (2x)	AFLP (10,365)	751 (maternal), 773 (paternal)	van Os et al. (2006)
F_1 (90)	*S. tuberosum* (2x)	cDNA-AFLP (700)	795	Ritter et al. (2008)

Table 2.3 Selected virus resistance genes, source species and anchor markers in potato

Virus	Resistance gene	Source species	Anchor marker	References
PLRV	*Rlr$_{etb}$*	*S. etuberosum*	TG443	Kelley et al. (2009)
PLRV	*Rl*$_{adg}$	*S. t.* Gp. Andigenum	UHD AFLP map	Velásquez et al. (2007)
PLRV	*Plrv.1* (QTL)	*S. chacoense*	GP125, GP185	Marczewski et al. (2001)
PLRV	*Plrv.4* (QTL)	*S. t.* Gp. Andigenum	St3.3.11, CP117, GP250	Marczewski et al. (2004)
PVS	*Ns*	*S. t.* Gp. Andigenum	GP126, GP189, CP16	Marczewski et al. (2002)
PVX	*Rx$_{acl}$*	*S. acaule*	GP21, TG432	Ritter et al. (1991)
PVX	*Nb$_{tbr}$*	*S. t.* Gp. Tuberosum	GP21, TG432	De Jong et al. (1997)
PVX	*Rx$_{adg}$*	*S. t.* Gp. Andigenum	GP34, CP60	Ritter et al. (1991)
PVY	*Ny$_{tbr}$*	*S. t.* Gp. Tuberosum	TG316, TG208	Celebi-Toprak et al. (2002)
PVY	*Ry$_{adg}$*	*S. t.* Gp. Andigenum	TG 508, CD 17, CP58, GP125	Hämäläinen et al. (1998)
PVY	*Ry$_{sto}$*	*S. stenotomum*	GP268, TG28, GP81	Song et al. (2005)

Table 2.4 Late blight resistance genes identified in potato species

Species (ploidy/EBN)	Resistance gene	References
S. berthaultii (2*x*/2EBN)	*Rpi-ber1*and *Rpi-ber2*	Park et al. (2009)
S. bulbocastanum (2*x*/1EBN)	*RB/Rpi-blb1, Rpi-blb2, Rpi-blb3, Rpi-abpt* and *Rpi-bt1*	van der Vossen et al. (2003, 2005)
S. demissum (6*x*/4EBN)	*R1, R2, R3 (R3 & R3b), R4, R5, R6, R7, R8, R9, R10, R11* and *Rpi-dmsf1*	Huang et al. (2005), Bradshaw et al. (2006), Hein et al. (2009)
S. microdontum (2*x*/2EBN), 3x	*Rpi-mcd1*	Tan (2008)
S. mochiquense (2*x*/1EBN)	*Rpi-mcq1*	Smilde et al. (2005)
S. papita (4*x*/2EBN)	*Rpi-pta1* and *Rpi-pta2*	Wang et al. (2008)
S. paucissectum (2*x*/2EBN)	*QTLpcs10*, *QTLpcs11*, *QTLpcs12*	Villamon et al. (2005)
S. phureja (2*x*/2EBN)	*Rpi-phu1*	Śliwka et al. (2006)
S. pinnatisectum (2*x*/1EBN)	*Rpi-pnt1*	Kuhl et al. (2001)
S. stoloniferum (4*x*/2EBN)	*Rpi-sto1* and *Rpi-sto2*	Champouret (2010), Wang et al. (2008)
S. enturi (2*x*/2EBN)	*Rpi-vnt1.1, Rpi-vnt1.2* and *Rpi-vnt1.3*	Foster et al. (2009), Pel et al. (2009)
S. verrucosum (2*x*/2EBN)	*Rpi-ver1*	Jacobs et al. (2010)

2.8 Marker-Assisted Breeding

Germplasm characterization is the foremost important part of molecular breeding. In addition to novelty, important characteristics for the release of new varieties are distinctness, uniformity and stability. Therefore, integration of marker-assisted selection (MAS) in conventional breeding is inevitable for the rapid release of new varieties. MAS method has been applied mostly in simply inherited traits like late blight, viruses, and potato cyst nematodes resistance, but limited in case of complex inherited traits like yield contributing traits. Moreover, gene pyramiding through MAS has been executed to accelerate the use of genetic resources in potato breeding. PCR-based markers are the best for MAS due to their ease in application, especially in a resource-poor developing country. Advances in molecular marker technology, large-scale whole genome sequencing and an expanding genetic map of potato chromosomes have progressed significantly. In the future, it would essentially improve the prospects of identification of resistance gene clusters with common sequence motifs for mapping and cloning of more *R* genes. Thus, it may lead to the development new diagnostic markers for MAS for biotic stress traits in potato.

MAS is particularly useful in the case of introgression breeding from the donor (e.g., wild) to the recipient (e.g., cultivated) genotype. In usual practice, this is achieved by recurrent backcrossing and selection cycles. Markers tightly linked to the gene of interest are used to identify progenies at the seedling stage, thereby decreasing the number of breeding cycles. Tightly linked markers for many qualitative and quantitative traits have been published and made available for MAS such viruses and late blight resistance (Tiwari et al. 2012, 2013) (Tables 2.5 and 2.6). For example, markers linked to extreme resistance to PVY and late blight resistance were validated in triplex parental lines and their progeny. Breeding of multiple disease resistance is a major priority in most potato-growing counties. Recently, MAS has been deployed to develop a new variety Kufri Karan at ICAR-CPRI, Shimla. This variety is highly resistant to late blight, viruses and moderately resistant to potato cyst nematode. MAS can also be performed in programs based upon interspecific

Table 2.5 A few molecular markers of virus resistance genes in potato

Gene	Virus	Marker name	Marker type	References
Rl_{adg}	PLRV	$E35M48_{192}$	AFLP	Velásquez et al. (2007)
Ry_{adg}	PVY	$ADG2_{310}$ (*Bbv*I)	CAPS	Sorri et al. (1999)
Ry_{adg}	PVY	$RYSC3_{321}$	SCAR	Kasai et al. (2000)
Ry_{sto}	PVY	$GP122_{406}$ (*Eco*RV)	CAPS	Heldák et al. (2007)
Ry_{chc}	PVY	38–530 (OPC-01)	RAPD	Hosaka et al. (2001)
Ny-1	PVY	$SC895_{1139}$	SCAR	Szajko et al. (2008)
Ny-1	PVY	$GP41_{443}$	SCAR	Szajko et al. (2008)
Nb	PVX	GP21 (*Alu*I)	CAPS	De Jong et al. (1997)
Nb	PVX	SPUD237 (*Alu*I)	CAPS	De Jong et al. (1997)

Table 2.6 Some molecular markers of late blight resistance genes for MAS in potato

Gene	Marker/primer	Marker type	References
R1	R1-1205	SCAR	Sokolova et al. (2011)
R3 (*R3a* & *R3b*)	R3-1380	SCAR	Sokolova et al. (2011)
R3 (*R3a* & *R3b*)	*R3b*F4/*R3b*R5	AS	Rietman (2011)
RB/Rpi-blb1	RB-629/638	SCAR	Sokolova et al. (2011)
RB/Rpi-blb1	RB-1223	SCAR	Pankin et al. (2011)
Rpi-sto1	Ssto-448	SCAR	Sokolova et al. (2011)
Rpi-snk1.1 and *Rpi-snk1.2*	Th21 (*Mbo*I)	CAPS	Jacobs et al. (2010)
Rpi-ver1	CD67 (*Hpy*CH4IV, *Ssi*I)	CAPS	Jacobs et al. (2010)
Rpi-vnt1.1, and *Rpi-vnt1.3*	TG35(*Hha*I/*Xap*I)	CAPS	Pel et al. (2009)
	NBS3B	AS	Pel et al. (2009)

hybridization when markers are not associated with the trait under selection. In these cases, MAS can be employed to estimate the wild genome content of the recurrent parent at each backcross, and can help to identify hybrids combining useful traits with the lowest percentage of wild genome content. This type of MAS, termed negative assisted selection, has been successfully applied at various steps of breeding programs aimed at transferring resistance traits from *S. commersonii* into *S. tuberosum* (Carputo et al. 2002).

2.9 Genomics-Aided Breeding

2.9.1 The Potato Genome

Unlike diploid crops, tetraploid potato varieties have four copies of each of the 12 chromosomes. This makes it very difficult to follow inheritance patterns, especially concerning the many complex traits with which breeders are compelled to work with. Moreover, the *S. tuberosum* genome high heterozygosity makes it recalcitrant to current sequencing technologies and bioinformatics programs. Therefore, the genes affecting many important agronomic traits remain still undiscovered and their locations on the 12 chromosomes are often imprecise. In 2011, the "Potato Genome Sequencing Consortium" (PGSC)—formed by 26 international institutes belonging to 14 countries—successfully solved these problems by sequencing a homozygous doubled monoploid (DM 1–3 516 R44, referred as to DM) of *S. tuberosum* Group Phureja ($2n = 2x = 24$), in which there were only two copies of each chromosome and, more importantly, each copy was identical. The PGSC deciphered 840 Mb of the potato genome in 2011. This represented the first genome of a plant belonging to the Asterid clade of eudicot, representing 25% of flowering plant species. A total of

39,031 protein-coding genes were predicted in the 840 Mb genome size of the potato. Numerous publications witness the usefulness of DM genome, but undoubtedly its quality and potential are limited by the technology exploited a decade ago. Now the advanced sequencing technologies and the improved software are enabling the generation of a high-quality potato genome assembly that will facilitate research aimed at improving potato agronomic traits and understanding genome evolution (Zhou et al. 2020). At present, a chromosome-scale long-read reference genome assembly of the potato genome has been constructed (Pham et al. 2020). In recent years, a few more wild or cultivated potato genotypes have been sequenced. They highlight evolutionary relationship, adaptation mechanism and novel resistance/tolerance genes in wild species such as *Solanum commersonii* (Aversano et al. 2015), tuber-bearing *Solanum* species (Hardigan et al. 2017), *S. chacoense* 'M6' (Leisner et al. 2018), cultivated potato taxa (Kyriakidou et al. 2020), and *S. pinnatisectum* derived somatic hybrid (Tiwari et al. 2021). Further genome analyses have identified markers for agronomically important traits (Li et al. 2018).

2.9.2 Functional and Comparative Genomics

The potato genome sequence has opened up new vistas in potato research. Functional genomics allows the mining of novel genes in potato germplasm/varieties for traits of economical and industrial importance through transcriptome analysis. Further, genome sequence and reference map allow association of genes to target traits in the genome. Such regions can then be used to define further markers for fine-scale mapping; alternatively, candidate genes can be sought directly from the genome sequence and associated annotation data. This step-change, facilitating sequence-based genomics and aiding molecular breeding in potato, would accelerate trait-gene discovery and gene isolation. This would further shorten the time to breed new varieties and also significantly improve parental genotypic assessment. Genome tagged molecular marker studies would be more meaningful and enable more accurate estimates of population genetic and linkage disequilibrium parameters. The shift towards sequence-based polymorphism rather than fragment-based will virtually replace centiMorgan position by sequence co-ordinates and greatly increase the information output and accuracy of mapping procedures. The integrated potato genetic and physical reference map forms an important resource for genetic mapping efforts and will alleviate many of the complicating aspects of potato genetics. With the release of the genome of the other economically important Solanaceous crop, i.e., the tomato, comparative genomics and sequence-based synteny analysis among *Solanaceae* have been made feasible. Given the biological and economic importance of many Solanaceous species and the diversity of their phenotypes/products (agriculturally useful parts tubers, berries, etc., growth habits, wide geographical growing range, clonal propagation, regeneration), comparative genomics provides a fundamental framework for tackling both applied and basic questions.

With the completion of genome sequencing of more and more organisms, research focus has now been shifted from sequencing to delineating the biological functions of genes. Methodologies of biological research are evolving from the "one gene in one experiment" to the "multiple genes in one experiment" paradigm. It is not possible to perform analysis on a large number of genes using traditional methods. Earlier DNA microarray and now RNA sequencing are the technologies that enable researchers to investigate and address issues that first were thought to be non-traceable. Functional genomics involves the use of high-throughput methods for the study of large numbers of gene set in parallel. Indirect information on cellular or developmental function can be obtained from spatial and temporal expression patterns. For example, the presence of mRNA and/or protein in different cell types, during development, during pathogen infection, or in different environments. The subcellular localization and post-translational modifications of proteins can be informative as well. The potato genome sequence can be used for functional validation of gene function. The techniques used for functional genomics in potato include RNA-Seq and microarray at the whole genome level and reverse genetic approaches, like gene knockout by RNAi (RNA interference) and VIGS (virus-induced gene silencing), at the gene level. They allow to analyze the expression of many genes in a single reaction quickly and efficiently. Besides, allele mining application has shown discovery in novel alleles of the genes for late blight resistance in wild potato species (Tiwari et al. 2015).

2.9.3 Next-Generation Potato Breeding

Potato improvement through the application of next-generation breeding techniques is essential to shorten the usually long period (over 10 years) required for developing a new variety. Successful completion of potato genome sequencing enables discovering a large number of genes regulating multiple traits like biotic stresses and yield attributes etc. Besides, bi-parental linkage mapping, population genetics by GWAS, genome editing (GE) and genomic selection (GS) coupled with GBS and SNP array chip platform with integrated high-throughput genotyping (HTG) and high-throughput phenotyping (HTP) facilities have emerged as powerful techniques for completion of breeding cycles in shorter time. There is immense potential to apply these new breeding techniques for rapid potato improvement.

2.9.3.1 Genomic Selection (GS)

Potato breeders have to deal with more than 50 characters (biotic stress, abiotic stress, quality traits, yield attributing and tuber traits) to develop a new variety. Although MAS has been a powerful tool in plant breeding and potato has been applied to improve resistance traits, it has limitations for complex inheritance traits, like yield. With the availability of the potato genome, there is immense opportunity to work at the whole genome level. Hence, GS or genome-wide selection, or genomic-assisted

breeding can enable the integration of phenotyping and high-throughput genotyping data of pedigree/segregating generations to enhance the selection of superior genotypes and accelerate breeding cycles. GS works on the principle of linkage disequilibrium (LD) with a minimum of one marker per locus in the breeding population without gene mapping. GS accelerates breeding cycles with an increase in genetic gain per unit time and reduces costs as well. It combines molecular and phenotypic data in a training population (TP) to acquire the genomic estimated breeding value (GEBV) of individuals in a TP that have been genotyped but not phenotyped. GS determines genetic association and diversity in different landraces/cultivars/varieties/breeding lines/wild species with variation in topography and ecology. With the identification of genome rearrangements and SNP discovery at whole-genome level, GS can be efficiently applied in the near future. GS has been successfully applied in animals and reported to some extent in plants like maize, wheat, sugar beet. In potato its application has been very limited so far, a few like resistance to late blight and common scab (Enciso-Rodriguez et al. 2018). This might be due to the unavailability of SNP markers distributed throughout the genome, trait association, SNP calling rate and software uses. However, the rapid advancement in genotyping techniques (SNP and haplotypes), high-throughput phenotyping and trait association would lead to reality potato GS in the near future.

2.9.3.2 Genotyping by Sequencing (GBS)

GBS is one of the high-throughput techniques currently being used to generate genotyping data for several crop species including potato (Bastien et al. 2018). With the reducing NGS costs, a considerable amount of high-throughput data has been developed. GBS has been designed for several studies, including genetic analysis, population studies, molecular characterization of germplasm, SNP discovery. To breed varieties, knowledge about genes and environment and their interaction is essential for using GBS to select advanced breeding lines with desirable traits (Schönhals et al. 2017). Besides GBS, SNP chip-based markers are an additional high-throughput genotyping platform available in potato for genotyping. Various platforms such as 20 K SNPs Affymetrix Axiom (SolSTW array) (Vos et al. 2015), Infinium 12 K V2 Potato Array (Illumina platform) (Ellis et al. 2018), and 8 K SNPs (Illumina Infinium BeadChip) (Obidiegwu et al. 2015; Schönhals et al. 2017) are currently available.

2.9.3.3 Genome-Wide Association Studies (GWAS)

GWAS (or linkage disequilibrium mapping) has been applied in potato and many other crops to examine simple and complex traits taking advantages of linkage disequilibrium. It is a family-based linkage mapping approach to identify the link between genotyped markers and phenotypes of interest scored in a large number of individuals with broad genetic and phenotypic diversity (landraces, wild and cultivated species, varieties, core collection). Usually, the rationale behind GWAS is to assess SNPs

that influence phenotypes. Currently, the use of Next Generation Sequencing (NGS) allows to genotype large populations with a higher density of markers. This offers the unique opportunity to increase mapping resolution. In line with this, specialized mapping populations have also been developed that significantly enhance the power and efficiency of these association studies. GWAS requires a detailed understanding of population structure to minimize false-positive and false-negative associations; for this purpose, various statistical methods have been developed over the years. Softwares like STRUCTURE and EIGENSTRAT are very popular within the scientific community working with GWAS. Recently, additional software specifically tailored for the tetraploid potato (i.e., GWASpoly) has been made available (Rosyara et al. 2016). In potato, GWAS has been conducted for various traits like common scab resistance (Yuan et al. 2020), *Verticillium* resistance and quality traits (Khlestkin et al. 2019).

2.9.3.4 Genome Editing (GE)

Genome editing is a targeted alteration in a genome that creates new allelic variation. Sequence-specific nucleases (SSNs) have been applied for genome editing and genetic manipulations. The SSNs technology is rapidly becoming important in plants, which uses three major nuclease systems like Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR- associated proteins (CRISPR/Cas9). Among these, CRISPR/Cas9 is the most widely used today for genome editing and is based upon an RNA-guided method to target DNA sequence. This is being used widely due to its simplicity, multiplexing capability, cost-effectiveness, and high efficiency. An important issue with this technology is the off-target mutations due to mismatch base pairing between gRNA and DNA. Genome editing is an essential tool to create new variants with desirable gene combinations. Unlike genetic transformation methods (see in later section), which creates stable integration of cisgenes or transgenes, genome editing provides crop improvement opportunities where no foreign gene is introduced. In potato, TALENs and CRISPR/Cas9 have been used for site-directed mutagenesis and gene silencing (Nadakuduti et al. 2018). The main targets were traits like cold-induced sweetening and glycoalkaloid (solanine and chaconine) content, acetochalactate synthase, granule bound starch synthase (review by Dangol et al. 2019).

2.9.3.5 High Throughput Phenotyping (HTP)

High-throughput precision phenotyping is essential to utilize ther ultimate potential of a genotype. Present methods of phenotyping are often slow, time consuming, laborious and inaccurate, often destructive or with limited phenotyping ability. High-throughput phenotyping platforms are essential for precision phenotyping and modern breeding applications. They are usually based on automation, sensors,

high resolution imaging capability, robotics etc. In potato, a few technologies have been applied to roots and shoot traits. For example, Phenofab and Keytrack System (KeyGene, The Netherlands) have been developed for measuring plant growth and other traits using multiple imaging systems and thermal sensors with automated handling under controlled environments. However, high correlations between pots and field-grown plants are essential.

2.10 Genetic Transformation

Genetic transformation has many advantages for plant breeding, and these advantages are even more striking in crops with complex polyploid inheritance, such as the potato. While conventional breeding manipulates genomes in a mostly uncontrolled fashion, requiring generations of selection to assemble and fix the maximum number of desirable traits, genetic transformation offers a direct approach, allowing introgression of a single, distinct gene without linkage drag. Thus, it enables rapid and often powerful improvement of crop plants, and is not limited by compatibility barriers. In cases where genetic diversity among sexually compatible relatives of crop species is insufficient for a particular trait, genetic transformation may represent the only possibility for trait improvement. It offers a highly effective means of adding a single gene to existing elite potato clones with no or very minimal disturbances. Potato, being highly amenable to genetic transformation, has been subjected to genetic transformation to confer resistance to a wide range of diseases (late blight, viruses, bacterial wilt and soil and tuber borne diseases) and pests (aphids, white fly, potato tuber moth). It should be pointed out that genetic transformation is not simply a faster alternative to conventional breeding. Rather, it is a complementary way to exploit plant genetic diversity that may require time to create and evaluate the most desirable expression of the transgene.

The availability of a suitable regeneration protocol is a pre-requisite for undertaking genetic transformation. A rapid and efficient *Agrobacterium tumefaciens* mediated transformation protocol based on direct organogenesis from inter-nodal stem explants of in vitro potato plants is available in potato. On the other hand, biological balistics (i.e. gene gun) plant transformation methods have also been applied. In these cases, either tungsten or gold particles coated with DNA are accelerated to a high speed to bombard target tissues. Although potato is highly amenable to *Agrobacterium* mediated transformation, the use of gene gun is necessary for plastid transformation and enhances transformation efficiency. The technique has been successfully used to transfer the plastid specific cassette for tuber-specific expression of *cry1Ab* and a fused *cry1Ab* + *cry1B* genes to develop transgenic potato resistance to potato tuber moth.

Many efforts have been devoted to the characterization, mapping, and cloning genes or resistance breeding in potato. For example, the *RB* gene (Bradeen et al.

2009) and an osmotin-like gene were cloned and sequenced from wild *S. bulbocastanum* and S. *chacoense* and used for developing transgenics with late blight resistance. The *RB* gene has been found the most effective to confer durable resistance against late blight in the F_1 progenies (Sundaresha et al. 2018). Similarly, virus and potato cyst nematode-resistant genes were cloned, the sequence was characterized and utilized for transgenics development. Post-transcriptional gene silencing (PTGS) or RNA interference (RNAi) are mechanisms of gene regulation in eukaryotes. In plants, PTGS acts as surveillance system against the invading molecular parasites like viruses, transposons and transgenes. PTGS is being utilized for transgenics development against pathogens and for functional genomics aimed at elucidation of gene functions. PTGS has been targeted for the *avr3a* gene for late blight resistance, and phosphatidic acid phosphatase 2 (*PAP2*) gene for bacterial wilt resistance. Viral gene sequences were also cloned and characterized. The coat protein gene from an Indian isolate of potato leaf roll virus (PLRV) was targeted through PTGS. Similarly, the coat protein gene of PVY, PLRV and potato apical leaf curl virus (PALCV), replicase associated protein gene of PALCV, and movement protein gene of potato stem necrosis virus were cloned, sequenced and used for the development of virus-resistant transgenics by RNAi technology. Technologies are available for easy and efficient transformation and protocols are applicable to carry out the southern, northern and western blotting for characterizing transgenic events.

2.11 Bioinformatics

Bioinformatics is now an inevitable tool in plant science with increasing advancements in genomics technologies. Bioinformatics plays significant role in generating new tools and databases; it increases the efficiency and precision of data analysis of huge genomics information. Genomics and post-genomics research requires modern bioinformatics tools to integrate genomics, transcriptomics, proteomics, metabolomics and phenomics data. The development of modern and customized bioinformatics tools and advanced databases has become mandatory to handle the increasingly enormous amount of large datasets in crop species. This helps us to systematically store, organize, and analyze large amounts of biological information computationally. With the advent of bioinformatics tools, an enormous amount of DNA, RNA and protein sequences are currently stored in gene data banks. Major public gene banks of the DNA and protein sequences are GenBank NCBI (National Centre of Biological Information) in USA (http://www.ncbi.nlm.nih.gov), EMBL (European Molecular Biology Laboratory) in Europe (http://www.ebi.ac.uk/embl/), and DDBJ (DNA Data Bank) in Japan (http://www.ddbj.nig.ac.jp).

The potato genome sequence was deciphered initially in 2011 by the PGSC and now the database is maintained by the SpudDB, Potato Genomics Resources, Michigan State University, USA (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml). It represents one of the most important tools for potato biotechnologists and breeders worldwide. This database has been recently updated with

the genome sequence of wild tuber-bearing *S. chacoense*. Additional bioinformatics resources are available. Genome sequences of a few more wild potato species are available at the NCBI. Solanaceae Genomics Network (http://solgenomics.net/) is a collection of maps, genomes, and tools for Solanaceae species. Sol*R*gene database (http://www.plantbreeding.wur.nl/SolRgenes) provides a comprehensive dataset to explore disease resistance genes in *Solanum* species. PoMaMo (Potato Maps and More) (https://gabi.rzpd.de/PoMaMo.html) contains molecular maps of all potato chromosomes with about 1000 mapped elements, sequence data, gene functions, BLAST search, SNP and InDel information etc. The PlantGDB (http://www.plantgdb.org/StGDB/) database describes genomes, gene models, alignments, gene structure annotations, annotated protein alignments etc.

2.12 Social, Political and Regulatory Issues

Conventional breeding is commonly practiced without any concern to develop new potato varieties. Transgenics development has raised serious biosafety issues in public and therefore, transgenics products are fully regulated worldwide with some exceptions. Recently, genome editing technology has emerged as a safer strategy to produce new, improved genotypes. All the technologies result into varieties having desirable agronomics traits. Hence, right of the inventors needs to be protected through legal means. Intellectual property rights (IPRs) have been created to protect these rights. Usually, IPRs are protected under categories such as patents, copyrights, trademarks, trade secrets, geographical indicators, design and layout design of integrated circuits. Patents are the most important form of protection for research and developmental activities. Another important example of intellectual property is given by Plant Breeders' Rights (PBRs), which refer to the legal protection offered to a breeder or developer or owner over a newly developed variety. Thus, it prevents any third party from the commercial exploitation of the new variety without a developer's authorization. For example, India has enacted the "Protection of Plant Varieties and Farmers' Rights" (PPV&FR) Act, 2001 as *sui generis* system of plant variety protection, which was based on the UPOV Act 1991. The PPV&FR Act 2001 protects plant varieties such as newly bred varieties, extant varieties (released but not completed 15 years on the date of application), farmers varieties (traditionally cultivated or landraces or wild/native) and essentially derived varieties (derived from an initial variety but little difference). For a registration of a new variety, the criteria of novelty (N), distinctness (D), uniformity (U), and stability (S) must be met. Plant variety protection is allowed for 25 years for trees and vines and 20 years for others (while 20 years for tree and vines and 15 years for others by PPV&FRA in India). Moreover, researchers' rights have been provided to use the protected varieties for research purpose as parents in breeding to develop new varieties. Overall, social, political and regulatory issues need to be addressed before acceptance of any crop variety.

2.13 Future Perspectives

Biotic stresses are one of the major limiting factors of yield reduction in potato. Moreover, in the climate change scenario, management of these biotic stresses would be more challenging where emergence of new pathotypes/strainal variations is common in pathogens and pests. The increasingly interest in genomics research combined with available knowledge on genetics, breeding (conventional and marker assisted selection) have enabled better scope for potato improvement. With the knowledge on a huge genetic diversity in the genus *Solanum*, phylogenetics relationship, molecular markers, gene mapping and cloning have also paved pathways for genetic enhancement of this crop at molecular level. The potato genome sequence alongwith a few more potato genomes sequences, and functional genomics provide immense opportunities to discover new genes and markers for breeding and biotechnological applications. With the next-generation breeding tools like high-throughput genotyping coupled with high-throughput phenotyping (phenomics), SNP array, GBS, GWAS, GS and genome editing provide powerful technologies fast development of climate resilient potato varieties resistance to biotic stress. Taken together, there is a need of genome wide characterization of whole germplasm collection at global level using robust SNP arrays or other technologies, robust phenotyping under controlled as well as natural environments on multiple locations, marker-trait association analysis, develop trait-specific driven molecular markers or haplotype-based next-generation potato breeding. Simultaneously, discovery of novel genes using transcriptomics approaches and other modern omics/biotechnological tools like proteomics, metabolomics etc. would strengthen the management of biotic stresses in potato.

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Chapter 3
Genomic Designing for Breeding Biotic Stress Resistant Pepper Crop

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Abstract Pepper is one of the most important spice crops in the world today with an enormous economic value. The pepper fruits are rich in pharmaceutically important compounds such as carotenoids and capsaicinoids. Over the years, crops of pepper have suffered significant losses in terms of yield and quality due to a myriad of pathogen infections including fungi, viruses and bacteria. More often, broad host ranges, novel pathogen strains and simultaneous infections due to multiple pathogens lead to resistance breakdown of host plants. An increased virulence of pathogens also results in exacerbated disease symptoms and yield losses. Coevolution of pathogens and crops allows them to harden each other's defense responses, however the whole process remains skewed in favor of the pathogens. Genomic designing of Capsicum genotypes which are more resilient to the imminent threats of rapid climatic changes and biotic stresses is now the major focus of current research. Hence, it becomes critical to understand the pathogens and their pathogenic properties in details to incorporate this knowledge into future breeding programs on disease resistance. Traditional breeding programs have met with little success due to the polygenic control of resistance, wide variability in the pathogen range along with complex pathogenicity mechanisms. Marker-assisted selection allows indirect selection of desired resistance alleles in the early stages of life cycle of the plant. The development of resistant commercial pepper varieties and host plant resistance are the permanent, effective and eco-friendly substitutes to the chemical and physical control methods and cultural practices for management of various biotic stresses. The multiplicity of abiotic and biotic stresses are the warning signs to initiate serious and concerted efforts towards making the crops more resilient and resistant to these stresses and

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to achieve desired crop breeding goals. Present chapter assembles the recommendations, details of the resistance sources, genes, QTLs and other resources available to diminish the effects of different biotic stresses towards genetic improvement of *Capsicum* species with modern, time critical and scalable scientific methods.

Keywords *Capsicum* · Fungi · Virus · Bacteria · Resistance genes · QTLs

3.1 Introduction

Pepper (*Capsicum* species) belongs to the Solanaceae family and is one of the most important horticultural crops grown worldwide which is used both as a spice and vegetable. In the past years, pepper has suffered major yield losses due to pathogen infections and related diseases. This could be attributed due to many reasons such as advancement and expansion of pepper cultivation around the world, increasing globalization and trade of fresh pepper produce, all of which serve as carriers for a range of pathogens and vectors and introduce them to new geographical locations. Climate change also remains a key factor leading to expansion of geographic ranges of the pathogens. The world produced approximately 38–42 million tons of green and dry chili pepper, with India being the top producer with a production of 1.74 million tons of chili pepper (FAOSTAT 2019). Pepper however needs urgent attention from the plant researchers and breeders in order to reduce current crop losses (Chhapekar et al. 2018). The range of pathogens infecting pepper species is very broad and includes bacteria, fungi, viruses and insects (Parisi et al. 2020). The broad and overlapping host ranges along with an unpredictability of the pathogen outbreaks pose serious challenges in the process of designing and implementing disease management programs. Novel pathogen strains elevate the chances of co-infection, which in turn leads to exacerbated disease symptoms and the resulting yield losses. This is often accompanied by resistance breakdown of host plants and increased virulence of pathogens. In addition, the indiscriminate use of insecticides in the fields for controlling vector organisms has raised concerns over the irreversible consequences on the environment and overall well-being of both the cultivators and the consumers. Also, for most of the pathogen organisms no chemical control methods exist which are highly effective in reducing the yield losses. Despite these challenges, notable progress has been made in the fields of molecular biology to decipher host–pathogen and pathogen-vector interactions, identification of risk factors that lead to increased vulnerability to diseases, and several disease management strategies and control measures are currently in practice to alleviate the impact of biotic stresses. Tangible and pragmatic solutions that integrate traditional practices, sustainable use of insecticides, application of natural biochemical products and target gene resistance should therefore be employed for prevention and control of pathogen infections.

Conventional breeding programs have met with little success due to the polygenic nature of resistance, wide variability of pathogen range and complex pathogenicity

mechanisms. Thus, development of resistant commercial pepper varieties and host-plant resistance are a permanent, effective and eco-friendly source in management of biotic stresses. Techniques like ecotype target induced local lesions in genomes (EcoTILLING) and gene pyramiding can help analyze multiple accessions of pepper for identifying allelic polymorphisms in the candidate resistance genes in the natural germplasm, and to impart durable resistance against diverse pathogens. Eventually, marker-assisted selection (MAS) will allow selection of desired traits especially when the traits show recessive or polygenic inheritance. Molecular markers also offer a cost-effective, time saving and rapid way to detect the desired resistance alleles in the early stages of life cycle of a plant. Codominant markers can even detect homozygous and heterozygous resistant plants without phenotypic assessment.

3.1.1 Economic Importance of Pepper

Pepper is an important crop in the Indian subcontinent being used both as a vegetable and spice, and also has many important metabolic compounds. As a crop whose center of origin is believed to be Mexico, pepper is currently grown in different parts of the globe. The maximum diversity, however, is reported to exist in Peru and Bolivia, the primary center of diversity for the cultivated genotypes of pepper (Zonneveld et al. 2015).

India is the largest producer of dry chillies, with a production of around 2 million tons annually. Pepper plants easily adapt to a wide range of climatic conditions and exhibit remarkable diversity in plant architecture, fruiting flavors and ornamental appeal. The pepper crop has high economic importance as a great ornamental crop, due to ample variegation in foliage, flowers, diversity in fruits and the unique flavors ranging from sweet to fiery hot forming a continuous gradient. Several interesting variations in fruit shape have been observed in pepper such as erect, habanero type, cherry, pendant type, jalapeños, conical, and blocky, among the many other classified fruit morphologies. The commonly marketed forms of pepper include fresh fruits, dried whole fruits, powdered form, paste and sauces. Globally, pepper farmers fetch good revenue due to the growing food processing industry and rising awareness towards nutraceuticals, which have consequently led to an expansion in the crop area. Beneficial metabolites found in pepper, such as vitamin C and E, carotenoids (provitamin A), flavonoids and capsaicinoids are recognized for their health benefits and their nutraceutical applications. Studies undertaken in mice with direct administration of Ghost chili extracts have also indicated its antioxidant, genotoxic and apoptotic activities (Sarpras et al. 2018).

3.1.2 Reduction in Yield and Quality Due to Biotic Stresses

Although pepper plants have high adaptability and general resilience to most stresses yet the crop is susceptible to several biotic stresses that ultimately impact the overall quality as well as net yield, and significant damages have been reported even at post-production and storage stages (Lownds et al. 1994; Samira et al. 2013). Biotic stresses are much more persistent than abiotic stresses under cropping systems, and heavy yield and quality losses are reported with prolonged exposure, as a result productivity and quality downfall. Reduction in yields due to damages in vital tissues are very common with effects such as leaf discoloration, chlorosis, curling, insect damages, which are therefore the most common causes of yield losses. The yield losses can be incurred in many forms, even before the crop grows in field conditions; there are early losses in nursery stages such as root rot, stem rot, etc. Frequent encounters with biotic stresses at the seedling stage itself lead to significant crop management and economic issues particularly for the exotic seeds or rare genotypes. Assessment of quality of the consumption-ready fruits is an important point of active research along with the molecular assessment of pesticide residues, both of which are of great interest to the pepper breeders. It is an acceptable realization that varietal resistance may not be durable, and therefore external measures of stress management will become inevitable to achieve the end goals of better-quality pepper fruits. Golge et al. (2018) conducted health risk assessment of residual pesticides in peppers and cucumber, and made startling revelations that 12.9% of peppers and 13.5% of the cucumbers sampled had at least one detectable chemical residue from among the 170 pesticides used for screening 725 vegetable samples.

Pepper is known to be a highly responsive crop to greenhouses, surpassing yield thresholds of many other comparable crops due to good response to nutrients and ambient growth conditions, yet yield losses have been reported of higher orders (Parisi et al. 2020). Under greenhouse conditions, pest infestations such as due to whiteflies, aphids and thrips, all lead to increased viral attacks. High humid conditions even for brief periods are also conducive for many fungal and bacterial infections which often are more severe than those in the open fields. An outbreak of powdery mildew on peppers resulted in a loss of 100% plants in six out of the 12 fields evaluated in Ontario in 2005 (Cerkauskas et al. 2011), and upto 40% loss in the Pacific Northwest in 2009 (Glawe 2008; Glawe et al. 2018a, b). Direct damage to fruits accrues a considerable loss to their market value by compromising their quality.

Anthracnose disease lesions appearing as black concentric rings also cause serious damages to pepper production worldwide. The lesions, starting as sunset yellow and ultimately turning as gray spots cause considerable quality loss, as well as transitions to several other severe infections. Frog eye spots due to *Cercospora* species (spp.) are prevalent across tropical and subtropical climates appearing on leaf, stem, petiole and peduncles, as circular spots with water-soaked appearance which ultimately dry out to look as frog eyes causing passive losses attributed to reduced photosynthesis, while also serving as gateway to multiple successive infections.

Wilts are major diseases of peppers caused by multiple organisms, and unforeseen crop losses due to wilts have become common sightings across pepper fields. Wilts are soil-borne infections, mostly manifested under warm days with a sudden drop of all leaves and eventually the whole plant, sometimes leaving only a single chili if the fruiting stage has already been attained. Wilt caused by the fungus *Verticillium dahliae* characterized under field conditions of the central coast of California reported a mean incidence rate of 6.3–97.8% wilted plants per field with Anaheim, jalapeño, paprika or bell peppers (Bhat et al. 2003). The economic yield losses due to *Fusarium* spp. have been estimated to be 68–71% (Gabrekiristos and Demiyo 2020). Growing conditions of warm soil temperature, low soil moisture, susceptible host and pH in the range of 5–6, were ideal factors leading to massive losses attributed to Fusarium wilt. *Ralstonia solanacearum* is another major wilt causing bacteria, and is described as the most destructive disease-causing pathogen of not only the peppers, but rather whole of the Solanaceous crops which therefore suffer great yield losses worldwide (Mamphogoro et al. 2020; Thakur et al. 2021). Waxy skin of peppers lacks lenticels or stomata, and hence is relatively resistant to water loss, but a loss of 5% or more becomes evidently visible. In a study, a total loss of 28.6% in weight was observed under dry season, while 38.7% under humid conditions in Trinidad (Mohammed et al. 1992). Accompanied losses in quality were also incurred during prolonged storage in peppers including fresh weight loss, increased acidity, vitamin C content degradation and loss of fruit firmness under ambient conditions.

3.2 Description of Different Biotic Stresses

Extensive cultivation of pepper as a crop along with its expansion to wide geographical conditions exposes the pepper plants to many biotic stresses not encountered before. There is a great degree of sharing of pathogen profiles among the species belonging to Solanaceae and interspecies infections via the same pathogen are frequently observed. It also makes research results greatly exchangeable and translatable among members. In plants, resistance to most of the potential invaders is attained through an integrated transcriptional activation of pathogenesis related (PR) genes followed by a hypersensitive response (HR) and systemic acquired resistance (SAR) (Ryals et al. 1996; Dangl and Jones 2001). In brief, whenever a pathogen attacks, specific receptors trigger the warning signals to prevent the spread of the infection by inducing HR and programmed cell death (PCD). But sometimes, pathogens bypass these systems by releasing chemicals that inhibit these receptors or circumvent the membrane system by using a vector host (Liu et al. 2020). Upon recognizing the pathogen, plants activate numerous defense related genes, produce reactive oxygen species (ROS), undergo phosphorylation of proteins and change their ionic flux to induce SAR (Knogge 1996).

Diseases are molecular level disturbances, often having genetic manifestations, while disorders are physiological in nature, manifested at genetic levels after a certain condition persists for long. Emerging environmental patterns and projected changes

over the years have made a profound impact on the future of our crops. Pepper being distributed all across the globe is exposed to widely contrasting climatic conditions, and hence there is a greater challenge as well as the accompanying opportunity to get real insights on the dynamic influence of climate over disease resistance.

3.2.1 Range of Pathogens and Insects Afflicting Peppers

3.2.1.1 Fungi

Peppers encounter various fungal pathogens in nature. Pepper fungal pathogens are devastating in nature and directly attack internal tissues, thus affecting the physiology and growth of plants. The mycotoxins released by fungi affect the seed germination, viability and root growth. This physiological impairment is accelerated by prevailing environmental factors viz. nutritional substrate, water mismanagement, temperature and pH of the soil (Costa et al. 2019). Fungi spread among plants by contamination through wind, harvesting and mechanical pruning, besides being also carried by insects. They enter the plant tissues through the stomata or through exposed physical injury sites and directly affect the foliar tissues, roots, stems, fruits, vascular systems, causing physiological stress and serious impairment in the normal growth of plants. Plants normally respond to the biotic stress upon recognition of appropriate stimuli.

Peppers suffer infection from many common fungi present in the soil (Mandeel 2005). Species of *Aspergillus, Mucor* and *Rhizopus* mainly affect the organoleptic properties of processed pepper and create risk to the consumer's health (Costa et al. 2019). In fields, fungal pathogens mainly include, *Phytophthora, Fusarium* and several others (Table 3.1). A severe outbreak of *Choanephora cucurbitarum* was observed for the first time in bell pepper (*C. annuum* cvs. Aristotle, Crusader and Sentry) in Southwestern and Northern Florida, with an incidence of 40% and substantial fruit infection predominantly around the calyx (Roberts et al. 2003). The list of important diseases caused by fungal pathogens includes powdery mildew, fruit rots, root rot, necrotic spots, vascular wilt and leaf spots.

Fruit Rot of Pepper

Powdery mildew in peppers is caused by *Leveillula* spp. which affect many other crops also including cereals, legumes, onions and model organisms such as Arabidopsis and tobacco. The disease is characterized by the leaf underside turning grayish white in patches and appearance of yellowish green lesions on the opposite sides of leaves. Main causative agent is *Leveillula taurica* or *Oidiopsis taurica* (asexual stage). Powdery mildew in pepper was first reported in Florida in 1971 (Blazquez 1976), Puerto Rico in 1992 (Ruíz Giraldo and Rodríguez 1992), Idaho (in greenhouse grown pepper) in 1998 (Ocamb et al. 2007), in Canada (Cerkauskas and Buonassisi 2003), Bolivia (Correll et al. 2005), Oklahoma (Damicone and Sutherland 1999) and Maryland (Jones et al. 2009). *C. annuum* L. infected with *L. taurica*

Table 3.1 The common fungal diseases, causative organisms and symptoms in *Capsicum* spp.

Fungal disease	Pathogen	Symptoms	References
Powdery mildew	*Leveillula taurica*	White patches and lesions on adaxial as well as abaxial surface of leaves	Smith et al. (1999), Jones et al. (2009)
Anthracnose fruit rot	*Colletotrichum* spp.	Stem and leaf drooping, softening and rotting of fruits	Sun et al. (2015), Mongkolporn and Taylor (2018)
Verticillium wilt	*Verticillium* spp.	Browning of vascular tissues, wilting of leaves and stem, necrosis, foliar epinasty	González-Salán and Bosland (1991)
Fusarium wilt	*Fusarium* spp.	Drooping and yellowing of leaves, stunted growth, wilting of flowers	Lomas-Cano et al. (2014)
Pepper canker	*Rhizoctonia solani*	Root and stem rot, fruit canker	Muhyi and Bosland (1995), Mannai et al. (2018)
Necrotic root rot	*Pythium* spp.	Crown rot, Necrotic rot of root tips	Chellemi et al. (2000)
Pepper gray mold	*Botrytis cinerea*	Gray mould in fruits resulting in rot	Kamara et al. (2016)

(Lév.) G. Arnaud was reported for the first time in western New York in 1999 and Long Island, New York in August 2000 (McGrath et al. 2001).

L. taurica is an obligate biotrophic ascomycete, with mycelia spanning on the whole epiphytic surface, as well as haustorial structures exclusively in epidermal layers feeding on mesophyll cells. The visible infection occurs as powdery white patches on the leaves mainly stemming from the lower undersides of the abaxial surface. Eventually, infection progresses and affects the whole leaves and other parts of the plant. The fungus prefers to grow in leaves that are in moderate temperatures, high humidity and a moist environment. Affected leaves turn brown and defoliate, affecting the photosynthetic rate of the plants that results in a slow growth. PCR assays have been developed for the rapid and exact detection of damage and spread pertaining to the early and late stages of infection of *L. taurica* in peppers using primers from the rRNA internal transcribed spacer (ITS) regions of *L. taurica* (Zheng et al. 2013a). This relative quantification was done for rapid experimentation and assessment in the plant–microbe interaction domain.

Capsicum germplasm resistant to *Leveillula* has been reviewed by Parisi et al. (2020). Resistant varieties include *C. annuum*—H3, H-V-12 ['H3' x 'Vania' (susceptible)]; *C. baccatum*—CNPH36, CNPH38, CNPH50, CNPH52, CNPH279,

CNPH288, KC604, KC605 and KC608; *C. frutescens*—IHR 703; *C. chinense*—KH616; and *C. pubescens*—KC638, KC640, KC641, KC642, KC643, KC644 and CNPH279 (Anand et al. 1987; Daubeze et al. 1995; Souza and Café-Filho 2003).

Anthracnose of Chili

Anthracnose in chili is caused by the *Colletotrichum* spp. *Colletotrichum* is responsible for major crop losses and its pathogenicity is extremely diverse across different crop plants of Solanaceae, Malvaceae, Fabaceae and Brassicaceae (Jayawardena et al. 2016).

Worldwide, *Colletotrichum* affects up to 80% of crops in various countries viz. Vietnam (Don et al. 2007), Korea (Kim et al. 2008a, b; Park Sook-Young; Choi 2008), Thailand (Than et al. 2008), India (Ramachandran and Rathnamma 2006), Pakistan (Tariq et al. 2017), Brazil (Almeida et al. 2017), Australia (De Silva et al. 2017) and China (Diao et al. 2017) etc. Among the species, *C. truncatum* (previously known as *C. capsici*), *C. acutatum* and *C. gloeosporioides* are common in chili and are the most virulent. Highly virulent *C. truncatum* isolate (UOM-02) has reportedly caused severe losses under favorable conditions (Naveen et al. 2021). *C. javanense* and *C. scovillei* show great damages compared to other species after inoculation on intact fruits (De Silva et al. 2021). Infected plants suffer from sunken necrotic lesions resulting in both pre- and post-harvest rotting of fruits (Rao and Nandineni 2017). The pathogen is seed-borne and therefore can infect the next generation of plants also (Singh et al. 2018). The pathogen can be detected by loop mediated isothermal amplification assay (LAMP) (Aravindaram et al. 2016) or can be characterized using sequence characterized amplified regions (SCAR) (Srinivasan et al. 2014).

Several *Capsicum* spp. resistant varieties are reported that include *C. annuum* resistant against *C. truncatum* and *C. siamense* viz. Jinda, Bangchang, 83–168, Acchar lanka, CA-4, Pant C-1, Punjab Lal and Bhut Jolokia BS-35 (Mongkolporn et al. 2010; Mishra et al. 2018); *C. frutescens* against *C. siamense* viz. Khee Noo and Karen (Mongkolporn et al. 2010); *C. chinense* against *C. truncatum*, *C. scovillei* and *C. siamense* viz. PBC932, CO4714, PRI95030, CO4714 (Montri et al. 2009); *C. baccatum* against *C. truncatum* and *C. scovillei* viz. PBC80, PBC81, CA1422 (Montri et al. 2009) and *C. baccatum* var. *pendulum* against *C. scovillei* viz. UENF 1718, UENF 1797 (Silva et al. 2014).

Pepper Gray Mold

Pepper gray mold disease is caused by a polyphagus fungal pathogen *Botrytis cinerea.* This pathogen has a broad range of distribution affecting vegetable and crop plants viz. tomato, chickpea, strawberry, castor, tulips and ornamental plants like chrysanthemum, rose and lily (Pande et al. 2006; Petrasch et al. 2019; Kumar et al. 2020). *Botrytis* affecting peppers was reported in some Middle East and Asian countries viz. Taiwan (Huang and Sung 2017) and Pakistan (Naz et al. 2018). In India, the gray mold caused by *B. cineria* Pers. Fr. in *C. annuum* var. *grossum* was first reported in Jammu and Kashmir (Kamara et al. 2016). The fungus develops both in warm and cold temperatures and remains latent in the fruits and later affects post-harvest produce which makes it difficult to control the infection rate (Droby and Lichter

2007). Pathogenicity of *B. cinerea* is partially attributed to a phytotoxin Botrydial, however its role as a primary determinant is not established. Highest concentration of botrydial on the ripe fruit samples and open wounds with induced inoculation, correlates with strain's overall virulence (Deighton et al. 2001).

Genetic diversity present in *B. cinerea* among isolates studied from Southern Turkey revealed two distinct gene pools and five genetic clusters indicating that presence of the ample diversity can be exploited to design gray mold disease management breeding strategies (Polat et al. 2018).

White mold

Fungus *Sclerotinia sclerotiorum* was first observed in Korea infecting peppers (*Capsicum annuum* var. *grossum*) and was identified using ITS rDNA regions ITS1, ITS2 and 5.8S sequences which were 100% similar to the ones that infected lettuce (Jeon et al. 2006). Twelve commercial pepper cultivars and 110 *Capsicum* accessions were tested for their resistance to *S. sclerotiorum* (Lib.) de Bary out of which 58 showed some resistance (Yanar and Miller 2003). The results indicated that the Sclerotinia stem rot resistance existing among the *Capsicum* spp. could be used to transfer resistance to commercial pepper cultivars.

Root rot of pepper

Fusarium spp. cause decaying of roots, stems and leaves along with brown sunken cankers visible at the base of the plant. *Fusarium oxysporum* induced crown and root rot was first reported in Italy on sweet pepper plants (Gilardi et al. 2019), while *F. semitectum* was first reported in China affecting greenhouse pepper (*C. annuum*) (Li et al. 2018). Several other isolates of *Fusarium* have been reported in pepper viz. *F. solani* (Ramdial and Rampersad 2010), *F. oxysporum* f. sp. *vasinfectum*, *F. redolens*, *F. oxysporum* f. sp. *capsici*, *F. verticillioides* and *F. pallidoroseum* (Lomas-Cano et al. 2014). *Fusarium* strains are more complex and are pathogenic to many plants. *F. oxysporum*, the main pathogenic species, impacts onion in Japan and Indonesia (Dissanayake et al. 2009; Sasaki et al. 2015), cotton (Cianchetta and Davis 2015) and melon (Imazaki and Kadota 2019) etc. Among Solanaceae, it affects tomatoes (Srinivas et al. 2019), potatoes (Du et al. 2012), eggplant (Ishaq et al. 2019) and peppers (Gabrekiristos and Demiyo 2020). However, not all *Fusarium* are pathogenic with some of them being beneficial endophytes or soil saprophytes, and even antagonists of other fungus like *Verticillium*. In *Fusarium* spp. molecular characterization was carried out using ITS of the fungus ribosomal region in the affected pepper (*C. annuum*) (dos Anjos et al. 2019). Earlier, protein profiles of a resistant (Mae Ping 80) and susceptible (Long Chili 455) cultivars identified NADPH HC toxin reductase, serine/threonine protein kinase and 1-aminocyclopropane-1-carboxylate synthase 3 that were involved in plant defense mechanism (Wongpia and Lomthaisong 2010).

Necrotic spot and Vascular wilt

Verticillium affects plants viz. cotton, alfalfa, watermelons, chili and some ornamental plants like petunia, chrysanthemum and rose. *Verticillium* causes stunting and yellowing of leaves leading to leaf shedding, permanent wilt and plant death.

The epidemic was first reported in 1937 in California in pepper fields with about 20% crop losses (Bhat et al. 2003). *V. dahliae* is cross pathogenic and infects crops during rotational cycle of growth.

V. dahliae usually affects the temperate crops. The leaf and vascular wilt in pepper caused by *V. dahliae* leads to dropping of the leaves as a result of dehydration or increased transpiration exceeding water intake by plants. *V. dahliae* is restricted to the infection of the vascular tissues of plants and plugs the xylem and phloem tissues, thus resulting in leaf wilt as the plant is unable to transport water to its sink (Reusche et al. 2012).

Early studies in pepper have uncovered 125 novel accessions of *C. annuum* and *C. baccatum* and identified 27 *Capsicum* accessions that were resistant to *Verticillium* wilt. Plant introductions (P.I.) PI215699 and PI 535616 that included *C. baccatum* var. *microcarpum* and *C. annuum* showed the highest resistance (González-Salán and Bosland 1991). Later on, 397 *Capsicum* accessions were screened for resistance against two isolates Vdca59 and VdCf45. These accessions included *C. annuum, C. chinense* and *C. frutescens* varieties. Eight accessions, namely, Grif 9073, PI 281396, PI 281397, PI 438666, PI 439292, PI 439297, PI 555616 and PI 594125 were resistant to *V. dahliae* (Gurung et al. 2015). In another study, a total of 97 pepper accessions from Bulgaria, Serbia and Romania were studied, of which 12 were reported to be resistant to *V. dahliae*. Among these breeding lines, Buketen 3, Buketen 50, Gorogled 6, IZK Rubin and, IZK Kalin were found to be highly resistant (Vasileva et al. 2019). Changes observed in lignin composition and higher deposition of bound phenolics in infected stems seem to contribute to the reinforcement of cell walls and the impairment of *V. dahliae* colonization, and hydroxycinnamic acidamide N-feruloyltyramine was reported in response to *V. dahliae* infection (Novo et al. 2017).

Damping off and Root Rot

Pythium spp. cause a disease in plants known as "damping off" where the newly emerging seedlings wilt and die (Sutton et al. 2006). They constitute a range of species including *Pythium aphanidermatum, P. myriotylum, P. helicoides* and *P. splendens*, reported to cause significant root rot and reductions in root biomass of bell pepper, with *P. aphanidermatum* and *P. myriotylum* being the most severe (Chellemi et al. 2000). They commonly affect plants grown in greenhouses. They are generalists and unspecific in their range of hosts and are more dangerous than *Phytophthora* or *Rhizoctonia* which prefer specific hosts (Owen-Going et al. 2003). Their spores are motile and therefore commonly affect waterlogged or hydroponically grown plants. *Pythium* also causes serious losses in agricultural production worldwide. *Pythium* does not influence the photosynthetic activity of the plants but rather directly reduces the biomass (Wu et al. 2020). Damping off can result in heavy losses in crop yields as has been shown in a study where 5–80% of the seedlings were affected, and caused serious economic losses to the farmers (Lamichhane et al. 2017).

Rhizoctonia is a soil-borne pathogen responsible for causing root rot, collar rot and damping off related to stem wilt in various crops including Capsicum (Mannai et al. 2018). It was first observed in potato tubers in 1858 and was named *Rhizoctonia*

solani. In *Capsicum*, *R. solani* affects multiple growth stages and causes seedling damping off, necrotic spots at the hypocotyl and tap roots and root rot (López-Arredondo and Herrera-Estrella 2012). Genetic resources in pepper showing resistance against this pathogen are rare. Pepper accessions that develop resistance to *R. solani* have been found in *C. annuum, C. baccatum, C. chinense and C. frutescens* against a virulent strain of Mexican PWB-25 isolate (Anaya-López et al. 2011). Screening of 74 *Capsicum* accessions representing these four species for resistance against *R. solani* identified 19 accessions that were resistant (Muhyi and Bosland 1995).

Chili leaf spot/Gray leaf spot

Stemphylium solani (or *Stemphylium lycopersici* for the ones that infect tomatoes) first described by G. F. Weber in 1930, is a pathogenic ascomycete that causes gray leaf spot in plants. Its distribution varies, with *S. lycopersici* reported in Japan causing fruit rot even in peppers (Tomioka and Sato 2011), *S. solani* reported in Malaysia (Nasehi et al. 2012), and *S. lycopersici* in China (Xie et al. 2016). Infected plants have white spots and sunken red or purple lesions on leaves that finally necrose. The pathogen severely affects important vegetable crops like tomato, brinjal, chili, potato, onion, cotton etc. (Zheng et al. 2008). It causes secondary infections among the cycle of rotational crops and spreads through wind or air, and is even transmitted through seeds (Zheng et al. 2010).

Chili leaf spot caused by *Cercospora capsici* is prevalent in the tropics. Optimal conditions for infection are a relative humidity of 77–85% and temperatures close to 23°C. Assessment of the survival ability of the fungus on soil surface, infected debris and in refrigerator (4°C) showed their broad adaptability (Swamy et al. 2012). Infected leaves turn dark brown with a distinctive sporulating gray center, hence called the "frog eye" spot. It was first isolated from bell peppers and described by Heald and Wolf (1911). Later, sightings of *Cercospora* were studied in peppers for their virulence and pathogenicity by Meon (1990) in Malaysia. The *C. capsici* isolate reduced the photosynthetic ability of the infected plants resulting in consequent yield losses.

Resistant varieties have not been reported as yet for *C. capsici*. But, the responses of different *Capsicum* genotypes viz. *C. chinense* (Jacq.) cv. Rodo, *C. frutescens* L. cv. Ata wewe, *C. frutescens* cv. NHVI-AB and *C. frutescens* cv. Sombo were observed to be moderately resistant in field experiments conducted under tropical conditions to assess the effects of genotype, season and the genotype × season interaction (Afolabi and Oduola 2017). Some variants of the species infect peppers viz. *C. apii* affecting *C. chinense* grown in Brazil (Nicoli et al. 2011) and *C. tezpurensis* affecting Naga king chili in north-eastern states of India (Meghvansi et al. 2013).

3.2.1.2 Bacteria

Bacterial spot

Bacterial spot (BS) initially observed on tomato in South Africa in 1914, is a condition caused by a gram-negative bacterium formerly called *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), which is presently classified into *X. euvesicatoria*, *X. vesicatoria*, *X. gardneri*, and *X. perforans* on the basis of homology of DNA sequences and the phenotypes (Obradovic et al. 2004; Jones et al. 2005; Hamza et al. 2010). The occurrence of BS has been reported all over the world, such as the USA, north-western Nigeria and Saudi Arabia (Jones et al. 2005; Ibrahim and Al-Saleh 2012; Jibrin et al. 2014).

The bacteria have a short life span in the soil, but can persist for longer periods in association with infected debris or diseased plants or weed species. Bacteria can gain entry through stomata on the surfaces of the leaves and injured leaves and fruits. Extended spells of high humidity intensify the infection and disease development. Bacteria infect the stems and fruits, forming lesions on fruit and the peduncle, adversely affecting the crop productivity due to shedding of blossoms and developing fruits, while the fruits that remain lose commercial value because of poor quality.

Bacterial wilt

Bacterial wilt is one of the most common diseases in members of the Solanaceae family. It is caused by a soilborne, aerobic gram-negative bacteria named *Ralstonia solanacearum*. The disease is also known as 'Green wilt' because even though the infected plant wilts, the leaves remain green. Symptoms are usually seen on the young foliage and include necrosis and browning of vascular tissues. Use of resistant varieties remains the most effective, economical and environmentally safe method to control the disease (Yuliar et al. 2015).

3.2.1.3 Viruses

The number of incidences of viral diseases has increased considerably in pepper producing areas over the last few years. Earlier catalogues suggested some 35 viruses affecting pepper species (Green and Kim 1994). Till date, more than 45 viruses have been reported to infect chili peppers causing severe losses in production and quality (Arogundade et al. 2020). Of the viruses that threaten pepper over the past are—*Potato virus Y* (PVY), *Tomato spotted wilt virus* (TSWV) and *Pepper mild mottle virus* (PMMov), and among these, PVY and TSWV fall under top ten in the list of most detrimental plant viruses (Scholthof et al. 2011).

Most of the virus infections result in distortion of foliar tissues, chlorosis and necrotic spots, and sometimes these spots appear on other tissues such as of fruits. A comprehensive study on incidences of viral diseases in *C. chinense* var. Bhut Jolokia from Assam concluded that most of these were infected with Potyvirus, followed by Cucumovirus, Tospovirus and Begomovirus (Talukdar et al. 2017). PVY

is distributed worldwide and is transmitted by a large number of aphid species that cause global yield losses in Solanaceae members including pepper (Janzac et al. 2008). Several leaf curl begomoviruses associated with beta satellites were reported in chili pepper plants in Pakistan (Yasmin et al. 2017). A serological survey conducted in different altitude zones of Rwanda confirmed the presence of at least one virus from among—*Cucumber mosaic virus* (CMV), *Pepper veinal mottle virus* (PVMV), PVY, *Tobacco mosaic virus* (TMV), PMMoV and *Pepper vein yellows viruses* (PeVYV) (high to low incidence), in 73% of Capsicum plants (Waweru et al. 2021).

Most of the pepper-infecting viruses are transmitted by vector groups belonging to aphids, thrips and whiteflies (Kenyon et al. 2014). More often than not, the synergistic effects of more than one virus infection are seen in plants that further increase disease severity (Murphy and Bowen 2006). Aphids transmit nearly 30% of plant viral species known till date (Brault et al. 2010). Whiteflies are very resistant to most insecticides and also cover long distances over foliage and spread many viruses. Poleroviruses (Luteovirideae) is a phloem-restricted RNA plant virus exclusively transmitted by aphids, while *Pepper whitefly-borne vein yellows virus* (PeWBVYV) is *Bemisia tabaci*-transmitted polerovirus or whitefly-borne vein yellows virus (Ghosh et al. 2019).

Orthotospoviruses

***Tomato spotted wilt virus* (TSWV)**

Tospoviruses pose a major constraint in the production of vegetable crops, including pepper in various parts of the world due to their wide host range and propagative transmission by thrips (Pappu et al. 2009). Since the end of the 20th century, the spread of the invasive western flower thrips (*Frankliniella occidentalis*) from the western United States and local reemergence have led to major TSWV outbreaks worldwide (Moury and Verdin 2012). Temperatures greater than 30°C promote the incidences of TSWV infections (Llamas-Llamas et al. 1998; Roggero et al. 1999). The typical symptoms in in *Capsicum* spp. include stunting and yellowing or browning of leaves or of the whole plant, mosaic or necrotic ringspots on leaves and fruits, necrotic streaks on stems and curling of the leaves. Deformed fruits exhibit necrotic ring patterns along with discolored arabesque-like areas.

***Tomato chlorotic spot virus* (TCSV)**

TCSV was first reported to infect bell pepper in Spain but it could not be transmitted experimentally to healthy plants (Lozano et al. 2004; Wintermantel and Wisler 2006). TCSV causes irregular chlorotic, interveinal yellowing, mild leaf curl, necrotic ring spots and stunting along with deformed leaves as the common symptoms. Out of the four thrips species—*F. kelliae*, *F. schultzei*, *F. bruneri* and *Thrips palmi* that were detected in pepper growing areas (Webster et al. 2013), *F. schultzei* was an efficient vector for TCSV (Nagata et al. 2004).

***Capsicum chlorosis virus* (CaCV)**

It is a serogroup IV virus species infecting *Capsicum* and was first reported in 2000 in Queensland, Australia (McMichael et al. 2002). In the same year, CaCV was first detected in chili pepper fields in Karnataka, India (Krishnareddy et al. 2008). Recently, incidences of CaCV were also reported in glasshouse grown *C. annuum* var. *annuum* in Greece (Orfanidou et al. 2019). Symptoms include mottling and distortion of leaves, chlorotic and necrotic ring spots on leaves and apical necrosis.

***Groundnut ringspot virus* (GRSV)**

Distortion of leaves and fruits, chlorotic and necrotic spots on newly developed leaves, terminal necrosis and mottle were observed in GRSV infected *C. annuum* L. (Webster et al. 2011). *F. schultzei* is observed to be a better vector for GRSV than *F. occidentalis* and has contributed to recent outbreaks in Brazil and North America (Webster et al. 2013).

Potyvirus

***Chili veinal mottle virus* (ChiVMV)**

ChiVMV is a destructive potyvirus found mostly in Asia and causes systemic mosaic, vein-banding and leaf mottling and chlorosis (Tsai et al. 2008). The concurrent double recessive mutations—$pvr1^2$ in *eIF4E* and *pvr6* in *eIF(iso)4E*, respectively, provide resistance to ChiVMV, and double silenced plants showed reduced viral accumulation (Hwang et al. 2009). Recombination events and geographical locations drive most of the genetic variations, diversity and environment adaptability among the ChiVMV isolates as studied in China (Rao et al. 2020).

***Pepper veinal mottle virus* (PVMV)**

PVMV is mostly common in Africa and Asia causing major setbacks in chili pepper yield and quality. Recently, PVMV was reported in Rwanda along with Pepper Yellow Virus (PeYV) (Skelton et al. 2018). The prevalent symptoms observed for PVMV infected chili plants are mosaic, vein mottling and stunted growth. Aphid species like *Aphis gossypii* are the potential insect vectors for non-persistent transmission of PVMV (Shah et al. 2009). Six Japanese isolates of PVMV in *C. annuum* were characterized by whole genome sequencing and found to have similar molecular and pathological impacts (Laina et al. 2019). The cDNA clone used to study the molecular etiology of PVMV in *C. chinense* cv. Yellow Lantern was associated with floral chlorosis and rugosity (Hu et al. 2020).

***Pepper severe mottle virus* (PepSMoV)**

The symptoms of PepSMoV infection include deformed leaves and stunted growth. The coat protein gene from PepSMoV was isolated from chili pepper plants in

Colombia that showed high sequence similarity with the PepSMoV strain from Venezuela (Rivera-Toro et al. 2021).

Cucumovirus

***Cucumber mosaic virus* (CMV)**

Symptoms include curling, mosaic, vein banding, leaf mottling and malformation. Monogenic recessive resistance was found in a multiple disease resistant pepper variety, Punjab Lal, against CMV and other mosaic tobamoviruses (Bal et al. 1995). The gene expression analysis could confirm the presence of CMV causing disease symptoms in pepper plants in Malaysia (Azizan et al. 2017). The viral coat protein gene of 800 bp was isolated from leaf tissues of CMV infected chili peppers in Tamil Nadu also showed high sequence similarity with other Indian CMV isolates (Rajamanickam and Nakkeeran 2020). Higher incidences of CMV in various accessions of king chili in Manipur were reported alongside mixed infection with ChiVMV (Chanu et al. 2004).

Tobamovirus

The Tobamovirus pathotypes are named by the type of *L-gene* mediated resistance they break, for example, P_0, P_1, $P_{1.2}$ and $P_{1.2.3}$. The *L4* HR mediated resistance, which previously had the broadest resistance spectra, was overcome by a new PMMoV pathotype $P_{1.2.3.4}$ in *C. annuum* (Genda et al. 2007). Susceptible allele L^0 carrying *Capsicum* plants are infected by any Tobamovirus pathotype.

***Pepper mild mottle virus* (PMMoV)**

PMMoV has been found to be transmitted through hydroponic systems in pepper with 100% incidence (Choi et al. 2004). The infection cycle of PMMoV was traced in developing seedlings of infected *C. annuum* cv. Shosuke up to the seed development stage, and in seeds to cotyledon stage via immunofluorescence of viral coat protein (Genda et al. 2011). PMMoV specific virus screening tests were developed based on double antibody (Anti-PMMoV) sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for advanced detection of soilborne PMMoV, which allows preventing possible damage to the crops (Ikegashira et al. 2004).

Geminivirus

Geminiviruses, being the largest family of plant viruses, pose a major threat to economically important crops throughout the world especially in developing countries (Boulton 2003). Among all, *Begomovirus* is the most notorious genus of the family Geminiviridae which affects a wide range of host plants. Geminiviruses are

mostly transmitted by the B-biotype of the polyphagous whitefly vector. Recently, *Pepper yellow leaf curl virus* (PepYLCV) and PeVYV were reported for the first time in Malaysia with serious implications in pepper production (Sau et al. 2020). Several attempts to characterize the chili plants infected with *Pepper leaf curl virus* (PepLCV) at the molecular level have been carried out to isolate the viral amplicons (Nigam et al. 2015). In India, the viral genome sequence of chili infecting Begomoviruses like *Tomato leaf curl Joydebpur virus* (ToLCJV), *Chili leaf curl Vellanad virus* and *Chilli leaf curl Gonda virus* have been successfully characterized (Kumar et al. 2012; Shih et al. 2007; Khan and Khan 2017). *Cotton leaf curl Multan virus* (CLCuMuv) and *Tomato leaf curl beta satellite* (ToLCPaB) with genetic recombination sites were found to be associated with ChiLCV disease in Bhut Jolokia accessions from Manipur state of north-east India (Yogindran et al. 2021).

***Pepper leaf curl virus* (PepLCV)**

PepLCV is also one among the most destructive viruses affecting chili peppers and causes heavy yield losses in pepper production in India and globally. New variants of *Chilli leaf curl virus* (ChiLCV) were reported from districts of Uttar Pradesh in North India (Rai et al. 2010). The histopathological characterization of ChiLCV and associated *Tomato leaf curl Bangladesh betasatellite* (ToLCBDB), revealed elevated levels of stress-related biological compounds like proline and polyphenols and defense enzymes like Superoxide dismutase (SOD) along with overall deterioration of fruit quality in sweet pepper plants (Kumar et al. 2018).

***Tomato yellow leaf curl virus* (TYLCV)**

Pepper is an asymptomatic host to TYLCV, which is primarily a tomato pathogen, and may act as an alternative host and a natural reservoir for acquisition and transmission of TYLCV (Kil et al. 2014). Some reports suggest that pepper is a dead-end host in the epidemiological cycle of TYLCV, while others speculate that it may serve as a source of TYLCV for healthy tomato plants via whitefly (Morilla et al. 2005; Polston et al. 2006). The acquisition, path of translocation in vector body, transmission between vector organisms and to host plants, and retention of pathogen components in the vector organisms have been studied for TYLCV that offer alternative solutions to resistance gene breeding (Czosnek et al. 2002). In a remarkable incidence of synergistic interaction of four viral components—ChiLCV, ToLCBDB, *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Tomato leaf curl Gujarat virus* (ToLCGV) were found to be associated with severe leaf curl disease, increased viral DNA and suppression of NBS-LRR gene expression in resistant *C. annuum* cv. Kalyanpur Chanchal (Singh et al. 2016). Recently, ToLCNDV was reported to infect sweet peppers for the first time in Europe which may thus affect the genetic variability and virus prevalence (Luigi et al. 2019).

***Tobacco mosaic virus* (TMV)**

TMV, the first ever virus to be identified infects more than 350 plant species, including tobacco, tomato, pepper, eggplant, potato and cucumber (Kumar et al. 2011). The virus subsists in diseased plants for a long duration. It can reproduce in living plant

tissues but remains inactive in dead tissues, retaining without any loss in its ability to infect (Damiri et al. 2017). TMV propagates mostly through contact among plants, infested seeds and by mechanical means. Typical symptoms include leaf chlorosis, mosaic leaves, leaf distortion and arrested growth accompanied with small-sized fruits.

3.3 Management Strategies—Cultural, Chemical, Biocontrol and Integrated Pest Management

Different cultural, chemical, biocontrol and Integrated Pest Management (IPM) practices are currently being used by farmers to control pathogens and pests of peppers. The pre-sowing cultural practices include deep summer ploughing, fallow, crop rotation with non-host crops and destruction of the alternate host plants. Timely sowing of the pepper crop should be ensured at the seed sowing/transplanting stage, cultivation with resistant/tolerant varieties, and use of healthy, certified and weed free seeds are some important approaches to minimize yield losses. Other practices implemented at this stage include removal and destruction of infected plants, growing pest repellent plants like Ocimum/Basil, and crop rotation with a non-host cereal, cucurbit, or cruciferous vegetable crop. Common cultural management practices at the vegetative stage of the pepper crop include adoption of the recommended spacing for adequate air circulation, judicious use of fertilizers, collection and destruction of crop debris, sufficient irrigation at critical stages of the crop, ensuring minimal waterlogging and other field sanitation methods. Some of the common cultural and traditional methods for controlling disease organisms and their vectors are listed in Table 3.2.

Table 3.2 Common cultural methods of control of disease pathogens and vector organisms in *Capsicum* spp.

Method	Effective against	Remarks	References
Leaf pruning	Aphididae	Leaf pruning coupled with application of natural predator *Macrolophus pygmaeus* effectively controls aphids in sweet pepper	Brenard et al. (2020)
Yellow sticky traps	*Trialeurodes vaporariorum*	Significant reduction in oviposition of greenhouse whitefly in *C. annuum*	Moreau and Isman (2011)
Vegetable extracts	*Cercospora*	*Momordica charantia* and garlic-pepper sprays were significantly effective in reducing the green peach aphid abundance on pepper	Oke et al. (2010)

Chemical methods of control like soil fumigants were used in the early days viz. MeBr (Methyl Bromide), to control the rate of epidemic, which was observed to be biocidal and cost-effective, but was not practical (Xie et al. 2015). Prolonged ozone exposure was sufficient to prevent PepMOV infection at lower PepMOV concentrations, but chemical treatments like trisodium phosphate (TSP) were more efficacious at higher concentrations (Stommel et al. 2021). Treatment with fungicide seems to ameliorate their growth; however, growing concerns of using synthetic chemicals have prompted the use of a natural resistance approach. Some chemical methods of control are summarised in Table 3.3.

The biological control or biocontrol methods for defending the pepper crop from various phytopathogens are progressively eliciting interest among the farmers because it is environment-friendly. In a study on biocontrol of pepper seedling wilt disease, three natural substances called lipopeptides, with antifungal properties—surfactin, iturin and fengycin produced post *B. subtilis* infection in the host were shown to be effective against *R. solani* infection (Wu et al. 2019). The results

Table 3.3 The chemicals effective against pathogen organisms and their vectors along with their working mechansims

Chemical	Effective against	Remarks	References
Spinosad, indoxacarb, methoxyfenozide	*Ostrinia nubilalis* (European corn borer)	–	Chapman et al. (2009)
Thiamethoxam (TMX)	*Bemisia tabaci*	Assessed optimal application of doses	Mei et al. (2019)
Novaluron	*Liriomyza trifolii*	Effective against leafminer	Hernández et al. (2011)
Spiromesifen	*Bactericera cockerelli*	Reduction in oviposition and egg hatching against tomato-potato Psyllid	Tucuch-Haas et al. (2010)
Spiromesifen	mites and whiteflies	Foliar application of Oberon/spiromesifen shows effective control against whiteflies in *C. annuum* even after 36 days with no residual phytotoxicity	Fanigliulo et al. (2010)
Azadirachtin and methoxyfenozide	*Spodoptera littoralis*	Reduction in adult longevity by 2.3 d at high concentration; significant impact on population dynamics of pest by oviposition deterrence on *C. annuum* plants pretreated with Azadirachtin	Pineda et al. (2009)

obtained in the study also indicated that *B. subtilis* SL-44 triggered the induced systemic resistance in the seedlings against *R. solani* wilt through the jasmonic acid-dependent signaling pathway. Moreover, *B. subtilis* SL-44 also produced antifungal compounds—lipopeptides, which could further inhibit or even damage the mycelial growth of *R. solani*. Biotrophic bacteria and arbuscular mycorrhiza are other alternatives to control fungal pathogens. They are natural and their effect is permanent. Some Arbuscular mycorrhizal fungi (AMF) have shown the potential in providing resistance against *V. dahliae* in *C. annuum* L. pepper cv. Piquillo by delaying the disease symptoms buildup by improving a balanced antioxidant metabolism in leaves during early inoculation, and reducing the photosynthesis in *Verticillium* inoculated tissue to conserve resources, adding up to final yield outcomes. Biocontrol is also a practical approach for mitigation of the blight of *Rhizoctonia* like several others (Huang et al. 2017). Some biotrophic fungi like *Trichoderma*, *Gliocladium* and *Rhizobacteria*, *Pseudomonas* and *Bacillus* are natural bio-antagonist of *R. solani* (Mannai et al. 2018). Antagonistic rhizobacterial and epiphytic species viz. *B. cereus*, *P. putida*, *B. subtilis*, *Paenibacillus macerans*, *Serratia marcescens*, *B. pumilus* and *P. fluorescens*, compete with and inhibit the growth of *R. solani* (Mamphogoro et al. 2020).

Some fungi viz. *Trichoderma harzianum*, *T. viride* and *Gliocladium virens* control damping off caused by *P. aphanidermatum* and *P. ultimum* in pepper seedlings, showing improved seedling emergence and length up to 25% relative to control, respectively (Sivan et al. 1984; Lumsden and Locke 1989; Mannai et al. 2020). The rhizobacteria, *P. aureofaciens, P. fluorescens*, *P. putida* and *B. pumilus* have been shown to increase the length of the seedlings and biomass in pepper (Hahm et al. 2012). Control of *Pythium* root rot was mostly based on fungicides in the early days (Cook et al. 2009), but there is a growing concern for health issues and ethical considerations. Some of the *Pythium* species themselves have received interest as potential biocontrol agents and include *P. oligandrum*, *P. nunn*, *P. periplocum* and *P. acanthicum*. Different biocontrol measures have been summarized in Table 3.4.

The IPM approach relies on the optimal usage of every applicable management solution to achieve pest management goals with ecologically sustainable goals in mind. A mixed application of cultural, biocontrol and chemical means at minimal levels, often provides much better results than individual applications of each of these crop practices. Usage of chemical controls is discouraged in IPM approaches till necessary. Even in the least preference cases, all reliance is held upon the use of biorational pesticides, with low toxicity, easy degradation and consumption safe doses. Efficacy of such pesticides in most cases is really insufficient to moderate pest populations, but in mixed proportions with other milder pesticides or conventional one, achieves the goals sustainably.

3.4 Genetic Sources of Resistance to Biotic Stresses

Among the 35 characterized species of the genus *Capsicum*, only *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens* are widely domesticated.

Table 3.4 The biocontrol methods adopted and their molecular mechanisms in *Capsicum* spp.

Species	Biocontrol species/bioactive compounds	Summary	References
B. cinerea	*B. licheniformis*	–	Márquez et al. (2020)
B. cinerea	*F. oxysporum* f. sp. *lycopersici* (FOL) insoluble protein free fraction	Induction of defense related genes such as chitinase (*CACHI2*), a peroxidase (*CAPO1*), sesquiterpene cyclase (*CASC1*) and basic *PR1* (*CABPR1*)	Veloso and Díaz (2012)
B. cinerea	*Beauveria bassiana*	Antifungal properties against *B. cinerea* infection	Barra-Bucarei et al. (2019)
B. cinerea	Capsaicinoid–N-Vanillylnonanamide	Lateral chain of capsaicinoids has more inhibitory activity than the phenolic part; confers systemic protection to the upper leaves of pepper	Veloso et al. (2014)
Leveillula taurica	Bicarbonate, sulphates and phosphates-KH_2PO_4, $KHCO_3$, $MgSO_4$, $MnSO_4$	Salts control the growth and infection rate probably by disrupting the osmotic balance for the growth of fungus	Dik et al. (2003)
Colletotrichum gloeosporioides	Antimicrobial peptides (AMPs)	Inhibition of trypsin and α-amylase activity of fungi	da Silva Pereira et al. (2021)

(continued)

Major evolutionary and historical events often lead to loss or gain of desired allele copies from domesticated populations. To incorporate novel alleles for disease resistance, breeders have to regularly survey the crop wild relatives (CWRs). Expansion of crop germplasm resources with CWRs is crucial for development of varieties suitable for climate change affected production systems (FAO 2015).

Table 3.4 (continued)

Species	Biocontrol species/bioactive compounds	Summary	References
Colletotrichum coccodes	Compost water extracts (CWEs)	In vitro inhibition of conidial germination and appressorium formation and enhanced expression of PR proteins CaBPR1, CaBGLU, CaCHI2, CaPR-4, CaPO1, CaPR-10	Sang and Kim (2011)
Rhizoctonia solani	*B. subtilis*	Production of fungicidal compounds surfactin, iturin and fengycin	Wu et al. (2019)
F. oxysporum, *F. culmorum*, and *F. moniliforme*	*Beauveria bassiana* (strain NATURALIS) and *Metarhizium brunneum* (strain BIPESCO5)	Antagonize the persistence of crown and root rot	Jaber (2018)
Verticillium dahliae	*Arbuscular Mycorrhizal Fungi (AMFs)*	Balanced antioxidant metabolism in leaves, deposition of higher lignin, induction of new isoforms of chitinases and superoxide dismutases and enhanced *PAL* expression in roots	Goicoechea et al. (2010)
Verticillium dahliae	*B. chitinosporus, B. megaterium, B. pumilus, B. subtilis, B. thuringiensis, P. fluorescens* and *P. putida* induced by Chemicals (IRCs) Bion (BTH), chitosan and salicylic acid	Increase in photosynthetic pigment and Vitamin C	Abada et al. (2018)
Stemphylium solani	*Kluyvera cryocrescens* and *Brevibacterium iodinum*	Activation of defense related *CaPR* and *CaChi2* genes and induction of SAR (Systemic Acquired Resistance) by the whole plant	Son et al. (2014)

(continued)

Table 3.4 (continued)

Species	Biocontrol species/bioactive compounds	Summary	References
Xanthomonas campestris pv. *vesicatoria*	*Ascophyllum nodosum*	Foliar applications of 0.5% *A. nodosum* extract (AN) at 10-day intervals resulted in significant ($p < 0.05$) increase in plant growth parameters, including plant height (40%), leaf number (50%), plant dry biomass (52%), root length (59%) and chlorophyll content (20%) compared to control	Ali et al. (2019)

Table 3.5 summarizes the various viral pathogens affecting *Capsicum* spp. under broad classes along with their symptoms and the available sources of resistance against each viral organism. In Florida, the asexual stage of *S. solani* was used to infect 33 breeding lines of pepper in order to study their pathogenicity, and it was found that all plants were susceptible (Blazquez 1971). Early screening for pepper resistant varieties were done in Korea where 467 accessions of peppers were screened for their resistance to *S. solani* and *S. lycopersici* (isolated separately). Accessions KC320, KC220, KC208, KC47 (PI244670), KC43 (PI241670), KC380 and KC319 showed highest resistance to both the pathogens (Cho et al. 2001). *S. solani* and *S. lycopersici* (Enjoji) Yamamoto were identified in the northern provinces of Korea, Gyoengbuk and Gangwon (Kim et al. 2004), and were reported to be prevalent since 1994.

Two *C. annuum* lines 'Perennial' and 'Vania' showed no symptoms upon CMV inoculation but the yield and specific infectivity of the virus was lower when extracted from Perennial than from Vania (Nono-Womdim et al. 1993). The Indian hot pepper accession Perennial was used to develop CMV resistant pepper varieties which were able to recover from high viral titers (Lapidot et al. 1997). The inheritance was found to be polygenic and incompletely dominant. A *C. frutescens* accession, BG2814-6, represented incomplete penetrance of resistance towards six isolates of CMV via at least two recessive genes (Grube et al. 2000a). The resistance to CMV_{KOREAN} and CMV_{FNY} strains is controlled by a single dominant gene *Cucumber mosaic resistance 1* (*Cmr1*) in *C. annuum* with three single nucleotide polymorphisms (SNP) markers linked to this gene (Kang et al. 2010). Hybrids—PBC1354 and PBC378 were crossed

Table 3.5 The various viral pathogens under broad classes affecting *Capsicum* spp. along with their symptoms. The available sources of resistance against each viral organism in *Capsicum* germplasm have also been listed

Virus	Symptoms	Sources of resistance		
		Accession	Species	Reference(s)
Thrips trasmitted orthotospovirus				
Tomato spotted wilt virus (TSWV)	Chlorotic and necrotic rings on leaves, stunting	PI 159,236 (CNPH 679) x Magda (CNPH 192), PI 152,225, Panca, AC09-207, ECU-973, PIM261, AVRDC C00943	*C. chinense* x *C. annuum, C. baccatum*	Boiteux and de Ávila (1994), Cebolla-Cornejo et al. (2003), Sherwood et al. (2003), Hoang et al. (2013), Soler et al. (2015)
Tomato chlorotic spot virus (TCSV)	Chlorosis, necrosis, mottle/mosaic, bronzing	–	–	Batuman et al. (2014)
Capsicum chlorosis virus (CaCV)	Necrotic ringspot, leaf mottling	–	–	–
Groundnut bud necrosis virus (GBNV)	Mosaic with ringspots and necrosis	IIHR4360, IIHR4577, IIHR4578, IIHR4582, IIHR4585, IIHR4587, IIHR4588 and EC631810	*C. annuum*	Pavithra et al. (2020)
Groundnut ringspot virus (GRSV)	Ringspots, chlorotic and necrotic areas	–	–	–
Aphid trasmitted potyvirus				
Potato virus Y (PVY)	Mosaic, mottling	PI2664281, SC46252	*C. annuum*	Kyle and Palloix (1997)
Tobacco etch virus (TEV)	Vein clearing, chlorotic and necrotic spots	–	–	–
Pepper yellow mosaic virus (PepYMV)	Leaf curling, yellow green mosaic, fruit deformation	UENF 1616 × UENF 1732	*C. baccatum*	Bento et al. (2013)
Chilli veinal mottle virus (ChiVMV)	Leaf mottling, mosaic, mottle, yellow vein banding	CV3, CV8 and CV9	*C. annuum*	Shah et al. (2009), Tsai et al. (2008), Lee et al. (2017)

(continued)

Table 3.5 (continued)

Virus	Symptoms	Sources of resistance		
		Accession	Species	Reference(s)
Pepper veinal mottle virus (PVMV)	Foliar chlorosis, rugosity, mosaic, vein banding	–	–	–
Aphid trasmitted cucumovirus				
Cucumber mosaic virus (CMV)	Mosaic-mottling, necrosis, yellow ringspots, leaf deformation, stunting	Punjab Lal, Perennial, BG-2814–6	*C. annuum, C. frutescens*	Bal et al. (1995), Nono-Womdim et al. (1993)
Contact trasmitted tobamoviruses				
Pepper mild mottle virus (PMMoV)	Mottling, chlorosis, curling, stunting	PI159236, CM334, 9093	*C. chinense*, *C. annuum*	Venkatesh et al. (2018)
Paprika mild mottle virus (PaMMV)	Yellowing, light and dark green mottling	–	–	–
Pepper severe mottle virus (PepSMoV)	Mosaic, leaf deformation	–	–	–
Tobacco mosaic virus (TMV)	Mosaic, mottle, necrosis, yellowing, stunting	PI315008, PI315023, PI315024	*C. chinense*	Boukema (1980), Scholthof (1997)
Whitefly transmitted geminivirus				
Pepper leaf curl virus (PepLCV)	Stunted growth, upward leaf curling, crowding of leaves, swelling of veins, puckering of intervenous regions, blistering	GKC-29, BS-35, Bhut Jolokia, EC-497636, Japani Longi, Punjab Lal, Pant C-1, S-343, SL 456, SL 475, DLS-Sel-10, WBC-Sel-5, PBC-142, BJ001	*C. chinense, C. annuum*	Kumar et al. (2006), Rai et al. (2014), Srivastava et al. (2017), Thakur et al. (2018, 2019, 2020)
Tomato yellow leaf curl virus (TYLCV)	Curling and yellowing	–	–	–
Pepper golden mosaic virus (PepGMV)	Interveinal chlorosis of young leaves, apical necrosis	BG3821, BG3820, BG3819	*C. chinense, C. annuum*	Anaya-López et al. (2003), Holguín-Peña et al. (2008), García-Neria and Rivera-Bustamante (2011)

(continued)

Table 3.5 (continued)

Virus	Symptoms	Sources of resistance		
		Accession	Species	Reference(s)
Pepper huasteco yellow vein virus (PHYVV)	Yellowing of veins, mosaic, leaf curl, stunting	BG3821, BG3820, BG3819, UAS12, El Reparo, Yecorato	*C. chinense, C. annuum*	Hernández-Verdugo et al. (2001), Holguín-Peña et al. (2008), García-Neria and Rivera-Bustamante (2011)

with CMV tolerant parents to generate fifteen backcross populations, which were characterized for morphological traits and CMV resistance. Nine genotypes including B3A29-13, B3A24-20, B3A29-22, B3B12-13, B3B12-25, B3B37-9, B3C16-16, B3C16-5 and B3C16-5, and six genotypes including B3D11-17, B3D11-8, B3D12-17, B3D38-5, B3E31-19 and B3E20-22 resembled the two parents, PBC378 and PBC1354 in tolerance to CMV, respectively (Herison et al. 2012). A single recessive *CMV resistance gene 2* (*cmr2*) was identified which provides resistance to CMV-P1 along with other pathotypes (Choi et al. 2018).

Eight *C. annuum* genotypes from Karnataka (India) showed a HR to *Groundnut bud necrosis virus* (GBNV) without systemic infection and can be utilized as natural sources of resistance in breeding programs (Pavithra et al. 2020). The wild *C. annuum* populations from El Reparo and Yecorato region of Northwest Mexico showed neither the presence of viral DNA nor any symptoms upon mechanical and biolistic inoculation of *Pepper huasteco virus* (PHV) (Hernández-Verdugo et al. 2001).

Genes that provide broad spectrum resistance to viruses in *Capsicum* have been studied using genetic analysis. Two genes—*Pr4* (dominant) and *pr5* (recessive) provide resistance to all the known and common strains of PVY, respectively, in *C. annuum* variety 'Serrano Criollo de Morelos 334' (SCM334), while another dominant gene *Pn1* is involved in systemic necrotic response (Dogimont et al. 1996). Afterwards, the potyvirus resistance genes were designated by the symbol *pvr* followed by chronological order of the identified locus, and alleles at the locus were differentiated using subscripts (Kyle and Palloix 1997). The recessive allele *pvr2* provides resistance to PVY strains—$pvr2^1$ to PVY-0 and $pvr2^2$ to PVY-0 and PVY-1, respectively, and encodes a translation eukaryotic initiation factor 4E (*eIF4E*) in pepper (Ruffel et al. 2002). It was reported that *eIF4E* interacts with the potyviral genome-linked protein (VPg) to cause viral production and breaking of resistance during potyvirus infection (Léonard et al. 2000). Mutations in the *eIF4E* lead to incompatibility in host-virus interaction, without compromising the plant life cycle and resistance systems against several RNA viruses (Lellis et al. 2002).

3.5 Breeding Objectives and Methods

Chili pepper is becoming an increasingly important crop for being both a vegetable and a spice crop with diverse applications and considerable socio-economic importance. Keeping these points in mind a comprehensive strategy must be evolved which has a guided purpose to serve the objectives of pepper breeding in order to obtain genotypes that meet the demands of the growers and consumers. While briefly touching upon its use as a flavoring agent, as a reservoir of antioxidants and nutraceuticals, a vegetable and many other uses due to its great therapeutic value, the principal focus of this chapter is on the aspect of breeding for biotic stress resistance.

The highly versatile nature of pepper crop makes it adapted to very divergent conditions of cultivation as well as cultural practices, leading to entirely exclusive preferences in terms of end usage. Preferences of the pepper growing countries and assorted cultures for hot or sweet pepper varies, leading to totally isolated domestication paths; hence, a suitable breeding strategy has to be accountable to address those specific needs by choosing most acceptable parental pools.

Resistance breeding has been emphasized for the need of *Capsicum* breeding. Identifying the suitable resistant hosts as well as focusing on pathogens is extremely important in *Capsicum* as there is a very broad spectrum of choices to make owing to very rich and diverse morphologies. Some earlier work on the classification of major *Capsicum* pathogens is discussed in details in Sect. 3.2. Identifying and understanding the genetics and crossability of novel (wild sources) or established (characterized lines) resistance sources with host is a very vital step to achieve effective introgression of desired characters.

Several diseases of interest in the present scenario have been successfully addressed by utilization of wild resistance sources. Many viral, fungal and bacterial diseases, and pests such as whiteflies, thrips, mites and nematodes have been characterized for their source of plant resistance genes involved in important defense complexes. Two important aspects need to be clearly established before designing a resistance breeding program, by making a distinction between the qualitative as well quantitative nature of trait of interest, and to understand linked traits by sourcing inputs from genetic mapping and verification with suitable markers, as undesirable traits are also very likely to introgress, especially when the source is a wild relative. Further, it should be equally important to have continuous efforts to track resistance breaking pathogens along with a constant search for novel resistance sources.

Other major objectives with indirect relationship to biotic stresses are yield, marketability traits such as colour, aroma, flavour etc., desired chemicals, pungency, oleoresin, flavonoids etc. However, the major breeding objective of *Capsicum* breeding is to increase overall productivity by increasing yields and secondary morphological traits such as branching habits, height, nutrient use efficiency and stress tolerance. Heterosis breeding programs are gaining popularity in *Capsicum* breeding as a targeted solution to multiple end goals. Targeted efforts made in the identification of male sterility-based hybrid development systems will be very useful in saving time as well as labour. For hybrid seed development, both kind of

male sterility systems—genetic (GMS) and cytoplasmic (CMS) have been utilized in *Capsicum* breeding. The CMS system which is being widely explored in *Capsicum* breeding is mainly dependent on the well characterized maintainers as well as diversified germplasm. Priority areas in the development of CMS based hybrids will consist of identification of suitable restorer lines with good general and specific combining ability, and exploiting them by introgressing resistance genes for easy transferability.

Capsicum is a vegetable crop also revered for its ornamental properties, and accessory features such as fruit colour, fruit length, and overall glossiness also play an important role in marketability and consumer preferences. Along with the features promoting the economic value, there are several other horticultural and biochemical traits demanding a breeder's attention, e.g., pungency, which is an important commercial attribute in peppers and is mainly governed by capsaicinoid complexes. Most abundant capsaicinoids are capsaicin and dihydrocapsaicin, while 71% of pungency in all varieties is a manifestation of capsaicin alone (Kosuge and Furuta 1970). Total capsaicin content is an important quality parameter of breeder's interest in the development of new commercial varieties.

Effective breeding for fruit dry matter content refers to improvement in the powder formation qualities as well as color and pungency. Major characteristics desirable for export quality produce include high dry matter content, but in practice there is no positive correlation between the capsaicin levels and dry matter obtained (Dhall 2008). The thin pericarp of fruits assures quicker drying times, while thick skin fruits are severely shriveled and dull upon visual inspection after drying. A growing trade among countries enforces certain quality standards, which are always to be met with locally available and adapted germplasm for inclusive growth of all stakeholders. Genomic designing along with improved breeding practices can assure uniformity and desired throughput in emerging climate change scenarios, and stresses.

Blocky fruit shape and colour variations at unripe stages of sweet peppers are also a desired objective of *Capsicum* breeding. Sweet peppers are primarily consumed for their high levels of antioxidants and vitamins, such as ascorbic acid, flavonoids and phenolic compounds, carotenoids including vitamin A precursor like alpha and beta-carotene, beta-cryptoxanthin (Tomlekova et al. 2009). Sweet pepper breeding traits of secondary importance include stability and sustainability of carotenoids content unaffected by the photooxidation damages and varied storage conditions. Multiple pathogens infecting the sweet peppers include *Phytophthora*, anthracnose, viruses, and bacteria under field conditions. Therefore, breeding for genotypes with wider adaptability is highly desirable for cold as well as tropical climates to ensure the survival of crop in areas with excessive biotic and abiotic stresses, and also for the expansion of pepper crop to non-traditional areas. Under protected and curated conditions, many of the field stresses become obsolete, and traits including indeterminate growth habits, manageability to training and pruning, marketable fruit shapes such as blocky, and resistance to soil borne pests such as nematodes are therefore the major goals (de Swart 2007).

3.5.1 Traditional Breeding Methods

Mendelian principles of heredity and inheritance have been the leading concepts in resistance breeding throughout the past century. Acknowledging critical limitations of classical breeding methods is however the need of hour under changing climatic conditions and biotic factors outpacing our crops. Traditional breeding is the art and science of aggregating all favorable traits in a plant from two compatible parents. Mass selection, pedigree selection, single seed descent, recurrent selection and backcrossing are the common breeding methods. Selection is the most vital and distinguishing aspect of conventional versus modern breeding methods. Few notable limitations to conventional methods while breeding for biotic stress resistance are as follows: (1) a disconnect of genotype vs. phenotype: conventional breeding selection cycles heavily depend upon the major traits where, gene x environment interactions govern the final phenotypes, but environment components are nearly impossible to account for without compromising significant error margins and thus create a lot of inherent selection bias, thus allowing undesired genes; (2) hybridization to achieve heterosis is the common goal with expectation of a fair introgression of desired traits, particularly sexually incompatible crosses give undesirable results due to linkage drag, disrupting the Mendelian assumptions, and therefore very limited control on the process can be achieved via conventional means; (3) lack of control over the expression in crossed progenies is also a major concern with conventional approaches, in resistance breeding it is often desirable to completely express an introgressed gene complex.

The major objectives in breeding of pepper genotypes focus on yield, earliness and vigor, superior fruit quality, resistance against pathogens, and high stress tolerance. Classical plant breeding techniques have proven to be very useful for improvement of pepper crop for yield and quality traits as well as enhancing disease resistance properties. Traditional breeding involving the use of various crossing schemes and periodic selection of suitable plants reflecting traits of interest, is mostly based upon easily recognizable morphological characters.

Among some of the classical methods exploited in *Capsicum* breeding, mass selection which is based on phenotype of traits with high heritability has been used by some breeding groups in Portugal and Brazil. In comparison, the pedigree method based on hybridization was used to breed the cultivars, BRS Sarakura and BRS Garça, adapted to Central Brazil (Carvalho et al. 2009). The backcross method was used to transfer virus resistance from *C. chinense* to *C. frutescens* (Greenleaf 1986). Recurrent selection, which can be used to select traits of low heritability was used by Palloix et al. (1990a, b) in the development *C. annuum* genotypes showing resistance against *V. dahliae* and *P. capsici*. The single seed descent method for the development of recombinant inbred lines (RILs) was employed by Moreira et al. (2013) to obtain *Capsicum* lines resistant to bacterial spot, and by Villalon (1986) to fix recessive genes conferring resistance to potyvirus.

Of the several plant breeding procedures, heterosis breeding is expected to play a crucial role in increasing the yield of pepper crop and improving other important

traits with commercial value. In heterosis breeding, genetically diverse inbred lines of chili showing good combining ability are utilized. Two cultivars, Branang (resistant) and Lembang1 (susceptible) were crossed and their F_1 hybrid was analyzed for *CaChi2* gene expression patterns after infection with *F. oxysporum*. Results showed an increased expression in the F_1 hybrid by qRT-PCR (Ferniah et al. 2018). JNA2 × ACB1 × 9608D and Rajaput × P3 hybrid lines were obtained by Maruti et al. (2014) against *F. solani*. Monogenic and dominant resistant lines were also observed in the hybrids—SNK × P3, KA2 × P3, and RAJPUT × P3 (Manu et al. 2014). Good sources of resistance against *F. verticillioides* and *F. pallidoroseum* viz. Masalawadi, SC-120, Phule C-5, SC-335, SC-415, SC-1 07, SC-348, SC-108, LCA-304, Arka Lohit, Pusa Jwala and Pant C-2 for *C. annuum* are also available (Khan et al. 2018).

3.5.2 *Limitations of Traditional Breeding and Rationale for Molecular Breeding*

Traditional breeding methods have generated many useful results in terms of better varieties and a knowledge-base of mapping information. However, there are some major limitations of these methods. Classical plant breeding methods require longer periods and several generations for identifying useful genotypes. The basis of selection in traditional breeding is always on major phenotypic traits, which as they allow rapid visual selections, but on the other hand they fail badly for identification of undesirable genes, which in later cycles of selection may reappear or even remain unidentified for whole breeding cycles. Another important issue relates to the problematic incompatible crosses, e.g., across genera. Such morphological as well physiological barriers are hard to overcome.

In contrast, molecular breeding allows selection for both qualitative and quantitative traits at all stages of plant's life cycle and thus reduces the time required for accurate phenotyping of a plant. It also allows identification of undesirable genotypes, which can be easily eliminated by marker-assisted selection (MAS). Furthermore, as molecular markers are not affected by the environment, selection can be undertaken in all types of environmental settings—greenhouses, nurseries or field conditions. Thus, traits that are conditional upon favorable conditions of a particular environment, e.g., disease/pest resistance and stress tolerance, can also be selected with precision. Genomic designing of modern stress resistant crops involves precise selection with the help of genetic markers and genetic maps. Polygenic traits with known linkages can be efficiently mapped and targeted via simple and accessible genetic markers. Genetic maps of fine details are nowadays a reality achieved via incremental steps of progress, and a vast body of work generated with markers such as RFLP, RAPD (as low resolution), SSRs as (mid-resolution) and SNP markers with the finest resolutions to aid in the screening and selection stages of breeding programs. Robust genotyping possibilities allow efficient and guided understanding of linkage patterns at genome wide scales and help find associations such as QTLs

and/or through association mapping of traits of interest. Genomic designing is therefore the way forward for *Capsicum* crops with modern biotechnological tools such as restriction enzymes-based engineering, transgenics as well as pyramiding of genes of interest.

3.6 Molecular Genetics and Breeding of Biotic Stresses Related Traits

The *L* locus genes (*L3* and *L4*) which provide resistance to PMMoV in *Capsicum* spp. have been widely used in breeding programs. Several DNA markers closely linked to the *L4* genes have been screened for their applications in cost and time effective selection of markers in the PMMoV-resistance breeding (Kim et al. 2008a; Matsunaga et al. 2003). Resistance allele *L1a* was found to be involved in PaMMV (Japanese strain) resistance in bell pepper (Sawada et al. 2004). Unlike the other *L* alleles, *L1a* is temperature insensitive and is elicited by the viral coat protein of the P_0 pathotype of tobamoviruses (Matsumoto et al. 2008). *Pr4* (*Pvr4*) gene also provides resistance to all the known pathotypes of PeMV (Dogimont et al. 1996). Cleaved amplified polymorphic sequence (CAPS) markers for three recessive alleles of *pvr* locus—*pvr*, $pvr1^1$ and $pvr1^2$ on chromosome 3, were developed for selection of potyvirus resistance in *Capsicum* (Yeam et al. 2005).

Salicylic acid accumulation and reactive oxygen species (ROS) production were induced in PepGMV and PHYVV resistant BG3821 pepper plants carrying at least two genes with recessive epistatic effects (García-Neria and Rivera-Bustamante 2011). Three *C. annuum* varieties—DLS-Sel-10, WBC-Sel-5 and PBC-142 were found to be resistant to leaf curl causing begomoviruses (Srivastava et al. 2017). Genetic inheritance of PHYVV resistance in three wild pepper varieties from Mexico—UAS12, UAS13 and UAS10 showed that at least two genes govern the PHYVV resistance (Retes-Manjarrez et al. 2017). The *C. annuum* line, UAS12 showed high resistance towards PHYVV with lesser symptoms, longer incubation time, lower viral DNA levels and stable inheritance, and therefore can be a promising genetic resource for pepper improvement programs against begomoviruses (Retes-Manjarrez et al. 2018). Resistance for LCVD in a population developed from a cross between resistant DLS-Sel-10 and susceptible Phule Mukta pepper varieties was found to be monogenic recessive (Maurya et al. 2019). The phenolic content and peroxidase (POD) activity in resistant pepper variety 9853–123 was observed to be higher than the susceptible variety (KKU-P31118) upon PepYLCThV inoculation (Thailand) (Kingkampang et al. 2020). At least 7 genes, including *Pvr4* control the resistance to PepYMV in *C. baccatum* (Bento et al. 2013). Sixteen RILs in the F_6 population of the *C. baccatum* var. *pendulum* were resistant for PepYMV when tested via phenotyping and agronomic performance. A highly resistant line did not give good agronomic performance, while four other lines were resistant and productive, and suitable for field tests in resistance breeding programs (da Costa et al. 2021).

3.6.1 *Genetic Mapping in* Capsicum *Spp.*

Interspecific variability among 21 accessions of cultivated and wild pepper (*C. annuum*, *C. baccatum*, *C. chacoense*, *C. chinense* and *C. frutescens*) and later on intraspecific variability was examined among four *C. annuum* cultivars (NuMex R Naky, Jupiter, Perennial and Criollo de Morelos 334) to study DNA polymorphisms utilizing restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers. Important findings suggested that any two pepper accessions can be utilized as parents to create a good segregating population for RFLP analysis (Prince et al. 1995).

A genetic map of *Capsicum* spp. based on an intra specific cross was developed with a total length of 720 cM. The map was based on 192 molecular markers consisting of RFLP and isozymes, and comprised of 19 linkage groups. At least a genetic distance of 228 cM (31.7%) covered by the markers reflected a high level of conservation with respect to the tomato genome in terms of order (Prince et al. 1993) (Table 3.6). Authors also concluded that the mechanism for genome evolution in Solanaceae is primarily via centric fusions and resulting chromosome breakage events.

RFLP and RAPD markers were also utilized to construct an intraspecific linkage map of segregating doubled haploid (DH) progenies. Spanning an approximate length of 820 cM, a total of 85 markers were mapped on to 18 linkage groups which were assigned to 4 chromosomes eventually (Lefebvre et al. 1995). Genes responsible for fruit pungency were precisely located; meanwhile segregation data also labelled the genomic regions with evident segregation ratios favouring particularly big fruited parents, suggesting available selection of DH progenies for mapping. Also, two new genes of breeder's interest for controlling hypersensitive resistance to TMV and controlling the erect growth of fruits were located (Lefebvre et al. 1995).

Tomato specific probes were utilized to create a genetic linkage map from an interspecific F_2 population in *Capsicum*, with a total coverage of 1,245.7 cM. Eleven large (76.2–192.3 cM) and two small (19.1 and 12.5 cM) linkage groups were identified. Comparisons with genetic maps of tomato reflected a high degree of conservation, and 18 homologous linkage blocks covered 98.1% of tomato and 95.0% of the pepper genome (Livingstone et al. 1999).

An intraspecific consensus map of *C. annuum* was constructed using three populations comprising 215 DH lines and 151 F_2 individuals. Each individual map comprised 16 to 20 linkage groups with lengths ranging from 685 to 1,668 cM. The consensus map contained 100 known functional gene markers as well as loci of plant breeder's interest such as disease resistance locus *L*, *pvr2*, *pvr4* and *C* locus determining capsaicin content and the erect fruit locus. Additional linked loci related to disease resistance such as *Tsw*, *Me3*, *Bs3* and *Y* locus for fruit color were also identified in the same study (Lefebvre et al. 2002).

RILs of PSP11 (susceptible) crossed with PI201234 (resistant), and F_2 lines of Joe E. Parker (susceptible) × CM334 (resistant) were used to create two independent linkage maps. The RIL map spanning a distance of 1,466.1 cM consisted of a total

Table 3.6 The mapping populations and genetic markers used for the development of genetic maps*Capsicum* spp

Population	Markers	References
Interspecific F_2 Hybrid from *C. annuum* (CA133) X *C. chinense* (CA4)	RFLP and Isozymes (192)	Prince et al. (1993)
Three intrapsecific *C. annuum* DH populations	RFLP and RAPD (85)	Lefebvre et al. (1995)
(*C. annuum*) BG 2816 (*frutescens*) derived Interspecific BC2 population constructed by crossing the C. annuum cv. Maor (recurrent parent) with A C. frutescens wild accession BG 2816	RFLP (92)	Rao et al. (2003)
Intrapsecific C. annuum F_2 *population derived from* CM334/Chilsungcho cross	RFLP (202), WRKY (6), SSR (1)	Kim et al. (2008a)
Populations derived from cross between ChiVMV resistant and susceptible varieties	SNP (1466)	Lee et al. (2017)
C. annuum (DLS-Sel-10 x Phule Mukta)	–	Maurya et al. (2019)
Intraspecific C. baccatum F_2 *population derived from a cross between UENF 1616 (female parent) and UENF 1732*	SSR (42), ISSR (85), RAPD (56)	Moulin et al. (2015)
Interspecific F_2 *population derived from crossing C. annuum (TF68) and C. chinense (Habanero)*	EST-SSR (150)	Yi et al. (2006)
Doubled haploid C. annuum population derived from crossing California Wonder and LS2341	SSR (106), AFLP (253)	Mimura et al. (2009, 2010)
F_2 mapping population derived from a cross between the inbred lines BA3 (*C. annuum*)and YNXML (*C. frutescens*)	SSR (95)	Tan et al. (2015)
F_2 mapping population developed by selfing the F_1 hybrid of the inbred lines FL201 (*C. annuum*) and TC 07245 (*C. galapagoense*)	SSR (400)	Arjun et al. (2018)

of 144 markers including 91 Amplified fragment length polymorphism (AFLPs), 34 RAPDs, 15 SSRs, 1 SCAR and 3 morphological markers (erect fruit habit, elongated fruit shape, and fasciculate fruit clusters) across 17 linkage groups. Meanwhile, F_2 map covered a total of 1,089.2 cM with 113 markers (51 AFLPs, 45 RAPDs, 14 SSRs and 3 SCAR) distributed across 16 linkage groups (Ogundiwin et al. 2005).

A linkage map with a total genetic length of 54.1 cM was constructed with 7 AFLP and one CAPS marker. AFLP markers detected by bulked segregant analysis of 8 markers were linked to fertility restorer locus (Rf), while one AFLP marker (AFRF8) was converted to CAPS marker in this study. The AFRF8 CAPS marker was located close to the *Rf* locus within a genetic distance of 1.8 cM (Kim et al. 2006a, b).

A RIL population consisting of 297 individuals was used to construct a high-resolution intra-specific linkage map of *C. annuum* using the parents 'Yolo Wonder' and CM334 as source of resistance to a number of diseases. A total of 587 markers (507 AFLP, 40 SSR, 19 RFLP, 17 sequence-specific amplified polymorphisms, and 4 sequence tagged sites) were used, which assembled into 49 linkage groups. With an average inter-marker distance of 5.71 cM, spanning over 1,857 cM, 69% markers covering 1,553 cM were assigned to 1–12 chromosomes, while 26 LGs remained unassigned (Barchi et al. 2007).

An integrated map developed from four genetic maps of two interspecific (*C. annuum* 'TF68' and *C. chinense* 'Habanero') and two intraspecific (*C. annuum* 'CM334' and *C. annuum* 'Chilsungcho') populations of pepper, was construed using 169 SSR, 354 RFLP, 23 STS from BAC-end sequences, 6 STS from RFLP, 152 AFLP, 51 WRKY, and 99 rRAMP markers on 12 chromosomes of *Capsicum.* A total map distance of 1,858 cM with 805 markers for interspecific population, and a total map distance of 1,892 cM with 745 markers were covered in the intraspecific population (Lee et al. 2009a, b).

A total of 288 conserved orthologous set II (COSII) markers spanning 12 linkage groups which corresponded to 12 chromosomes were characterized. Aforementioned map represented genomes of cultivated *C. annuum* and wild *C. annuum* as well as other related *Capsicum* spp. differing by reciprocal chromosome translocations. This high resolution COSII map identified 35 conserved syntenic segments (CSSs) between tomato and pepper, wherein gene/marker order was well-preserved (Wu et al. 2009).

The *C. baccatum* genetic map of the F_2 population (203 progenies) was constructed based on 42 SSR, 85 inter-simple sequence repeat and 56 RAPD markers. A total of 12 major and 4 minor linkage groups covering a total genome distance of 2,547.5 cM, with an average distance of 14.25 cM in between markers were inferred from the map. Sixty-two SSR markers out of 152 already available for *C. annuum* were successfully transferred to *C. baccatum*, generating polymorphisms of which 42 were directly mapped, allowing further studies with other members of the genus *Capsicum* (Moulin et al. 2015).

3.6.2 Molecular Mapping of Biotic Stress Related Loci

Marker-assisted selection (MAS) has proved to be a very useful technique in classical as well as the post genomic era. Breeding objectives turn towards finer traits as molecular information about traits of interest stack up. The ability to do so for selection even before plants see the field saves a lot of screening time and personal human biases while evaluating major morphological traits. In *Capsicum,* MAS has been successfully utilized for biotic stress resistance breeding. Available marker resources can be effectively utilized in MAS since well-characterized and markers tightly linked with the locus of interest are very effective at narrowing down selection and screening efforts.

In Solanaceae, resistant genes were found only for tomatoes at the *Ve* locus. The linked genes, *Ve1* and *Ve2* in the locus cause H_2O_2, peroxidase and *PAL* expression in the roots of inoculated plants (Gayoso et al. 2010). Further, in *Capsicum* (New Mexico variety), an ORF (open reading frame) was identified by WGS (whole genome sequencing) with homology to the *Ve* locus of tomato. Sixteen SNPs were identified between the resistant and the susceptible cultivars (Barchenger et al. 2017). A CAPS marker developed from the coding region of *CaVe* was used to screen diverse germplasm that was resistant to Verticillium wilt. The CAPS marker could identify accessions with resistance against the New Mexico *V. dahliae* isolate with 48% accuracy.

A partially dominant gene *L* has been identified, isolated and employed for broad resistance to Tobamoviruses like TMV, ToMV and PMMoV in pepper breeding programs. Different alleles of the *L* locus on chromosome 11 determine the resistance for TMV strains in five *C. chinense* accessions (Boukema 1980). The major alleles at the *L* locus—L^1, L^{1a}, L^{1c}, L^2, L^{2b}, L^3 and L^4 have different resistance spectra determined by multiple sub-regions of the leucine rich repeats (LRR) domain of the L proteins in *Capsicum* spp. (Tomita et al. 2011). The *L3* and *L4* were suggested to be closely linked genes instead of different alleles based on SNP markers (Yang et al. 2009). The mutation studies demonstrated that the functional coat protein, and not the viral RNA is required to induce the L^2 allele mediated HR in resistant *Capsicum* varieties (de la Cruz et al. 1997). L^3 gene was able to provide resistance to most of the Tobamoviruses including PMMV-S isolate, to which a local hypersensitive response is induced in *Capsicum* plants (Berzal-Herranz et al. 1995). *L* allele specific markers like L4segF&R have been developed based on the LRR region of the L^4 allele, which however did not completely segregate with the L^4 allele (Yang et al. 2012).

The *Pvr4* from *C. annuum* CM334 and *Pvr7* from *C. chinense* variety PI159236 provide completely dominant resistance to PepMoV. Eight AFLP markers linked to the *Pvr4* gene were mapped and a tightly linked codominant marker was converted into CAPS marker using sequence alignment of the allelic sequences (Caranta et al. 1999). The molecular mapping of *Pvr7* gene from *C. annuum* resistant variety '9093' using SNP markers of *Pvr4* region and further sequence analysis revealed that *Pvr4* and *Pvr7* are the same genes on chromosome 10 (Venkatesh et al. 2018).

The dominant, additive and epistatic effects were observed for the genes responsible for ChiLCV resistance in the F_1 and F_2 population of a cross between *C. annuum* L. and *C. frutescens* L. (Anandhi and Khader 2011). Pepper genotypes were screened using artificial inoculation in a microarray and a recessive monogenic inheritance pattern against PepLCV was revealed in Bhut Jolokia (*C. chinense*) (Rai et al. 2014). Three *C. annuum* genotypes—S-343, SL 456 and SL 475 were tested for ChiLCV resistance using natural and artificial inoculation that was found to be controlled by a single dominant gene (Thakur et al. 2019). Two SSR markers, *Ca516044* and *PAU-LC-343–1* were found to be linked to the ChiLCV resistance gene on chromosome 6 of the pepper genome (Thakur et al. 2020). *Solanum pseudocapsicum* was found to be a symptomless carrier of ChiLCV when field tested for ChiLCV resistance via inoculation challenge and could therefore serve as a source of resistance for pepper species (Srivastava et al. 2021). Nine *Capsicum* genotypes were screened for ChiLCV resistance and three genotypes exhibited lower viral incidences—Punjab Lal, Pant C-1 and Japani Longi (Singh et al. 2021). The combination of two recessive alleles—*pvr6* and *pvr2*[2] provided complete resistance to PVMV (Caranta 1997).

A new source of resistance in the form of a single dominant resistance gene at the ChiVMV locus was discovered linked to two AFLP and one CAPS marker on chromosome 6 in *Capsicum* spp. (Lee et al. 2013). Further, three ChiVMV resistance genes—single dominant gene *Cvr1* on chromosome 6, single recessive gene *cvr4* and one oligogenic resistance gene—*Cvr2-1* and /*Cvr2-2* on chromosomes 6 and 10, respectively, were identified using population analysis in four *Capsicum* varieties from Hong Kong (Lee et al. 2017).

A RFLP based linkage map derived from F_2 generation (100 lines) of a cross of *C. annuum* cv. CM334 and *C. annuum* cv. Chilsungcho detected a QTL associated with *Phytophthora capsici* resistance (Kim et al. 2008b). Bulked segregant analysis performed with 400 RAPD markers identified three capsaicinoid content related loci that could distinguish the two bulks in *Capsicum.* QTL mapping for individual and total capsaicinoid content detected a major QTL, which could explain more than 30% of the phenotypic variation for this trait (Blum et al. 2003). Four disputed *C. annuum* samples were differentiated with 17 Inter-simple sequence repeat (ISSR) markers (Kumar et al. 2001). An intraspecific F_2 population of *C. baccatum* var. pendulum and *C. baccatum* 'Golden-aji' was used for QTL identification for anthracnose resistance with 175 AFLP markers (Kim et al. 2010). A total of 197 AFLP markers were developed in the introgression population of *C. annuum* cv. SP26 and *C. baccatum* cv. PBC81 to identify QTLs for resistance against anthracnose caused by *C. scovillei* and *C. dematium* (Lee et al. 2010). Genetic variability was studied in six *Capsicum* spp. with the help of 8 ISSR markers (Thul et al. 2012).

A total of 95 SSR markers were validated against a genetic map developed using *C. annuum* cv. BA3 and *C. frutescens* cv. YNXML. The map was used to identify the QTLs for initiation of flower primordia (Tan et al. 2015). A total of 28 SSR markers were mapped in the F_2 population of a cross between *C. annuum* cv. FL201 and *C. galapagoense* cv. TC07245, from a survey panel of 400 SSR markers (Arjun et al. 2018). The molecular markers developed in pepper populations are summarized in Table 3.7. To effectively characterize the potyvirus resistance locus recessive alleles

Table 3.7 The molecular markers and their respective linked loci in *Capsicum* mapping populations for viral resistance

Population	Marker	Linked locus	Derived from	References
F_2 progenies developed from a cross between *C. annuum* L. cv. 'Yolo Wonder' and an accession Criollo de Morelos 334 (CM334)	AFLP and CAPS	*Pvr4*	AFLP (E41/M49-645)	Caranta et al. (1999)
F_2 progenies developed from a cross between a *C. frutescens* accession (PI 195301) and a *C. chinense* accession (PI 152225)	RAPD and CAPS	*TsW*	RAPD ($OPAC10_{593}$)	Moury et al. (2000)
F_2 population derived from a cross between *C. annuum* inbred variety (Maor) and a *C. frutescens* line (BG 2816)	RFLP and CAPS	*C* locus	RFLP (TG 205)	Blum et al. (2002)
Germplasm representing C. annuum and C. chinense	CAPS	*Pvr1* + , *pvr1*, $pvr1^1$, $pvr1^2$	Sequences of exon1, exon2, and intron1 at the *Capsicum pvr1* locus	Yeam et al. (2005)
F_2 segregating population of *C. annuum* developed from a cross of TS502 (CMS line) and HK6T (Restorer line)	AFLP and CAPS	*Rf*	AFLP (AFRF8)	Kim et al. (2006a)
F_2 mapping population consisting developed by crossing PepMoV-resistant *C. annuum* '9093' and the PepMoV-susceptible *C. annuum* 'Jeju'	SNPs	*Pvr7*	SNP-H2.3 and SNP-H1.7	Venkatesh et al. (2018)
F_2 population derived from pepper CMS line BA3 and restorer line B702	SNPs	*Rf*	SNP-H2.3 and SNP-H1.7	Venkatesh et al. (2018)

$^{+}$*pvr1*, *pvr1*^{1} and *pvr1*^{2}, three CAPS markers viz. *Pvr1-S*, *pvr1-R1*, and *pvr1-R2* were developed in *Capsicum* spp. (Yeam et al. 2005). Among eight AFLP markers used for mapping the *Rf* locus, the closest marker at 1.8 cM, AFRF8 was converted to a CAPS marker named as AFRF8CAPS in *C. annuum* L. (Kim et al. 2006a). AFLP maker E-AGC/M-GCA112 positioned at 1.8 cM from partial restorer (pr) locus was used to develop CAPS marker PR-CAPS in pepper (Lee et al. 2008). RFLP marker CT211, linked to *P. capsici* resistance has also been converted to a CAPS marker in *C. annuum* (Kim et al. 2008b).

Powdery mildew sensitive (Saengryeg) and resistant (PRH1) were sequenced to develop 6,840,889 and 6,213,009 SNP markers respectively (Ahn et al. 2018). Additionally, 6281 SNPs associated with 46 resistance genes that were related to the NBS-LRR family were mapped to chromosomes 4 and 5, respectively, in the PRH1 line, and were validated using high-resolution melting (HRM) assay in 45 F_4 populations, and correlated with the phenotypic disease index (Ahn et al. 2018).

Genotyping by sequencing (GBS) identified 2,831,791 SNP markers from a panel of 142 *Capsicum* genotypes from Ethiopia. A total of 509 were significantly associated with fruit, stem and leaf related traits (Solomon et al. 2019). A total of 10,307 SNPs were observed in a core collection panel (256) of pepper accession upon GBS (Tamisier et al. 2020). A high-density genetic map was constructed with 7,566 SNP markers from the F_2 population to study the pepper restorer-of-fertility (*CaRf*) gene in *Capscium* spp. (Cheng et al. 2020). A total of 35 different *C. annuum* lines were sequenced to identify 92 perfectly polymorphic SNPs (Du et al. 2019). F_5 population of 188 plants derived from AR1 (powdery mildew resistant) × TF68 (powdery mildew susceptible) was subjected to GBS, generating a total of 41,111 polymorphic SNP markers, of which a filtered set of 1,841 markers was further used for linkage map construction (Manivannan et al. 2021). A total of 66,750 high-quality SNPs with homogenous distribution among 12 chromosomes were identified using GBS in *Capsicum* spp. for the purpose of a diversity study (Lozada et al. 2021).

Other markers linked to resistance were identified in different studies viz. SCAR, SNPs and InDels that were tightly linked to the *PMR1* (Powdery mildew resistance) region on chromosome 4 (Lee et al. 2001; Jones et al. 2009; Rajesh and Madhukar 2018). The powdery mildew resistance locus, *PMR1*, was identified in the 4 Mbp region between two markers, CZ2_11628 and HRM4.1.6 in the pepper genome (Jo et al. 2017). GBS analysis revealed one SCAR and 5 SNP markers to be closely linked to *PMR1*. The comparative analysis of *C. baccatum* specific markers and SNP markers linked to *PMR1* locus revealed that the resistant variety 'VK515R' may have the alien resistance source from *C. baccatum*. In addition to *PMR1* on chromosome 4, QTL *Lt6.1* on chromosome 6 (Lefebvre et al. 2003) was reported to confer resistance against powdery mildew.

Several QTLs have been identified for peppers that resist *C. truncatum* and *C. gloeosporioides* using interspecific populations derived from varieties of *C. annuum* and *C. chinense* (Voorrips et al. 2004). Pepper accession PBC932 (*C. chinense*), PBC80 and PBC81 (*C. baccatum*) with resistance against *Colletotrichum* were used to introgress anthracnose resistance (Yoon et al. 2009). The PBC932 (*C. chinense*) showing resistance in green and mature fruits against *C. acutatum* is associated with

QTLs on the P5 chromosome (Sun et al. 2015). Two pepper populations—Bangchang (*C. annuum*) × PBC932 (*C. chinense*), and PBC80 (*C. baccatum*) × CA1316 (*C. baccatum*), were used for the identification of two and three major anthracnose resistance QTLs flanked by SNP markers on LG2 and LG4, respectively (Mahasuk et al. 2016). Two anthracnose resistant *C. annuum* introgression lines derived from PBC932 and PBC80 were crossed to a susceptible parent, and the resistance was found to be individually controlled by a major recessive gene. The resistance genes were selected by SCAR-InDel and SSR-HpmsE032 with a combined efficiency of 77% (Suwor et al. 2017).

Pvr4 locus provides resistance to PVY and PepMoV. Eight AFLP markers in an interval of 2.1 ± 0.8 to 13.8 ± 2.9 cM were mapped in pepper, followed by shortlisting of one co-dominant AFLP marker, with verified polymorphic sequence converted into CAPS marker, based on two related allele sequences (Caranta et al. 1999). A total of 78 *C. annuum* var. *annuum* L. genotypes were studied for gene effects for six generations, prior total genetic variability estimation with variance analysis of half-diallel crosses for over-dominance genes and their distribution along chromosomes. Progeny generations F_1, F_2, B_1 and B_2 outperformed for fruit traits, and were much better than parents P1 and P2. This direct application of screening and selection for significant gene pairs among diverse choices of breeding populations resulting from heterosis, backcrossing, multiple crossing and pedigree breeding greatly facilitates exploitation of desired gene effects and genetic components to develop new varieties (Marame et al. 2009).

A RAPD marker linked to *Pvr4* was transformed into a SCAR marker ($SCUBC19_{1432}$) to facilitate its use in developing PVY resistant pepper varieties (Arnedo-Andrés et al. 2002). Besides *Pn1*, *Pvr4* and *pvr5*, two more PVY resistance genes were identified at the *pvr2* locus in SCM334, a recessive gene *pvr8* and a codominant gene which expressed only in the absence of *Pvr4*. The genetic analysis revealed that the *pvr2*/*pvr5* locus for resistance to PVY and TEV in the pepper genome shares orthology with the *pot-1* gene for resistance to both potyviruses on chromosome 3 of tomato (Parrella et al. 2002). The SNPs in four different alleles of *pvr2* locus were detected using tetra-primer ARMS-PCR procedure to make them useful in breeding for potyvirus resistance (Rubio et al. 2008). Single gene resistance by $pvr2^3$ was defeated in a susceptible genetic background without the partial resistance QTL which suggested that polygenic host resistance will be more durable than monogenic resistance and should be favorably incorporated in breeding strategies (Palloix et al. 2009). EcoTILLING analysis of variability in the coding sequence (CDS) of *eIF4E* and *eIF/(iso)/4E* led to identification of five new mutants at the *pvr* locus—$pvr2^{10}$, $pvr2^{11}$, $pvr2^{12}$, $pvr2^{13}$ and $pvr2^{14}$ related to PVY resistance in *Capsicum* spp. (Ibiza et al. 2010).

QTL mapping in *Capsicum* also identified 84 RAPD and 51 RFLP markers on three linkage groups significantly associated with partial resistance to CMVY in *Capsicum* lines (Caranta et al. 1997). The population obtained from the cross between susceptible 'Maor' and resistant 'Perennial' varieties of *C. annuum* led to the identification of four QTLs governing CMV resistance, out of which *cmv11.1* was also

linked to the *L* locus for TMV resistance, indicating some association between CMV resistance and TMV susceptibility (Chaim et al. 2001).

A functional codominant marker—*PR-Bs3* was established, which allowed the identification of bacterial spot resistance *Bs3* lines by detecting nucleotide polymorphism, and was thus considered to be useful for marker assisted selection of *Bs3* resistant lines in resistance breeding programs (Römer et al. 2010).

Chili genotypes that were resistant to *Fusarium* were screened using RAPD markers to identify mutants in M_2 and M_3 generation that included P3 T1 1–26, P3 T2 1–26 and P3 T3 1–14 (Tembhurne et al. 2017).

3.6.3 Gene Pyramiding

Gene pyramiding involves the aggregation of related alleles governing the same trait from multiple parental lines. Gene pyramiding has been an effective tool to aggregate multiple alleles or complete QTLs for traits of interest in *Capsicum.* Availability of quality genetic maps enriched with multiple markers including classical and next generation markers can be an integrated approach for improved Genomic selection (GS) and pyramiding of resistance traits in *Capsicum.* Additionally, gene pyramiding offers the recycling of the broken genes as well as introduction of new alleles.

In case of breeding for resistance traits, it is desirable to have broad spectrum resistance against all subsequent mutations of the pathogen, which on the plant's side is mostly governed by the effective recognition by the resistance complexes. Gene pyramiding can thus assist to have multiple allelic variants of all major or minor associated QTLs for increasing the range of response. Gene pyramiding has also been a useful approach to introduce multiple gene pairs in a single breeding cycle. It has thus helped to develop several resistant phenotypes with great yield and quality traits. Prior characterization of interactions among alleles and genes of many polygenic as well as traits with complex linkage patterns is helpful to achieve successful screening and selection with carefully designed markers.

Tamisier et al. (2020) observed natural gene pyramiding in *C. annuum* against PVY accumulation at systemic levels. By using 10,307 SNPs, generated from GBS (256 genotypes), GWAS was performed and crucial observations were made on resistance alleles found at different loci stacking up together with unlikely frequency indicating pyramiding events.

A marker assisted backcrossing (MABC) scheme was proposed for introgression of new traits into elite lines by using 412 evenly spread locus specific SNP markers in *Capsicum* on a diversity panel of 27 accessions. The SNP markers were able to clearly distinguish each accession suggesting that these SNP loci will be useful for MABC, genetic mapping and comparative genome analysis in *Capsicum* spp.

3.7 Association Mapping Studies

Linkage disequilibrium (LD) is an important measure to understand genetic variability of the population. Confounded by the inherent limitations of size of the experimental populations and captured allelic diversity which tend to be really limited in terms of power of resolution, LD mapping is an improved and scalable direct sampling approach from the populations. Association mapping helps to identify significant marker-trait associations by exploiting the naturally present high genetic diversity of the population under equilibrium state compared to observed disequilibrium (Flint-Garcia et al. 2003; Myles et al. 2009). A wide range of resistance genes or factors are highly conserved as well as distributed across the Solanaceae, one such locus *P11* showing linkage to *L* locus confers resistance to TMV (Lefebvre et al. 1995; Thabuis et al. 2003).

In contrast to linkage mapping based approaches, association mapping directly probes the available polymorphisms, and the simple marker-trait associations give important insights to effectively target polygenic traits, and thus a limited representative set of makers is sufficient to select relevant traits.

Under ideal conditions, linkage operates proportionately to physical distance on chromosomes, which has been the basis of many assumptions in genetics. While in practice, linkage never exists in equilibrium state, hence called LD. It is an important metric to study the composition of populations and individual members, reflected as arbitrary grouping sharing common allele frequency profiles, called haplotypes. Genetic drift, mating system, high levels of selfing and selection history are important factors influencing the LD in plants (Flint-Garcia et al. 2003).

Transcriptome sequencing of progenies of *C. annuum* cv. YCM344 (*P. capsici,* resistant) and Teaen (*P. capsici,* susceptible), labeled as TF68, revealed many polymorphic linked loci, and 7 resistance related genes were identified by putative locus SLch11. A total of 1,500 high confidence SNPs, validated against NCBI dbEST (ID: 23,667) were also identified (Lu et al. 2011).

Sharp differences in the distribution patterns of crossover points were observed for *L3* locus in two mapping populations, viz. NK and YB. NK reflected a selfed F_1 of an intraspecific cross between two *C. annuum* genotypes (KOS and NDN), while YB reflected an interspecific cross of *C. chinense* (PI159236) and *C. frutescens* (LS1838-2–4); a high discrepancy among the number of recombinants among NK and YB, for respective markers suggested a presence of strong LD (Tomita et al. 2008).

Fruit mass gene in tomato, encoding for ortholog KLUH, SIKLUH, a P450 enzyme of CYP78A subfamily, regulates enlarged pericarp and septum tissue size by increasing cell numbers. Role of SIKLUH is also ascertained in plant architecture traits, such as side shoots, and ripening time. Down-regulation of SIKLUH dramatically reduces fruit mass. Association mapping has been successfully applied to find a polymorphic SNP locus in the promoter of the fruit mass gene, indicating an important regulatory mutation. This association has been observed in *C. annuum*, emphasizing on the idea of fruit mass gene orthologs to be generated in an independent domestication event (Chakrabarti et al. 2013). An association was also found

among six promoter region SNPs of the *Pun1* gene among *Pun1, CCR, KAS and HCT* with capsaicin metabolite levels. Candidate gene *Pun1* can therefore be an effective design target for resistance breeding.

3.7.1 Genomewide LD Studies

Covering a physical distance of 2,265.9 Mb from the 3.48-Gb hot-pepper genome, SSR markers were used to model population structure and LD of *C. annuum* cultivars. Five population clusters were identified and cross-confirmed by diversity analysis based on SSR dataset covering the hot pepper genome. Seventeen LD blocks were characterized across chromosomes with spans ranging from 0.154 Kb to 126.8 Mb. Significant association of *CAMS-142* was reported with capsaicin (CA) and dihydrocapsaicin (DCA) levels. A fairly large LD (98.18 Mb) encasing the *CAMS-142* gene was observed, with alleles of 244, 268, 283 and 326 bp. Among all, alleles with band sizes of 268 and 283 bp were found to have positive effects on CA (R^2 = 12.5%) and DCA (R^2 = 12.3%) levels. Eight markers across seven chromosomes were also shown to be significantly associated with fruit weight, with three major QTLs, CAMS-199 (chromosome 8), HpmsE082 (chromosome 9) and CAMS-190 (chromosome 10) from data across two years (Nimmakayala et al. 2014).

Population structure was characterized by utilizing the 36,621 polymorphic SNPs for *C. annuum* and *C. baccatum*. A population bottleneck was identified among both populations based on the estimated mean nucleotide diversity (π) and Tajima's D, observed as a biased distribution towards negative values across all but chromosome 4 in *C. baccatum*, while for *C. annuum* the same measures showed a bias towards positive values except chromosome 8, indicating that domestication events at multiple sites have contributed to its wider genetic base (Nimmakayala et al. 2016).

It was noted that selection for different goals within domesticated *C. annuum* types might have fragmented the genetic diversity into narrow pools (Pickersgill 1997). Despite the great economic and cultural importance of *C. annuum*, the population structure of worldwide collections is little known (Aguilar-Meléndez et al. 2009).

3.7.2 Future Potential for the Application of Association Studies

High-throughput genotyping and low-cost marker generation with the help of modern sequencing-based technologies have enabled genomewide association studies (GWAS) in plants. Novel genes and alleles identified with GWAS greatly facilitate modern crop breeding for pathogen resistant and climate resilient traits. One common inference from above-mentioned studies can be derived as, choice of and number of markers (SSRs, AFLP, RFLPs) heavily influence scope of final outcome,

classical markers prepared by laborious screening processes present an inherent limitation of scalability. Modern NGS-based marker development has greatly accelerated the population level marker-trait association studies.

3.8 Genomics-Aided Breeding for Resistance Traits

Intensified crop production and better stress management is the new realization for crop breeders owing to rapidly evolving priorities of feeding a massive human population in the coming years. An integrated and combinatorial approach using modern OMICS tools such as genomics, transcriptomics, proteomics, and metabolomics have proven to be effective. Successful genomic designing of crops revolves around two fundamental aspects, (1) identify and discover available resources such as diversity and novel alleles in the population; (2) maximize the efficiency of breeders with information, and scalable modern technologies. Recent genomic scale approaches have shifted the focus on the second aspect, which was severely lagging behind since decades. Now with novel resources such as high-density genomic scale maps, whole genome sequences, annotated resources and data services, and modern tools to scale up the data, analysis capabilities have greatly enabled genomic designing in crops. Low-cost GBS led marker development has not only accelerated the discovery process but scaled it towards whole population. Pangenomic scale experimental planning has enabled discovery of novel alleles from large populations, while the genomewide association studies have helped in providing an unparalleled support to stress tolerant crops breeding (Scheben et al. 2017).

Transcriptomics

Underlying mechanisms of biological processes, excluding the regular housekeeping processes, are mostly condition-specific such as growth stages, stress response, response against external inputs such as pesticides, and resistance responses which all are very contrasting with mean housekeeping expression. Transcriptome analysis enables the study of expression differences in a robust way to understand such phenomena, in an empirical manner (Ashrafi et al. 2012). These techniques employ absolute/relative quantification of RNA present in the sample, primarily by means of hybridization e.g., microarrays or by a variety of sequencing techniques which later on can be compared by simple counts e.g., RNAseq.

The merits of RNA sequencing of whole transcriptomes using next-generation-sequencing (NGS) approaches have been emphasized enabling coverage of all expressed transcripts, without any prior knowledge of any sequence information (Wang et al. 2009). RNA-seq has been effectively extended to capture quantitative as well as qualitative expression of almost all kinds of RNA species observed in a cell, such as mRNAs, miRNAs, LncRNAs, and small interfering RNAs (Marioni et al. 2008). Recently, isolates of *Bacillus spp.* LBF-01 in pepper indicated resistance against *F. oxysporum* (Silvar et al. 2009). Besides quantitative profiling in temporal and spatial dimensions, across various developmental stages, ecological

influences, treatments and tissues, RNA-seq is also very helpful to identify intron–exon structures, full transcripts diversity and annotation of structural as well as functional features of genomes. These insights are directly useful in genome annotation and refinement of gene definitions as well as variant identification and marker development.

Genotyping By Sequencing

NGS-based approaches also allow marker generation, and multiple in silico prior quality checks on polymorphisms and potency of markers can be applied on such datasets. Classical markers are however based on random probing of genomic locations and are characterized to be useful only after showing some linkage to recognizable traits, but coverage cannot be assured to be homogeneous across the whole genome, while the costly and labour-intensive nature can be excused however in modern age of lab automation. Sequencing based marker development and genotyping allows surpassing many abovementioned limitations of classical markers, such as RFLP, AFLP, ISSR and SSR etc., by allowing more targeted marker development with good reproducibility and very high coverage. Genotyping by such markers enables full population scale mining of genomic patterns such as linkage, LD and most consistent haplotypes, at very low cost but with high accuracy.

Sequencing Based Trait Mapping

Studying and identifying trait introgression is an immensely useful approach to understand complex linkage behaviour of various alleles, while in practice designing effective markers using traditional approaches was an important bottleneck. Further, it suffered a huge reproducibility problem and overall number of candidate genes identified was also very less. Data repositories providing sequencing information such as reference genomes, BAC sequences, ESTs and RNA-seq data, serve as a valuable resource to design and refine high-density linkage maps, and after sufficient coverage these maps can also lead to candidate gene identification in *Capsicum.* However, GBS platforms have furthered these studies in terms of vast scale and reproducibility.

3.8.1 Genome Sequencing in Capsicum *Spp.*

Capsicum species have around nine genome assemblies available as of now, covering species such as *C. annuum*, *C. chinense* and *C. baccatum.* Early sequencing efforts in *Capsicum* were focused on assembling a reference quality genome, hence critical attention was paid towards quality control using the short-read sequencing datasets, cross-verified with BAC libraries with at least 99% match. However, since *Capsicum* spp. are too diverse, no single reference could qualify as best representative even when representing the same genera of plants under study. *Capsicum* genome is four-fold in terms of size, compared to its near relative members from the Solanaceae (tomato).

Majority of plants in Solanaceae share the same number of chromosomes (n = 12), yet considerably differ in size.

Early sequenced genotypes belonged to *C. annuum.* A Mexican landrace Criollo de Morelos 334 (CM334), characterized for its *Phytophthora* spp. resistance properties, was sequenced at 186.6X coverage (650.2 Gb), with an effective genome size estimated to be of 3.4 GB (based on 19-mer analysis), of which 80% region consisted of repetitive sequences, yet a fair number of genes (~35,000) were mapped in the first draft alone. Sequenced reads (GAIIx and HiSeq2000) were subjected to filtering and only good matches (identity >98%, coverage >50%) were used for assembly, discarding all low-quality reads, as well as potential duplications, along the pipeline. Assembled reads were anchored to genetic maps generated for this purpose exclusively. RILs from a cross between *C. annuum* cv. Perennial and *C. annuum* cv. Dempsey were used to generate high-density linkage and physical maps (Kim et al. 2014).

After a short interval, *C. annuum* cv. Zunla-1 and its progenitor and wild relative Chiltepin (*C. annuum* var. *glabrisculum*) were also sequenced. Zunla-1 is an inbred line (F_9 generation), from a cross of two *C. annuum* cultivars from China, while Chiltepin belongs to North-central Mexican wild selection landrace. Zunla-1 was sequenced at 146.43X coverage (477.37 Gb; 6PE and 5MP libraries) and Chiltepin to a 96.37X coverage (295.85 Gb), using the Illumina genome analyser platform II (Qin et al. 2014).

Another genome assembly was published based on F_1 progeny of CM334 (hot pepper) and a non-pungent blocky pepper using Illumina HiSeq10 sequencer (Hulse-Kemp et al. 2018). A single "pseudohap" composed of 83,391 scaffold sequences for 3.21 GB size demarcated a reference assembly. With 123 KB (contig), 3.69 Mb (scaffolds) and 227.2 Mb (pseudo-molecules) average N50 lengths, a total of 83% data (~2.67 GB) was anchored to 12 chromosomes, with only 541 Mb of unplaced sequences.

Resequencing is often done for refinement or gap filling in early drafts, sometimes with assistance of better BAC libraries, and assemblies are improved or coverage is extended for poorly represented genomic regions. In some cases, newer and latest technologies with better accuracy or longer read length are employed to address the repeat regions. These projects have led to identification of many novel genes as well as helped to improve the understanding of evolutionary lineage in *Capsicum* with sequencing of another genome *C. baccatum* cv. PBC81, known for broad spectrum resistance against multiple fungal and bacterial pathogens. Publication of reference assembly of the *Capsicum* genome has led to many other genetic and genomic scale studies. Several aspects of Capsicum research have been influenced by the downstream exploration of the genome by characterizing the architectural and functional aspects. Genomic scale understanding of genetic variation and regulations has enabled study of many comparative and evolutionary interrelations among related crops from Solanaceae and the genus Capsicum itself. Table 3.8 summarizes the sequence assemblies of the pepper genomes.

Table 3.8 Details of sequencing projects completed for *Capsicum* accessions

Assembly	Prot	Genes	Unplaced	Scaffolds/N50 (Mb)	Size (Mbp)	Cultivar	References
C. annuum							
Zunla 1 Ref_{v1}.0; GCA000710875.1	45,410	36,784	4,589	6,478/1,483	2,935	Zunla-1	Qin et al. (2014)
UCD10Xv1.0; GCA002878395.2	–	–	–	81,378/227,195	3,212	CM334 x Blocky; F1	Hulse-Kemp et al. (2018)
ASM51225v2; GCA000512255.2	35,845	31,600	4,245	35,797/250,930	3,064	CM334	Kim et al. (2014)
SNU_{ECW1}.0; GCA011745845.1	36,937	36,937	–	44,080/385	2,880	ECW	SAMN12612368 (NCBI)
SNU_{SF1}.0; GCA011745865.1	36,854	36,854	–	44,704/418	2,882	SF	SAMN12612367 (NCBI)
Chiltepin Ref_{v1}.0; GCA000950795.1	–	–	–	16,998/919	2,768	Chiltepin	Qin et al. (2014)
C. baccatum							
ASM227188v2; GCA002271885.2	35,853	29,592	6,261	23,261/58	3,216	PBC81	Kim et al. (2017a)
C. chinense							
ASM227189v2; GCA002271895.2	34,974	31,592	3,382	87,978/103	3,071	PI159236	Kim et al. (2017b)

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

Transcriptomes and Gene Discovery for Biotic stresses

Plants respond in a variety of manners when exposed to a biotic stress. An understanding of these responses by genomewide expression studies opens up a new and holistic outlook of the underlying processes. Stressed versus non-stress conditions when compared in terms of differentially expressed transcripts provide a fair understanding of the ongoing interactions based on principles of guilt by association. Those involved in the common processes are supposed to reflect common expression profiles. This sort of profiling helps to identify behavior in stressed vs. normal or controlled conditions. Functional genomics approaches are an important resource to identify and understand disease resistance mechanisms and to design successful breeding programs.

Earlier studies based on functional genomics and expression analysis of *Capsicum* have relied on microarrays. To elucidate the defense mechanisms in hot pepper (*C. annuum*), a total of 8,525 expressed sequence tags (ESTs) were generated for an in silico expression study (Lee et al. 2004). A total of 613 hot pepper genes were found to be responsive to non-host soybean pustule pathogen *Xanthomonas axonopodis* pv. *glycines* (*Xag*). Early infection of *Xag*, induced functional genes involved in cell wall modification/biosynthesis, transport, signalling pathways and many other diverse defense reactions, and revealed a clear contrast of expression of chloroplast biogenesis proteins, photosynthesis and carbohydrate metabolism genes to be downregulated in later stages of *Xag* infection. The expression profiles corroborated with almost similar profiles which are displayed when *Capsicum* suffers fungal, wounding, cold, drought and high salinity stresses. The authors also elucidated the role of gibberellin deactivation as a defense reaction in hot peppers.

Non-host resistance sources are also an important reservoir of knowledge to understand defense mechanisms (Lee et al. 2016). Microarray analysis also helped to identify the molecular mechanisms for induction of *cytosolic pyruvate kinase 1* (*CaPK(c)1*) gene after inoculation by TMV in *C. annuum.* Inoculated leaves of *C. annuum* cv. Bugang with TMV-P (0) showed upregulated response for HR genes. The expression of the cloned *CaPK(c)1* gene was also reported to increase, specifically in the incompatible interaction with TMV-P(0). *CaPK(c)1* also showed triggered response to hormones such as salicylic acid (SA), ethylene, methyl jasmonate (MeJA), and also to NaCl and wounding, indicating a role of (*CaPK(c)1*) as defense response under various TMV infection and many abiotic stresses (Kim et al. 2006b). The TMV resistance locus *L* in pepper is homologous to I2 in tomato in the *R*-like gene cluster region on chromosome 11 (Grube et al. 2000b). A WRKY transcription factor CaWRKYb is involved in positive regulation of immune response to TMV-P_0 pathotype infection by binding to the CaPR-10 promoter (Lim et al. 2011). Another transcription factor CaWRKYd was found to bind to the W-box containing promoters of *PR* genes and causes HR mediated cell death during TMV-P_0 infection (Huh et al. 2012a). *Capsicum annuum* basic transcription factor 3 (CaBtf3) also regulates the

expression of *PR* related genes during hypersensitive response upon TMV infection in *C. annuum* (Huh et al. 2012b). In high temperature conditions, the antiviral immune response in *C. annuum* is conferred via specific vsiRNAs based on RNA-i mediated resistance (Kim et al. 2021).

In another study, *C. annuum* cv. Bukang, inoculated with *X. axonopodis* pv. *glycines* 8ra showed increased expression of *C. annuum* cytochrome P450 (*CaCYP1*). Expression of *CaCYP1* has earlier been observed to increase under salicylic acid (SA) and abscisic acid responses; however, the authors established the role of *CaCYP1* under non-host defense response also, which was confirmed by gene silencing studies. The silencing of *CaCYP1* under the same inoculation results in a down-expression of defense-related genes such as *CaLTP1*, *CaSIG4* and *Cadhn* (Kim et al. 2006b). The transcriptomic profiling of the susceptible (IVPBC535) and resistant (BS-35) pepper varieties led to the identification of 234 genes that were upregulated during TYLCV resistance (Rai et al. 2016).

Pepper hypersensitive induced reaction protein gene (*CaHIR1*) is proposed to be a positive regulator of cell death in plants and has been functionally associated with non-specific basal disease response against multiple pathogens. *CaHIR1* was verified for involvement in defense response against *Pseudomonas syringae*, *Hyaloperonospora parasitica* and *B. cineria* as well as osmotic stress. Genomewide comparative expression profiling revealed 400 differentially expressed proteins, and 11 of them directly mapped to many key metabolic pathways (Jung et al. 2008).

Bacterial TALE proteins (*Xanthomonas* spp.) bind with host plant susceptibility genes to induce diseases, and many of the plant defense mechanisms revolve around the recognition of TALE and with the help of TALE binding sites often found in upstream regions of resistance (*R*) genes. They also comprise a hallmark expression pattern, with expression only invoked under the specific TALE binding events. RNA-seq based transcriptome profiling has been used to identify a candidate of *BS4C*, a resistance gene from peppers mediating the recognition of *Xanthomonas* TALE protein AvrBs4. RNA-seq was also effectively used to identify the major *Bs4C* transcripts and it's uniquely encoding *R* genes (Strauss et al. 2012). Negative regulation of *bcbrn1* and *bcpks13*, which encode polyketide synthase and tetrahydroxynaphthlane (THN) in *B. cinerea* can be utilized for regulating the overall virulence and melanization.

Virus induced gene silencing experiments with *Mildew Resistance Locus O* (*MLO*) established a new functional role for the loss of function of *CaMLO2* gene in *C. annuum*, which is transcriptionally induced in response to *X. campestris* pv. *vesicatoria* and salicylic acid. It is a membrane bound amphiphilic Ca^{2+}-dependent calmodulin binding protein known to accelerate cell-death and rapid bacterial growth, however, silenced allele conferred increased resistance by disrupting the downstream communications in pepper and Arabidopsis (Kim and Hwang 2012).

Disease Resistance

Resistance against a variety of plant pathogens and insect pests is among the major objectives of crop improvement. Constant exploration of sources of diversity against pathogen resistance is very useful to achieve durable resistance. Pathogens on

the other hand are also constantly under evolution towards having increased virulence. Therefore, for a future ready and successful breeding program, knowledge of available genetic variation in germplasm for resistance, evolutionary potential of pathogens, and a comprehensive application of modern methods are required. A large number of pathogens are known to impart biotic stresses in *Capsicum* plants by means of a variety of damages and cause quality loss impacting global productions.

A short-read genome assembly of *L. taurica* detected up to 92,881 transposable elements covering 55.5 Mbp from the total sequenced 187.2 Mbp assembly from a sweet pepper (*C. annuum*) in Hungary, and predicted the occurrence of 19,751 protein coding gene models (Kusch et al. 2020). Genomes of some species of *Colletotrichum* were comparatively sequenced to detect a class of pathogenesis related genes that affect chili (Rao and Nandineni 2017). A compendium of genomic resources is now available for several species in different stages of pathogenicity (Weir et al. 2012; Baroncelli et al. 2014; Zampounis et al. 2016).

Effectors like FAD oxidases, subtilisins, pectin lyases, metabolic enzymes like carbohydrate-active enzyme (CAZyme) family of pectinases and cutinases along with several proteases were key factors associated with *Colletotrichum* infection (Baroncelli et al. 2016). Many of the genes expressed under *Colletotrichum* infection are usually chemically induced, defense responsive, pathogenesis related proteins and transcription factors that relay signaling transduction to induce systemic acquired resistance. An expression analysis by qRT-PCR under infection in Bhut jolokia demonstrated the accumulation of jasmonic acid and ethylene responsive genes (Mishra et al. 2017). Expression of genes—*Lipoxygenase 3* (*Lox3*), *Allene oxide synthase* (*AOS*), *Plant defensins 1.2* (*PDF 1.2*) for JA biosynthesis, and *ACC synthase 2* (*ACS2*) for ethylene biosynthesis were associated with *C. truncatum.* Transcription factors, *WRKY33*, *CaMYB*, *CaNAC* and *bZIP10* were upregulated in response to *C. truncatum* infection (Mishra et al. 2017). With regard to mitigation, melatonin has been shown to increase transcription of *CcChiIII2* chitinase genes and confer resistance against anthracnose (Ali et al. 2021). Extracts of the common tropical plants *Eupatorium odoratum* L. also inhibit anthracnose and are shown to be more effective than synthetic biofungicides (Indrawati 2021). Antimicrobial peptides (AMPs) from pepper accession UENF1381 inhibit trypsin and amylase activity and significantly reduce the growth of *C. scovellei* (da Silva Pereira et al. 2021). Recently, 79 C_2H_2 Zinc Finger transcription factors were identified in *C. annuum* out of which 18 of them were differentially expressed in response to *C. truncatum* infection (Sharma et al. 2021).

A loss of function mutation in *SlMlo1* was reported in tomato to confer resistance against *Oidium neolycopersici,* another powdery mildew causing pathogen. The investigation was extended to study *C. annuum*, *CaMlo1* and *CaMlo2* genes which were isolated by a homology based cloning approach to study their relationship with *L. taurica* infection. Both *CaMlo1* and *CaMlo2* played a role in susceptibility of the plant when infected with the pathogen though *CaMlo2* was phylogenetically more related to *SlMlo2*, and overexpression of *Mlo* restored the susceptibility of the plant (Zheng et al. 2013b).

Increasing the disease resistance by a modified promoter pCaD has also been explored. Sesquiterpene phytoalexin capsidiol (produced as defense response to fungal pathogen attack) is catalyzed by two final-step enzymes—a sesquiterpene cyclase (EAS) and a hydroxylase (EAH), which are genetically linked and present in head-to-head orientation in the genome, and are governed by a common bidirectional promoter pCAD in *C. annuum.* Promoter deletion analysis showed that the 226 bp of the adjacent promoter region of EAS and GCC-box in EAH orientation were determined as critical regulatory elements for the induction of each gene (In et al. 2020). Pepper shows local resistance against *Botrytis* infection in response to wounding, but manifests systemic susceptibility (García et al. 2015). This was proved using inhibitors of hormonal regulators at the cotyledonary stage of the plant where differential expression of plant defense genes *CaBPR1* and *CaSC1* were observed locally but reduced systematically (García et al. 2015).

Pepper plants infected with *Botrytis* have reduced floral anthesis and the flowers drop automatically with increased inoculation (Le et al. 2013). The production of ethylene promotes the growth of *Botrytis,* and changes in cell wall composition reflected by polygalacturonase activity are associated with infection (Rha et al. 2001). The leaves of *C. annuum* form free radicals at positions remote from the site of infections (Muckenschnabel et al. 2001). Some cultivars of pepper grown in Egypt upon treatment with BC-3 isolate displayed both tolerance and susceptibility correspondingly; in turn upregulating defense related enzymes *PPO*, *POD* and *PAL* in response to salicylic acid, methyl jasmonate, abscisic acid and calcium chloride treatment (Kamara et al. 2016). Some extracts of *F. oxysporum* have also been shown to reduce the infection rate of *Botrytis* in peppers (de Lamo and Takken 2020). SAK1, a Stress-Activated Mitogen-Activated Protein Kinase is involved in vegetative differentiation and pathogenicity in response to *B. cinerea* infection (Segmüller et al. 2007). In *Arabidopsis,* the membrane anchored *BOTRYTIS-INDUCED KINASE1* (*BIK1*) plays a distinct role in resistance to necrotrophic and biotrophic pathogens and could also be reflected in *Capsicum* (Veronese et al. 2006).

Role of PdeR transcription factor in virulence of *B. cinerea* has been established by comparing expressions of deleted and complement strains of *B. cinerea.* Deleted strain showed impaired polysaccharide hydrolysis by reducing amylase and cellulase expression. Fungus grows normally yet without surface penetration in case of the deletion strain (Han et al. 2020). Vanillyl nonaoate (VNT) treatment imparts a systemic resistance to *B. cinerea*, both symptoms and colonization of pathogen are reduced via induction of two pathogenesis-related and another phytoalexin biosynthesis gene, and increased lignification via peroxidase gene's hyperexpression (García et al. 2018).

No genes for resistance to *Stemphylium* have been reported, but, a single dominant resistance gene *Sm* locus located on chromosome 11 in tomato has been mapped and reported to be responsible for conferring resistance to *S. lycopersici* (Su et al. 2019). The *Capsicum* pectin methylesterase inhibitor protein *CaPMEI1* provides basal disease resistance to pathogens including *P. syringae* pv. *tomato* (An et al. 2008).

Peppers infected with *C. coccodes* among other pathogens showed increased transcription predominantly in the phloem areas of vascular bundles in the stems and fruits (Cannon et al. 2012). *C. coccodes* was first reported causing chili anthracnose in India (Sharma et al. 2011). *CaChi2*, a pepper basic class II chitinase gene is constitutively expressed in leaf, stem, fruit and root endodermis of peppers infected with *C. coccodes* (Hong and Hwang 2002).

MLO, primarily associated with powdery mildew susceptibility in plants is also known to be a positive regulator in response to high temperature and high humidity but negatively regulates *R. solanacearum* infection led damages, partially moderated by CaWRKY40 (Yang et al. 2021). A novel MYB transcription factor *CaPHL8* provided clues about evolution of pepper immunity against soil borne pathogens. *C. annuum HsfB2a* positively regulates the response to *R. solanacearum* infection or high temperature and high humidity forming transcriptional cascade with *CaWRKY6* and *CaWRKY40*. Three receptor-like proteins CaRLP264, CaRLP277 and CaRLP351 in *C. annuum* provide broad spectrum resistance to multiple biotic stresses like viruses and bacteria including *R. solanacearum* (Kang et al. 2021).

Multiple breeding programs for developing pepper varieties resistant to viruses have been undertaken and genes from resistant varieties have been introduced into commercial varieties. The *pvr1* locus in *Capsicum* lines is responsible for viral infection and susceptibility via complex interaction between elF4E and VPg. This locus has been used in breeding programs for more than 60 years for broad spectrum resistance to potyviruses including TEV. Two recessive alleles of the *pvr1* locus—*pvr11* and $pvr1^2$ with narrow resistance spectra were identified in *Capsicum* that encode elF4E homologs that failed to bind to the VPg and therefore resulted in resistance and reduced susceptibility (Kang et al. 2005).

Highly polymorphic and closely linked markers have assisted in the selection of resistance traits in pepper varieties. One of them led to the development of a superior pepper line resistant to three viruses-PVY, TSWV and PMMoV using molecular markers linked to *Pvr4*, *Tsw* and L^4 locus (Özkaynak et al. 2014). The markers associated with *Tsw*, L^4 and *Pvr4* genes have been assessed for useful selection of resistant *Capsicum* genotypes (Dato et al. 2015). *Capsicum* accessions have also been field tested for their resistance to viruses, for instance, five *Capsicum* accessions showed resistance to CMV-Y but were susceptible to TSWV (Suzuki et al. 2003). A detailed pepper linkage map located the three disease resistance loci—L, *pvr2* and *pvr4* using linked markers (Lefebvre et al. 2002). The survival mechanisms for plant viruses have been laid down in several studies. Incidences of transmission of CMV and PMMoV via contaminated soil with debris of previous crops have been reported in *Capsicum* plants grown in glasshouse conditions (Pares and Gunn 1989). Five NBS-LRR resistance gene analogues (RGAs) were characterized in a pepper multiple disease resistant variety 'IHR 2451' that provided helpful insights into the identification of other resistance genes for marker assisted breeding in pepper plants (Naresh et al. 2017). The evolutionary phenomenon of gene duplication and divergence has led to the emergence of a plethora of resistance genes in plant immune response that though sharing a common ancestral origin and high sequence similarity, differ in the effector viral targets and functional specificity (Kim et al. 2017a, b). Often

wild *Capsicum* varieties carry lower viral diversity than the commercial varieties under natural conditions, and are a potential resource for resistance genes (Vélez-Olmedo et al. 2021).

Three TSWV resistant lines belonging to *C. chinense*—PI 159236, PI 152225 and AVRDC C00943 showing concentric local necrosis were earlier identified (Black 1991; Black et al. 1996). A single dominant gene located at the *Tsw* locus that provides resistance to TSWV was identified using segregation and allelism studies in *C. chinense* accessions 'PI 159236', 'PI 152225' and 'Panca' (Boiteux and de Ávila 1994; Boiteux 1995). The *Tsw* gene codes for a *NB-LRR* (Nucleotide binding and leucine rich repeats) gene on chromosome 10 of the pepper genome for which the non-structural (NS) proteins encoded by S-RNA of the TSWV are the effector molecules. The resistance hypersensitive response was characterized by local necrotic lesions and premature leaf abscission in other *C. chinense* accessions (Moury et al. 1997). However, high temperatures and the heterozygosity at the *Tsw* locus increase the chances of systemic symptoms and decrease the resistance in the plants (Moury et al. 1998). The corresponding locus in tomato—*Sr-5* shares phenotypic and genetic similarity with *Tsw* in pepper, however, the genome segments responsible for overcoming *Tsw* and *Sr-5* resistance are different in TSWV (Grube et al. 2000b). When 29 *Capsicum* accessions were tested for TSWV resistance, a *C. chinense* accession ECU-973 showed 100% resistance upon inoculation and vector transmission (Cebolla-Cornejo et al. 2003). Often there is sympatric occurrence of TSWV, GRSV and TCSV due to common routes and concurrent introduction of these three viruses in peppers as reported in South Florida (Webster et al. 2011). However, the *Tsw* resistance is only effective against TSWV isolates and not against other tospoviruses (Boiteux 1995). A unique resistance gene at the *Tsw* locus was identified in *C. chinense* resistant variety, AC09-207, that showed highly different immune responses from the previously identified resistant varieties, PI152225, PI159236 and PI159234 (Hoang et al. 2013). A *C. baccatum* variety, PIM26-1 showed a similar level of resistance and very high tolerance to TSWV resistance breaking isolates as compared to PI159236 (Soler et al. 2015).

At the same time, resistance-breaking pathotypes of TSWV were isolated from a few *C. chinense* lines with systemic necrotic symptoms which posed fresh challenges for *Capsicum* breeding. Three resistance breaking isolates—TSWV-LE, TSWV-YN18 and TSWV-YN53 caused systemic necrosis, ring spot and chlorotic mottling, respectively, and could suppress RNA silencing in the *C. chinense* accession PI152225 (Jiang et al. 2017). Sometimes, the resistance breaking and non-resistance breaking TSWV isolates showed a synergistic infection characterized by systemic necrosis, stunting and chlorosis in resistant pepper varieties (Aramburu et al. 2015). The phylogenetic analysis of resistance breaking strains of TSWV reported in Hungary revealed the closest similarity with the wild type and no common mutations in the NS effector proteins with those of other resistance breaking strains indicating separate evolution (Almási et al. 2016). Another TSWV strain, RB-TSWV-CA-P-1 was reported to break *Tsw* resistance and caused stunting and mottling in resistant and susceptible commercial sweet pepper varieties in California, USA (Macedo

et al. 2019). Recently, a *Tsw* resistance breaking strain TSWV-P1 was isolated from a commercial *C. annuum* variety in South Korea (Yoon et al. 2021).

Certain isolates of PMMoV like PMMV-I were able to break the resistance by the L^3 gene in *C. chinense* which is due to a single amino acid substitution in the coat protein gene (Berzal-Herranz et al. 1995). Point mutation and deletion studies in the replicase (REP) gene and pseudoknots in the 3' non-coding region (NCR) could determine the major pathogenicity domains of PMMoV (Yoon et al. 2006). Two amino acid substitutions in the PMMoV coat protein reversed the L^3 mediated resistance in *C. annuum* (Hamada et al. 2002). Similarly, two amino acid substitutions in the coat protein of PMMoV pathotype $P_{1,2,3,4}$ enabled overcoming L^4 resistance in *Capsicum* varieties (Genda et al. 2007). Further characterization of $P_{1,2,3,4}$ revealed severe mosaic symptoms associated with it and unique restriction cleavage sites for its differentiation from other *L* gene resistance breaking PMMoV isolates (Antignus et al. 2008). Two Korean isolates—S47 and J-76 of PMMoV produced mild symptoms in *C. annuum* whereas very severe symptoms in *Nicotiana benthamiana* (Han et al. 2017). There has been an expansion of *Tsw* and L^3 resistance breaking pepper TSWV and PMMoV isolates over the years. As much as the resistance breaking virus isolates raise an alarm for agriculturists, they also serve as models for plant-virus interaction and coevolution studies.

Mature plant resistance or age-related resistance has been a well adopted mechanism against viruses and was demonstrated in bell pepper plants in response to CMV (Garcia-Ruiz and Murphy 2001). Therefore, the resistance in plants that are infected at an early growth stage can easily be overcome by evolution of resistance breaking isolates. A more dangerous CMV pathotype Ca-P1-CMV is able to break the resistance of the P0-CMV resistant pepper cultivar variety (Lee et al. 2006).

Transcriptome profiling of CaCV inoculated susceptible and resistant bell *Capsicum* varieties revealed several differentially expressed genes that were either upregulated or downregulated such as *PR* genes like *PR1* and thionins, disease resistance genes (*Rg*) like *NB-LRR* and Coiled-coil at N-terminal (CNL) and secondary metabolism-related genes like *5-epi-aristolochene synthase* (*EAS*) (Gamage et al. 2016). Polyclonal antibodies against the recombinant nucleocapsid proteins of CaCV were produced in rabbits that could successfully detect natural and artificial CaCV infection (Haokip et al. 2018).

3.9 Recent Concepts and Strategies Developed

3.9.1 Gene Editing

Recent advancements in gene editing have enabled targeted site-specific modifications in genomic regions. Engineered or bacterial nucleases have extended this to almost every type of eukaryotic cell and across organisms. Direct gene editing has

accelerated designing more resilient and resistant crops for the future. Choice of suitable vector, transformation mediator and protocol standardization are very crucial aspects of any cloning or point editing exercise. Rigorous optimizations are often conducted to achieve optimal and replicable results. Many such vectors and protocols have been standardized in *Capsicum* for resistance loci as well and have shown good applications in molecular characterization of pathogenicity mechanisms of various pathogens.

Gene editing mediated via *Agrobacterium tumefaciens* has been utilized in *C. annuum* cv. CM334 and bell pepper cultivar Dempsey. Efficacy of multiple *A. tumefaciens* strains such as AGL1, EHA101, and GV3101 has been investigated by assessing the number of calli induced by each strain in both *Capsicum* cultivars. The sweet pepper cultivar Dempsey reported the highest number of calli with GV3101, while no difference was observed in case of CM344 for any strain. Diligent screening of transformed calli with phosphinothricin (PPT) to select CRISPR/Cas9 binary vector (pBAtC) was done prior to screening. Target locus *C. annuum MLO* gene (*CaMLO2*) showed consistent 1-bp deletion at primary indel region, however all other screened calli reflected different indel frequencies from transformed calli. Sensitivity levels of CM334 and Dempsey against *A. tumefaciens* mediated callus induction with pBAtC binary vector are different and carefully accounted while designing future gene editing experiments (Park et al. 2021).

Soil grown leaf—or callus-derived protoplast for *Capsicum* gene editing has been utilized in CM344 and Dempsey cultivars to screen efficient guide RNAs for CRISPR/Cas9 or CRISPR/Cas12a (Cpf1). Purified ribonucleoproteins (RNPs) and endonuclease mixed complexes of CRISPR/Cas9 or Cpf1 and single guide RNA targeted towards conserved *CaMLO2* locus were delivered (PEG-mediated) to *C. annuum* cvs. CM334 Dempsey. Differential editing was observed in both cultivars upon targeted deep sequencing, depending on the applied CRISPR/RNPs (Kim et al. 2020). Alteration in susceptibility gene *CaERF28* (anthracnose resistance) was performed through CRISPR/Cas9 mediation (Mishra et al. 2021).

3.9.2 Nanotechnology

Nanotechnology has been a powerful tool in recent years and many novel products have been developed with the help of nanomolecular transformations to already potent compounds. Usage and application of nanotechnology in crop research is an underexplored area. Many potential areas are emerging for nanomolecules in *Capsicum* research, apart from effective transformation potential by effective delivery of DNA into protoplast, increasing pharmaceutical availability (Choi et al. 2013), many other alternate areas such as new product creation out of many nutraceuticals from *Capsicum*, novel pesticides against a variety of pathogens, quality assessment of *Capsicum* produce for residues of harmful chemicals, heavy metal contamination detection (Gupta et al. 2021) and fruit quality assessment (Vidak et al. 2021). Nanotechnology has the potential to enhance the industrial application of

the *Capsicum* crop, improving its already diversified usage profile, which might not be directly involved into biotic stress resistance itself, but this secondary usage allows, a novel kind of breeding approach leading to targeted breeding for desired molecules, such as capsaicin.

Cobalt and nickel ferrite nanoparticles ($CoFe_2O_4$ and $NiFe_2O_4$) have been successfully tested as potential fungicides for antimycotic activity against *F. oxysporum*, *C. gloeosporioides* and *Dematophora necatrix* (Sharma et al. 2017). Another important application of lecithin nanoemulsion of Oleoresin Capsicum (OC) extract has been characterized as a potential food grade surfactant effective against *Escherichia coli* and *Staphylococcus aureus* (Akbas et al. 2019).

Bioactive selenium nanoparticles (SeNPs) of mycogenic origin from *Trichoderma atroviride* displayed excellent in vitro antifungal activity against *Pyricularia grisea* and inhibited infection of *C. capsici* and *A. solani* on chili and tomato leaves at concentrations of 50 and 100 ppm, respectively. Also, an aggregation and binding with zoospore of *P. infestans* was reported at 100 ppm (Joshi et al. 2019). *B. licheniformis* encapsulated in alginate-chitosan nanoparticle (CNPs) beads supplemented with rice starch demonstrated antifungal activity against *Sclerotium rolfsii,* and also reflected plant growth promoting and biocontrol properties in *C. annuum* (Panichikkal et al. 2021).

3.9.3 Gene Stacking

Gene stacking is the practical solution to the problem of not finding desired genetic diversity to select suitable parents. In such cases, a breeder has to look out for external sources of available allelic diversity to bring desired genes into close linkage so that subsequent crosses do not lose the desired gene. Though the term is frequently used to indicate transgenic compilation of desired genes into a single plant, classical backcrossing to introduce more parental genes is also a valid example of gene stacking. Molecular gene stacking or more generic version of it is called gene pyramiding when targeting multiple genes into a single plant. Many pathogenic responses have evolved in specific plants based on evolutionary exposure towards it, many times the best resource for resistance lies outside the gene pool of the host plant in such cases. Stress response is more often governed by highly polygenic traits, showing disproportionate linkage patterns, which are also cumbersome to map and inherit; marker-assisted selection is a good solution in such cases. A few examples are listed in Table 3.9.

3.10 Future Perspectives

Global demand for *Capsicum* production has been steadily rising owing to rising awareness of health and nutrition. Besides being an excellent source of important

Table 3.9 Transgenic genes introduced into *Capsicum* varieties

Source	Host	Gene of interest	Effective against	Method	References
Solanum bulbocastanum	*C. annuum*	Broad host resistance gene *RB*	*Phytophthora capsici*	*Agrobacterium tumefaciens* mediated transformation	Bagga et al. (2019)
A. thaliana	*C. annuum* L	*AtEDT1/HDG11* and *Cry2Aa2*	*Prodenia litura*	*Agrobacterium tumefaciens* mediated transformation	Zhu et al. (2015)

metabolites, *Capsicum* is also an important culinary enhancement to most of the global cuisine. Compounds from *Capsicum* are finding their importance in cosmetics as well as nutrient supplement industry which is a rising phenomenon.

Fungal stress at the seedling stage influences growth potential and eventually lowers the resistance barrier of the plant leading to multiple attacks and significant plant mortality as well, emphasizing the development of fungal stress tolerant genotypes. Sources of biotic stress resistance have been identified through rigorous screening of available germplasm of *Capsicum* spp. *C. baccatum* has been identified as a great source of resistance genes against various fungal as well as bacterial attacks. Identified loci have been successfully transferred as well as expressed in other related *Capsicum* members to confer similar or enhanced resistance. Major challenge for the *Capsicum* producing nations is to mitigate the rising demand for nutraceuticals by achieving inexpensive and sufficient quantity as well as quality. Classical breeding methods severely fall short to meet the rising expectations of the industry, and hence a major overhaul in production capacity as well as quality is only achievable through modern biotechnological as well as bioinformatics-based interventions. Changes as well as frequent exposure to climate extremes is likely to decrease major crop yields and will simultaneously affect all dimensions of crop production.

There is an alarming realization that conventional breeding methods do not account for a sufficient amount of genetic variation and are incompetent to address rising biotic stresses and to compensate for quality and yield losses. Immediate incorporation of superior biotic stress traits should be prioritized to address climate change and its effects on the pathogenicity of biotic stress causing organisms. Crop improvement programs incorporating highly diverse parents can help to design widely resistant *Capsicum* varieties for the majority of biotic stresses through identification and integration of resistance associated QTLs utilizing marker-assisted selection in genetically adapted backgrounds.

3.10.1 Potential for Expansion of Productivity

Enhancing the disease resistance of *Capsicum* genotypes towards the most common pathogens can be prioritized for immediate increase in production as a major share of total yield is wasted while in field and also due to post-harvest losses. Current rising trends of adoption of polyhouses with precision nutrient delivery systems have played a major role in ensuring quality in urban areas. However, selling prices are not that competitive to sustain a major share of *Capsicum* production in such facilities. Multi-pronged approach with a general focus on productivity as well as disease management can be realized with efficient and improvised use of agricultural inputs and methods. Quality seed availability of resistant cultivars and adoption of better crop and nutrient management, resource conservation and precision farming coupled with crop contingency planning can be adopted. *Capsicum* has heavy yield losses on field as well as post-harvest, hence its production as well as marketing is a challenging task to be handled by marginalized farmers.

Climate change has been an important factor in deciding overall production cost, and adoption of high-quality germplasm has the potential to curtail overall pathogen loads on the pepper crop. Furthermore, a large number of rare *Capsicum* spp. germplasm can be utilized for screening of both biotic and abiotic stress resistance and identification of important genes with great yield potential and response to nutrients dosage.

3.11 Conclusions

The past three decades have seen massive losses in crop production, yield and quality due to plant disease causing organisms. The wide use of naturally occurring resistance genes for the improvement of plant varieties have also triggered the emergence of resistance breaking pathogen isolates which urge the discovery of new resistance genes (Turina et al. 2016). Genetic recombination and the presence of satellite DNA molecules have led to increasingly new epidemics due to emergence of resistance-breaking new strains and new species, altogether, of viruses and other pathogens that may prove detrimental to food and agricultural production. Vector management strategies, like, growing plants in vector-free periods and covering plants with row covers have long been used as sustainable solutions to plant diseases, but, breeding for resistance has always been a priority. To control the crop losses caused by biotic stresses, there is a rapid need to identify and characterize the causative organisms via extensive genetic mapping, transcriptome analysis and expression profiling, understand their epidemiology and etiology, and to develop effective integrated and practical solutions. Detailed characterization of receptor molecules in vector organisms will promote strategies like transgenic expression of receptor blocking molecules in plant hosts to avoid pathogen transmission. RNA-interference mediated gene silencing of viruses has several advantages over traditional pesticides such as zero crop-residue, minimum off-target effects and lower chances of resistance (Nilon et al. 2021). Innovative eco-friendly methods and biocontrol strategies are therefore urgently needed for sustainable management of diseases in *Capsicum* spp.

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Chapter 4
Breeding and Genome Mapping for Resistance to Biotic Stress in Eggplant

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Abstract Eggplant (*Solanum melongena* L.) is a major vegetable crop widely grown in tropics and subtropics. However, eggplant production is subject to high losses from biotic and abiotic stress. Eggplant is exposed to a broad range of biotic stresses such as nematodes, wilt diseases, eggplant fruit and shoot borer, two-spotted spider mites, whitefly and aphids. These biotic challenges reduce significantly yields, fruit quality, shelf-life, and nutritional content in eggplants. Farmers mostly rely on pesticides to control biotic stress. Breeders from public and private sector have performed some efforts for the development of pest-resistant varieties. To date, some disease resistance genes have been utilized in commercial cultivars, but much less progress has been achieved for arthropods resistance. In this book chapter, we review the basic information of the crop, major different biotic stresses in eggplant, genetic resources of resistance, traditional and marker assisted breeding, molecular mapping and cloning of resistance genes, genomics-assisted breeding, as well as genetic engineering for resistance traits. Furthermore, brief accounts on social, political and regulatory issues and future perspectives for this crop are highlighted.

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4.1 Introduction

Eggplant (*Solanum melongena* L.) is an economically important vegetable crop worldwide, with a total production of more than 52 million tonsand net value of over $21.4 billion according to statistics from the Food and Agriculture Organization of the United Nation (FAOSTAT 2017). Asia alone produces more than 90% of the global production of eggplant. As of FAOSTAT (2017), China (32.8 mill tons), India (12.5 mill tons), Egypt (1.3 mill tons), Turkey (0.82 mill tons) and Iran (0.75 mill tons) are the five leading eggplant producing countries in the world.

Eggplant ($2n = 2x = 24$), also known as brinjal eggplant, is native to the Old World and was first domesticated over 4,000 years ago in South East Asia (Meyer et al. 2012). Two other cultivated eggplants (*S. aethiopicum* and *S. macrocarpon*), which are members of the nightshade (Solanaceae) family are also grown. Brinjal eggplant (*S. melongena*) is a well known species worldwide, but the scarlet eggplant (*S. aethiopicum* L.) and the gboma eggplants (*S. macrocarpon* L.), have local importance in tropical Africa (Daunay and Hazra 2012). Solanaceae family includes approximately 3,000 species distributed in 90 genera. *Solanum* is the largest genus, which includes around 1,500 species such as eggplant and other globally important crops like potato (*S. tuberosum* L.) or tomato (*S. lycopersicum* L.). *Solanum melongena* and *S.macrocarpon* belong to section *Melongena* (Lester and Daunay 2003; Lester et al. 2011), whereas *S. aethiopicum* belongs to section *Oliganthes* (Lester 1986).

Eggplant is a low-calorievegetable crop and contributes to a healthydiet of consumers. It is a very good source of dietary fiber (both soluble and insoluble) and considered amongst the healthiest vegetables for their high content in vitamins, minerals and bioactive constituents for human health (Taher et al. 2017). The phytonutrients found in eggplant fruits include various phenolic compounds, such caffeic and chlorogenic acid and flavonoids such as nasunin. The benefits attributed to these compounds include antioxidant and antimicrobial activity. In many African countries, leaves and roots of African eggplant are used as medicines for the treatment of several ailments such as high blood pressure; cure wounds, diabetes, or inflammatory tumours (Oboh et al. 2005).

Eggplant production is severely constrained by biotic and abiotic stresses in tropics and subtropics. The most common abiotic stress includes high and low temperatures, salinity, and drought, and biotic stress includes nematodes, wilt diseases caused mostly by *Ralstonia solanacearum*, *Fusarium oxysporum* f. sp. *melongenae*, *Verticillium dahliae*, and insect pests such as eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenée), whitefly (*Bemisia tabaci* Gennadius), two-spotted spider mites (*Tetranychus urticae* Koch), leafhopper (*Amrasca devastans* Distant), and aphids (*Aphis gossypii* Glover) (Taher et al. 2017). Cultivated eggplants are related to a large number of wild species which are rich sources of variation for breeding programs,

in particular for traits related to adaptation to climate change including disease and insect resistance (Rotino et al. 2014).

Little breeding efforts have been made in eggplant compared to other major Solanaceous crops such as tomato (*S. lycopersicum*), potato (*S. tuberosum*), and pepper (*Capsicum annuum*) (Daunay and Hazra 2012), mostly because production is overwhelmingly concentrated in developing countries where investments in breeding are often reduced. Current eggplant breeding programs focuses on development of F_1 hybrids with high-yield and fruit quality as well as resistance to abiotic and biotic stresses.

4.2 Description on Different Biotic Stresses

Eggplant is one of the most important vegetables in tropics and subtropics, but production is subject to high losses due todiseases and insect pests. In addition, biotic stress reduces fruit quality, shelf-life, and nutritional content of eggplant. Farmers often depend on pesticides to control diseases in the absence of resistant cultivars. Intensive pesticide use in eggplant poses health hazards to growers and their families, the environment, and consumers; as well as increases the cost of production, which makes this vegetable expensive for poor consumers (Ramasamy 2009).Resistant cultivars are among the cheapest, simplest, and most environmentally safe ways to manage diseases and insect pests. The major biotic stress include bacterial wilt, Fusarium wilt, Verticillium wilt, eggplant leaf spot, brinjal little leaf, eggplant fruit and shoot borer, spider mites, leafhopper and whitefly.

4.2.1 Bacterial Wilt

The disease is caused by *Ralstonia solanacearum* (Smith) Yabuuchi et al., which has many hosts in the nightshade family including tomato, pepper and potato. It is a soil-borne bacterium and infection takes place through roots and especially damaged root tissue. Infection may follow infected planting material, contaminated tools or irrigation water. High temperature increases the growth of the pathogen and therefore the bacterial wilt is a problem mainly in the tropics and subtropics. Symptoms are seen as wilting but then the roots and stems are already damaged and the losses can be fatal Genin et al. (2012). Cultural methods as crop rotation and hygiene measures are important, as chemical methods are not effective due to the location of the pathogen deep inside the xylem or the soil. Usingresistant cultivars and rootstocks for grafting has been a more reliable strategy, but requires access to such germplasm and a screening and breeding pipeline (Huet 2014; Keatinge et al. 2014). At the World Vegetable Center, accessions from eggplant and its wild relatives have been screened for resistance to bacterial wilt with promising results (AVRDC 1999; Namisy et al. 2019). Especially the wild relatives are interesting here, which

have beenconfirmed in several studies (see Genetic Resources Section for details). A challenge however is how to move from trait discovery to resistant cultivars, as it is hampered by polygenic inheritance and linkage drag with traits associated with the wild species (e.g., Boshou 2005).

4.2.2 Fusarium Wilt

The disease is caused by *Fusarium oxysporum* f. sp. *melongenae* (Fomg), which is a soil-borne pathogen that also is a problem in other Solanaceous crops. Symptoms are discoloration of leaves that later develops into wilting plants with severe stem and root damages. Cultivation methods with raised beds that promote soil water drainage may reduce the infection. The pathogen can survive as chlamydospores over time in soil and plant debris. Crop rotation with non-hosts will however reduce the population. Disinfection of equipments and removal of infected derbies is important hygiene measures if infection is found. Fungicide treatment is difficult to apply as the pathogen is located deep inside the root and stem. Fusarium wilt resistant varieties is a way forward and resistance genes have been identified in both cultivated and wild eggplants and a review of these sources is provided under the Genetic Resources Section in this chapter. Resistant rootstocks could be of great value, as grafted plants are presenting a good level of resistance.

4.2.3 Verticillium Wilt

The disease is caused by the fungus *Verticillium dahliae* Kleb., which has many host plants and can survive for several years as microsclerotia in the soil. Symptoms are curling leaves with discolouration, and early senescence and dieback of plants. Crop rotation with at least two years of non-host crops like wheat, corn and barley may help, as removing plant derbies and other hygiene measures like clean planting material. Soil fumigation or pre-plant fungicide treatments are methods used. Resistant varieties would be a good alternative, but so far, there are not many varieties released with proper resistance. Resistance sources have been identified but these are found in wild eggplant species, which means that a lot of breeding effort is needed. Grafting eggplants onto resistant tomato rootstocks to suppress the infection is an alternative tested (Liu et al. 2009).

4.2.4 Eggplant Leaf Spot

The disease is caused by *Pseudocercospora egenula* (Syd.) U. Braun & Crous, which is a fungus. Symptoms start as round yellow spots, as they grow larger, the shape

becomes irregular, merge and turn brown, and the older leaves die. Spores are spread by wind and rain splash. Locally, the disease can be a large problem and fungicides are applied. Some differences in tolerance among varieties arereported but the disease is not fully mapped (Liang et al. 2016; Vaghefi et al. 2016).

4.2.5 Brinjal Little Leaf

Brinjal little leaf is caused by a phytoplasma. Symptoms are small leaves that turn yellow and later the whole plant is affected, including fruit setting and yield causing considerable economic losses (Rao et al. 2010). Phytoplasma belonging to six groups, and from different parts of the world, have been reported to infect eggplant (Kumar et al. 2017; Kumari et al 2019). The pathogen is transmitted via leafhoppers. Other *Solanum* crops but also weeds may serve as host plants. To reduce the problem, vectors but also certainweeds should be controlled, but there are no direct chemical treatments of plant phytoplasmas. Tolerant varieties have been developed (Chakrabarti and Choudhury 1975) but not many publications on resistance screening and recent breeding are available.

4.2.6 Eggplant Fruit and Shoot Borer

Eggplant fruit and shoot borer is major pest on eggplant throughout the tropics in Asia and Africa. The larva feeds on the tender shoots, flower buds, flowers, and fruits. It also tunnels inside the shoot and feeds on the inner contents, resulting in wilting of young shoots, followed by drying and dropoff, which slows plant growth. In addition, the larva feeds inside the fruit and creates tunnels filled with frass and fecal pellets, which makes the fruit unmatketable and unfit for consumption. Farmers are mostly applying large quantities of insecticides to control the pest, which has resulted in the development of pesticide resistance in insects. Tolerance to this pest has been reported in some local varieties in India such as Pusa Purple Long, Pusa Purple Cluster, Pusa Purple Round, Aushey, Shyamla Dhepa, Banaras Long Purple, Arka Kesav, Arka Kusmakar, Punjab Barsati, Punjab Chamkila, Kalyanpur-2 and Gote-2 (Parker et al. 1995; Alam et al. 2003; Shivalingaswamy and Satpathy 2007).

4.2.7 Spider Mites

The two-spotted spider mite [*T. urticae* Koch (Acari: Tetranychidae)] is a very distructive pest in eggplant worldwide. This pest mostly prefers to live in colonies on the underside of leaves, and high temperatures and dry conditions are suitable for the multiplication and reprodution. Adults suck the chlorophyll, nutrients, and water

from the leaf cells with their piercing and sucking mouthparts which causes foliar damage with tiny white or yellow spots on the leaves. Under heavy infestation, the mites move to the tip of the leaf or top of the plant and result in leaf discoloration, often called bronzing and drop off, and will finally lead to stunting or plant death. Chemical control of spider mites is often costly, and the excessive use of pesticides harms human health and environment. Resistant cultivars are not available and biological control by natural enemies could be used in protected cultivation but is not feasible in the field (Taher et al. 2019).

4.2.8 Leafhopper

The leafhopper [*Amrasca biguttula* (Ishida) (Hemiptera: Cicadellidae)] is an eggplant pest in several countries in Asia. Both nymphs and adults suck the sap from the undersides of leaves causing small and yellow patches, followed by crinkling, curling, bronzing, and drying, or "hopper burn" in severe attacks. High infestation also causes a reduction in yield. Leafhoppers may transmit viruses and phytoplasma that causes the so-called little leaf disease. Management of leafhopper might include use of tolerant eggplant varities such as Manjari Gota, Vaishali, Mukta Kesi, Round Green, and Kalyanipur T3, growing okra as a trap crop along the borders of an eggplant field, and using natural predators such as ladybird beetles and green lacewings which are highly efficient in preying on leafhopper nymphs and adults.

4.2.9 Whitefly

The sweetpotato whitefly (*Bemisia tabaci* Gennadius) can cause considerable direct and indirect damages in eggplant. This pest causes direct damage on leaves that result in reduced leaf photosynthetic efficiency, as well as on the fruits which increases the number of unmarketable fruit (Schuster et al. 1996; Rakha et al. 2017; Taher et al. 2020). Through indirect damage, whitefly transmitsmany species of plant viruses as well. The control of whitefly is highlydifficult due to high reproductive capacity and it can quickly develop resistance against insecticides (Rakha et al. 2017). Eggplant farmers apply a lot of insecticides, particularly in developing countries, which is often costly. Biological control by natural enemies is not sufficient in the open fieldconditions and no eggplant cultivars are resistant to whitefly (Taher et al. 2020).

4.3 Genetic Resources of Resistance Genes

Crop wild relatives of eggplant are rich sources of variation for pre-breeding and breeding programs, particularlyfor traits related to adaptation to climate change

(Taher et al. 2017, 2020). These wild relatives mostly produce small, bitter, multi-seeded fruits, almost always inedible and with prickly calyx. Some of the eggplant wild relatives contain high levels of chlorogenic acid and other bioactive compounds, which may have potential interest for human health (Meyer et al. 2015). The wild species were classified into three genepools based on crossability relationships and following the biological concept of species (Harlan and de Wet 1971). For instance, in eggplant, *S.insanum* can be crossed easily and produce normal fertile hybrids whichis considered underprimary genepool (GP1) of eggplant (Plazas et al. 2016; Syfert et al. 2016). More than 40 African and Southeast Asian species are classified assecondary genepool (GP2) ofeggplant based on crossability relationships and phylogenetic studies. However, some interspecific hybrids derived from GP2 were partly sterile or weak due to reproductive barriers such as *S. dasyphyllum*, *S. linnaeanum* or *S. tomentosum* (Rotino et al. 2014; Kouassi et al. 2016). The tertiary genepool (GP3) includes more distantly related species and the crosses with cultivated eggplant generally failed or need specific breeding techniques such as embryo rescue to succeed (e.g., *Solanum torvum* and *S. elaeagnifolium*) (Kouassi et al. 2016; Plazas et al. 2016; García-Fortea et al. 2019).

Resistance to biotic stress has been reported in crop wild relatives of eggplant (Table 4.1). At World Vegetable Center, about 200 accessions were evaluated for resistance to bacterial wilt (*Ralstonia solanacearum*) under greenhouse using root wounding and soil drenching inoculation methods and 38 accessions were identified with high levels of resistance (AVRDC 1999).

Resistance to whitefly was detected in the eggplant wild relatives *Solanum dasyphyllum*, *S. campylacanthum*, *S. tomentosum* and *S. pyracanthos* (Taher et al. 2020).In addition, resistance to spider mite was detected in African eggplant *S. macrocarpon* as well as wild relatives such as *S. sisymbriifolium*, *S. dasyphyllum* and *S. torvum* (Taher et al. 2019). Resistance to leafhopper and aphids was found in eggplant accessions VI034971, VI035822, and VI035835. These results show that crop wild relatives of eggplant are very promising materials for breeding pest tolerant and resistant varieties can be developed.

4.4 Glimpses on Traditional Breeding

4.4.1 Focus of Traditional Breeding

The classical breeding of this crop has focused on the development of high yielding varieties with larger and more uniform fruits with less flesh browning, a characteristic that greatly reduces the quality of the fruit (Hurtado et al. 2013). Eggplant is highly variable in shape, color and size, and this has allowed the selection of a broad array of improved cultivars adapted to local preferences for fruit size, shape, and color; however, the genetic diversity of the cultivated eggplant genepool is narrow (Muñoz-Falcón et al. 2009a, b). The focus aimedatbreeding for tolerance to stresses has been

Table 4.1 Genepool and species of genetic resources for resistance/tolerance to biotic stress in eggplant

Genepool	Species	Resistance traits	References
GP1	*Solanum melongena*	*Phytophthora capsici* L. and *Ralstonia solanacearum*	Naegele et al. (2014), AVRDC 1999,
	S. insanum	*Ralstonia solanacearum*	Namisy et al. (2019)
GP2	*S. incanum*	Resistance to *Pseudomonas solanacearum*, *Leucinodes orbonalis*, *Phomopsis rexans* and tolerance to drought	Bletsos and Olympios (2008)
	S. anguivi	Resistance to *Ralstonia solanacearum*	Schippers (2000)
	S. campylacanthum	Whitefly (*Bemisia tabaci*)	Taher et al. (2020)
	S. dasyphyllum	Whitefly (*Bemisia tabaci*)	Plazas et al. (2016), Taher et al. (2020)
	S. lichtensteinii	Tolerance to drought	Vorontsova and Knapp (2012)
	S. linnaeanum	Tolerance to salinity and resistance to verticillium wilt (*Verticillium dahliae*)	Liu et al. (2015)
	S. pyracanthos	Tolerance to verticillium wilt (*Verticillium dahliae*), Whitefly (*Bemisia tabaci*)	Bletsos and Olympios (2008), Taher et al. (2020)
	S. tomentosum	Whitefly (*Bemisia tabaci*)	Taher et al. (2020)
GP3	*S. torvum*	Resistance to verticillium wilt, bacteria, and *Fusarium oxysporum,* nematodes and spider mite	Bletsos et al. (2003), Taher et al. (2020)
	S. sisymbriifolium	Resistance to nematodes and verticillium wilt, spider mite	Bletsos et al. (2003), Taher et al. (2020)

GP1 Primary gene pool, *GP2* Secondry gene pool; *GP3* Tertiary gene pool

much less intense than in other major Solanaceae crops, like tomato, potato or pepper. In this way, although tolerance to abiotic stresses have also been sought (Plazas et al. 2019), more works have been performed on finding resistance to biotic stresses, like root-knot nematodes, bacteria, fungus and some insects (Kalloo 1993; Rotino et al. 2014; Miyatake et al. 2016). Other recent works have focused on the study of the functional and nutraceutical characteristics attributed to this crop, rich in antioxidant compounds (Plazas et al. 2013; Kausik et al. 2016). The development of seedless varieties has also been a major focus of eggplant breeding, and some highly parthenocarpic materials have been obtained (Miyatake et al. 2012; Du et al. 2016; Li et al. 2012).

The feasibility of obtaining hybrids with many wild relatives (Daunay et al. 2012; Rotino et al. 2014; Kouassi et al. 2016; Plazas et al. 2016) holds an immense potential to genetically improve eggplant. This makes possible the transfer of genes of interest

from wild relatives into the genetic background of eggplant. In the last decades some examples of genetic transfer of interest from wild species to the genetic background of the eggplant have been established. These include the development of introgression lines with several wild species such as *S. incanum* (Gramazio et al. 2017a, b) for adaptation to climate change, as well as backcross generations with *S. linnaeanum* and *S. tomentosum* for resistance to Fusarium, Verticillium and nematodes (Toppino et al. 2018).

4.4.2 Limitations of Classical Endeavors and Utility of Molecular Breeding

The increasing population in the world, climate change and the high incidence of new diseases in crops has posed a challenge for modern agriculture and breeding (Ray et al. 2013; Brooks and Blandford 2019). In eggplant, as in most major crops, classical breeding methods, with a selection of traits of interest, and the subsequent hybridization, selection and fixation in the cultured materials has been very efficient in delivering improved cultivars; however, nowadays other approaches must be used to accelerate the processes. The speed at which cultivars become obsolete has led breeders to develop breeding methods in a shorter time, where marker-assisted selection is essential. Access to the eggplant genome (Barchi et al. 2019a) and the syntenic relation with the tomato genome (Wei et al. 2020), together with the large number of wild species with which the eggplant can obtain fertile hybrids, is making the speeding of the breeding process possible.

The application of modern molecular marker based approaches carried out have accelerated the process of introduction and localization of genes of interest, including genes from related wild species, in the eggplant genepool. In this way, Wei et al. (2020) found 210 markers associated with around 71 traits of interest in eggplant. Furthermore, the new methodology suggested by Prohens et al. (2017) called "introgressiomics", proposes the massive introduction of genes from wild species in the genetic background of the crop of interest, where the generated materials, with wild introgressions, will be tested and genotyped when necessary to solve the problem that arises. This approach greatly benefits from the use of marker assisted selection for forward and background selection.

The potential of molecular markers for the development of a new generation of eggplant cultivars comes through its application for genetic mapping and detection of quantitative trait loci (QTLs) in natural (germplasm collections) and experimental (biparental, multiparental, advanced backcrosses and introgression lines sets) populations. The use of genome-wide association studies (GWAS) in germplasm populations as well as in biparental populations has allowed the identification of severalQTLs for morphological and agronomic traits of interest (Ge et al. 2013a; Cericola et al. 2014; Portis et al., 2015; Toppino et al. 2016). One of the potentially most useful

experimental populations in eggplant will be a multiparent advanced generation intercross (MAGIC) population which is under development at the Universitat Politècnica de València. In these populations, eight eggplant parents (including a *S. incanum* wild accession) have been intercrossed and recombinant inbred lines (RILs) which are an admixture of the eight genomes are under development (Arrones et al. 2020). The use of experimental populations in eggplant can go beyond the identification of QTLs, and thanks to the fine mapping of a segregating eggplant population, a deletion has been found in the genome of eggplant that could be related to the prickliness trait (Miyatake et al. 2020). Also, Gramazio et al. (2014) found some interesting genes related with polyphenol oxidases involved in chlorogenic acid biosynthesis pathway in a first backcross population.

4.4.3 Positive and Negative Selection in Eggplant Breeding

Traditionally, the breeding objectives in eggplant have been to improve the size and uniformity of the fruit, an increase in yield, as well as to eliminate the bitter taste that characterizes the fruit of primitive varieties and wild ancestors of eggplant. In this way, some traits, such as yield and fruit size have been under positive selection, while others have been under negative selection. Regarding traits under negative selection, eggplant, as other *Solanum* species, contains solasonine and solamargine, two glycoalkaloids with a toxic effect on humans and that give this crop a bitter taste, limiting its use at a commercial level (Cham 2012; Ranil et al. 2017). The presence of saponins also contributes to the bitter taste (Toppino et al. 2016) and has also been selected against its presence. Another undesirable trait is the oxidation that occurs when cutting the fruit and that depreciates its quality, known as browning (Plazas et al. 2013). Selection against this trait has indirectly led to the negative selection of phenolic acids content, and therefore accessions with low browning and at the same time with low antioxidant content. Also, in the case of eggplant, the reduction in the number of seeds results in decreased browning (Maestrelli et al. 2003). Another characteristic that has been under strong negative selection is the presence of prickles both on the plant and on the fruit calyx, as this makes the management difficultof the crop and the marketing of the fruits (Miyatake et al. 2020). Another important trait under negative selection has been the presence of dormancy, very common in eggplant wild relatives, and that is clearly detrimental in the cultivated eggplant.

Some traits that have been under positive selection include the high yield and fruit set. Other traits in which a great emphasis has been paidis the intense dark color, resulting from the combination of chlorophylls and anthocyanins in the eggplant peel. Some recent breeding programs have also been aimed at improving the tolerance to biotic and abiotic stresses, although up to now the results have been less impressive, as well to improving the bioactive properties of the eggplant fruit by improving the content in bioactive phenolic acids of interest for human health.

4.4.4 Classical Breeding Achievements and Limitations

Many of the efforts made to improve the cultivation of eggplant have been carried out using classical breeding methods, where much importance has been given to yield and the external characteristics of the fruit, as well as to tolerance to some biotic and abiotic stresses (Kalloo 1993).

Taking advantage of the wide phenotypic diversity that this crop has, today it is possible to find eggplants of different sizes, from a few g to more than 1 kg, colors, such as white, black, purple, or green, with different patterns of colorations, and shapes including long and narrow (even serpentiform), long and wide, round, oval, and flattened. Depending on the target market, some types are more demanded than others. A wide variability can also be found in terms of texture and taste, as well as on response to biotic and abiotic stresses, which are one of the most important sources of economic losses.

Most of the modern eggplant cultivars have been developed using only intraspecific diversity. Given the potential of wild species as sources of variation for many traits and the limitations that have been found in the use of traditional breeding, multiple strategies have been developed to foster the use of wild species in eggplant breeding. In the "introgressiomics" approach (Prohens et al. 2017) the objective is to develop materials and populations that contain a large number of fragments of wild species, distributed throughout the genome of cultivated species. The application of this approach in eggplant is generating a large array of materials that can be used to address present and future challenges in this crop. For this new approach we will need to have identified the introgressions need to be identified taking advantage of the high throughput molecular markers.

4.5 Brief on Diversity Analysis

4.5.1 Phenotype-Based Diversity Analysis

A main challenge with phenotype-based characterization is to avoid environmental bias. Standardized sets of descriptors and methods are developed for eggplants (IBPGR 1990; van der Weerden and Barendse 2007). In addition, the phenomic tool developed for tomato (Rodríguez et al. 2010) has been applied for eggplants with good results (Prohens et al. 2012; Hurtado et al. 2013; Kaushik et al. 2016). Coming to how to express phenotypic diversity and relationships, different measures are used, from *Coefficient of variation* and *Shannon diversity index* to more multivariate tools (Everitt 1998; Spellerberg and Fedor 2003).

Brinjal eggplant, and especially its fruits, exhibit large phenotypic variation, as people have been selecting for traits as color, shape, texture and size. This adaptive evolution has produced many cultivars with a seemingly high diversity, but based on a limited number of traits. Vegetative traits have been less emphasized. Nevertheless,

they are of major importance for photosynthesis and plant arrangement, and for producing high yielding and robust plants. Regarding phenotypic diversity within a population or an accession, modern varieties are per definition uniform and stable, in contrast to traditional cultivars (landraces) that are more diverse, and wild eggplants that are even more diverse. A study in black-fruited brinjal eggplant clearly showed such a pattern (Muñoz-Falcón et al. 2009a, b). Furthermore, round-fruited cultivars have been found to be more diverse than semi-long or long-fruited cultivars (Prohens et al. 2005; Tümbilen et al. 2011).

Crop diversity is safeguarded in public seed banks (gene banks), in breeders' collections and in-situ in farmers' fields and in the wild. Gene banks provide information on phenotypic characters, and this on accession (seed sample, cultivar) level. Data include plant growth habit, plant height, branching and leaf- flower- and fruit trait characters (e.g. Boyaci et al. 2015; Taher et al. 2017). The largest ex-situ collection of eggplants is maintained at World Vegetable Center, with more than 2,700 accessions (Taher et al. 2017). This collection houses large phenotypic diversity. Regarding abiotic and biotic stress resistance, less data are available. Nevertheless, the information is increasingly important. An old study at the World Vegetable Center examined two hundred brinjal eggplant accessions for bacterial wilt (*Ralstonia solanacearum*) resistance and 38 were identified as promising (AVRDC 1999). Other trials have examined resistance for eggplant fruit and shoot borer (*Leucinodes orbonalis*), two-spotted spider mite (*Tetranychus urticae*), leafhopper (*Amrasca devastans*), and aphids (*Aphis gossypii*). Some brinjal eggplant accessions showed high level of resistance (Ramasamy 2009). For two-spotted spider mite, resistance was detected in accessions of scarlet- and gboma eggplants (Taher et al. 2019).

4.5.2 Genotype-Based Diversity Analysis

Over the years, different marker systems have come. In the early 1990s, restriction fragment length polymorphism (RFLP) markers were used in eggplant research but they were not so reliable (Sakata and Lester 1997). Later, random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers for eggplant were used (e.g.,Furini and Wunder 2004; Sing et al. 2006), as well as simple sequence repeats (SSRs) or microsatellites (Stàgel et al. 2008; Li et al. 2010; Tümbilen et al. 2011) and most recently came the single nucleotide polymorphism (SNP) markers (Gramazio et al., 2017a, b). The latter, with a panel of 5 k SNPs for coding regions and introns/UTRs has now been applied in single primer enrichment technology (SPET) for high-throughput genotyping in tomato and eggplant (Barchi et al. 2019a, b). A set of 422 eggplant accessions (including wild relatives and cultivated material) generated 30,731 high confidence SNPs, respectively. The authors concluded that this representsa robust; high-throughput technology for genetic fingerprinting that can be used to study genetic relationships among accessions and species, and that also could be useful in identification of mislabelled accessions and duplicates in genebanks. Many studies have been conducted of genotype-based diversity

analysis. Details on a few studies are presented in the sections that follow. Here we restrict us to emphasizethat major efforts have been based on identifying and introducing resistance genes and on developing molecular markers for breeders. For Fusarium wilt, a resistance gene (*Rfo-sa1*), identified in scarlet eggplant, was introduced to brinjal eggplant (Toppino et al. 2008). Cleaved amplified polymorphic sequence (CAPS) markers could link the resistance to one major QTL (Miyatake et al. 2016; Barchi et al. 2018). Two other minor QTLs have been reported but for other Fusarium resistance genes (Miyatake et al. 2016; Mutlu et al. 2008). Verticillium vilt resistance has been introduced to brinjal eggplant from the wild species *S. linnaeanum* (Liu et al. 2015). Three QTLs were identified for resistance to this disease (Barchi et al. 2018). For bacteria wilt, a linkage map based on SNP markers has been developed, and one major specific and two broad-spectrum QTLs have been identified associated with this resistance (Salgon et al. 2017). Coming to other traits, markers have been developed for parthenocarpy (Daunay and Hazra 2012). Markers have also been associated to fruit contents of glycoalkaloids, sugars and organic acids (Toppino et al. (2016).

4.5.3 Relationship with Other Cultivated Species and Wild Relatives

Wild relatives are important gene sources for new diversity, and especially for pest- and disease resistance (Toppino et al. 2008; Daunay and Hazra 2012; Rotino et al. 2014; Liu et al. 2015). In addition, many of the wild species grow in extreme environment and can be valuable for climate adaptation (Knapp et al. 2013; Rotino et al. 2014; Syfert et al. 2016). Brinjal eggplant closest relative is *S. insanum* that grows wild in South- and Southeast Asia and is very prickly and weedy (Lester and Hasan 1991; Knapp et al. 2013; Ranil et al. 2017). A genetic similarity as high as 0.947 between the species was found in an old study using 52 accessions and RAPD markers (Karihaloo et al. 1995). The authors concluded that even despite morphologically different, it is not appropriate to distinguish *S. melongena* and *S. insanum* into different species. However, other studies do not agree (e.g. Iwata et al. 2008) and different relationships between the different eggplant species have been presented (Mace et al. 1999; Meyer et al. 2012). Iwata et al. (2008) compared brinjal eggplant to eight related species using inter-simple sequence repeat (ISSR) markers to evaluate the phylogenetic relationship, and identified seven groups: (i) *S. melongena*; (ii) *S. aethiopicum* and *S. anguivi*; (iii) *S. incanum*; (iv) *S. violaceum* and *S. kurzii*; (v) *S. macrocarpon*; (vi) *S. virginianum* and (vii) *S. torvum.*

With regardsto wild relatives that are close to brinjal eggplant, one also finds *S. incanum* L. and *S. linnaeanum* Hepper & P-M. L. Jaeger, as well as *S. lichtensteinii* Willd (Vorontsova et al. 2013; Acquadro et al. 2017). These species can be intercrossed with brinjal eggplants. In addition, the cultivated scarlet eggplant (*S. aethiopicum* L.) and gboma eggplant (*S. macrocarpon* L.) can also be hybridized with

eggplant. These, in addition to their wild relatives *S. anguivi* Lam., *S. dasyphyllum* Schumach. & Thonn. and to some extent also *S. tomentosum* L., can be valuable gene sources for brinjal eggplant but are not closely related (Kouassi et al. 2016; Plazas et al. 2016). Intercrosses can however be made with intermediate fertility. Especially scarlet eggplant has served as a gene source for disease resistance in brinjal eggplant breeding (Prohens et al. 2012). More distant are the American species, *S. sisymbrifolium* Lam. and *S. torvum* Sw. (Vorontsova et al. 2013; Acquadro et al. 2017) but they could harbor potential genes of interest.

4.5.4 Relationship with Geographical Distribution

Brinjal eggplant can be divided into an oriental group traditionally grown in southern and eastern Asia and an occidental group traditionally grown in the Mediterranean basin including the Middle East, Europe and northern Africa. Several studies have pointed to geographical differentiation based on morphology (Chadha 1993; Daunay and Janick 2007) and genetics (Hurtado et al. 2012; Vilanova et al. 2012). For example, Vilanova et al. (2012), by using UPGMA procedures on SSR data, showed that 15 out of 16 accessions from the Mediterranean, Central Europe and Africa clustered together and another cluster had 5 out of 6 accessions from Eastern and Southeastern Asia. On a more detailed level, differentiation within regions is seen. For example, Gramazio et al. (2019a, b) examined landraces from Greece and found differentiation between accessions from the island and from the mainland. They concluded that Greece was part of a Mediterranean secondary center of diversity. We should say that the above-mentioned studies were in landraces, which are local varieties developed over time by farmers. What seems logical is that, as cultivars are becoming broader and with the use of genetic resources from different sources, geographical distribution patterns will be less clear. Liu et al. (2018) exemplifies this by showing that the accessions only partly clustered according to geographic origin. They examined 287 accessions and from around the world and included both inbred lines, cultivars and landraces.

4.5.5 Extent of Genetic Diversity

Genetic diversity can be expressed as Nei's genetic diversity index, expected heterozygosity (He) and/or Shannon's Information index (I). In addition, number of alleles per polymorphic locus, polymorphism information content (PIC) and observed heterozygosity (Ho) are measures used in genetic diversity studies.

Although morphologically diverse in fruits, cultivated eggplants have a much more narrow genetic background than its wild ancestors (Tümbilen et al. 2011; Vorontsova et al. 2013). This is a typical "bottleneck" effect of domestication (Meyer et al. 2012). To illustrate this, Kaushik et al. (2016) characterized 21 accessions from 12 different

wild relatives to brinjal eggplant. In addition, they included interspecific hybrids and cultivars. The result clearly demonstrated that wild specieswere more variable than cultivars and the interspecific hybrids were in between.

Regarding genetic diversity within different types of brinjal eggplant accessions, Hurtado et al. (2012) examined 52 landraces (or selections within landraces) from three recognized secondary centers of diversity, China, Sri Lanka and Spain. They applied 12 highly polymorphic SSR markers that resulted in average PIC of 0.574 and totally 110 alleles were identified with 4.3, 5.3 and 4.2 alleles per polymorphic locus for accessions from China, Sri Lanka and Spain, respectively. The calculated genetic diversity was relatively high, with He of 0.494–0.540 for the Chinese and Sri Lankan landraces and somewhat lower for the Spanish landraces. Muñoz-Falcón et al. (2011) examined 42 landraces of brinjal eggplant from Spain, which included 25 striped accessions most of them of the popular Listada type but also non-striped landraces. They applied 17 SSR and 32 EST-SSR markers and the SSRs had greater polymorphism and polymorphic information content (PIC) than EST-SSRs. A considerable level of diversity was found, with a mean Nei's genetic diversity (He) value of 0.323 (varied from 0.195 to 0.441) and a Shannon's information index (I) value of 0.570.

Ge et al. (2013a) examined 92 cultivars (pure lines) of brinjal eggplant collected from 21 provinces in China. They applied a set of 100 SSR markers with a mean PIC value of 0.285, and found 311 polymorphic alleles. The Nei's genetic diversity (He) was 0.323 and the average Shannon's Information index (I) was 0.570, but ranged from 0.060 to 1.341. The levels of eggplant genetic diversity decreased from south to north. Overall, the genetic diversity was lower in these cultivars than reported for landraces from China by Hurtado et al. (2012). Vilanova et al. (2012) examined 22 brinjal eggplant cultivars from around the world. They used 55 SSR markers with average PIC at 0.47. In total 203 alleles were detected, with an average of 4.7 per locus. The mean expected heterozygosity was 0.52 but the observed heterozygosity was as low as 0.06 and ranged from 0.00 for 16of the markers to 0.24 for one of the markers. Overall, the lowest diversity was found in non-hybrid cultivars compared to hybrid cultivars. The results demonstrate that inbreeding seems to be common in non-hybrid cultivars and that a very narrow genepool is applied in breeding programs. Liu et al. (2018) examined 287 of brinjal eggplant accessions and included inbred lines, cultivars and landraces from around the world. They applied 45 SSR markers and resolved 242 alleles and that ranged from 2 to 14 alleles per locus with an average of 5.38. Shannon information index ranged from 0.276 to 1.903 with an average of 1.055. Observed heterozygosity varied from 0.104 to 0.832 with a mean value at 0.558. There were big differences among all the 45 markers in polymorphism detection. The PIC value ranged from 0.102 to 0.815 with a mean value of 0.507.

4.6 Association Mapping Studies

4.6.1 Extent of Linkage Disequilibrium

Linkage disequilibrium (LD) is defined as allelic (neighbor variants) association at various genomic regions within the investigated population (Ramakrishnan 2013). In case of linkage disequilibrium, haplotypes do not happen at the expected rates when the alleles were independent (Goode 2011). On the contrary, LD takes place when haplotype frequencies are equal to the product of their corresponding allele frequencies. A haplotype is known to be the linked set of genes connected with one haploid genome. It is usually used to describe the linked genes of the major histocompatibility complex, where one haplotype is inherited from each parent. Linkage disequilibrium can be formed as a cause of selection, admixture, or bottlenecks. Studies of linkage disequilibrium patterns can contribute in branches like plant breeding, identification of genes responsible for a disease, and population history (Ramakrishnan 2013). There are plentiful ways to estimate deviation of LD which is referred to as D which was introduced in 1918 and is defined as comparing the observed and expected frequency of one haplotype, then the deviation is the difference between these two. LD can be positive or negative depending on when two alleles occur together on the same haplotype more or less often than expected (Goode 2011). The perception of LD is very important in genome-wide association studies (GWAS) where it helps in the identification of genetic markers that label the actual causal variants. A single-nucleotide polymorphism (SNP) is dissimilarity in a single nucleotide that arises at a specific position in the genome. In GWAS, markers for known complex traits, including some diseases, can be distinguished using SNPs across the genome. In population model LD is highly dependable for the success of GWAS using SNPs as genetic markers. LD is different from gametic phase disequilibrium which explains the non-random connotation of alleles within gametes (even for physically unconnected loci on different chromosomes) (Joiret et al. 2019). In eggplant, LD for some agronomic traits like anthocyanin pigmentation and fruit color was investigated with pair-wise r^2, $r_s{}^2$ and r^2_{sv} where global LDlevel was 3.4 cM (Cericola et al. 2014). Additionally, Ge et al. (2013b) reported that analysis of LD revealed an extensive long-range LD of 11 cM within 141 accessions of eggplant using 105 microsatellite markers.

4.6.2 Target Gene Based LD Studies

Solanaceae species are considered a well-defined family regarding identification of quantitative trait loci (QTLs) as well as other genetic and genomic research (Gebhardt 2016). High-quality genetic linkage map and LD of eggplant genome aresubstantial approaches to identify target genes associated with biotic and abiotic stresses that are quantitatively inherited or qualitative among various germplasm collections. Target

genes research based on LD concept is an advantageous pathway in molecular plant breeding programs and analysis of biotechnological processes for the development a valuable methods for crop improvement (Collonnier et al. 2001). Moreover, genetic linkage map construction representing quantitative genetic and genomics dataset that allows identifyingnew markers tightly related to the desirable horticultural traitsplays a key role in crop improvement and sustainable development. In eggplant, RAPD and AFLP markers have been used for a linkage map construction of fruit shape and color (Nunome et al. 2001). On the other hand, many linkage maps have been utilizedregarding a lot of horticultural traits and improvement of the level of resistance to various plant pathogens and insects that restrict production of eggplant (Lebeau et al. 2013; Portis et al. 2015; Barchi et al. 2016; Gramazio et al. 2017a, b). Genomic research strategies played a key role in smart plant breeding programs to detect particular trait through marker-assisted selection approach. The advantages of these DNA-based markers provide better chance for genetic variability assessment and mapping of QTLs among tested germplasm due to their cost, speed and reproducibility (Thapa et al. 2015). At present, there are many DNA-based markers for instance AFLP, RAPD, RFLP, microsatellite, SCoT (start codon targeted), etc. that can be applied in genetic and genomics studies including characterization, taxonomy, genetic diversity of wild and domesticated crop species.

4.6.3 Genome-Wide LD Studies

Despitethe economic importance of eggplant, omics research approaches including phenomic, genomics, transcriptomic, metabolomics and proteomic are still limited compared to other species of the Solanaceae family. Interestingly, the strategy of GWAS is considered a powerful substitutional strategy for investigating the genetic background of various agronomic traits. Furthermore, GWAS can be applied with many samples and accessions to represent the association between phenotypic and genotypic data as well as acceleration of plant breeding programs and crop improvement. Recently, GWAS approach was involved in eggplant research to identify multiple agri-horticultural traits such as fruit color, size and shape; serious biotic stress resistance, and productivity under difficult environmental conditions (Rotino et al. 2014). The genome-wide association (GWA) analysisis efficient in supporting and confirming QTLs and targeted genomic regions with different unique DNA markers. Concerning germplasm sets used for GWAS, LD may be specified by plant hybridization system, frequency of recombination, level of mutations, structure of tested populations, genetic linkage, alteration of gene sequence and natural selection and domestication (Rafalski and Morgante 2004). Globally, the genome-wide association mapping approach has been accomplished on eggplant populations with plenty of traits of interest (Fig. 4.1). Additionally, 191 accessions of eggplant including cultivars, lines and landraces were genotyped for association of fruit color and anthocyanin pigmentation where 338 SNPs were identified (Cericola et al. 2014).

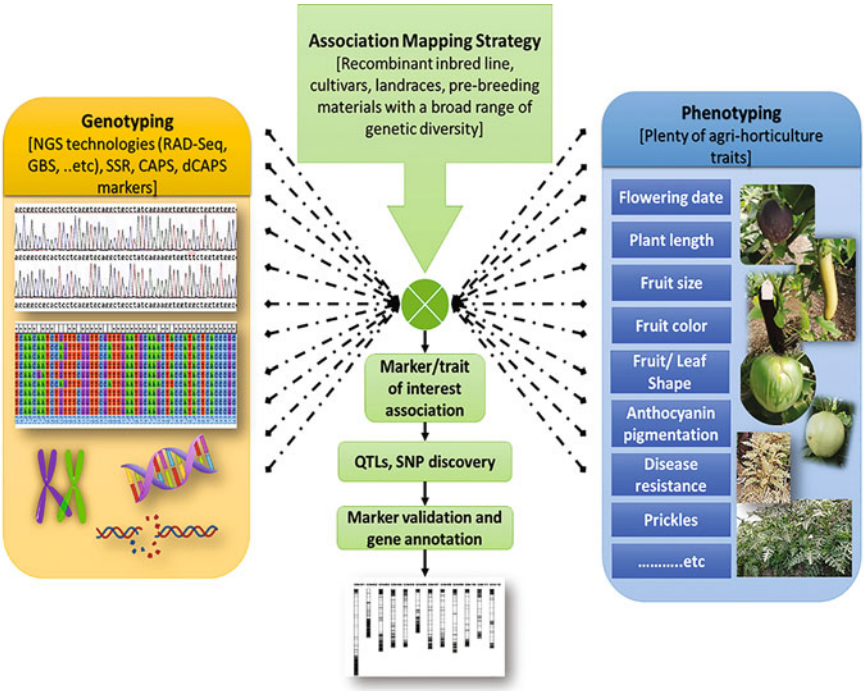

Fig. 4.1 Overview of association mapping in eggplant species to exploiting the available genetic resources for single nucleotide polymorphisms identification and gene annotation

4.6.4 Future Potential for the Application of Association Studies for Germplasm Enhancement

Association mapping is a very useful tool in modern agricultural and breeding programs to identify genomic regions that are responsible for traits of interest. Genomic association research in crop species are still ongoing where convenient trait measurements methodology and genotyping platforms with powerful analysis software are still the great challenges regarding crop improvement and molecular breeding. To maximize the future benefitof association mapping studies, all historical information about the population structure, size and marker density have to be known and informed (Álvarez et al. 2015). Moreover, advanced computational tools and bioinformatics analysis can play a crucial role in genetic resources and germplasm enhancement in the next decades. Furthermore, the next-generation sequencing technologies and whole-genome resequencing approaches are a great opportunity to improve association studies not only in model crops but also in non-model plant species. In the future, network of artificial intelligence can be applied in association research by large scale to minimize the genome-wide error rate and get more accuracy dataset. On the other hand, the future approaches of association mapping can be

selected upon LDhistory in the tested germplasm, targeted traits complication, the provided historical knowledge of pedigree, availability of the reference genome of the target crops and structure of population under the study.

Analysis of GWA of 191 eggplant accessions demonstrated that 79 SNPs were mapped on 39 genomic regions and scattered over all 12 chromosomes of eggplant (Portis et al. 2015). Specific length amplified fragment sequencing (SLAF-Seq) strategy was applied for construction of high-throughput SNP markersin the genome of eggplant. Subsequently, GWA and QTL analysis were conducted within F2 population of 121progynies where 2,122 SNPs, 12 linkage groups and 19 QTLs were generated for leaf morphology, height of stem and fruits (Wei et al. 2020). Moreover, Toppino et al. (2020) genotyped 163 RILs using GBS strategy where 7249 SNP markers were assigned to the 12 eggplant chromosomes and spanning 2169.23 cM. This association revealed the molecular bases of seven horticultural traits associated with anthocyanin and seed vigor where from 7 to 17 QTLs for each trait were detected as well as development of MAS for further research.

4.7 Brief Account of Molecular Mapping of Resistance Genes and QTLs

4.7.1 Brief History of Mapping Efforts

Genome mapping is a widely uasedmeansto investigate the genetic information of an organism for the genomic regions/genes that are associated with a desirable trait. Two main groups of genome mapping have been obviously reported which are genetic map and physical map. Actually, genetic map refers to the Mendelian rules of segregation and recombination to decide the distance between varied genomic sites within a gene or between different genes on the same chromosome with cM (centi-Morgan) units, where the linkage idea is important in genetic map construction. The connotation of genetic linkage mapping has been reported earlerin 1913 where five sex genes on the chromosome Y of *Drosophila melanogaster* were linked (Sturtevant 1913). On the other hand, physical map is a molecular biology strategy to determine the order of DNA fragments at the level of chromosome comprising of the whole genome or a specific genomic region that is responsible for trait of interest and can be a count as a real map expressed in million base pairs (Mbps). It is noticeable that great efforts have been successfully done regarding genomic and genetic research in eggplant. Many QTLs for horticulturally desirable traits such as fruit weight (fw), fruit shape (fs), fruit calyx prickliness (fcpri), number of seed locules (slon), plant growth habit (hab), leaf prickliness (lepri), etc. have been effectively identified in eggplant (Portis et al. 2015; Toppino et al. 2020; Wei et al. 2020). GWAS hasobviousadvantages than other approaches for QTL mapping and SNPs discovery. Furthermore, GWAS enables phenotypic/genotypic variation within and between an array of accessions to identify targeted genes and for crop improvement (Portis et al. 2015). The high-quality

reference genome of eggplant (Barchi et al. 2019a) will provide new insights with relevance to resequencing research, domestication and evolutionarly mechanisms of eggplant population. Addtionally, other genomic approaches and bioinformatics tools play substantial role in association studies, smart breeding programs, ecology and geographical origin, and climate-resilient cultivars in order to face the potential challenges (Gramazio et al. 2019b).

4.8 Marker-Assisted Breeding for Resistance Traits

4.8.1 Germplasm Characterization

Identification of sources of tolerance or resistance to stresses is a first step for conventional or marker-assisted breeding of these traits. Throughout the last decades multiple screenings for some of the most important stresses, mostly biotic (diseases and pests), involving both intraspecific and interspecific genetic resources have been performed (Toppino et al. 2021).

The most damaging soil-borne pathogens of eggplant are *Ralstonia solanacearum*, *Fusarium oxysporum* f. sp. *melongenae* and *Verticillium dahliae*, which are the respective causal agents of bacterial wilt, Fusarium wilt, and Verticillium wilt. Screening of collections of genetic resources against the different phylotypes of *R. solanacearum* have allowed the identification of several sources of resistance in the cultivated eggplant and in eggplant relatives (Lebeau et al. 2011; Namisy et al. 2019). Within *S. melongena* some resistant accessions have been discovered and so far the most studied are EG203 and AG91-25 (Lebeau et al. 2013; Salgon et al. 2018), although many others have been identified (Barik et al. 2020). Interestingly, AG91-25 derives from the hybridization between *S. melongena* and *S. aethiopicum* (Ano et al. 1991; Salgon et al. 2018). Amongst the eggplant relatives that can be successfully hybridized with eggplant via sexual crosses (Daunay et al. 2019), resistances have been found in several species, such as *S. aethiopicum*, *S. anguivi*, *S. incanum*, *S. insanum*, and *S. torvum* (Namisy et al. 2019; Barik et al. 2020). In addition, high levels of resistance have been found in other *Solanum* wild species. However, given the high genetic variability of *R. solanacearum*, resistance levels often depend on the strain used (Lebeau et al. 2011; Namisy et al. 2019). Resistance to Fusarium wilt has been found both in the cultivated species and in wild and cultivated eggplant relatives such as *S. aethiopicum, S. incanum, S. linnaeanum*, *S. sisymbrifolium, S. torvum*, *S. viarum* or *S. violaceum* (Cappelli et al. 1995; Boyaci et al. 2012; Altinok et al. 2014). Some of the resistances found are stable against a wide range of isolates (Altinok et al. 2014) providing sources of resistance of great value for the breeders. In particular, the resistance derived from *S. aethiopicum* has proved of great interest for eggplant breeding (Toppino et al. 2008). Different levels of resistance and tolerance to Verticillium wilt have been identified in the eggplant germplasm genepools. However, reports of high levels of tolerance within the cultivated species are scarce.

In this respect, the best sources of resistance have been found in wild species such as *S. anguivi*, *S. incanum*, *S. linnaeanum*, *S. tomentosum*, *S. torvum*, *S. sisymbriifolium*, or *S. viarum.*

Resistance to diseases other than the three major ones mentioned above has been found both in the cultivated and wild eggplant genepools (Toppino et al. 2021). In this way, resistance has been found either in cultivated eggplant and/or in eggplant relatives to fungal pathogens that may affect eggplant such as *Alternaria melongenae*, *Cercospora solani*, *Cercospora solani-melongenae*, *Colletorichum coccodes*, *Colletotrichum gloesporioides* f. sp. *melongenae*, *Fusarium solani*, *Leveillula taurica*, *Phomopsis vexans*, *Phytophthora parasitica*, *Phytophthora capsici*, *Sclerotinia sclerotiorum*, *Sclerotinia rolfsii*, or *Verticillium albo-atrum* (Daunay and Hazra 2012; Rotino et al. 2014; Toppino et al. 2021). A number of studies have also screened cultivated and wild germplasm for resistance to root-knot nematodes (*Meloidogyne* spp.), mostly *M. incognita.* Although most of the cultivated eggplant materials have been found to be susceptible, some lines have found to be tolerant or partially resistant to root-knot nematodes (Colak-Ates et al. 2018), or in the case of line *A-264-A* from the Philippines, fully resistant to *M. javanica* (Boiteux and Charchar 1996). The most promising materials have been found in the related species genepool. In this way, high levels of resistance to nematodes have been found in accessions of *S. torvum*, *S. viarum* and *S. stramonifolium* (García-Mendivil et al. 2019).

Regarding viruses and phytoplasma, a large screening for resistance to tobamoviruses (Rast 1991) resulted in the identification of sources of resistance against *Bell pepper mottle virus* (BPMV), *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus* (ToMV). Resistance to *Pepper mild mottle virus* (PMMV) has also been found in some accessions of *S. aethiopicum* (Tzortzakakis et al. 2006). However, no resistance against *Potato virus Y* (PVY) was found in a screening of 77 eggplant accessions (Colak-Ates et al. 2018). Screening for little leaf disease, caused by phytoplasma, has been found within eggplant germplasm, as well as in *S. aethiopicum.*

Compared to disease resistance, the screening of sources of resistance against pests has been less intense. One of the major pests in Southeast Asia is the fruit and shoot borer (*Leucinodes orbonalis*). Few materials of potential interest have been found in the cultivated eggplant genepool against this insect, although it has been suggested that varieties with high contents of phenolics, glycoalkaloids, dietary fiber, ash, starch and polyphenol oxidase activity are less susceptible (Doshi 2004; Prasad et al. 2014). Some resistances to *L. orbonalis* have been found in some eggplant relatives, such as *S. aethiopicum*, *S. incanum*, *S. macrocarpon*, and *S. violaceum.* A few research works have been performed on the identification of sources of resistance to other eggplant pests. Recently, Taher et al. (2020) have identified several sources of resistance to the whitefly *Bemisia tabaci* in one eggplant accession (MEL2) as well as in the wild species *S. campylacanthum*, *S. dasyphyllum*, *S. pyracanthos*, and *S. tomentosum.* Hasanuzzaman et al. (2018) found that eggplant varieties with lower contents of nitrogen, glucose and aminoacids and higher contents of phenolics were less susceptible to *B. tabaci*. Leaf hopper (*Amrasca devastans*) resistance has been

identified both in the cultivated and wild germplasm, associated to higher leaf hairiness and thin leaf lamina, as well as to higher content in phenolics and sugars (Ali et al. 2016). Also, sources of resistance to *A. devastans* have been identified in *S. aethiopicum*, *S. insanum* and *S. violaceum* (Warade et al. 2004). Certain levels of resistance or tolerance have been found against other insect pests affecting eggplant, such as thrips, aphids, or spotted beetle has been identified. Regarding spider mites, differences among accessions in susceptibility have been described, and high levels of resistance against the two-spotted spider mite (*Tetranychus urticae*) have been identified in *S. dasyphyllum*, *S. macrocarpon*, *S. sisymbriifolium*, and *S. torvum* (Schaff et al. 1982; Taher et al. 2020).

A few germplasm characterizations have been performed for the identification of sources of tolerance to abiotic stresses such as drought, salinity, or extreme temperatures. Although no large screenings have been performed, some differences among eggplant cultivars have been found for tolerance to drought or salinity (Hanachi et al. 2009; Saracanlao et al. 2016; Tani et al. 2018; Kiran et al. 2019; Plazas et al. 2019). Some wild species have proved to be more tolerant than the cultivated species. In this way, *S. insanum* and *S. torvum* have been found to be more tolerant to salinity than *S. melongena* and this may be associated to a higher accumulation of proline as well as of the ions Na^+ and Cl^- (Brenes et al. 2020a, b). García-Fortea et al. (2019) also been found *S. elaeagnifolium*, a particularly drought tolerant species, had a root system that explored a larger volumen than that of *S. melongena.* Although data on tolerance to stresses is lacking for many wild eggplant relatives, some of them grow in highly stressful environments indicating that they are highly tolerant to the stresses they suffer (Vorontsova and Knapp 2016). In this way, *S. incanum*, which grows in desertic and semi-desertic areas, has been identified as highly promising for breeding for tolerance to drought (Gramazio et al. 2017a, b). Kouassi et al. (2021) found that *S. sisymbriifolium* and the interspecific hybrids of *S. melongena* with *S. anguivi*, *S. dasyphyllum* and *S. insanum* were tolerant to drought, being the interspecific hybrids heterotic for the tolerance to this abiotic stress. Differences in eggplant cultivars have been observed for tolerance to low temperatures (Boyaci et al. 2009; Yang et al. 2020), and some wild species such as *S. aculeatissimum*, *S. grandiflorum* and *S. mammosum* have been reported as cold tolerant (Toppino et al. 2021). Differences among eggplant cultivars have been observed for tolerance to high temperatures, with several promising varieties having been identified (Santhiya et al. 2019).

4.8.2 Marker-Assisted Gene Introgression

The success of marker-assisted gene introgression of the resistance or tolerance to abiotic stresses depends on the availability of markers closely linked to the gene/s that have to be introgressed. The success of introgression of the trait also depends on the genetic control and the expression of the gene/s in the recipient genetic background. Genetic analysis of resistance to diseases has revealed different patterns

of inheritance to the major eggplant diseases. In this way, for bacterial wilt resistance, different mechanisms have been found including, monogenic and polygenic inheritance, with different gene action mechanisms and interactions (Barik et al. 2020). Through the use of segregating generations some molecular markers have been identified associated to resistance to *R. solanacearum.* In this way, Bi-hao et al. developed a SCAR marker associated to a dominant resistance to bacterial wilt from resistant accession E-31. Lebeau et al. (2013) also found a major dominant gene (*Ers1*, subsequenlty renamed *EBRW9*) for resistance against three strains of *R. solanacearum* from accession AG91-25, which has been positioned in LG9 (Salgon et al. 2017). More recently, Salgon et al. (2018) detected several QTLs associated to resistance to phylotypes I and III coming from accession EG203, although the expression of the QTLs was highly influenced by environmental conditions. Despite the availability of these markers, there are no reports of introgression of resistance to bacterial wilt in eggplant élite genetic backgrounds. In the case of Fusarium wilt resistance, a major dominant resistance gene derived from *S. aethiopicum* (*Rfo-sa1*) has been introgressed by Toppino et al. (2008) in the genetic background of cultivated eggplant. Several molecular markers, such as CAPS (Toppino et al. 2008), associated to *Rfo-sfa1*, which maps in chromosome 2 (Barchi et al. 2018), are available. Using a recombinant inbred line (RIL) population using a parent with resistance introgressed from *S. aethiopicum*, Barchi et al. identified a major QTL cosegregating with *Rfo-sfa1*. Apart from this major QTL, these authors also identified a minor QTL, accounting for 11% of the variation, in chromosome 11. By using a bulked segregant analysis (BSA) strategy, Mutlu et al. (2008) also developed SCAR markers linked to a dominant gene of resistance derived from the eggplant resistant line LS2436. This resistance has been introgressed into eggplant (Boyaci et al. 2020). In another study (Miyatake et al. 2016), using the two eggplant resistant varieties, one of which is LS2436 (as in Mutlu et al. 2008), found two resistance alleles ($Fm1^L$ and $Fm1^E$) that mapped in the same genomic region as*Rfo-sa1*. In addition, Miyatake et al. (2016) also found an additional QTL in chromosome 4 derived from LS2436. Regarding Verticillium wilt, the high levels of resistance to this disease found in *S. linnaeanum* have been introgressed into the eggplant genetic background by Acciarri et al. (2004) and Liu (2015). Sunseri et al. (2003) using AFLP markers found two tentative QTLs for resistance to Verticillium wilt in segregating populations using the same source of resistance than Acciarri et al. (2004). Liu et al. (2015) found that the resistance to Verticillium of the *S. linnaeanum* accession PI388846 could be selected with a molecular marker for the homolog of the tomato *Ve* resistance gene. In addition to the resistance introgressed from *S. linnaeanum*, Barchi et al. (2018) identifyied three QTLs for tolerance to Verticillium wilt in chromosomes 5, 8 and 9 in a RIL population. Interestingly, Barbierato et al. (2016) found that after inoculation with *Fusarium oxysporum* f. sp. *melongenae*, materials carrying the *Rfo-sfa1* gene expressed improved tolerance to *Verticillium* wilt. Marker-assisted breeding to other biotic, either diseases or pests, and abiotic stresses is still in its infancy in eggplant and introgression of resistance into the eggplant genetic background has not been reported yet. However, the availability of introgression lines with wild species that may harbor genes for tolerance of stresses, such as the one with *S. incanum*

(Gramazio et al. 2017a, b) may facilitate the identification of lines with tolerance to stresses introgressed from the donor wild species. Also, some genes involved in tolerance to stresses have been identified. For example, Zhou et al. (2018a) found a gene from the eggplant wild relative *S. aculeatissimum* potentially involved in resistance to the root-knot nematode *M. incognita.* Also, Li et al. (2019) found that the SmAKT1 K^+ transporter gene contributed to higher tolerance to salinity in eggplant, while Zhou et al. (2018b) found that three C-repeat binding factor genes (*SmCBF1*, *SmCBF2* and *SmCBF3*) were involved in tolerance to cold, drought and salinity in eggplant.

To our knowledge, no gene pyramidimg works have been performed aimed at developing eggplant varieties with tolerance to several eggplant biotic and abiotic stresses. However, the availability of markers for genes and QTLs associated to some of these traits would facilitate this task. Similarly, hybrids resistant or tolerant to several stresses could be easily obtained in eggplant by crossing complementary parents for the resistance or tolerance (Sidhu et al. 2005).

4.9 Map-Based Cloning of Resistance Genes

Several cultivated accessions and varieties and wild relatives have traits useful for breeding new and robust eggplant varieties. Traditionally this is done by hybridization and backcrossing methods. This works as long as the species are cross compatible and time allows. Gene editing and cloning can speed up the process, and allow introduction of genes from species further away in the taxonomy system. Still there are limitations, especially when it comes to risks and public acceptance. Several genes have been identified, characterized and cloned, and these are especially genes encoding for disease- and insect pest resistance (Table 4.2).

For root-knot nematode resistance, the gene *SacMi* was recently cloned (Zhou et al. (2018a, b). The full-length DNA is 4,014 bp and enhances the production for a protein of 1,338 amino acids. The gene has been cloned into *S. aculeatissimum,* where tobacco rattle virus was used as a vector, and from where the plasmids were transformed into *Agrobacterium tumefaciens*. Interspecific hybridization between *S. aculeatissimum* and *S. melongena* gives opportunity to utilize this, and other resistance genes, from *S. aculeatissimum*, through traditional breeding as well as further gene editing research. Another nematode resistance gene, but from tomato (*Mi-1.2*), has also been transferred to eggplant (Goggin et al. 2006). Furthermore, a modified rice cystatin gene, *OC-IΔD86*, which is controlling nematodes, has been introduced to eggplants (Papolu et al. 2016) but further research is needed before it can be taken into fields.

A Verticillium wilt resistant gene, *Ve*, has been isolated in the wild relative *S. linnaeanum* (accession number PI388846). Through hybridization and backcrossing it was introduced into eggplant (Liu et al. 2014). Another resistance mechanism is ove-expression of a yeast desaturase gene done through transgenic introduction (Xing and Chin 2000). The gene increases the production of 16:1 and 16:3 fatty acids that

Table 4.2 Overview of some interesting genes and their encoding traits if expressed in eggplant

Gene and source plant	Trait/resistance	References
Ve gene, *S. linnaeanum*	*Verticillium* wilt	Liu et al. (2014)
Yeast Δ-9 desaturase gene	*Verticillium* wilt	Xing and Chin (2000)
Chitinase genes, rice	*Verticillium* wilt	Singh et al. (2014)
Glucanase gene, alfalfa	*Fusarium oxysporum*	Singh et al. (2014)
Dm-AMP1 gene, *Dahlia merckii*	*Botritys cinerea*	Turrini et al. (2004)
Defensin gene, wasabi	*Alternaria solani*	Darwish et al. (2014)
cry1Ac gene (Bt-gene)	Eggplant fruit and shoot borer	Shelton et al. (2018)
Mi-1.2 gene, tomato	Root-knot nematode	Goggin et al. (2006)
SacMi gene, *S. aculeatissimum*	Root-knot nematode	Zhou et al. (2016, 2018)
OC-1ΔD86 gene, rice	Root-knot nematode	Papolu et al. (2016)

again inhibit the Verticillium wilt pathogen. Resistance genes to Verticillium wilt and Fusarium has also been introduced by expressing a glucanase gene from of alfalfa and a chitinase gene from rice, respectively (Singh et al. 2014). Furthermore, the gene *Dm-AMP1* from a *Dahlia* species has been introduced to eggplant to increase the resistance to *Botritys cinerea* and some other fungi through a protein release via the root exudates (Turrini et al. 2004). Resistance to *Alternaria solani* has been introduced to eggplant by expressing a defensingene from wasabi.

Coming to insect pest resistance, a *Bacillus thuringiensis* (Bt) *cry1Ac* gene, which has been introduced into several important crops, has also been introduced into eggplant to reduce the damage from eggplant fruit and shoot borer (*Leucinodes orbonalis*) and other insect pests (Shelton et al. 2018). Modified eggplant varieties with the gene are now used in South Asia since 2014 (ISAAA 2019).

4.10 Genomics-Aided Breeding for Resistance Traits

4.10.1 Structural and Functional Genomic Resources Developed and Applications

The fact that eggplant is one of the most cultivated *Solanaceae* crop, which has received little attention open a huge range of possibilities for the scientific community. It has been observed that at least 40% of the most important agronomic traits of this crop are controlled by the same genes that have been previously described in other *Solanaceae*crops such as potato, tomato and pepper (Doganlar et al. 2002b). This feature will be very useful for genotyping characters on genetic maps and for generating comparative maps between species.

The first interspecific linkage map developed in eggplant was constructed using an interspecific cross *S. linneanum* × *S. melongena* (Doganlar et al. 2002a). For the development of this map, markers previously described and located in tomato and potato was used. Several years later this map was improved by Wu et al. (2009). Later on, a new linkage map based on a different interspecific cross (*S. melongena* × *S. incanum*) was developed by Gramazio et al. (2014). In this map, the prickliness trait as well as the genes involved in the chlorogenic acid pathway and genes of the polyphenol oxidases were located in different chromosomes. A new intraspecific map was developed and used to anchor the genome sequence (Hirakawa et al. 2014). More recently, another linkage map developed using 114 RILs between the "Ramnagar Giant" eggplant variety and the wild species *S. incanum* "W-4", locating 1443 polymorphic markers between the two species of diverse molecular nature (Mishra et al. 2020).

The first intraspecific map of eggplant was constructed by Nunome et al. (2001) and improved by Nunome et al. (2009). Subsequently, Barchi et al. (2010) developed two intraspecific mapping populations from the cross between the breeding lines '305E40' and '67/3'. This map was improved using RAD-seq approach and is composed mainly of SNPs (Barchi et al. 2012). Finally, two high-density intraspecific genetic maps were developed using a cross between *S. melongena* MM738 and *S. melongena* AG91-25 (Salgon et al. 2017) and a double haploid population from the cross EG203 × MM738 (Salgon et al. 2018).

The genome-wide association mapping approach (GWA) was also implemented in eggplant. The first attempt was carried out by Ge et al. (2013a), whom analysed the association of several fruit traits. Taking advantage of the high-throughput SNP technologies, new association studies were performed by Cericola et al. (2014) and Portis et al. (2015). The authors were able to associate SNPs to several traits related to fruit, plant, and leaf morphology.

Some works have attempted to unravel the relationship between eggplant and some of its related species, seeking to find the reason for the resistance or tolerance present in wild species and related cultivated species (Lebeau et al. 2013; Toppino et al. 2008; Yang et al. 2014). QTLs related to fungal resistance have been found (Barchi et al. 2018) and also some rootstock cultivars were developed to be resistant

to bacterial wilt (Rakha et al. 2020). The mechanisms involved in the development of the eggplant ovary when it is crossed with other cultivated species (*S. aethiopicum*) and with different wild species have been studied. Performing a comparative analysis of the transcriptome, more than 1600 transcription factors involved in interspecific hybridization have been located (Li et al. 2020). In addition, the mechanism that regulates anther dehiscence has been studied in depth. Using fertile and sterile eggplant accessions, relevant information has been obtained that makes it possible to clarify what reactions are taking place in the flower (Yuang et al. 2021).

4.10.2 Details of Genome Sequencing

The first version of the genome was published in 2014 by Hirakawa et al. (http://eggplant.kazusa.or.jp/) and it was a giant step in the improvement of the crop, since the improvement processes were going to accelerate, for the development of this genome an Asian type eggplant was used, elongated and purple. Despite this, this genome was just a first draft and only reached scaffold assembly level and only covers 70% of the genome.

The availability of the genome of the eggplant has allowed carrying out synteny studies with respect to other nightshades (Portis et al. 2015; Gramazio et al. 2016) of interest, such as tomato and potato. This has made possible to annotate a multitude of genes (more than 800 in the work by Barchi et al. 2019a), some of them previously described in otherrelated species, and to locate multiple translocations. In this sense, the potential of using wild species in breeding was observed (Acquadro et al. 2017). Wild relatives of eggplant separated into four clusters species that have been used successfully in breeding programs (Plazas et al. 2016).

The new version of the eggplant genome developed and published by Barchi et al. (2019b). This new genome was sequenced using a combination of Illumina and single molecule optical mappingreaching chromosome-anchored genome assembly. The annotation of the genome detected 34,916 high-quality protein-coding genes; whichis similar to the previously annotated Solanum genomes (http://www.eggplantmicrosatellite.org/). This genome information was used for resequencing seven eggplants and its wild relative *S. incanum* to develop useful breeding tools (Gramazio et al. 2019a, b).

A new genome is currently available, developed by Wei et al. (2020). Its coverage is around 91% compared to 74% of the genome developed in 2004 (Hirawaka et al. 2014). The HQ-1315 accession was used, obtaining a genome of 1.17 GB in size with more than 36,582 protein-coding genes. This study has made it possible to locate genes that control important crop traits. In addition, a QTL has been located on chromosome 3 involved in the length of the fruit, and more specifically, a gene from the SUN family (Smechr0301963) that regulates the length of the fruit in eggplant. In addition, more than 200 markers have been identified associated with 71 morphological and physiological characters (size and color of the fruit, leaf morphology and some nutritional components). Of these genes, at least 1009 are listed. Comparison

of this new genome with the previous ones has made it possible to locate some variations and confirm that the eggplant is phylogenetically speaking, closer to the potato and tomato than to the pepper.

The currentgenome of the eggplant developed by Li et al. (2021) has found slightly fewer high-quality protein-coding genes. Despite this, one of its potentialities is that, within the specific genes of the family (646 genes), the genes related to the bacterial spot of the eggplant and the genes involved in the synthesis pathway of chlorogenic acid have been located. This feature makes this new genome a very useful tool for the scientific community.

All the phenomic and genomic information available has allowed a more efficient improvement in horticuoral traits. Shortening the development and adaptation times of new cultivars is a primary objective, since mainly due to climate change and the disproportionate increase in the world population, agriculture has had to accelerate its objectives. It took more than sevn years to develop markers associated with resistance to Fusarium (Toppino et al. 2008), however, it is possible to reduce these times with new information and technologies (Barchi et al. 2018).

4.10.3 Impact on Germplasm Characterization and Gene Discovery

At this time, having the phenotypic and genotypic information of the cultivated species in the same database would save a lot of time and work for the breeders (Raubach et al. 2020). Focusing on this objective, the "Germinate" database (https://ics.hutton.ac.uk/cwr/eggplant/#/) was developed, which is a repository that collects both the passport data of the material, genotypic data and phenotypic characterization information, among others. One of its most important uses is to be able to search for sources of resistance in related species, and thus improve the species more efficiently. Many times, that variability is present, but that information is unknown, so for germplasm banks to be more useful and accessible, they must have that information collected and easily accessed.

Throughout the process of developing eggplant materials with crop wild relative (CWR) introgressions, different evaluations have been made for biotic and abiotic stresses (Barchi et al. 2018; Brenes et al. 2020a, b; Kaushik et al. 2016; Kouassi et al. 2020). Some interspecific hybrids were found to be very vigorous and exhibited a powerful root system, which may explain greater tolerance to drought, as well as greater stem vigor when eggplant is grafted onto vigorous interspecific aubergine hybrids (Mangino et al., 2020). Therefore, the direct use of interspecific eggplant hybrids with wild relatives as rootstocks is promising (Somvanshi et al. 2020). Global warming, in addition to the multiple consequences it has had on crops, has accelerated the search for sources of resistance or tolerance. Trying to find eggplant accessions that are better adapted to very high temperatures, 315 upregulated genes and 342

downregulated genes have been located when different accessions of this cropwere subjected to stress due to high temperatures.

Selection that has traditionally been made towards the shape of the fruit has had a considerable impact on the genetic structure and diversity of the eggplant (Liu et al. 2019; Stagel et al. 2008), an impact that has been detected with unique changes distributed throughout the genome. The variability found in the Asian center of diversity (Miyatake et al. 2019) has been represented using almost 900 molecular markers and 893 eggplant accessions, from which a nuclear collection of 100 accessions was developed. The increase in cystatin represented a considerable advance in the development of rootstocks tolerant to nematodes (Papolu et al. 2016), as well as the silencing of the *Mi-msp-1* gene (Chaudhary et al. 2019). These advances have occurred thanks to having the genome of the eggplant available (Chapman 2020).

4.11 Genetic Engineering

4.11.1 Brief on Genetic Engineering for Resistance Traits

Gene silencing (GS) is the switching off a certain gene expression in the plant cell through transcription or translation process that possibly developed as a molecular defense system against biotic stress (Waterhouse et al. 2001). Gene activity suppression in plant species can be controlled by various molecular and biochemical mechanisms such astranscriptional gene silencing (TGS), virus-induced gene silencing (VIGS), RNA interference (RNAi) and micro RNAs. Transgenic plants may be released as a result to manipulation of gene silencing approach that switch off genes of interest. These indispensable strategieshaveopened new insights in front of plant scientists (breeders, pathologist, entomologist, agronomist, etc.) forcrop improvement as well as to accelerate and facilitate plant breeding programs in model and non-model species. Moreover, GS technology has been significantly applied in different research objectives like resistance to biotic stress (Scorza et al. 2001), food quality (Ogita et al. 2003), protein value in tomato and maize (Segal et al. 2003), quantity and quality of forest trees. In eggplant, VIGS tool has been successfully used as an authoritative technology regarding gene function investigation. To accomplish VIGS tool, a lot of vectors have been widely utilized for functional genomics analysis like *Tomato golden mosaic virus* (TGMV), *Tobacco mosaicvirus* (TMV), *Potato virus X* (PVX) and *Tobacco rattle virus* (TRV) where TRV is particularly excessively applied in Solanaceae species (Fu et al. 2005). Interestingly, four genes *PDS*, *Chl H*, *Su* (*Sulfur*), *CLA1* were silenced in eggplant by VIGS (Liu et al. 2012). Wang and Fu (2018) applied VIGS approach to chalcone synthase gene (*SmCHS*) expression through fruit ripening process of eggplant where *CHS* gene expression was related to a negative gravitropic response.Unfortunately, the gene silencing research on eggplant is lack, thus many investigations are needed in the future regarding an array of horticultural traits.

4.11.2 Bt Eggplant

As indicated above, the eggplant fruit and shoot borer (*Leucinodes orbonalis*) is a major constraint to eggplant production in Southeast Asia. An alternative to conventional plant breeding against this pest is the development of transgenic plants expressing the *Bacillus thuringiensiscry1Ac* gene (Shelton et al. 2018). This strategy has proved as very efficient in other crops, such as cotton or maize, where other Lepidoptera borers are major pests (Hautea et al. 2016). The Indian company Maharashtra Hybrid Seeds Co. Pvt. Ltd. (Mahyco) transformed eggplant with the *cry1Ac* gene under the control of the constitutive 35S CaMV promoter and the transformation event called EE-1, which proved successful to control the eggplant fruit and shoot borer, has been introduced in several lines and hybrids (Shelton et al. 2018). While in India the cultivation of Bt eggplant was cleared for commercial cultivation in 2009, since then it is under a moratorium (Hautea et al. 2016). However, in Bangladesh commercial cultivation of several Bt eggplant varieties were approved and nowadays there are over 1,000 ha cultivated of this transgenic eggplant with excellent results in terms of yield and revenue, which was 19.7% and 21.7% higher compared to non-Bt varieties (Shelton et al. 2020), demonstrating the high potential of transgenic eggplant for dealing with pests such as the eggplant fruit and shoot borer.

4.12 Gene Stacking

Gene stacking is the process of combining two or more desirable genes (multigene) into a single crop plant. The newly produced traits from this strategy can be called as stacked traits and the crop species is known as biotech stacked (Singh et al. 2018). Genetic variability plays a crucial role in crop improvement, breeding programs and gene pyramiding. The source of variation may be derived from landraces, wild species, RILs, etc. that support plant breeders to generate new cultivars and hybrids with genes of interest through interspecific or intergeneric hybridization. Biotech or stacked cultivars have advantages more than mono-trait varieties and theyallow farmers to increase their crop production and overcoming the biotic (insects, diseases, weeds) and abiotic (high temperature, salinity, drought) problems. Gene stacking can be accomplished via two main approaches: hybrid stacking and molecular stacking. Hybrid stacking aaproachcan be conducted by hybridization between the two parental lines that possess multi-agronomic traits of interestin order to introduce new populations with desirable traits. Although, the aforementioned tool is simplest and earliest method of developing stacks, however there are some drawbacks like high cost, time consuming, long process, selectable markers requiredand difficult to gain homozygous crop plants for all transgenes. In molecular stacking approach, all genes of interest can be inserted into the target plant jointly or sequentially using *Agrobacterium* mediated transformation as a standard transfer technique or biolistic method (Que et al. 2010).In eggplant, gene pyramiding strategy can be applied for crop

improvement where transgenic plants with a modified rice cystatin and root-knot nematodes resistance were developed (Papolu et al. 2016). Interestingly, many future advanced studies that associated with gene stacking to improve quality, quantity, and biotic and abiotic stress resistance of eggplant particularly in omics era are indispensable.

4.13 Protection of Eggplant Plant Material

Usually, plant breeders exert their intelectual property rights by protecting the eggplant varieties they developed according to the International Union for the Protection of New Varieties of Plants (UPOV) requirements (Sanderson 2017). This is usually done by filing an individual application for each variety with the authorities of UPOV members granting breeders' rights. New varieties have to conform to the Disctinctness, Uniformity, and Stability (DUS) criteria according to the descriptors used for evaluation of eggplant, which in the case of the UPOV guidelines for eggplant include 43 morphological descriptors (UPOV 2002). New eggplant varieties can have different genetic constitutions, typically being lines or hybrids and nowadays, only in the European Union there are 405 registered eggplant varieties (European Commission 2020). An special case of protection in the European Union for landraces with distinctive features, is including them in the register of conservation varieties. In the case of eggplant, there is one eggplant conservation variety, which is the 'Almagro' eggplant, grown in a limited geographical area in the center of Spain, and used for pickling (Hurtado et al. 2014).

4.14 Future Perspectives

Eggplant is a major vegetable crop with an increasing global cultivation. However, eggplant is exposed to many abiotic and biotic stresses. Abiotic stress includes salinity, drought and high tempratuers, and biotic stress includes soil-borne diseases and many pests such as nematode, spider mite, whiteflies, aphids, eggplant fruit and shoot borer, leafhopper, and thrips, in particularly tropics and subtrobics (Taher et al. 2017, 2020). Eggplant wild relatives are richer than cultivated eggplant in genetic variations and could provide a great source for resistance to biotic and abiotic stresses (Daunay and Hazra 2012). Commercial varieties containing wild relative introgressions are not yet available and more research is required on using wild relatives in eggplant breeding. In the past, researchers had limited molecular markers to work with and could not easily introgress resistance genes into cultivated genetic backgrounds because of wild characteristics linked at other resistance loci. Now with functional genomics we have saturated markers, and such crossovers can be detected in each region. From that point forward, these genes could be easily deployed in pest resistant cultivars in the future. Of course if disease and insect resistance genes are

combined in a single vaieity it would likely be a durable resistance package for biotic stress especially under climate change.

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Chapter 5
Genomic Design for Biotic Stress Tolerance in Vegetable Brassicas

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Abstract Vegetable Brassica species comprise various agro-economically significant crops that offer nutrition and health-promoting elements to humans globally. In recent years, the major constraint of the *Brassica* crop production is constantly evolving fungi, virus, bacteria and insects causing variety of diseases, ultimately affecting quality and quantity of plant products. Among many of them, major threats to crop productions are clubroot, Fusarium wilt, stem rot, black leg, downy mildew, diamondback moth and TuMV disease. Traditional approaches of disease management are largely expensive, offer incomplete efficacy, and cause, in some cases, environmental harm; however, the best strategy is to identify resistant genetic resources, mining of genes/loci and deploy them in *Brassica* crop improvement programs. Combination of molecular breeding tools with advanced next generation sequencing (rapid and cost-effective) derived methods enables quick detection of resistant genes, and development of molecular markers that can be utilized in resistant breeding. Altogether in this chapter, we present a review on how vegetable Brassicas can be improved to address diverse biotic stresses, their plant genetic resources, genetic and genomics tools for their introduction into the cultivated *Brassica* crops and subsequently developing *Brassica* crops resistant to the adverse biotic conditions.

Keywords Biotic stress · Resistant genes · Genomics · Gene mapping · Marker-trait association · Marker assisted selection · Bioinformatics

5.1 Introduction

The genus *Brassica* is an important member of the Brassicaceae (Cruciferae) family which consists of about 39 species with high economic importance [source http://

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www.theplantlist.org/ (Al-Shehbaz et al. 2006)]. Six of these *Brassica* species establish U's Triangle (Nagaharu and Nagaharu 1935) containing three diploid species, viz. *Brassica rapa, B. nigra* and *B. oleracea* and the remaining three allotetraploid viz. *Brassica juncea, B. napus* and *B. carinata* (Fig. 5.1). The widely accepted U's triangle not only offer information on fundamental relationship between *Brassica* species but also act as a basis of evolutionary research for intra- and interspecific hybridization for gene/loci exchange. Each of these six species consists of major economic crops that are globally cultivated for vegetable (leafy and root), oilseed, condiments and forages (detailed representation in Fig. 5.1). Briefly, *B. rapa* is mainly cultivated for leafy (Chinese cabbage and pakchoi) and root (turnip) vegetables, *B. oleracea* includes commercially significant leafy vegetables such as cabbage, broccoli, cauliflower and kale however, *B. napus* majorly include oilseed crops like oilseed rape and canola. *B. carinata*, *B. nigra* and *B. juncea* comprise important oilseed crops such as Ethiopian mustard, black mustard and Indian mustard, respectively (Fig. 5.1).

Global climate change, variation in pathogen infection, use of inappropriate farming practices leads to disease onset which are major constraints for yield and production of *Brassica* crops worldwide. The loss of production in *Brassica* crops are due to various pathogens including diverse fungi, virus, bacteria and oomycetes. These pathogens lead to variety of diseases to crops plants but major constraints to crop productions are due to clubroot, Fusarium wilt, stem rot, black leg, downy mildew, and Turnip mosaic virus (TuMV) diseases (Fig. 5.1). Globally, these diseases received more attention and studied extensively due to their severe infestation and devastating nature to crops from many years. Several traditional practices to avoid disease infection such as physical, cultural, biological, or chemical practices, or a combination of these practices along with integrated pest management (IPM) are

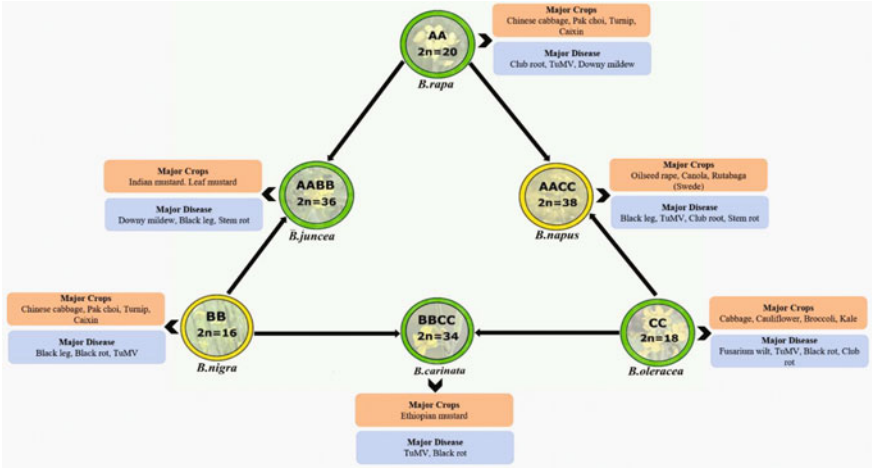

Fig. 5.1 Description of major representative *Brassica* crops and diseases in Brassicaceae family displayed in the form of U's triangle. Each crops are mentioned with their ploidy nature and chromosome numbers along with representative crops and associated diseases

being implemented. Among which, IPM is a well-studied and implemented practice with some successful example for few diseases. Nonetheless these methods are inadequate to provide resistance as they are complex, costly and environmentally harmful. However, natural resistance in *Brassica* for these diseases are appropriate way for durable resistance which is cost-effective.

In genomic design for future crops, 'disease resistance' is an important trait to avoid yield loss and maintain quality of crop products. This disease resistance governed by either pathogen/microbe-associated molecular pattern (PAMP/MAMP)-triggered immunity or effector triggered immunity elicited by resistant (R) genes. In genomic era, several R genes/loci have been identified for these diseases and deployed for resistance however, in actual applied sense, the effective utilization of a novel R gene/locus into a crop plant is subject to the (1) detection of a positive phenotype, (2) the development of genetic/molecular markers for marker-assisted selection (MAS) breeding, and (3) understanding the nature of novel resistance as it will work in diverse genetic backgrounds and pathogenic atmosphere in the field. In this chapter, we review the major diseases of vegetable Brassicas, recent information of identified molecular markers and quantitative trait loci (QTL)/R genes developed, MAS breeding, genomics aided breeding and use of transgenic technologies for several important diseases in Brassicas.

5.2 Description on Different Biotic Stresses in Vegetable *Brassica* Species

Brassica crops are continuously attacked by several pathogens and insect pests leading to reduction in quality and yield of crops. In this section, we described some of the major diseases of vegetable *Brassica* crops worldwide.

5.2.1 Clubroot

The infection of obligate parasite namely *Plasmodiophora brassicae* Woronin, causes clubroot (CR) which is widely known as a major devastating disease in Brassicaceae. Clubroot was first identified in 1878 in Russia and further quickly spread to Europe, South Africa, Brazil, China, South Korea, Australia, New Zealand, America and globally emerged as a major constraint in *Brassica* crop production (Perez-Lopez et al. 2018). *P. brassicae* demonstrates multifarious pathotypes and for the correct identification of pathotypes, two differentiation systems are widely used (1) William's system and (2) European clubroot differential set (Williams 1966; Donald et al. 2006). Use of traditional practices like chemical and physical methods are inadequate to gain control of the disease. Besides, the variation in pathogen and its persistence as a resting spore in the field, makes difficult to control as further crops are always at

high threat of secondary infection. The utilization of *P. brassicae* resistant cultivars in *Brassica* improvement is an ideal approach.

5.2.2 Fusarium Wilt

Fusarium wilt (FW) is a common vascular wilt fungal disease, caused by the fungus *Fusarium oxysporum* f. sp. *conglutinans* (Foc), is posing a threat to *Brassica* production worldwide, especially for vegetable *Brassica* plants (Bosland and Williams 1988; Enyaet al. 2008). The general symptoms in the infected plants include browning of vascular tissue, leaf wilting, marginal necrosis, and eventually death, resulting in serious crop yield loss and reduced commercial value (Li and Hartman 2003; Michielse and Rep 2009). *F. oxysporum* is a common soil saprophyte, which was found in many environments and survives for long periods in the absence of a suitable host plant. Since the chemical and biological control of Fusarium wilt is ineffective, the methods to control Fusarium wilt are mainly focused on breeding disease-resistant varieties (Kawamura et al. 2016).

5.2.3 Stem Rot

Stem rot is caused by the *Sclerotinia sclerotiorum* (Ss) which is the most endemic, omnivorous, soil-borne, destructive, and ubiquitous plant pathogen of *Brassica* and among over 500 other plant species. In 1837, the first time *Sclerotinia sclerotiorum*was reported as the pathogen of stem rot and now in the current scenario is found worldwide (Cheng et al. 2020). *S. sclerotiorum* can persist for numerous years in the soil and distributes throughout the tilled level of the soil. Till now, eight pathotypes of *Sclerotinia sclerotiorum* have been identified, the host range of this disease is so broad which makes it difficult to control it (Bolton et al. 2006; Mei et al. 2011; Barbetti et al. 2012). Stem rot is the most predominant and catastrophic disease affecting *Brassica* production, especially in oilseed rape (*Brassica napus* L.), the second most important oil crop worldwide. It can cause 10% to 80% yield to lose with low quality of oil (FAOSTAT, 2015; Qasim et al. 2020). The most cardinal approach for the control of this disease is the use of resistant cultivar (Derbyshire and Denton-Giles 2016), but grievously, there is no remarkably immune germplasm that has been found in *Brassica* species which makes it difficult to develop stem-rot resistant variety through breeding.

5.2.4 Black Leg

The *Leptosphaeria maculans (Lm)* is a disastrous and ubiquitous pathogen and causes blackleg or stem cancer, the most serious and deadly fungal disease in *Brassica*s, including *Brassica napus* (canola or rapeseed) worldwide (Fitt et al. 2006). The first time this disease was noticed in the stem part of dried red cabbage (*Brassica oleracea*) by Tode (1791) and later the fungus was named and cited by Henderson (1918). At the end of the nineteenth century and the beginning of the twentieth century, severe epidemics of blackleg has been reported in cabbage, cauliflower, and fodder *Brassica* growing areas, particularly in Australia, Europe, and Northern America (Henderson 1918), though in the mid of 20th-century blackleg has become the more problematic for oilseed rape in the term of mass infection (Hall 1992; Sivasithamparam et al. 2005). An average annual yield loss associated with this disease was noted around 10 to 20% in the main canola growing region (Toscano et al. 2003; Fitt et al. 2006; Van de et al. 2016; Zhang et al. 2018). When this disease in uncontrolled conditions, particularly in the early stage of plants mainly in the four- to five-leaf stage, losses range between 30 and 50% (Sprague et al. 2016). *Leptosphaeria maculans* is having very high diversification and has been described as different pathotypes/races, groups, and subgroups (Williams and Fitt 1999; Howlett et al. 2001; Balesdent et al. 2005). This fungal disease usually disseminates through rain and air, and through the production of fruiting bodies (pseudothecia and pycnidia), it can remain on infected crop residue for many years (West et al. 2001; Li et al. 2007; CCC 2021).

5.2.5 Downy Mildew

Downy mildew is a destructive and foliar disease of *Brassica oleracea* crops (Brussels sprouts, broccoli, cauliflower, cabbage, and, kale) and the causal agent is oomycete pathogen *Hyaloperonospora parasitica* (syn. *Peronospora parasitica*) (Göker et al. 2003; Vicente et al. 2012a, b). Primarily, in crucifers, 52 *Peronospora* spp. were identified based on morphological elucidations and cross-inoculation tests, and pathotype variation or physiological races are also illustrated in numerous studies, though, some studies have attained strong race differentiation and taxonomically corrected name of this pathogen in *Brassica* species is *Hyaloperonospora brassicae* (Jensen et al. 1999; Agnola et al. 2003; Göker et al. 2009). Worldwide this disease is dispersed wherever *Brassica* crops are cultivated especially, in Asia, Australia, and Europe (Yuen 1991; Vicente et al. 2012a, b). Higher disease frequency and a high degree of severity are observed especially in the autumn and spring season which is more conducive to attack of this pathogen (Yu et al. 2009). After infection, it can reduce the harvested yield by 16–20% and can infect up to 50–60% of cabbage seeds and it can damage all the stages of plant growth but the most damaging effect can be seen in young seedlings (Channon 1981; Saharan et al. 2017).

5.2.6 *Turnip Mosaic Virus*

Turnip mosaic virus (TuMV) belongs to the genus *Potyvirus* and is the second largest virus after *Cucumber mosaic virus* (CMV) that possesses wide range of plant hosts. This RNA virus is spread by lot of aphid species, and the disease causes great yield damage to many plant species worldwide (Edwardson and Christie 1991; Shattuck 1992; Ohshima et al. 1996). The TuMV has broad range of species diversity and more than 400 complete genomes from various parts of the world were sequenced and submitted to the public databases, GeneBank (Li et al. 2017; Yasaka et al. 2017). This viral disease causes great damage to many *Brassica* species including economically important crops, *B. rapa* (Lydiate et al. 2014), *B. oleracea* (Smith 1935) and *B. napus* (Li et al. 2019). Rapid genome variation and transmission by almost 89 aphid species, make difficult controlling and management of TuMV disease (Walsh and Jenner 2002).

5.2.7 *Diamondback Moth*

Apart from the above mentioned diseases, Brassica crops are affected by several minor insects leading to yield loss, one of such is Diamondback moth (DBM) *Plutella xylostella* L., The DBM is considered as one of the most damaging insect pests and major constraints for the production of cruciferous crops including vegetables like *B. oleracea* (cabbage, broccoli, collard), *B. rapa* (Chinese cabbage, turnip, pakchoi) and oilseeds such as *B. napus* and *B. juncea* worldwide (Eigenbrode et al. 1991; Talekar and Shelton 1993). After the use of insecticides in about 1950s, the widespread attack of DBM become prominent. DBM has ability to adapt quickly by generating resistance against insecticides and pesticides. The failure in implementation of biocontrol agents because of lack of natural enemies in environment helped to increase DBM population in *Brassica* crops. Additionally, DBM is one of the first insects to develop a resistance against *Bacillus thuringiensis* insecticide when sprayed in the open field (Tabashnik et al. 1990). Overall, present strategies for DBM management using chemical and biological methods are unsuccessful and there is urgent need to identify and deploy DBM resistant *Brassica* cultivars in cruciferous crop breeding programs.

5.3 Genetic Resources of Resistance Genes

5.3.1 *Primary Gene Pool*

Apart from the above mentioned diseases, other important diseases of *Brassica* vegetables are black rot [*Xanthomonas campestris* (Pam) Dawson (Xcc)], *Alternaria*

leaf spot (*Alternaria brassicae* and *Alternaria brassicicola*), black stem rot (*Pythium ultimum* Trow) and head rot of cabbage or wire stem of *Brassica* crops (*Rhizoctonia solani*) which cause huge yield losses. The resistant sources have been found with botanical varieties for immediate use in resistance breeding (Table 5.1).

In black rot disease, yellowing of leaves starts from leaf margin and extend in the direction of the midrib, followed by blackening of veins (vascular bundles). Cauliflower lines reported as resistant sources are Sn 445, Pua kea and MGS2-3 (Sharma et al. 1972); RBS-1, EC162587 and Lawyana (Sharma et al. 1995); Sel-12 (Gill et al. 1983); Sel-6–1-2–1 and Sel-1–6-1–4, Avans and Igloory (Dua et al. 1978). Saha et al. (2016) identified cauliflower genotypes BR-207, BR-202–2 and AL-15 as resistant to *Xcc1* (*Xanthomonas campestris pv. Campestris* 1).

Downy mildew can infect the crop at any stage of growth. It is systemic in nature and infection observed at seedling stage can reappear at curd and marketing stage (Coelho et al. 1997). It is prevalent in almost all countries of the world wherever

Table 5.1 Wild *Brassica* species as sources of tolerance to biotic stresses

Trait of interest	Source wild species
Leaf thickness/waxiness (insect and drought tolerance)	*Brassica cretica, Moricandia spp.*
Epicuticular wax columns	*Brassica alboglabra, B. bourgeaui, B. incana, B. hilarionis, B. macrocarpa, B. montana, B. insularis, B. rupestris, B. villosa*
Black leaf spot (*Alternaria spp.—Alternaria brassicae, A. brassicicola, A. raphani*)	*Alliaria petiolata, Barbarea vulgaris, Brassica nigra, Brassica fruticulosa, Capsella bursa-pastoris, Diplotaxis catholica, D. erucoides, D. tenuifolia, Brassica maurorum*
Blackleg (*Leptosphaeria maculans*)	*Brassica carinata, B. juncea, Brassica elongate, B. fruticulosa, B. nigra, Brassica insularis, B. atlantica, B. macrocarpa*
Downy mildew (*Hyaloperonospora parasitica*)	*Brassica oleracea, wild accessions*
Sclerotinia stem rot (*Sclerotinia sclerotiorum*)	*Capsella bursa-pastoris*
Black rot (*Xanthomonas campestris*)	*Brassica juncea, B. nigra, Brassica carinata*
Flea beetles [*Phyllotreta cruciferae* and *P. striolata*]	*Brassica incana, Brassica juncea, Brassica villosa*
Diamond-back moth (*Plutella xylostella*)	*Brassica juncea, Brassica oleracea*
Cabbage butterfly (*Pieris* spp.)	*Erysimumc heiranthoides (cardenolides), Iberis amara (cucurbitacin glycosides)*
Cabbage aphid (*Brevicoryne brassicae*)	*Brassica fruticulosa, B. spinescens, Brassica wild C genome: B. cretica, B. incana, B. macrocarpa, B. villosa, Erucavesicaria subsp. sativa, Sinapis alba*
Cabbage root fly or Cabbage maggot (*Delia radicum*)	*Brassica fruticulosa, B. incana, B. macrocarpa, B. spinescens, B. villosa*

crucifers are grown. The disease is serious during both rainy and winter seasons, and approximately 75–90% seedling mortality has been recorded (Gaikwad et al. 2004). Under favorable conditions, the pathogen affects 50–60% seed production of cole crops. Downy mildew resistance sources in cauliflower are BR-2, CC and 3-5-1-1; EC177283, Ec191150, EC191157, Kibigiant, Merogiant, EC191140, EC191190, EC191179, Noveimbrina, MGS2-3, 1-6-1-4,1-6-1-2 and 12C, KT-9, Early Winter Adam's White Head, CC-13, KT-8, xx, 3-5-1-1, CC, Perfection, K1079, K102, 9311 F1 and 9306 F1, Kunwari-7, Kunwari-8, Kunwari-4 and First Early Luxmi were reported moderately resistant to downy mildew (Sharma et al. 1995; Jensen et al. 1999; Trivedi et al. 2000; Pandey et al. 2001; Singh et al. 2013). The DNA markers linked to the *Ppa3* gene in cauliflower (Singh et al. 2012) and in *Brassica oleracea* (Carlier et al. 2011) were identified.

Stem rot has a very wide host range and can infect most of dicot crops, but is more severe in the seed crop of cauliflower, though, it may attack the crop at an early stage of its growth also. Moderate resistance to this pathogen was reported in EC131592, Janavon, EC103576, Kn-81, Early Winter Adam's White Head, EC162587 (non-winter type) and EC177283 (Sharma et al. 1995). They developed new lines from crosses involving 'Pusa Snowball I', 'Pusa Snowball K l' and resistant sources viz., 'EC 103,576', 'EC 131,592' and 'EC 162,587'. Of them, 'RSK 1301', 'Sel 25 (early)' and 'Sel 25 (late),] from crosses of 'EC 103,576', two lines i.e. 'RSK 1402' and 'RSK 1502' of 'EC 131,592' and two lines ('MRS l' and 'MRS 2') of' EC 162,587' were reported as promising for resistance and horticultural traits. Pandey et al. (2003) reported moderately resistant lines of early cauliflower to *Sclerotinia* rot, namely Kataki-6, Kataki-13, Patna Kataki, Deep Malika, Suryamukhi, Pusa Himkaran, Early Laxmi and PDVR early. However, Kataki-13 and Kataki-6 showed high degree of tolerance.

In cole crops, the black leaf spot (*Alternaria* spp.) disease is caused by *Alternaria brassicae* and *Alternaria brassicicola.* Brown to black, small to elongated spots appears on leaves, stems of older leaves. In younger plants, it may cause symptoms like *Rhizoctonia solani*. When the fungus infects the curd, especially in case of seed crop, the disease is called as inflorescence blight. The resistance sources in Indian cauliflower are MGS2-3, Pua Kea and 246-4, 23-7, 466, MS98, 210-21, Sel-9, 443-7 (Trivedi et al. 2000); IIHR142 and IIHR217 (Pandey et al. 1995) and Snowball KT-9 (Sharma et al. 1991). In cabbage, PI 291,998 was reported to be resistant to *A. brassicicola* (King and Dickson, 1994).

5.3.2 Secondary Gene Pool

Brassica oleracea is the major host for black rot (*Xanthomonas campestris* pv. *campestris*) (Vicente et al. 2001). This disease can cause severe damage, affecting up to 50% of the crop (Singh et al. 2011). Several resistant lines have been reported in *B. oleracea* (Lema et al. 2012; Saha et al. 2016; da Silva et al. 2020) and *B. rapa* (Lema et al. 2015), however, in these lines moderate level of resistance were detected.

Hence, search of black rot resistance in other sub-genomes has become a prerequisite to develop stable and strong resistance in commercial varieties. Tonguç and Griffiths (2004) reported no symptoms in *Brassica carinata* accessions A19182 and A19183, however, they observed segregation in plants of PI 199,947, PI 194,256 and PI 199,949 indicating that presence of susceptible and resistant genotypes. Since, transfer of genes/QTLs from these species into commercial cole crops may bring undesirable changes, hence precise location needs to be introgressed with maximum possible background genome recovery. Here, molecular biology tools have great role to identify, isolate and pyramid the target genomic regions into cultivated species (Prakash and Bhat 2007). The single gene resistance locus, *C*, was identified in *B. carinata*, located on linkage group B7 (Sharma et al. 2016a, b). Later on, introgression of this locus into *B. oleracea* was initiated using embryo rescue (Sharma et al. 2017).

5.3.3 Tertiary Gene Pool

Introgressions of desirable genes from wild species through sexual hybridization always encounter crossing barriers either at pre-or post-fertilization stage. Pre-fertilization barriers occur due to the inability of the pollen tube to reach the style and it can be overcome by following certain measures, namely grafting, mixed pollination, bud-pollination, stump pollination and in vitro fertilization (Kameya and Hinata 1970; Namai 1971). Post-fertilization barriers happen because of embryo abortion due to genetic incompatibility between the developing embryo and the endosperm. Embryo rescue is an effective way to overcome post-fertilization barriers. Shivanna (1996) reported that sequential culture (culture of ovaries, ovules and seeds/embryos) is more effective as compared to simple ovary or ovule culture. Further, somatic hybridization is the first choice used for introduction of desirable traits from alien species of secondary and tertiary gene pools. It has been extensively used in *Brassica* crops, since these are very amenable for tissue culture techniques. *Brassica* crops have vast pool of germplasm for use as source of novel genes/QTLs for important biotic challenges (Table 5.2). Sharma et al. (2002) identified *Brassica* coenospecies as rich reservoir for resistance to *A. brassicae*. They reported *Brassica desnottesii, Camelina sativa, Coincya pseuderucastrum, Diplotaxis berthautii, D. catholica, D. cretacea, D. erucoides*, and *Erucastrum gallicum* as completely resistant to *A. brassicae.* The cytoplasmic male sterile (CMS) system has been transferred from *Brassica juncea* with *Moricandia arvensis* cytoplasm and *B. napus* with *Erucastrum canariense* cytoplasm to *B. oleracea var. botryrtis* by embryo culture (Chamola et al. 2013). Sarmah and Sarla (1998) used *Erucastrum abyssinicum* to obtain CMS system in *Brassica oleracea* hybrids by ovary and ovule culture.

Table 5.2 Summary of different resistance gene (s) identified in *Brassica* vegetables and allied species

S. no	*Brassica* spp		Gene/QTLs	Linked/associated* markers	LG	References
1	*B. oleracea* var. *capitata*	Black rot	Two QTLs	RFLP	C01, C02	Camargo et al. (1995)
			Two QTLs, two minor QTL	SRAP & CAPS		Doullah et al. (2011)
			QTL-1 (Major), QTL-2 and QTL-3 (minor)	EST-SNP markers	C02, C09	Kifuji et al. (2013)
			150 unigenes	ESTs		Roohie and Umesha (2015)
			QTL-1 (Major) and three minor QTLs	SNP-based dCAPS	C2, C4 & C5, C01, C03, C06	Lee et al. (2015)
2	*B. oleracea* var. *botrytis*	Black rot	Single dominant gene	RAPD and ISSR	C3	Saha et al. (2014)
		Downy mildew	Single dominant gene *Ppa3*	RAPD and ISSR	NA	Singh et al. (2012)
			Single dominant gene *Ppa207*	SSRs (BoGMS0486 and BoGMS0900)	Chr 2	Saha et al. (2020)
3	*B. oleracea* var. *italica*	Black rot	Major QTL *XccBo*(*Reiho*) 2 and two minor QTLs *XccBo*(*GC*)1 and (*XccBo*(*Reiho*)1	pW, pX and BoCL	C8 and C9, C5	Tonu et al. (2013)
4	*B. rapa*	Black rot	Single major gene *R4*	RAPD WE(22)980	–	Ignatov et al. (2000a, b)
			One major QTL and two minor QTLs	AFLP, SSR	A06 and A02, A09	Soengas et al. (2007)
5	*B. carinata*	Black rot	Single dominant gene	RAPD	–	Tonguç et al. (2003)
			Single dominant gene (*Xca1bc*))	Two ILP flanking markers	B-7	Sharma et al. (2016a, b)
	B. napus	Black rot	Single dominant gene (Xca4)	pN215a and pN2dNP	N5	Vicente et al. (2002)

5.4 Glimpses on Classical Genetics and Traditional Breeding

5.4.1 Classical Mapping Efforts

Development of morphological, cytological, protein based markers have played key role in tracking of economic traits in crop plants. The first three sometimes designated as classical markers and mapping of economic traits using such set of markers is known as classical mapping. Qualitative morphological traits are the earliest and easiest set of genetic markers for genetic studies; however, the number of such usable markers is limited in crop species (Singh and Singh 2015). They also have limitations on stage specific expression, and sometimes, their expression has threshold requirement for expression (i.e. pathogen/insect-pest inoculum) and maintenance of such traits in vegetable *Brassica* crops is challenging due to highly cross-pollinated nature. Genetic analysis of progeny from inter-varietal crosses could reveal the inheritance pattern of traits which can be useful as morphological markers, namely annual plant habit (Detjen 1926; Dickson 1968; Baggett and Wahlert 1975; Pelofske and Baggett 1979), internode distance (Pease 1926; Dickson 1968; Pelofske and Baggett 1979), heading trait (Kristofferson 1924; Pelofske and Baggett 1979), flower color (Pearson 1929), curd color in cauliflower (Crisp et al. 1975; Crisp and Angell 1985), leaf characteristics (Kristofferson 1924; Pease 1926). Linkage analysis for genes controlling morphological traits using isozyme markers have been reported in *B. oleracea* by (Arus and Orton 1983) and in *B. campestris-oleracea* addition lines while monitoring the presence of specific alien chromosomes (Quiros et al. 1987).

The isozymes have been used in genetic diversity analysis of three most divergent species *B. rupestris, B. villosa,* and *B. macrocarpa* by Lazaro and Aguinagalde (1998) and they concluded the Sicilian region as a center of genetic diversity. Allen et al. (1986) employed isozymes to distinguish the origin of cauliflower stocks of United Kingdom and Australian types and also showed the European annuals and biennials as the parents of the Australian cauliflowers. The relationship between three diploids, namely *B. rapa* (A genome, $n = 10$), *B. nigra* (B genome, $n = 8$) and *B. oleracea* (C genome, $n = 9$) and three amphidiploid species, namely *B. juncea* (AB genome, $n = 18$), *B. napus* (AC genomes, $n = 19$) and *B. carinata* (BC genomes $n = 17$) which is known as the U-triangle (U, 1935). The cytological and isozyme markers have been useful to establish this relationship (Prakash and Hinata 1980; Coulthart and Denford 1982; Quiros et al. 1987). These markers could establish the two evolutionary pathways for three *Brassica* species: *B. rapa* and *B. oleracea* from one pathway having a common origin and *B. nigra* from another (Vaughan 1977; Attia and Robbelen 1986; Song et al. 1988; Warwick and Black 1991). The isozymes found effective in testing the purity and distinguish the F_1 hybrids in *B. oleracea* (Arus et al. 1985).

The isozymes were employed by Lamboy et al. (1994) in assessing the genetic diversity among *B. oleracea* (56 accessions) and found them partially useful. While, Van Hintum et al. (1996) could group 11 white cabbages and nine Brussels sprouts

using isozymes in crop specific clusters and Lanner-Herrera et al. (1996) reported similar results in wild cabbage germplasm from Spain, France and the United Kingdom. However, morphological markers have limitations in *Brassica* vegetables due to limited number of usable markers, biennial nature, complex nature of inheritance of economically useful traits, predominance of self-incompatibility (SI) and special techniques required for maintaining the genotypes. The usefulness of the isozymes was low as compared to DNA markers such as random amplified polymorphic DNA (RAPD) in genetic variability studies in *B oleracea* L. var. *acephala* DC. (Sawaza et al. 1997).

5.4.2 Breeding Objectives

The breeding objectives and methodology was earlier reviewed by Kalia (2009) and Kalia and Singh (2020). In *Brassica* vegetable crops, the breeding objectives can be grouped into following sections: (i) fundamental objectives i.e. crop uniformity for maturity, field appearance, shape and size of economic parts, high yield, resistance to common diseases (club root, Fusarium wilt, black leg, downy mildew, Alternaria leaf spot, Sclerotinia rot and black rot) and short crop duration, (ii) Special traits i.e. attractive colors of edible parts such as orange in cauliflower, head in broccoli and cabbage, sprouts in Brussels sprouts and knob in Knol khol, genotypes with wider curding plasticity for spatial and temporal expansion, tropical and sub-tropical flowering habit, since such colorful crops are gaining popularity in health-conscious consumers and super markets, (iii) Futuristic traits for consumers' health: selective glucosinolates profiles (high in glucoiberin and glucoraphanin, low in progoitrin), dietary minerals (Fe, Ca, Zn), vitamins (A, C), amino acids, and overall nutrient matrix to serve as functional food. Since it has been accepted that the glucosinolates in *Brassica* vegetables is crucial factor with anticarcinogenic properties (Verhoeven et al. 1997), preventive activities against Alzheimer, cataracts, aging associated functional declines (Granado et al. 2003) and also goitrogenic activities, (iv) Breeding genotypes with glucosinolates profiles to use as soil biofumigants and also as trap/repellent for specialist insects, (v) Breeding for specialty items such as sweet type in cauliflower and broccoli, sauerkraut type in cabbage, pickling type Knol khol and Brussels sprouts, (vi) Agronomical traits, such as rapid ground coverage to avoid weed infestation, adaptive to closer spacing levels and suitable for problem soils with low transport of harmful elements during partitioning in edible portions; (vii) Suitable for mechanical harvesting, tolerance to low light levels and amenable for hydroponic system, (viii) Breeding climate resilient genotypes in tropical and sub-tropical segments particularly, since the sensory and appearance traits of these crops are very sensitive to temperature fluctuations, (ix) Leaves are used as animal feed and breeding genotypes with high dry matter and low goitrogenic substance is a challenge, (x) Development of hybrids using available genetic mechanisms viz., self-incompatibility and cytoplasmic male sterility is need of the hour to meet the demand of uniformity in maturity and produce, yield and resistance to major diseases.

5.4.3 Classical Breeding Achievements

In *Brassica* vegetables, the classical breeding has been pivotal in development of a large number of varieties and hybrids across the world. It also helped in development of tropical and sub-tropical types in cauliflower, broccoli, cabbage and kale. Further, recurrent selection was employed for improvement of specific traits like heat tolerance, sub-tropical flowering and tolerance to common diseases (black rot, Alternaria leaf spot). In *Brassica* vegetables, Pusa Shubhra was developed as an Alternaria leaf spot resistant variety of Indian cauliflower, Pusa Mukta of cabbage for black rot, Pusa Snowball K-1 of cauliflower with moderate tolerance to black rot, Pusa Virat of Knol khol having tolerance to frost and Pusa Ageti of cabbage as a tropical flowering 'no chill' type. Besides, a number of varieties in individual crop of *Brassica oleracea* have been developed for yield and horticultural traits (Table 5.3). However, their breeding took long time which, of course not available with breeders due to changing consumer demand and growing stress situations. Therefore, the role of molecular tools and techniques is vital to take care of genetic complexities and dynamic consumer needs in shortest possible time.

5.4.4 Limitations of Traditional Breeding and Rationale for Molecular Breeding

Traditional breeding is primarily based on phenotypic selection of superior individuals among segregating progenies, which is, often, time consuming as breeding a new variety takes 8–12 years and even then, the release of improved variety is not guaranteed. Although, it has been instrumental in developing a range of varieties in crop plants including *Brassica* vegetables but, now it became impossible to match the pace of rapidly evolving consumer preferences and changing growing environments. For this, the modern, time saving tools such as molecular markers offer such a possibility by adopting a wide range of novel approaches to improving the selection strategies in *Brassica* crops. Since, these are powerful research tools that make it possible to determine the genetic makeup of plants; they also serve as reference points to compare differences in DNA sequence and consequently, the allele composition between plants. Genetic dissection of complex traits such as glucosinolate pathway in across *Brassicaceae species,* curding behaviour in cauliflower, flowering mechanism in broccoli and cauliflower, heading traits in cabbage, pigmentation in leaves of red Chinese cabbage and in cabbage as well as curds of cauliflower, evolutionary trend in *Brassica species*, resistance to black rot, downy mildew and club root using molecular tools could facilitate introgression of gene(s)/QTLs in desirable backgrounds, which, otherwise, was difficult through conventional breeding methods. Molecular markers have provided a rapid method to screen parental germplasm for genetic variation, develop genetic linkage maps and tag genes controlling important traits assisting in selecting breeding progeny carrying desirable alleles. Their role is supporting the

Table 5.3 Details of commercial varieties of Brassica vegetables developed through classical breeding in India

Crop	Variety	Year	Source	Days to maturity (DAT)	Head/tuber weight (g)	Yield (t/ha)	Head/tubershape	Remark
Cabbage	Pusa Ageti	2000	IARI, New Delhi	70–90	600–1200	11–33	Flattish round	1st tropical variety, produce seeds in plains
	Golden Acre	1976	IARI RS, Katrain	60–65	1000–1500	25	Round, compact	Early, interior white, excellent quality
	Pusa Mukta	1985	IARI RS, Katrain	70–75	1500–2000	20–30	Flattish-round, solid	Moderately Resistant to black rot, Heads burst if harvesting delays
	Pusa Drum Head	1970	IARI RS, Katrain	90–100	3000–4000	50–54	Flat, less compact	Field resistant to black leg
Cauliflower	Pusa Meghna	2004	IARI, New Delhi	70–75	200–400	8–12	Creamish white, compact, small curd	September end maturity (25–27 °C), semi-erect, dwarf plants, puckered leaves
	Pusa Ashwini	2016	IARI, New Delhi	80–90	500–600	14–16	White, compact, medium size curd	Semi-erect, semi-vigorous plants, October 2nd fortnight maturity (20–25 °C)
	PusaKartiki:	2016	IARI, New Delhi	85–95	500–700	16–20	White, compact, medium curd	Semi-erect, semi-vigorous plants, October end maturity (20–25 °C)
	Pusa Sharad	2002	IARI, New Delhi	75–90	600–800	22–25	White, compact, granular curd	Semi-erect, semi-vigorous plants, short stem, November mid to December mid maturity (16–20 °C)

(continued)

Table 5.3 (continued)

Crop	Variety	Year	Source	Days to maturity (DAT)	Head/tuber weight (g)	Yield (t/ha)	Head/tubershape	Remark
	Pusa Paushja	2008	IARI, New Delhi	80–90	800–1000	28–32	White, compact, full size curd	Semi-erect, medium dwarf plants, short stem, December 2nd fortnight maturity (12–16 °C)
	Pusa Shukti	2008	IARI, New Delhi	90–100	1000–1500	30–35	Creamish white, compact, full size curd	Erect, vigorous plants, December end to 1st fortnight of January maturity (12–16 °C)
	Pusa Snowball K-1	2002	IARI RS, Katrain	100–115	900–1000	25–30	White, compact curds	Medium vigorous, snowball group, maturity in January end to February mid
	Pusa Snowball Kt-25	2004	IARI RS, Katrain	95–105	900–1000	30–35	White, compact curds	Medium vigorous, snowball group, maturity in January end to mid February
	Palam Uphar	2007	HPAU, Palampur	90–100	850–900	20–25	White, compact curds	It is 25–30 days early than PSBK 1, Field resistant to black rot and downy mildew. Medium frame, bluish green leaves and sets seeds under mid hill conditions of HP

(continued)

Table 5.3 (continued)

Crop	Variety	Year	Source	Days to maturity (DAT)	Head/tuber weight (g)	Yield (t/ha)	Head/tubershape	Remark
Broccoli	Pusa KTS-1	1996	IARI RS, Katrain	90–100	300 -400	10–16	Semi-compact	Short duration, medium fine buds, Green sprouting broccoli
	Palam Samridhi	1995	HPAU, Palampur	80–90	300–400	15–20	Semi-compact	Short duration, medium buds, Green sprouting broccoli
	Palam Vichitra	2003	HPAU, Palampur	115–120	350–450	20–22	Compact	Late maturity, vigorous plant, fine buds, purple heading broccoli
	Palam Kanchan	2003	HPAU, Palampur	140–145	350–400	25–27	Compact	Very late maturity, medium vigorous, fine buds, Yellowish green heading broccoli
	Palam Haritika	2003	HPAU, Palampur	145–150	300–400	17–22	Semi-compact	Very late maturity, vigorous plant, medium fine buds, Green sprouting broccoli
Knol khol	Palam Tenderknob	2004	HPAU, Palampur	45–50	800–850	20–25	Flattish round	Small green foliage, round knobs, flat, free from stringless and fleshy. Early maturity by about one week than White Vienna variety

(continued)

Table 5.3 (continued)

Crop	Variety	Year	Source	Days to maturity (DAT)	Head/tuber weight (g)	Yield (t/ha)	Head/tubershape	Remark
	Pusa Virat	2008	IARI RS, Katrain	50–60	700–800	17–20	Round	Big round knobs with 13–14 cm diameter and stringless. Seed sowing may be done during April to October under hilly regions and October to December in the north Indian plains
Kale	Pusa Kale-64	2019	IARI RS, Katrain	–	–	30–35	–	It has highly serrated, purplish green leaves, 40–50 cm in length and 15–20 cm in width, plant height is 50–60 cm. It has high tolerance to cold and frost conditions
Chinese cabbage	Palampur Green	1992	HPAU, Palampur	140–150	–	25–30	Fan shaped leaves	Itis a non-heading variety which produces medium green and smooth leaves free from purple pigmentation but with a prominent cream coloured petiole, first harvest 3–4 weeks after transplanting, 5–6 harvests at an interval of 4 weeks and late bolting

traditional breeding to enhance the precision and expediting the traditional breeding processes. Further, adequate genetic and genomic information is available now in major *Brassicaceae* crops and its ortholog species which can help to design more efficient breeding tools and strategies tailoring trait specific genotypes.

5.5 Brief Account of Molecular Mapping of Resistance Genes and QTLs

5.5.1 Clubroot

Comprehensive genetic studies have been performed for clubroot (CR) disease resistance among all the diseases, generating enormous resistant loci in *B. rapa*, *B. oleracea* and other *Brassica* species. In *B. rapa*, significantly important resistant genes harboring complete tolerance to specific pathogen have been detected over 20 years. Till now, about 23 major clubroot resistance loci have been identified in which, one of the first mapped is *CRa* encoding toll/interleukin-1 receptor (TIR)-nucleotide binding site (NBS)-leucine-rich repeat (LRR) gene (Matsumoto et al. 1998; Ueno et al. 2012). The other loci are as follows, *CRb* from the Chinese cabbage (Piao et al. 2004; Chen et al. 2013), *CRc* and *CRk* (Sakamoto et al. 2008), *CRaki* (Kato et al. 2012, 2013), *Crr1* and *Crr2* (Suwabe et al. 2003, 2012), *CRd* (Pang et al. 2018), *Crr3* (Hirai et al. 2004), *Crr4* (Suwabe et al. 2006), *PbBp3.1*, *PbBp3.3* (Chen et al. 2013), *CrrA05* (Nguyen et al. 2018), *qBrCR38-1*, *qBrCR38-2* (Zhu et al. 2019), *CRs* (Laila et al. 2019), *Rcr1* (Chu et al. 2014), *Rcr2* (Huang et al. 2017), *Rcr4*, *Rcr8* and *Rcr9* (Yu et al. 2017) and *Rcr3*, $Rcr9^{wa}$ (Karim et al. 2020) distributed on seven different chromosomes in *B. rapa.*

In *B. oleracea,* the clubroot resistance is controlled by quantitative genes however, handful loci have been found to confer complete resistance. In broccoli, the first resistant QTL was mapped against *P. brassicae* race 7 (Figdore et al. 1993). Further, Grandclement and Thomas (1996) identified QTL with RAPD markers in the resistant kale line C10 while, Morugachi et al. (1995) and Nomura et al. (2005) detected two and three loci respectively in another resistant kale line K269. For the first time, Voorips et al. (1997) identified two QTLs namely *Pb-3* and *Pb-4* in cabbage, later Nagaoka et al. (2010) identified *pbBo(Anju)1* in cabbage and studied comparative analysis of CR genes. The identification of numerous CR loci in *B. oleracea* suggest that, CR resistance is regulated in a polygenic mode, signifying the complex molecular basis of the resistance, in which particular resistance locus is hardly sufficient to impart complete resistance (Tomita et al. 2013). The comparative QTL mapping is presently difficult due to an absence of common molecular markers between various studies and utilization of diverse clubroot resistant sources and pathotypes (Nagaoka et al. 2010; Lv et al. 2020).

In one of the first study in *B. napus,* two dominant QTLs *CR2a* and *CR2b*, were detected in rutabaga exhibiting resistance to race 2 of *P. brassicae* showing phenotypic variation from 15 to 58% (Landry et al.1992). Further, a major gene, *Pb-Bn1* mapped on A03 along with two minor QTLs on C02 and C09 in Darmor-bzh of *B. napus* (Manzanares-Dauleux et al. 2000). Werner et al. (2008) used doubled haploid (DH) population and identified 19 QTLs on eight different chromosomes that showed resistance to seven diverse pathotypes however none of them could show resistance to all of the isolates. In addition to this, genetic analysis in canola positioned, five previously identified QTLs in *B. rapa* and developed 12 markers associated to the *CRa* locus, signifying that this locus might have origin from A genome (Fedua-Agyeman and Rahman 2016). Diederichsen et al. (2006) identified single locus linked to *CRa* in Mendel and two other recessive genes in Mendel's progenies.Furthermore,a genomic region mapped on chromosome A8 was detected using rutabaga-derived populations harboring resistance to five pathotypes such as 2, 3, 5, 6, and 8 indicating that single or cluster of genes responsible for resistance to these CR races (Hasan and Rahman, 2016).

Li et al. (2016a, b) used genome-wide association (GWA) approach which quickly detect recombinants and variations via natural populations based on whole-genome SNP data of 472 accessions for clubroot resistance. Using integrative analysis, a total of nine loci were characterized and of which seven are novel and six belongs to C genome. Dakouri et al. (2021) deployed genome-wide association studies (GWAS) strategy to 177 *B. napus* accessions and analyzed their effect towards four different pathotypes namely, 5X, 3A, 2B and 3D. They could identify 13 significant SNP loci among which nine SNPs are mapped to the A-genome while four to the C-genome which further used for marker development.

5.5.2 Fusarium Wilt (FW)

Recently, many genetic studies on Fusarium wilt resistance in *Brassica* crops have been reported. QTL mapping approach was used to detect the resistance genes responsible for Fusarium wilt in Brassica crops. Resistance phenomenon has been mostly uncovered from the genome of *B. rapa* and *B. oleracea.* There were three important loci identified in *Brassica* crops thus far (Table 5.4). *Foc-Br1*, a dominant gene for pathotype race 1, was first located by Shimizu et al. (2014) in A03 chromosome based on gene expression which revealed presence and absence of sequence of the putative R-genes, *Bra012688* and *Bra012689* and further correlated with the resistance of six inbred lines and susceptibility of four inbred lines, respectively (Shimizuet al. 2014). Most FW resistance resources have been identified in *B. oleracea.* The FW resistance locus *FocBo1* was first mapped to linkage group seven using both BSA and QTL analysis by Pu et al. (2012). Further, *FocBo1* locus was fine-mapped within 1.00 cM between markers, BoInd 2 and BoInd 11, using 139 recombinant F_2 plants derived from resistant cabbage (AnjuP01) and susceptible broccoli (GCP04) DH lines (Shimizuet al. 2015). The gene *FocBo1* was found to be homologous with the

Table 5.4 Resistance genes/QTLs for Fusarium wilt resistance identified in *Brassica* species

Species	QTLs	Parental lines/population	Pathogen race/isolates	Position	Linked marker	References
B. rapa	*Foc-Br1*	RJKB-T21, RJKB-T23 (Resistance) and RJKB-T22, RJKB-T24 (susceptible) /F_2	Cong: 1-1 strain	A03	Bra012688m, Bra012689m, and focbr1-2 m	Shimizu et al. (2014); Kawamura et al. (2016); Miyaji et al. (2021a)
B. oleracea	*FOC1*	99–77 (Resistance) and 99–91 (susceptible)/F_1, F_2	FGL3-6strain	C06	InDel marker: M10, A1 and Frg13	Lv et al. (2013, 2014); Li et al. (2017)
B. oleracea	*Foc-Bo1*	Anju (Resistance) and Green Comet (susceptible)/ F_2	Cong: 1-1 strain	C07	SSR marker: KBrS003O1N10; InDel marker: MTK-1	Pu et al. (2012); Shimizu et al. (2015)

candidate resistance gene *Bra012688* in *B. rapa* (Fig. 5.2) (Shimizu et al. 2015). At the same time, the other locus *FOC 1* also mapped in 1.8 cM interval between two adjacent InDel markers on C06, based on a cabbage DH population (Lv et al. 2013) and further identified the candidate gene *Bol037156*, which encodes a TIR-NBS-LRR (Lv et al. 2014).

5.5.3 Stem Rot

Nearly most of the mapping work in this context has been done in *B. napus,* however, the studies on both *B. oleracea* and *B. napus* have aided to discover resistance-related QTLs in three genomes A, B, and C of monogenomic, and digenomic *Brassica* species, but none of them has been commercially exploited because of the poor level of resistance (Atri et al. 2019). In initial studies, Zhao and Meng (2003) were first to identify a total of six QTLs with leaf and stem resistance (3 of each) in the seedling and mature stage, but no common QTLs were found among them. In two segregating DH populations, the HUA and MS population, eight and one QTL(s) were identified, with each explaining 6–22% of the variance, but also, in this case, no common QTLs were identified (Zhao et al. 2006). Yin et al. (2010), identified ten, one, and ten QTLs using three inoculation methods, MTI, MPI, and IPI, respectively in one DH population, among this total two common QTLs were found. Wu et al. (2013), found a total of ten QTLs at the adult stage for stem resistance, and three QTLs for leaf resistance at the seedling stage. A candidate resistant gene *BnaC.IGMT5* homolog to *Arabidopsis* and related to the *SRC6* locus was first identified and two major QTLs were identified repeatedly. These studies conclude that there are plenty of QTLs identified but rarely common ones were found which

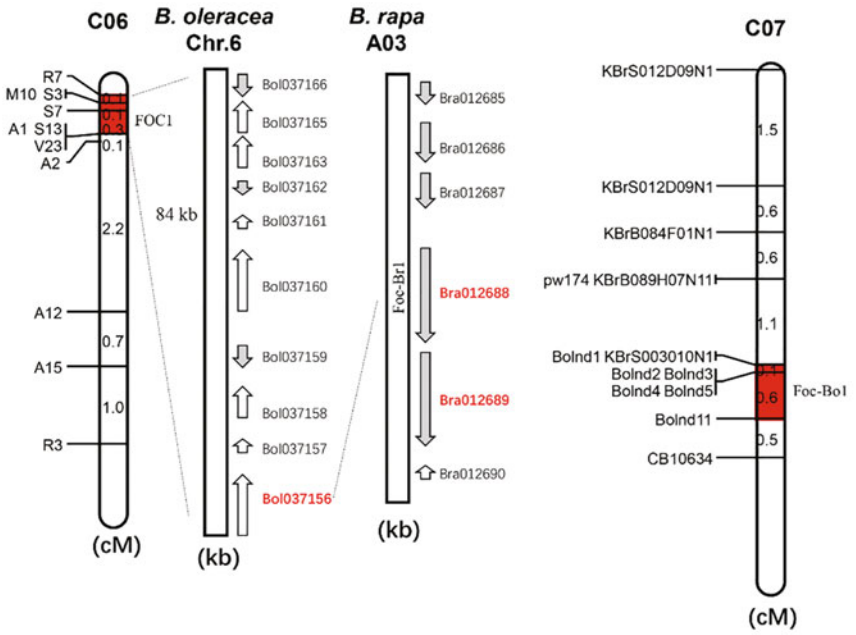

Fig. 5.2 The location of Fusarium wilt resistance loci in *B. oleracea.* The red box indicates *FOC1* QTL region mapped on C6 chromosome of about 86 kb region containing several important candidate genes including *Bol037156*. Foc-Bo1 locus mapped on C7 of *B. oleracea*; in *B. rapa* A03 represents the candidate genes in the *Foc-Br1* locus

describes the complication of the genetic structure of these plants. But lately, the release of the *B. napus* genome sequence made the mapping work slightly eased. Fomeju et al. (2014), used 116 varieties genotyped with 3,228 SNPs in a GWAS and specified that 321 markers, corresponding to 64 genomic regions were involved with resistance to stem rot. Wei et al. (2016) combined both the SNP array analysis and GWA analysis to elucidate the resistance genes, a total of 347 *B. napus* accessions were used and 17 significant associations were identified on chromosomes A8 and C6 for stem resistance. The SNPs which were found on the A8 chromosome were placed in a 409-kb haplotype segment. The same method was adopted by Wu et al. (2016), in this study 448 accessions genotyped were used and a total of 26 SNPs associated with stem rot resistance were found which were corresponding to three loci (*DSRC4, DSRC6,* and *DSRC8*) and a total number of 39 candidate genes were identified. Gyawali et al. (2016) used 152 accessions for GWAS analysis with microsatellite markers and identified 34 loci significantly associated with traits and out of which 21 lead to resistance, whereas the remaining contributed susceptibility. Qasim et al. (2020a), identified 17 QTLs associated with stem rot resistance using SNP markers, but no common QTLs were identified.

5.5.4 Black Leg

Since the 1990s, various mapping works have been performed regarding resistance genes and several cultivars are available which are blackleg resistant. In the previous studies mostly blackleg QTL/resistant genes were isolated from *B. napus* genome. In *B. napus* Ferreira et al. (1995) localized the main locus *LEM1* on N7 using a DH population of 101 lines and 138 RFLP markers. Later the same method was applied by Dion et al. (1995), who detected another major gene *LmFr1* using 98 DH lines. Mayerhofer et al. (1997) identified a major locus, *LmR1* using 13 RAPD and 2 RFLP markers in a segregating DH population and later using fine-scale mapping, co-segregating markers were developed (Mayerhofer et al. 2005). Delourme et al. (2004) conducted a study in which a resistant cluster containing five R genes (*Rlm1, Rlm3, Rlm4, Rlm7*, and *Rlm9*) and two genomic regions (on LG10 and LG16) were mapped and proposed as the candidate. After 2010, fine-mapping work was performed expansively. Long et al. (2011), discovered two resistant genes named *BLMR1* and *BLMR2*, and after fine mapping of *BLMR1* with 12 genome-specific markers, the closest marker with a genetic distance of 0.13 cM was identified. Jestin et al. (2011), discovered five novel alleles using 128 oilseed rape lines through the association mapping method. Raman et al. (2012), identified a new locus *Rlm4* on chromosome A7, and a further deposited region has been analyzed with many candidate genes (Tollenaere et al. 2012). Besides, blackleg-resistant loci have been introgressed from wild-type relative *B. rapa* and *B. oleracea* to *B. napus* through interspecific hybridization (Yu et al. 2012). Yu et al. (2005, 2008) located blackleg resistance *LepR1-LepR3* from wild type relative of *B. rapa.* Larkan et al. (2013, 2014) identified and cloned the first functional blackleg resistant gene *LepR3* using map-based cloning, they further isolated allele variant of *LepR370* as *Rlm 2* gene, located on chromosome A10 of the *B. napus* cultivar 'Glacier' (Larken et al. 2015). Recently the author discovered and cloned *Rlm9* genes from the *B. napus* cultivar Darmo which encodes a wall-associated kinase-like protein, a freshly emerging class of race-specific plant RLK *R* genes (Larkan et al. 2020). Raman et al. (2020) discovered two race-specific genes *Rlm3*, *Rlm4,* and 19 significant QTLs using 177 DH lines. Hossain et al. (2020), discovered blackleg resistance related domains in the collinear region of *B. napus* blackleg resistance locus *LepR2'* in *B. oleracea,* and high expression against the disease was observed in the LRR-MAP kinase gene *Bo9g126150* and the LRR-FBD gene *Bo9g111510*. Furthermore, some other blackleg resistance significant and stable QTLs (four and six in numbers) under different environments were also identified on some major locus in *B. napus* (Huang et al. 2016; Larken et al. 2016). Presently, R gene mapping work has been extensively studied which results in improved resistance of *Brassica* cultivars to blackleg disease.

5.5.5 *Downy Mildew*

Downy mildew resistance is supposed to be distinct with Brassica seedling and the adult period. The highly effective approach to control this disease is to breed genetically resistant varieties. Up till now, many R loci have been mapped and used in the breeding programs. Coelho et al. (2012), compiled six pathotypes and proposed five major-effect R loci associated with the observed phenotypes. Various R loci/genes have been identified through resistance mapping work. In *B. oleracea*, Giovannelli et al. (2002) identified the first locus providing resistance in the broccoli seedling stage in a linkage group, and later it was found near the glucosinolate linked gene *BoGsl-elong* and AOP gene family members *BoGSL-OH* and *BoGSL-ALK* (Gao et al. 2007). In Broccoli, one more single dominant resistant gene named *Pp523118* has been found expressed at the adult stage (Coelho and Monteiro 2003). The genomic region which contains this gene was further studied using cleaved amplified polymorphic sequence (CAPS) and sequence-characterized amplified regions (SCAR) markers, along with two bacterial artificial chromosomes (BAC) libraries (Farinhó et al. 2007; Carlier et al. 2012). In *B. rapa*, a key QTL conferring seedling resistance to downy mildew was identified, the main effect locus *BraDM* was mapped to a region spanning 2.9 cM on linkage group A8 using a genetic linkage map created with a DH population (Yu et al. 2009). Further, in *B. rapa*, a monodominant gene designated *BrRHP1*, which was localized on the A01 linkage group was detected for downy mildew resistance (Kim et al. 2011). In the time of modern technology, mapping methods based on high-throughput resequencing, offer abundant resources of R genes. For instance, a major locus, *sBrDM8*, was identified to a physical segment of ~228 kb on chromosome A08 and a serine/threonine kinase family gene, *Bra016457* through a high-density SNP-based map (Yu et al. 2016). Zhang et al. (2018) identified *Br-DM04* for downy mildew resistance in a region about 2.7 Mb mapped on chromosome A04 in *B. rapa.*

5.5.6 *Turnip Mosaic Virus*

Before the emergence of next generation breeding systems, traditional breeding (like chemical based methods) was the only approach that has been widely used to control TuMV or many diseases in *Brassica* breeding (Hughes et al. 2002; Rathore et al. 2018; Li et al. 2019). The chemical based approaches are hazardous, expensive and not effective. So many biotechnological techniques were introduced into breeding system to overcome these problems.

The first resistance gene mapped for resistance to TuMV was the dominant *Tu* gene in lettuce (*Lactuca sativa*) (Zink and Duffus 1970; Palukaitis and Kim 2021). Further many TuMV resistance genes were reported in *B. rapa* and *B. napus* compared to other Brassica species (Table 5.5). Of which, most of the genes happen to be dominant in nature. Till now five TuMV resistance genes were mapped including

Table 5.5 List of genes identified for TuMV resistance in *Brassica* species

Species	Gene	Chromosome	Effect on TuMv	References
B. napus	*TuRB01*	A6	Dominant	Walsh et al. (1999)
	TuRB02	linkage group N14	Dominant	Walsh et al. (1999); Walsh and Jenner (2002, 2006)
	TuRB03	chromosome N6	Dominant	Hughes et al. (2003)
	TuRB04; TuRB05	A. genome	Dominant	Jenner et al. (2002)
B. rapa	*ConTR01*	A08	Dominant	Rusholme et al. (2007)
	TuRBCH01	A06	Dominant	Xinhau et al. (2011)
	Rnt1-1	A06	Dominant	Fujiwara et al. (2011)
	TuMV-R	A06	Dominant	Chung et al. (2014)
	TuRB07	A06	Dominant	Jin et al. (2014)
	TuRB01b	A06	Dominant	Lydiate et al. (2014)
	TuRBCS01	A04		Li et al. (2015)
	retr01/retr02	A04	Recessive	Rusholme et al. (2007), Qian et al. (2013), Nellist et al. (2014)
	Trs	A04	Recessive	Kim et al. (2013)
B. juncea	*TuRBJU01*	–	Incomplete Dominant	Nyalugwe et al. (2015, 2016)
	retr03	–	Recessive	Shopan et al. (2017)

the *TuMV Resistance in Brassica 01- TuMV Resistance in Brassica 05* (*TuRB01-TuRB05*) and were reported in multiple studies (Walsh et al. 1999; Jenner et al. 2002; Walsh and Jenner 2006; Rusholme et al. 2007). Similarly, many dominant and few recessive genes (*ConTR01, TuRBCH01, Rnt1-1, TuMV-R, TuRB07, TuRB01b, TuRBCS01, retr01/retr02, and trs*) for various pathotypes were developed for TvMV in *B. rapa* (Xinhua et al. 2011; Fujiwara et al. 2011; Qian et al. 2013; Kim et al. 2013; Nellist et al. 2014; Lydiate et al. 2014; Li et al. 2015; Nyalugwe et al. 2016). And whereas in *B. juncea* two genes, a dominant, *TuRBJU01* and a recessive gene *retro03* have been reported (Nyalugwe et al. 2016; Shopan et al. 2017). A flanking dCAPS and a KASP markers were developed and applied in breeding programs (Li et al. 2016a, b). The alignment of TuMV disease' susceptible and resistant Chinese cabbage genotype specific gene (*retr02* and *Retr02*) sequences allowed to identify the nucleotide "G" variation between the genes. Similarly, other studies in Chinese cabbage used RFLP markers, *pN101e1* and *pW137e1* to map *TuRBolb* locus on Chromosome A06 (Lydiate et al. 2014). In addition, various molecular markers were developed and utilized for identification of genes related to TuMV disease in *B. rapa* (Hughes et al. 2003; Chung et al. 2014; Jin et al. 2014).

5.5.7 *Diamondback Moth*

Presently, inadequate data on genetic regulation of DBM in cruciferous crops are available. Initial studies in 1980 and 1990s identified resistant genotype named as Cauliflower Plant Introduction (PI) 234,599 and its progenies exhibited favorable resistance to DBM and other lepidopteron insects due to the glossy leaves structure (Dickson and Eckenrode 1980; Dickson et al. 1990; Eigenbrode et al. 1991). The waxy layer was responsible for glossy leaves trait that was inherited as single recessive gene which restricted DBM larvae feeding on leaves (Eigenbrode and Shelton 1990). Afterwards, many genes for glossy leaf traits from various sources were identified in *B. oleracea* (Stoner 1990).

In a rare genetic study, QTL mapping of DBM resistance loci in *B. napus* and *A. thaliana* was performed (Kliebenstein et al. 2002; Asghari et al. 2009). Kliebenstein et al. (2002) performed comparative analysis of QTLs for DBM resistance in *A. thaliana* and reported QTLs specific to one or more herbivore among which a two QTLs controlling DBM resistance were detected on chromosomes II and V. In *B. napus* three resistance QTLs were detected using SSR and RAPD markers on 180 $F_{2:4}$ populations (Asghari et al. 2009). However, no studies have been executed at the molecular level to detect QTLs that are tightly associated with DBM resistance traits. Ramchiary et al. (2015) identified eight genomic regions on five linkage groups in *B. oleracea* using QTL mapping harboring DBM resistance. Among these, one QTL *qDbm6* on LG7 was detected over three consecutive years and further developed molecular markers that could be used in marker-assisted selection. These discoveries indicate that genetic regulation of DBM resistance trait is complex phenomenon and governed by several genes, signifying that breeding of DBM resistance could be achieved with deployment of multiple loci or gene pyramiding in breeding program.

5.6 Marker-Assisted Breeding

Marker assisted selection (MAS) is the most effective approach of genotype selection (based on the sequence variation) linked to trait of interest using molecular markers. In maker-assisted breeding, combination of the marker and the other traditional breeding methods work together to facilitate the accelerated breeding process. Therefore, the molecular makers linked to the resistant loci need to develop for application of various resistant cultivars in breeding program. In MAS, a particular trait is selected based on a linked marker (morphological, preferably molecular).

5.6.1 Clubroot

Major limitation for clubroot resistance breeding is the existence of multiple CR pathotype and complex plant-pathogen interactions. However, utilization of diverse clubroot resistant genes in different combinations may result in increased resistance (Tomita et al. 2013; Doullah et al. 2011). For example, a clubroot resistant Chinese cabbage genotype, 'Akimeki', was developed by the introgression of *CRa*, *CRk*, and *CRc* genes. The study confirmed that, the accumulation of these CR genes through MAS not only increased the resistance but also provided resistance to the multiple pathotypes (six field isolates) of *P. brassicae* in *B. rapa* (Matsumoto et al. 2012). In *B. oleracea* several major and minor QTLs were introgressed to investigate their efficacy against *P. brassicae* infection (Nagaoka et al. 2010). In *B. napus* two genes namely, *CRb* and *PbBa8.1*, were combined through MAS and clubroot resistant homozygous lines were developed which showed increased resistance compared to heterozygous lines (Shah et al. 2019).

5.6.2 Fusarium Wilt

In *B. rapa*, based on the expression of at the whole genome level between resistant and susceptible inbred lines using RNA sequencing, two candidate genes (*Bra012688* and *Bra012689*) were detected for Fusarium yellows resistance in Chinese cabbage (Shimizu et al. 2014). Then two dominant DNA markers, Bra012688m and Bra012689m, were developed for predicting the Fusarium wilt resistance (Kawamura et al. 2016). However, as several lines were showing not identical to genotype information with resistance phenotypes by inoculation test, the new DNA marker focbr1-2 m was developed for molecular assisted breeding in Chinese cabbage (Miyaji et al. 2021a).

In *B. oleracea*, there were two types of resistance (Type A and Type B) against Fusarium wilt that have been reported (Blank 1937). Type A resistance is controlled by a single dominant gene, whereas Type B resistance is regulated by multiple genes. Initially, Type B integrated into cultivars, but the resistance to Fusarium wilt is unstable when temperatures is above 24 ℃ (Blank 1937; Walker 1953). Therefore, more studies were focused on Type A, the resistance locus *Foc-Bo1*, were detected on linkage group 7 while the association between this QTL and the closest simple sequence repeat marker (KBrS003O1N10) were analyzed in three F3 population (Pu et al. 2012) which found to be efficient for MAS of fusarium wilt breeding. After fine mapping and map-based cloning, the candidate gene *FocBo1* located in C7 which encodes the NBS-LRR protein was identified, the candidate gene-specific DNA markers (MTK-1) and phenotypes in F1 cabbage cultivars and their selfed F2 populations showed a perfect correlation (Shimizu et al. 2015). Further, other researchers also detected the Fusarium wilt resistance locus *FOC* in C6 of *B. olearacea,* the gene *re-Bol037156* showed gene sequence variation between the parental

lines, considered as the candidate gene for *FOC1* (Lv et al. 2013, 2014). Further, three InDel markers were M10, A1, and Frg13 can be used for molecular assisted breeding in cabbage (Lv et al. 2013; Li et al. 2017).

5.6.3 Stem Rot

Taking into account that *B. napus* is unable to give a high resistance to stem rot, researchers are likely to inspect other wild-type Brassica relatives for improved germplasm, like *Brassica cretica* and *Berteroa incana.* For resistance transfer, a combination of distant hybridization with MAS together plays a momentous role. For instance, Mei et al. (2011, 2013, 2015) successfully used phenotype evaluation and newly developed SSR markers for MAS and hexaploidy hybridization and introgressed resistance to *B. napus* from wild *B. incana*. The same strategy was applied on wild *B. oleracea* and a rapeseed variety 'Zhongshuang 9', and first-time *Sclerotinia*-resistant rapeseed lines were developed using several resistant loci from wild *B. oleracea* (Mei et al. 2020).

5.6.4 Black Leg

MAS also plays a very significant role in development of blackleg resistant cultivars through integration with another breeding program to reduce the breeding period. For example, Yu et al. (2012) developed a series of resistant cultivars resistant to blackleg disease through successful introgression of blackleg resistance from wild *B. rapa* subsp. *sylvestris* to *B. napus* using MAS and interspecific hybridization. Further, on the basis of identified QTLs and major genes, a combination of quantitative and qualitative loci could be included in the breeding program to deliver much durable resistance (Brun et al. 2010).

5.6.5 Downy Mildew

Markers that are closely located with R loci have been embraced for resistance breeding via MAS and have significantly contributed to resistance breeding. In the case of *B. rapa*, Yu et al. (2011) successfully converted a closely linked RAPD marker K14-1030 into a SCAR marker SCK14-825, which significantly improved the selection of high yielding and disease resistant lines.

5.7 Genomics Aided Breeding for Resistant Traits

Recent progress in genomics and computational biology offers better opportunities to associate the molecular and computational tools to better understand the regulation and functions of the genes. The sequencing-based approaches are also deployed in developing molecular markers like SNPs, InDels, DArT, and KASP. Various omics methods such as transcriptomics using global RNA-sequencing emerged as powerful tool which reveals the differentially and uniquely expressed genes underlying biotic stress of crops. Till now, several researches related to CR, Fusarium wilt, black leg in Brassica crops were reported.

GWAS of 472 lines using 60 K *Brassica* Infinium SNP arrays were employed to detect clubroot resistance (Li et al. 2016a, b). Through integrative analysis, nine loci were characterized, among which seven are novel and six are belonging to the C genome. Proteomics approach were also utilized in Chinese cabbage in response to *P. brassicae* infection and detected differentially expressed proteins (DEPs) among the resistant and susceptible genotypes (Lan et al. 2019). Proteins belonging to category of 'Glutathione transferase activity' was significantly enriched in gene ontology analysis, indicating involvement of glutathione transferase in the regulation of resistance.

Transcriptome analysis following *Foc* inoculation in *B. rapa* Fusarium wilt resistant and susceptible lines showed that activation of effector triggered immunity (ETI) such as salicylic acid (SA) dependent systemic acquired resistance (SAR) by recognition of avirulence (Avr) by the R protein is important for Fusarium wilt resistance, meanwhile, the SA-dependent SAR-related genes, *PR2*, *PR4*, and some *WRKY* family genes were detected as DEGs in resistant lines (Miyaji et al. 2017). Recently, RNA-sequencing of resistant (Nanene) and susceptible (Misugi) lines of Chinese cabbage treated with and without SA were investigated for differential transcriptional response (Miyaji et al. 2021b). The up-regulated genes in 'Nanene' were associated with SA response and down-regulated genes were associated with ethylene (ET)/jasmonic acid (JA) response however such DEGs were not detected in susceptible Misugi line indicating an antagonistic defense response to Foc in resistant and susceptible lines.

In *B. oleracea*, some studies on transcriptome profiling for Fusarium wilt were reported. This reveals firstly in early defense systems, Mitogen-activated protein kinase (MAPK) signaling pathway, calcium signaling and SA-mediated hypersensitive response (HR) were activated after pathogen infection. SA-dependent (SAR), ethylene (ET)- and jasmonic (JA)-mediated pathways and the lignin biosynthesis pathway play important roles in plant resistance (Xing et al. 2016). Secondly, Pu (2016) investigated the protein changes driven by Foc-infection in *B. oleracea* xylem sap in both the resistant and susceptible systems, and predicted 25 Foc proteins as candidates for avirulence factors in susceptible plant xylem sap infected by Foc (Pu et al. 2016). Recently, Many NBS-LRR genes and WRKY transcription factors were identified with different expression levels between the resistance and susceptible lines. Moreover, there were one potential effectors, two elicitors and six virulence

factors with increased or decreased transcript abundance among *Foc* DEGs (Liu et al. 2020).

5.8 Genetic Engineering for Biotic Stress Resistance

The development of transgenic plants through the genetic engineering approach is one of the best alternatives for gene transfer which specifically provides resistance against the pathogen, where traditional breeding approaches are ineffective. Several transgenic Brassica crops have been developed against pathogen diseases with a variety of genes from diverse organisms. Generally, these transgenes are derived from a plant source, while few of them have originated from the pathogen.

Several studies have been performed in transgenic *B. juncea* for TuMV disease resistance. Overexpression of pokeweed antiviral protein (PAP) gene shows resistance to TuMV in transgenic *B. juncea* (Zhao et al. 2008). Subsequently, an antisense *NIb* gene of TuMV was transferred into *B. juncea* and Brassica transgenics showed high resistance against TuMV (Zhao and Hao 2010). An alternative oxidase (AOX) gene, designated as *BjAOX1a* enhances the resistance to TuMV infection (Zhu et al. 2012). The overexpressed TuMV coat protein transgenic plants delay in appearance of symptoms and decrease the severity of symptoms compared to non-transgenic plants (Jafari and Shams-Bakhsh 2018). In *B. napus* the *Turnip mosaic virus* coat protein (TuMV CP) gene was integrated and transgenic plants showed resistance to virulent TuMV by varying degrees of virus infection (Lu et al. 1996).Overexpression of the coat protein (CP) gene shows enhanced resistance to TuMV in *B. napus* (Lehmann et al. 2003). In *B. rapa*, the overexpression of the EIF (ISO) 4E mutant confers resistance to multiple strains of TuMV (Kim et al. 2014). TuMV resistant transgenic plants were obtained through the *NIb* gene using marker-free *Agrobacterium tumefaciens* infiltration in *B. rapa* (Zhandong et al. 2007).

For Downy mildew, *Thkel1* gene from *T. harzianum* improves plant responses to this disease in *B. napus* through inducing systemic defense (Poveda et al. 2019). In *B. oleracea*, WRKY transcription factor gene *BoWRKY6* enhances the tolerance against downy mildew (Jiang et al. 2016). Oil radish superoxide dismutase gene designated *RsrSOD* confers tolerance to downy mildew in broccoli (Jiang et al. 2012). In *B. rapa*, the silenced seedling of *MSTRG.19915* showed increased resistance to downy mildew, apparently due to the upregulated expression of *BrMAPK15* (Zhang et al. 2021). In *B. napus*, overexpression of bacterial catalase exhibit enhanced resistance to downy mildew (El-Awady et al. 2008).

A *Pisum sativum* gene namely, *DRR206* enhances tolerance against blackleg (*Leptosphaeria maculans*) in transgenic *B. napus* (Wang et al. 1999). Overexpression of brassinosteroid biosynthetic gene *DWF4* provides tolerance to *B. napus* against blackleg (Sahni et al. 2016). In *B. napus*, a wall-associated kinase-like (WAKL) gene *Rlm9* gives race-specific resistance against blackleg disease (Larkan et al. 2020).

5.9 Brief Account on Role of Bioinformatics as a Tool

The introduction of cost-effective next generation sequencing technology generated a "big data" in the form of thousands of genome, transcriptome, methylome and protein sequences. Subsequently, bioinformatics and computational biology tools have become a fundamental basis of plant genetics and genomics. We have summarized a collection of database for major Brassica species with genome sequences, syntenic genes, comparative maps, markers and other genomics information in the form of Table 5.6 that could be utilized for disease resistance program.

Table 5.6 List of database used for Brassica crop improvement program

Database	Crops	Link	Description
BRAD	*B. rapa* *B. juncea* *B. napus* *B. nigra* *B. oleracea*	http://brassicadb.cn	The BRAD provides services for 35 genomes or genome versions from 25 species. The genomic data include mainly genome assemblies, predicted gene models and gene annotations
ensemble plant	*B. rapa* *B. oleracea* *B. napus*	ftp://ftp.ensemblgenomes.org	Information of protein-coding and non-coding genes, splice variants, cDNA and protein sequences, non-coding RNAs
NGDC	*B. rapa* *B. oleracea* *B. napus* *B. nigra* *B. juncea*	https://ngdc.cncb.ac.cn/	Multi-genome database for Protein-coding and non-coding genes, protein sequences
Plant GARGEN	*B. rapa* *B. oleracea* *B. nigra* *B. juncea* *B. napus*	https://plantgarden.jp/	Protein-coding and non-coding genes, splice variants, cDNA and protein sequences, RNA, non-coding RNAs
Brassica Genome	*B. napus pangenome* *B. napus* *B. oleracea pan genome* *B. rapa pangenome* *B. rapa*	http://www.brassicagenome.net/	Comprehensive annotation of *B. rapa* genome and pan genomes of *B. napus* and *B. oleracea* along with sequence similarity search portal against several Brassica genomes

(continued)

Table 5.6 (continued)

Database	Crops	Link	Description
brassica.info	*B. rapa* *B. napus* *B. oleracea*	https://www.brassica.info/tools/databases.html	Detailed information of Brassica genetics and genomics such as genome, phenome, several tools like genetic markers, tilling and production statistics
PlantGDB	*B. rapa*	http://www.plantgdb.org/BrGDB/	Comprehensive information regarding genome/gene models, gene annotation of *B. rapa*
Brassica IGF project	*B. rapa* *B. oleracea*	http://brassica.nbi.ac.uk/IGF/?page=body/database.htm	Provide information on physical maps of the Brassica 'A' and 'C' genomes by fingerprinting BAC libraries and further integrated these with the Arabidopsis genome sequence by hybridisation with selected gene anchor probes
CropSNPdb	*B. napus* *B. rapa* *B. oleracea* *B. juncea*	http://snpdb.appliedbioinformatics.com.au/	Data resource for crop variation identified using Brassica 60 K genotyping arrays

5.10 Future Perspectives

Brassica crops are important for human life, but their yield and quality are impacted by various external conditions, especially various diseases. Many types of R genes/QTLs have now been identified in Brassica for disease resistance and are being used to improve resistance in cultivars. Several genetic markers that are linked with disease resistance alleles have been developed, and they have been used for MAS in *B. rapa*, *B. oleracea* and *B. juncea* breeding programs. With the availability of the next generation based sequencing of whole-genome and transcriptome and other emerging functional genomics data, a detailed genome-wide comparison can be accomplished. The omics technology coupled with gene editing and gene pyramiding approach can offer new means to study the molecular mechanisms of plant disease resistance. These approaches will allow researchers to not only decipher the evolutionary history and genomic complexity of diseases but also facilitate transfer of R genes/loci to widely cultivated susceptible crop varieties. We will be able to further discover genomic models to elucidate the key genes or functional components

which regulate complex disease resistance traits. Next generation genomics methods like genomic selection and genotype prediction based on artificial intelligence can be deployed for Brassica crop improvement programs for complex disease resistance traits for biotic stress tolerance.

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Chapter 6
Allium Breeding Against Biotic Stresses

Anil Khar, Guillermo A. Galván, and Hira Singh

Abstract Among *Allium* species, onion (*Allium cepa* L., $2n = 2x = 16$) and garlic (*Allium sativum* L, $2n = 2x = 16$) are cultivated throughout the world for their culinary, medicinal and therapeutic values. The production, productivity and inherent nutritional potential of these crops is immensely affected by various biotic stresses before and after harvesting. Onion breeding techniques are in several aspects less developed than those available and employed in other horticultural and agricultural crops. The major biological limitations that hampers onion breeding programmes are its biennial nature, photosensitivity, outcrossing flowering behavior, combined with a high inbreeding depression. Apart from these, the huge genome size (16 GB) with highly repetitive non-coding DNA is also a big constraint to complement marker-assisted breeding. Recently, a garlic genome was completely sequenced, as the first Allium species. With the recent release of the first draft genome assembly of onion, hopefully this would help to augment onion breeding possibilities through developing more and reliable genomic resources for resistance breeding against various insect-pest and diseases. This chapter summarizes the main diseases and pests threatening onion production in tropical and temperate regions, the efforts in breeding for disease and pest resistance, the development of tools for marker assisted selection and the potential of genomic tools for the development of resistant cultivars.

Keywords *Allium cepa* L. · Biotic · Stress · Onion · Garlic

6.1 Introduction

Onion (*Allium cepa* L.) is the main *Allium* cultivated species and is grown throughout the world for its culinary and medicinal values. Onion ranks second after tomato

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among vegetable crops regarding the produce (FAOSTAT 2019). The production, productivity and inherent nutritional potential of onions is affected by many pre- and post-harvest diseases, pests, and viruses (Agrios 2005). This chapter summarizes the main diseases and pests threatening onion production in tropical and temperate regions, the efforts in breeding for disease and pest resistance, the development of tools for marker assisted selection and the potential of genomic tools for the development of resistant cultivars.

Plants live in nature in contact with a wide spectrum of microorganisms, arthropods, and a range of other potential enemies. Plants possess an immunity system to prevent that any microorganism can feed from live plant tissues (Niks et al. 2011). As a consequence of this general defense system, only a few microbes and arthropods co-evolved with specific *Allium* species to develop pathogenicity and virulence mechanisms to become pathogens, parasites, or pests. Pathogenicity may be either restricted to a specific plant species or taxa or extended to a broad spectrum of host species (Agrios 2005). As examples, onion is affected by downy mildew caused by *Peronospora destructor*, a pathogen able to infect only onion and few closely related *Allium* species (Kofoet and Zinkernagel 1989; Scholten et al. 2007). In contrast, *Botrytis cinerea*, a generalist pathogen able to cause brown stain in mature onion bulbs as well as flower blight at onion blooming (Steentjes et al. 2021), can cause grey mold in over 200 cultivated plant species, including tomato, grape vine, strawberry, and Cannabis (Williamson et al. 2007).

6.1.1 Passive Defenses

Plant immunity system involves different levels of defenses, briefly described in these sections. Some passive defense mechanisms are developed and present in plants as adaptative barriers (Niks et al. 2011). *Allium* species are characterized by the production of allicins, a family of cysteine-sulfoxides that upon damage are metabolized releasing thiosulfinates with antimicrobial activity against gram positive and negative bacteria (Ankri and Mirelman 1999, Reiter et al. 2020). To visualize its relevance, pathogenicity of *Pantoea ananatis* strains causing bulb rotting, requires the gene cluster *alt* (allicin tolerance), codifying for enzymes that confer tolerance against thiosulfinates (Stice et al. 2020).

Another pre-formed defense is given by the catechin, a skin phenolic pigment present in pigmented onion (yellow and red bulbs) with antioxidant activity (Beretta et al. 2017). Onion smudge caused by *Colletotrichum circinans* affects white onions, as small black flecks on the bulb surface with the sign of the pathogen. Smudge disease is not observed in pigmented onions (either with yellow or red skins) due to the inhibitory effect of catechin on spore germination and fungal growth, a ubiquitous example of passive defenses in plants (Link et al. 1929).

6.1.2 Active Defenses

In addition, active defense mechanisms are triggered once the presence of a potential pathogen or damage is perceived by plant tissues (Li et al. 2020). The early phase is a broad-spectrum resistance, triggered by the recognition of pathogen associated molecular patterns (PAMPs) by plant recognition receptors (PRR). This defense is called PAMP triggered immunity (PTI) (Jones and Dangl 2006). The main PTI signaling pathway is a cascade of mitogen activated protein kinases (MAPKs) leading to cellular responses that comprise the production of reactive oxygen species (ROS), synthesis of antimicrobial compounds like phytoalexins and phytohormones, reinforcement of the cell wall, cell wall appositions, and programmed cell death (Ponce de Leon and Montesano 2013). Some typical PAMP conserved molecules recognized by PTI are the bacterial flagellin and EF-Tu, and the fungal chitin (Panstruga et al. 2009).

Pathogens can hamper effective defenses by the release of effectors, proteins that, for instance, suppress early steps before the MAPK signaling pathway is activated (He et al. 2007). Then, the result is plant host susceptibility mediated by pathogen effectors (Jones and Dangl 2006). Like in an arm race, plants have developed the recognition of effectors by specific molecules (R proteins) triggering a resistance response called effector triggered immunity (ETI). This resistance occurs later in the infection process than PTI, when the plant and the pathogen establish an intimate contact (e.g., post-haustorial in obligate pathogens) (Niks et al. 2011). ETI is a highly effective resistance, typically leading to programmed cell death with no visible disease symptoms and controlled by a single R gene coding for the R protein, two reasons that make it very attractive for breeders. As constraints, effector mediated resistance is specific, only effective for those strains carrying the recognized effector, making a strong evolutive pressure in favor of other strains in the field or strains mutated at the recognized effector (Niks et al. 2011).

Plant recognition receptors and R proteins are a family of molecules with perception (ecological) activity. Most of them share the presence of a conserved region, like the nucleotide binding sequence (NBS), and a more variable region like the leucine rich repeats (LRR), the region with activity in the recognition of PAMP or effectors (Hammond-Kosack and Parker 2003). PTI is associated with the activation of jasmonic acid (JA) and ethylene (ET) activities, leading to high or partial resistance responses in plant genotypes either for necrotrophic or biotrophic pathogens. ETI is associated with the activation of salicylic acid (SA), leading to high resistance to complete resistance in plant genotypes (Panstruga et al. 2009). Throughout the action of mobile phytohormones like JA, ET, and SA, both PTI and ETI express additional systemic responses, activating defense reactions in plant tissues far apart from the infection points (Pieterse et al. 2009).

6.2 Breeding *Allium* Species

6.2.1 Rudimentary Genetics

Onion ($2n = 2x = 16$) breeding techniques are in several aspects less developed than those available and employed in other crops. Besides the minor economic relevance of *Allium* crops, some of the main biological constraints addressed in the literature are the biennial life cycle, the photothermal (seasonal) requirements, and the outcrossing flowering behavior of the crop combined with a high inbreeding depression (Shigyo and Kik 2008; Havey 2012; Khar and Singh 2020; Singh et al. 2021a). These onion features point Havey (2012) to qualify onion genetics as rudimentary, as only genes for a few agronomic traits were known and mapped at that time and until now. Among these mapped genes, few are disease resistance genes (Table 6.1).

The huge genome size for onion (*Allium cepa*, 32–33.5 pg·cell^{-1}), even larger than *A. roylei* (28–30 pg·cell^{-1}) and shallot (*A. fistulosum*, 22.5–23.5 pg·cell^{-1}) genomes (Ricroch et al. 2005), add to the list of constraints, has prevented and delayed the availability of fully sequenced genomes as a resource for molecular breeding. In comparison to other crops used as model plants, rice has a genome estimated in 490 Mb and tomato 1038 Mb, whereas onion genome is estimated at 17,500 Mb (Leitch et al. 2019). Fortunately, Finkers et al. (2021) have recently communicated the first draft genome available for onion, with 14.9 Gb assembled, and 2.2 Gb arranged in the eight pseudomolecules, with a high synteny with garlic (*Allium sativum*) genome (Sun et al. 2020). The advent of genomic resources is particularly good news and will accelerate the progress in molecular genetics and marker assisted selection.

Table 6.1 Few disease resistance genes identified and/or mapped for onion breeding

Disease resistance	Source	Gene	Chromosome	Marker system	References
Downy mildew	*A. roylei*	*Pd1*	3	SCAR	Kik et al. (1997)
				AFLP	Scholten et al. (2007)
				Simple PCR	Kim et al. (2016)
Fusarium basal rot	*A. cepa*	–	1	SNP	Taylor et al. (2019)
		–	6	SNP	
		–	8	SNP	
		–	Unmapped	SNP	
Purple blotch	*A. cepa*	*Apr-01*	Unmapped	STS, SSR	Chand et al. (2018)

Diverse molecular marker systems have been applied in genetic traits analysis and genetic diversity analysis in onion, summarized by Klaas and Friesen (2002) and Khosa et al. (2016): random amplified polymorphic DNA (RAPDs), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) (van Heusden et al. 2000). The development of simple sequence repeat (SSR) markers was successful only after based on expressed sequence tags (ESTs) (EST-SSR; McCallum et al. 2008; Khar et al. 2011). A step further was given by the upcoming of next generation sequencing (NGS) technologies, setting up the base for the development of highly dense linkage maps based on single nucleotide polymorphism (SNP) markers (Duangjit et al. 2013; Scholten et al. 2016).

6.2.2 Genetic Resources

Onion is a cultigen not found as such in nature. The center of origin as postulated by Vavilovi is Central Asia, where its close relative *A. vavilovii* is found (Fritsch and Friesen 2002). McCallum et al. (2008) studied a global panel of onion germplasm diversity based in EST-SSR markers and distinguished an Indian–Iranian gene pool separated from a SD and a LD gene pools of European and American germplasm, suggesting divergent adaptation of eastern and western onion gene pools. Similarly, Taylor et al. (2019) studied a world onion accessions panel using SNP markers developed by Duangjit et al. (2013), and accessions were grouped according to photoperiodic requirements and geographical regions.

A comprehensive strategy to identify sources of resistance is needed. Most of the research work has focused on Fusarium basal rot and downy mildew under temperate conditions. In tropical countries, research on purple blotch has only focused on management and phenotypic screening.

Wild relatives of Alliums have been evaluated and found carrying diverse degrees of resistance to various diseases and pests, as summarized in Table 6.2. Wild relatives of crops have been used to introduce genetic variation in crops for several plant families, mainly for diseases and pest resistance (Hajjar and Hodgkin 2007). Nevertheless, crosses between onion and resistant wild species have not been as successful as needed for introgression traits, due to pre- and post-fertilization barriers. Development of interspecific F1 having non-bulbing traits and poor to none male fertility have also hampered the interest of breeders to use these wild species in conventional breeding programs (Kik 2002). The only successful example of interspecific hybridization has been the crossing of *A. cepa* and *A. roylei* for the development of downy mildew resistant onions (Scholten et al. 2007).

The gene pool classification from Harlan and De Wet (1971) yields a very narrow gene pool 1 for onion (defined as viable crosses within the cultivated species and crosses with very narrow species producing completely fertile progenies). Viruel et al. (2021) proposed to consider crop wild relatives (CWRs), summing up information of phylogenetic distance and biological information on crossing compatibility. This kind of integrated information for onion related species was shown by van Raamsdonk

Table 6.2 Summary of some resistance reports against diseases and pests in onion (*Allium cepa* L.) and onion relatives

Resistance source	Disease resistance	Varieties	References
Allium cepa	Purple blotch	CBT-Ac77, Arka Kalyan	Nanda et al. (2016)
		Red Creole, Yellow Creole	Bock (1964)
		Red Creole, Red Shallot	Natural Resources Institute (1990)
		Red Creole	Montes (2004)
		Red Creole, Kaharda	Abubakar et al. (2006)
	White rot	Sweet sandwich	
	Thrips	VI038552, VI038512, AVON1067	Njau et al. (2017)
	Fusarium basal rot	Rossa Savonese	Galván et al. (1997)
		NMSU00-25	Gutiérrez and Cramer (2005)
		Ailsa Craig prinzewinner White Lisbon	Taylor et al. (2013)
	Downy mildew	Regia	Arias et al. (2020)
	Thrips	White Persian	Jones et al. (1934)
		IPA-3	Hamilton et al. (1999)
		Meshkan; Sefid-e-Kurdistan	Alimousavi et al. (2007)
Allium fistulosum	Anthracnose		Galvan et al. (1997)
	Stemphylium Blight		Pathak et al. (2001) Dangi et al. (2019)
	Fusarium basal rot		Holz & Knox (1974) Galvan et al. (2008) Rout et al. (2015)
	Pink root	Nebuka, Winterhecke, White Welsh	Porter & Jones (1933), Felix (1933) Ludwin et al. (1992), Netzer et al. (1985)
	Botrytis squamosa		Walters et al. (1996), Bergquist & Lorbeer (1971), Currah & Maude (1984)
	Smut	Nebuka	Jones et al. (1934)
	Thrips	Nebuka	Jones et al. (1934)
Allium roylei	Anthracnose		Galvan et al. (1997)
	Purple blotch		Nanda et al. (2016)

(continued)

Table 6.2 (continued)

Resistance source	Disease resistance	Varieties	References
	Fusarium basal rot		Rout et al. (2015)
	Downy mildew		Kofoet and Zingernagel (1989)
	Botrytis squamosa		De Vries et al. (1992) Walters et al. (1996)
A. schoenoprasum	Fusarium basal rot		Galvan et al. (2008) Rout et al. (2015)
	Purple blotch		Nanda et al. (2016)
Allium galanthum	Anthracnose		Galvan et al. (1997)
A. tuberosum	Root knot nematode		Huang et al. (2016)
A. aflatunense	Penicillium decay		Dugan et al. (2011)
A. atroviolaceum	Penicillium decay		Dugan et al. (2011)
A. stipitatum	Penicillium decay		Dugan et al. (2011)
A. telavinense	White rot		Bansal and Broadhurst (1992)

et al. (2003). Only few closer onion related species yield F1 viable seed in interspecific crosses with *A. cepa, e.g., A. vavilovii, A. galanthum, A. fistulosum, A. roylei*, but obtained interspecific F1 plants are frequently unfertile or of low fertility plants. Crossing *A. cepa* with other more distant *Allium* species may require embryo rescue techniques. The bridge cross concept is another applied approach to be exploited in introgression strategies (Khrustaleva and Kik 2000; Kik 2002).

6.3 Featured Examples in *Allium* Breeding for Resistance

6.3.1 Purple Blotch

Purple blotch caused by *Alternaria porri* (Ellis) Cifferi is an important onion disease throughout the world (Schwartz and Mohan 2008). This disease is widely prevalent in warm and humid environments (Suheri and Price 2000; Shahanaz et al. 2007), and therefore is relevant in tropical climates and as a late season disease in temperate climates. This fungus attacks leaves and flower stalks, and reductions in the range 62 to 92% in foliar production has been noticed (Bock 1964; Suheri and Price 2001). Purple blotch causes heavy yield losses in both bulb and seed crops ranging from 2.5 to 97% during *kharif* season (Nanda et al. 2016). Some reports suggest a yield loss of 30% (Everts and Lacy 1990) and 100% seed crop loss under favorable conditions (Schwartz 2004).

Research on identification of purple blotch resistance has been ongoing for several decades (Bock 1964; Pathak et al. 1986; Daljeet et al. 1992; Lakra 1999; Chethana et al. 2011; Behera et al. 2013). Breeding for resistance against purple blotch revealed that Red Creole (hybrid), Red Creole (open pollinated), Yellow Creole (Bock 1964), VL-1, PBR-1, PBR-5, PRR and Arka Niketan (Daljeet et al. 1992), Red Creole and Red Shallot from Ethiopia (Natural Resource Institute 1990), Red Creole from Honduras (Montes 2004), Red Creole and Kaharda from Nigeria (Abubakar et al. 2006) were identified as resistant cultivars. Abubakar and Ado (2008) demonstrated that onion hybrids resistant to purple blotch can be developed. Exploitation of heterosis in onion to develop resistant hybrids is one of the viable options (Singh and Khar 2021).

Field resistance can break down under artificial conditions due to high disease pressure. Screening under normal epiphytotic conditions and artificial conditions is important to identify the resistant cultivars. The onion variety 'Arka Kalyan' and the accession 'CBT-Ac77' were identified as highly resistant to purple blotch whereas *A. schoenoprasum* and *A. roylei* were identified as moderately resistant (Nanda et al. 2016). Studies on inheritance revealed that purple blotch disease is controlled by a single dominant gene christened as *ApR1* (Chand et al. 2018). Molecular mapping for disease resistance led to development of one SSR marker (AcSSR7) and one sequence tagged site (STS) marker (ApR-450) linked closely to the *ApR1* locus in coupling phase at 1.3 and 1.1 cm, respectively (Chand et al. 2018). These markers can be used for introgression breeding of resistant locus in onion accessions for development of resistant genotypes.

6.3.2 Stemphylium Blight

Stemphylium blight (*Stemphylium vesicarium*) was first reported by Miller et al. (1978) to cause significant damage in onions (Fig. 6.1). It is a potentially important pathogen in winter grown *Allium* crops (Suheri and Price 2001). Warm humid conditions with temperatures ranging from 18 to 22 °C and relative humidity (RH) above 85% favor disease development; but the pathogen can also cause infections at lower temperatures (10 °C), as well as can develop at higher temperatures (Suheri and Price 2000).

Screening of onion and *A. fistulosum* accessions revealed that onion is susceptible whereas some lines of *A. fistulosum* were resistant to Stemphylium blight (Pathak et al. 2001). A possible dominant gene control of the resistance was observed based on F2 and F3 generation. Pathak et al. (2001) first reported natural and controlled screening against Stemphylium blight and identified two resistant *A. fistulosum* accessions. Most of the research has focused on screening against purple blotch and Stemphylium blight under both natural (Dhiman et al. 1986; Behera et al. 2013; Tripathy et al. 2013) and controlled conditions (Nanda et al. 2016; Dangi et al. 2019). After the first report by Pathak (2001), Dangi et al. (2019) identified 'Pusa Soumya' (*A. fistulosum*) and 'Red Creole2' (*A. cepa*) as moderately resistant and 'Red Creole1'

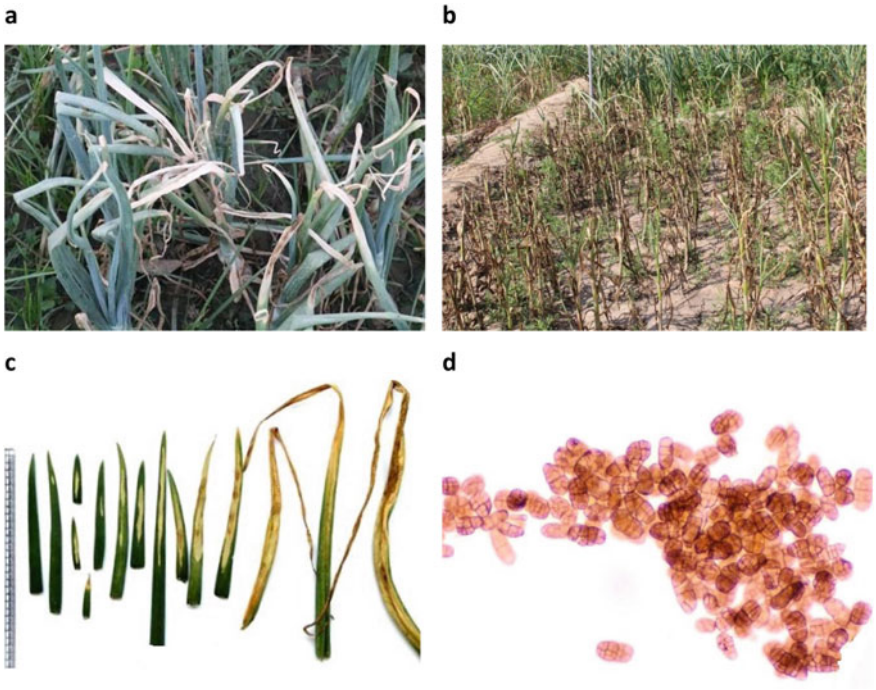

Fig. 6.1 (**a**) Stemphylium blight on onion in India; (**b**) Stemphylium blight outbreak in garlic; (**c**) different stages and levels of Stemphylium blight attack in onion as a scale in selection for resistance; (**d**) microscopical checking of *Stemphylium vesicarium* multicellular ovoid conidia, confirming field infections and symptoms in onion

as susceptible. Significant variation in morphological and biochemical traits was observed and it was suggested that dry matter and total foliar phenol content can be used as biochemical markers for high throughput screening against Stemphylium blight at preliminary screening stage.

In the absence of credible sources of resistance against Stemphylium, Kamal et al. (2008) advised application of benzothiadiazole (Bion®) and di-potassium phosphate salt (K_2HPO_4) to onion. Application of salicylic acid (2 mM) also suppressed 40.39% disease development after 15 days of inoculation under greenhouse conditions (Abo-Elyousr et al. 2009).

6.3.3 Anthracnose

Onion and shallot anthracnose or twister disease is a relevant cause of crop yield losses in tropical regions of Asia, Africa, and South America. The causal agent is

traditionally described as *Colletotrichum gloeosporioides* Penz. (teleomorph *Glomerella cingulata* (Stonem.) Spould & Schrenk). This airborne fungus is a saprophytic pathogen that infects onion leaves, but also seedlings and harvested bulbs (Maude 1990; Lopes et al. 2021). A collection of pathogen isolates from Brazilian regions were identified using sequencing of several genes (Lopes et al. 2021), and isolates were found to belong to the *C. gloeosporioides* and *C. acutatum* species complexes. The species *C. theobromicola* from the *C. gloeosporioides* cluster was predominant in the collection (Lopes et al. 2021).

Rodriguez and Hausbeck (2018) described that anthracnose caused by *Colletotrichum coccoides* is a relevant disease in Michigan. They tested favorable conditions for the disease and reported that the combination of high temperature (>25 °C) and extended (>24 h) high RH resulted in high (>20% leaf area affected) disease severity 28 days post-inoculation.

The scant breeding efforts within the genetic base of onion crop only revealed quantitative differences or partial resistance. Wordell Filho and Stadnik (2008) assessed the response of 20 commercial Brazilian cultivars and identified those with lower levels of disease severity after experimental inoculation in controlled conditions. Earlier, in São Paulo, Brazil, Melo and Costa (1983) evaluated the survival rate of onion cultivars affected by anthracnose. A cross between the highly resistant cv. "Barreiro" and the susceptible "Texas Early Grano 502" suggested that resistance was polygenic and quantitatively expressed.

Galván et al. (1997) screened shallot and its wild relatives for anthracnose (*Colletorichum gloeosporioides* Penz.). *Allium cepa* and *A. oschaninii* were most susceptible, whereas *A. altaicum*, *A. fistulosum*, *A. galanthum*, *A. psekemense* and *A. roylei* were partially resistant. Highly resistant reactions were observed in *A. galanthum* and *A. fistulosum* accessions (Fig. 6.2). Genetic analysis based on a cross *A. cepa* x *A. roylei* revealed that resistance from *A. roylei* was dominantly inherited and determined by more than one gene (Galván et al. 1997).

6.3.4 Fusarium Basal Rot

Fusarium basal rot (FBR) is an important soil-borne disease of *Allium* crops throughout the world, which can affect seedlings, mature plants, and stored bulbs as well. In onion the disease is caused mainly by *Fusarium oxysporum* f. sp. *cepae* (FOC). Other *Fusarium* species may cause FBR in onion and garlic. *Fusarium proliferatum* (du Toit et al. 2003; Valdez et al. 2004; Stankovic et al. 2007; Galván et al. 2008) calls the attention because of the potential fumonisins toxins production and the risks for human consumption. Other less frequent *Fusarium* species have also been identified (Entwistle 1990; Galván et al. 2008).

Field and storage losses up to 23% have been reported under soils naturally infested with *Fusarium* (Bacher et al. 1989). The fungus attacks seedlings leading to damping off, root rot and enters the basal plate of onion bulbs causing stem plate discoloration and bulb rot in the field and storage (Abawi and Lorbeer

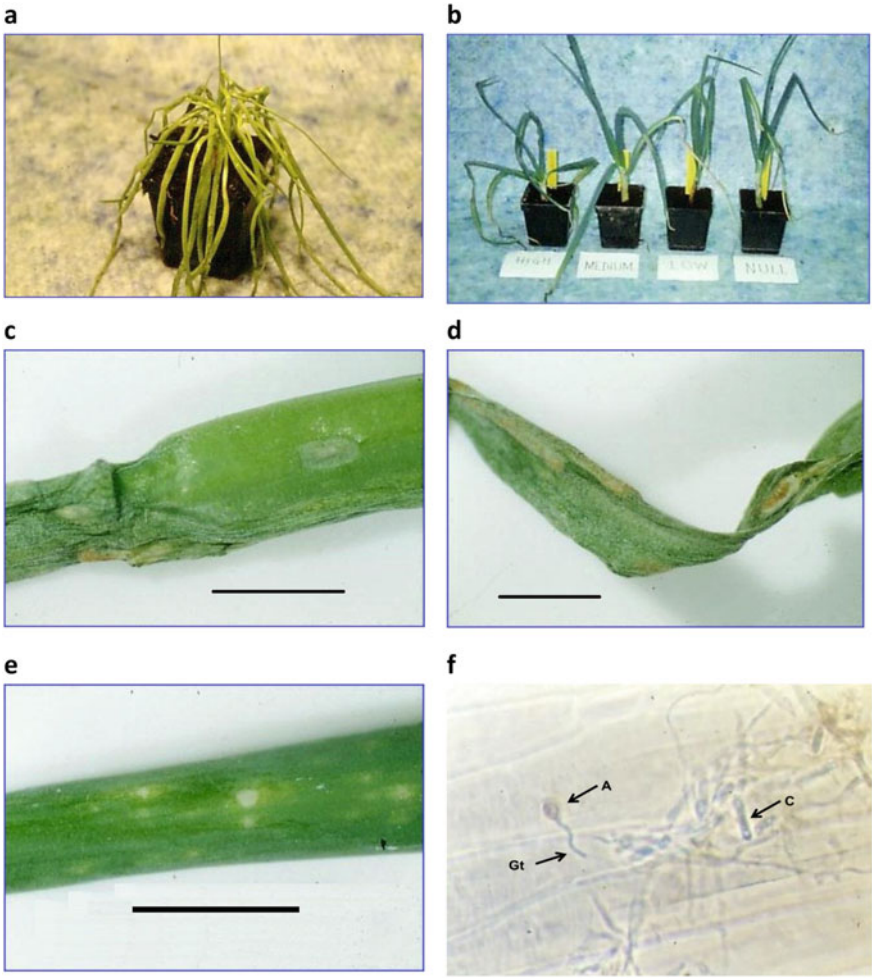

Fig. 6.2 Screening for anthracnose (*Colletotrichum gloeosporioides*) resistance under controlled conditions with experimental inoculation (Galván et al. 1997); (**a**) a severely affected shallot (*A. cepa*) pot plant; (**b**) evaluation scale from severely diseased (left) with typical curly leaves or '*vira cabeza*' symptoms, up to a non-affected healthy plant (right); (**c**, **d**) anthracnose spots on susceptible *A. cepa* genotypes with orange sporulation areas; (**e**) resistance reaction on *Allium fistulosum*, with small flecks and no sporulation; (**f**) microscopic observation 24 h after inoculation; the arrows point to cylindrical conidia (C) producing a germination tube (Gt) ending up an appressorium (A) attached to leaf epidermis. Pathogenicity in *Colletotrichum* species is characterized as hemi-biotrophic, with a brief biotrophic initial phase. Black bars depict 1 cm

1971). The first stages of emerging seedling and bulbing plants are the most susceptible phenological phases of the crop, suggesting an age-related resistance (defense) host system (Galeano et al. 2014). A temperature range of 25 to 28 °C is optimum for disease development (Sumner 1995) and used in experimental screening like the seedling test, with *Fusarium* inoculation during the germination phase (https://haveylab.horticulture.wisc.edu/wp-content/uploads/sites/66/2016/05/Fusarium-screening-in-onion.pdf).

FBR may be the only onion disease where systematic research on resistance has been conducted. Two recent reviews refer to FBR; Le et al. (2021) covering diverse aspects of the disease, whereas Cramer et al. (2021) point at the advances in breeding for resistance.

Fusarium oxysporum is a natural inhabitant in soils with non-pathogenic and pathogenic forms, and a generalist pathogen able to infect diverse plant families. Pathogenicity on a specific host or just the ability to multiply on a range of plant hosts is acquired in a quantitative manner (Dhingra and Cohelo Netto 2001; Leoni et al. 2013), with quantitative differences among isolates in virulence. Genetic diversity within FOC was reported with isolates distributed in two main clades by Galván et al. (2008) and three clades by Taylor et al. (2016), among those clades reported for this species complex by O'Donnell et al. (1998). Virulence differences among isolates are not linked to evolutionary genetic differences (Galván et al. 2008; Taylor et al. 2016). Pathogenicity related genes present in FOC were studied by Taylor et al. (2016) and ten effectors were identified. Seven secreted in xylem (SIX) genes out of 14 tested were identified, and their sequences were found specific for FOC, despite a high homology with corresponding six genes for other *forma specialis*. In addition, two genes with signal peptides and RxLR motifs (CRX1/CRX2) and a gene with uncharacterized domain (C5) are present in FOC isolates (Taylor et al. 2016).

Selection methods and the way to perform phenotypic evaluations have been under concern. Improvements in screening methods aim to increase heritability in recurrent selection for resistance. Gutierrez and Cramer (2005) developed a method of slicing the basal plate of the bulbs to quantify FBR infections, and identified 'NMSU00-25' as resistant cultivar with lowest disease severity and incidence in two years evaluation. A rapid, simple and repeatable seedling assay for high throughput screening of onion seedlings was employed by Taylor et al. (2013). Two onion cultivars 'Ailsa Craig Prizewinner' and 'White Lisbon' showed the highest level of resistance. In disease resistance programs, isolate and inoculum concentration are vital factors for identification of resistant germplasm. The use of low virulence isolates or low inoculum density for resistance breeding leads to false resistant reactions which prove to be susceptible under field conditions. Caligiori Gei et al. (2014) reported that an inoculum density of 10,000 microconidia/g of substrate was most effective for all tested *Fusarium* isolates.

Strong correlation between seedling and mature plant assays suggests that a high throughput phenotyping for resistance screening against FBR is a viable option (Taylor et al. 2019). Caligiori Gei et al. (2020) employed an integrated approach of laboratory screening complemented with field screening for resistance breeding

against FBR. This new technique not only minimizes the time to develop resistant material but also helps in selecting suitable material in a fast and cost-effective manner. At the same time, Mandal and Cramer (2020) implemented a successful inoculation method by placing on the basal plate of each bulb a plug of a growing media containing a suspension of conidia.

Resistance to onion *Fusarium* isolates was tested in several *Allium* species by Galvan et al. (2008). High levels of resistance were found in *A. fistulosum* and *A. schoenoprasum* against *F. oxysporum* and *F. proliferatum* isolates from onion. *Allium pskemense*, *A. roylei* and *A. galanthum* exhibited an intermediate level of resistance, as well as the Italian onion variety 'Rossa Savonese'. A counterintuitive result is how *A. fistulosum* accessions behaved resistant against onion isolates (Galván et al. 2008), but FBR is an important disease for Welsh onion (*A. fistulosum*) cultivation in Japan (Dissanayake et al. 2009), which suggest that pathogen divergent host specialization occurs. Preliminarily, a quantitative trait locus (QTL) from *A. fistulosum* for resistance against FOC was identified in the long arm of chromosome 8 (Galván 2009).

Selection for resistance in diverse onion breeding programs led to the obtention of resistant selections. Inheritance studies using onion segregant populations suggest a single major gene, two genes or polygenic control of resistance (Cramer 2000). However, the resistance response has not been stable in other regions, most likely due to conduciveness of the environment for the disease and differences in virulence factors in the *Fusarium* populations. A worldwide panel of onion accessions was tested for resistance by Taylor et al. (2019). Using SNP markers developed by Duangjit et al. (2013), three markers linked to FBR resistance on Chromosome 1, Chromosome 6 (linkage group 6B), Chromosome 8 and other two unmapped SNP markers were identified. In another approach, a set of monosomic addition lines was a tool to identify a steroidal saponin from shallot (*A. cepa*) on Chromosome 2 that plays a role in defense against FBR (Abdelrahman et al. 2017). Using RNA-seq analysis, 50 genes related to saponin synthesis were upregulated, and among these, some key genes are located on chromosome 2. The knowledge on genetics (QTLs), gene expression (transcriptome) and gene products (proteome) involved in FBR resistance can be integrated in ongoing onion breeding programs around the world, opening a new phase in FBR resistance breeding.

6.3.5 Downy Mildew

Downy mildew is an onion leaf devastating disease caused by *Peronospora destructor* Berk. (Casp.) prevalent in temperate to cold climates (Schwartz and Mohan 2008). The pathogen belongs to the Oomycete, a group of heterotrophic eukaryotic organisms with filamentous growth and spores as a means of dissemination and reproduction (Lamour and Kamaoun 2009). Oomycete cell walls are composed of polysaccharides like cellulose and glucans, but not chitin. The mycelia are coenocytic and diploid, except when gametangia are formed (Hardham 2007). The oomycete is a

group of important crop pathogens within the supergroup *Chromalveolata,* which also comprises autotrophic chromista algae (Kamoun et al. 2015). Although the monophyletic status of the supergroup has been under discussion (Lamour and Kamoun 2009; Kamoun et al. 2015), based on molecular sequencing and evidence from evolutionary anatomical comparisons, Beakes et al. (2012) postulates that *Oomycete* evolved from holocarpic marine parasites. The filamentous growth pattern as well as the gametangia and sexual reproduction were the main changes to adapt to land lifestyle, but parasitic ability was already present (Beakes et al. 2012).

Within the Oomycete, onion downy mildew belongs to the *Peronosporaceae* family, which comprises the important plant pathogenic genera *Peronospora* and *Phytophthora*, among others (Beakes et al. 2012). The family is characterized by obligate pathogenicity causing leaf blight on the whole plant, including young and turgent leaves, progressing dramatically in brief periods of time (Agrios 2005). Chemical management for onion downy mildew may be effective (Araujo et al. 2020), though may carry risks and negative consequences on laborers and consumers' health, the environment and farmers' profitability. Forecast systems like 'Downcast' (Jesperson and Sutton 1987; de Visser 1998) based on the environmental conditions required for pathogen sporulation and infection were developed to reduce the number of chemical interventions during the season (Lorbeer et al. 2002; Ullah et al. 2020). However, no significant spray reductions are obtained if environmental conditions for sporulation and infection frequently occur (Wright et al. 2002; Maeso 2005; Scholten et al. 2007).

Host resistance is an alternative disease management way, economically and environmentally sound. Early studies reported resistance to *P. destructor* in red onion lines (Jones et al., 1939; Warid and Tims, 1952). Recent studies have also identified and described highly resistant onion varieties (Galván et al. 2016a; Alves et al., 2018; Ullah et al., 2020). However, complete resistance was not available within the genetic base of the crop (Kofoet and Zingernagel, 1989) until the introgression of a simple dominant gene from *Allium roylei*. Among related onion species, an *A. roylei* accession was characterized as downy mildew resistant with a simple genetic control (Kofoet et al. 1990). The *Pd* gene was mapped to a telomeric position of Chromosome 3 (van Heusden et al. 2000), as proved also using cytogenetic tools (Khrustaleva et al. 2019). The gene was introgressed, overcoming dragged negative effects of lethal gene(s) linked to *Pd* from *A. roylei,* that caused distorted segregations. A recombinant with a crossover between the *Pd* gene and the deleterious effects was found, and homozygous *Pd* lines were obtained (Scholten et al. 2007). Currently, downy mildew resistant cultivars are available (Scholten et al. 2007), though the use of this resistance in onion cultivars adapted to diverse growing regions is a long-term process.

Specific molecular markers tagging *Pd* were initially developed as a sequence characterized amplified region (SCAR) marker (Kik et al. 1997; van Heusden et al. 2000) and AFLP markers (Scholten et al. 2007). More recently, Kim et al. (2016) developed a simple PCR marker to assist selection processes, based on cDNA sequences for the telomeric region of long arm of Chromosome 3 from the high-density linkage map developed by Duangjit et al. (2013) and transcriptome sequences

(RNA-Seq analysis). The nature of the codified molecule and mechanisms of resistance triggered by *Pd*-gene have not been studied. Programmed cell death is probably involved, as usually no symptoms at all are observed in comparison with susceptible or partial resistant varieties, except for atypical lesions observed in experimental inoculations (Galván 2011; Vu et al. 2012). The analysis of transcriptome and histopathological relationships may contribute to determining the mechanisms involved and the association with durability of resistance, an issue absolutely under concern for breeders and growers.

Partial levels of resistance in onion varieties might be due to basal resistance mechanisms triggered by the recognition of pathogen associated molecular patterns. These quantitative differences are expressed as epidemiological parameters leading to a slowdown of disease development (Niks et al. 2011), and those differences are exploited in onion breeding to reduce the negative effects on crop yields and contribute to an integrated crop management (Niks et al. 2011). The genetic basis of partial resistance is usually polygenic and has shown to be durable (Niks et al. 2011). Alves et al. (2018) evaluated a set of 46 onion cultivars in agroecologically managed experiments (organic agriculture) in Santa Catarina (Brazil) and selected two open pollinated experimental cultivars for their lower AUDPC for downy mildew, in combination with yield and storage ability.

A screening for sources of resistance in Uruguay led to the identification of 'Regia' as a highly resistant source to downy mildew (Fig. 6.3). Crosses 'Regia' x 'Pantanoso' for South Uruguay (Galván et al. 2016a), and 'Regia' x (Naqué x Casera) for North Uruguay (Galván et al. 2016b) aimed to combine the resistance of the former with favorable agronomic traits from the latter varieties. The analysis of initial steps of the infection process after experimental inoculation revealed a lower rate of successful infection and suggested that the lack of recognition as a potential host before the establishment of the infection could be a first mechanism of resistance in 'Regia' (Galván et al. 2016a). The resistance from 'Regia' has been proposed as determined by various genes with additive and eventually recessive effects (Arias et al. 2020). The segregation of resistance in six offspring from crosses between susceptible cultivars and 'Regia' resulted in skewed segregations towards susceptibility, with transgressive segregation in five of six progenies. Recessive inheritance was reported also in earlier studies by Warid and Tims (1952) in the USA, with 2.8 to 24% of resistant plants in F2 families. The recessive inheritance could be associated with loss of susceptibility mechanisms (Pavan et al. 2010), e.g., the lack of a target host receptor for a successful pathogenicity. Downy mildew severity was positively correlated with histological differences in the proportion of infected stomata, with 'Regia' presenting the lowest severity and the highest percentage of healthy stomata (Arias et al. 2020). The identification and selection of resistant F1S2 lines would allow the development of downy mildew resistant cultivars combining agronomic favorable traits like bulb yield, bulb quality traits and postharvest behavior (Arias et al. 2020).

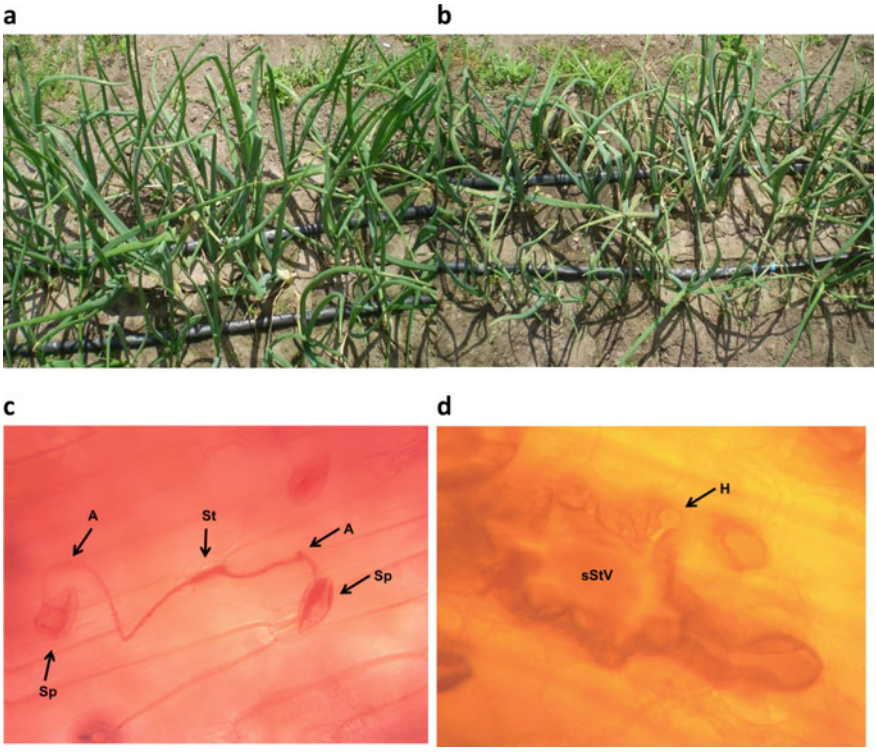

Fig. 6.3 Resistance against downy mildew (Arias et al. 2020). (**a**) Experimental plot of resistant onion 'Regia', (**b**) experimental plot of susceptible cv. 'Pantanoso del Sauce'. (**c**) Microscopical observation 24 h after experimental inoculation. Arrows point to lemon shaped sporangia (Sp), the appressoria (A) and the epidermal stomata (St) as penetration point. (**d**) Microscopic observation of a downy mildew infected leaf with a sub-stomatal vesicle (sStV) where the leaf parenchyma has collapsed, and a typical haustoria (H) with head and neck, as found in other Peronosporales pathogens

6.3.6 White Rot

White rot (*Scleotium cepivorum*) is highly specific to *Allium* species since their sclerotia germinate only in the presence of *Allium* specific root exudates (Entwistle 1990). It is thought to persist in soils for more than 20 years in the absence of host plants by means of sclerotia. White rot may become a devastating disease for both onion and garlic in farming systems with infected soils.

A fungal toxin (oxalic acid) secreted by the fungus degrades the plant cell walls and makes them amenable to this pathogen (Maude 2006). Licona-Juarez et al. (2019) developed a PCR based assay for *Sclerotium* detection in mycelia and infected garlic cloves. In vitro selection techniques using oxalic acid as the selective agent in the growth medium have led to initial success in resistance callus cultures of the variety Beheri Red (Sayed et al. 2016). Al-Safadi et al. (2000) started mutation breeding of

garlic to get mutants resistant to white rot using gamma radiation and successfully achieved resistant mutants. Utilization of induced mutagenesis could be another cheaper option to develop disease resistant mutants in Alliums (Khar et al. 2020; Singh et al. 2021b).

6.3.7 Rhizoctonia Seedling Stunt

In the cereal-onion cropping system, cereals like winter wheat (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.) are used as windbreak crops to protect onion seedlings against sand blasting during windy spring conditions. When herbicides are applied to kill the cover crop, the dying cereal roots provide substrate for the growth of saprophytic fungus *Rhozoctonia solani*. This fungus may infect onion seedling roots that result in significant stunting of onion plants in patches. Sharma et al. (2015) evaluated 35 onion genotypes for resistance to stunting and identified four genotypes that can be the base to develop cultivars partially resistant to *R. solani*.

6.3.8 **Pantoea** *sp. (Onion Center Rot)*

Onion bacterial diseases cause small flecks on onion leaves and seed stalks, leaf strep and even fully wet leaf rotting. As a postharvest disease, center bulb rot up to complete bulb rotting, among other diverse symptoms caused by bacteria constitute an economically relevant source of losses in onion storages (Schwartz and Mohan 2008). Besides environmental prevalent conditions like rainfall during the bulbing phase and the weeks before harvest, the occurrence of downy mildew and thrips damage will increase bacterial rotting during storage. Onion center rot was found to be caused by the genera *Pectobacterium* spp., *Pseudomonas, Dickeya (Erwinia)* and *Enterobacter* (Maude 1990). However, some strains from *Pseudomonas* spp. have been reclassified as *Burkholderia* (Yabuuchi et al. 1992), whereas *Dickeya* spp. strains were renamed as *Pantoea* (Gavini et al. 1989).

Among pathogenic bacteria, *Pantoea* species were identified as a relevant cause of onion center rot in Georgia and other regions in the USA. Center rot caused by *Pantoea ananatis* may appear as a leaf infection that later progresses towards the bulb. At harvest, onion plants may have a wetish bulb neck with a viscous content after pressuring the neck (Snowdon 2010).

Pantoea species identified as involved in center rot of onion are *P. agglomerans*, *P. ananatis*, *P. allii*, *P. dispersa* and *P. stewarti* subsp. *indologens* (Stice et al. 2021). Virulence in *Pantoea ananatis* has been extensively studied. The species lacks the typical bacterial virulence T2 and T3 secretion systems but holds the T6SS (De Maayer et al. 2017). Pathogenicity of *P. ananatis* on onion is supported by the HiVir cluster in combination with the *alt* (allicine tolerance) gene, leading to necrotrophic infection (Stice et al. 2020). The HiVir cluster is not extensively present in other

Pantoea species pathogenic to onion, and therefore Stice et al. (2021) suggested that different virulence mechanisms beyond HiVir are depicted in this genera.

Ongoing host resistance studies revealed quantitative differences among onion cultivars in leaf lesion length (de Armas et al. 2019), and open prospects for selection for resistance against bacterial diseases.

6.3.9 Thrips

Thrips are the major insect pests of onions throughout the world. Application of insecticides for thrips management is widely employed. As in fungicides, regular and indiscriminate use of pesticide has led to environmental pollution and risk of insecticides into our food basket. Thrips have direct damage, but also are vectors for IYSV, *Pantoea* species and give opportunity for *Alternaria* infections. Development of resistant plant material is a viable option. In onion, thrips resistance can be achieved through selection on family basis instead of single plant selection since the heritability is extremely low (Hamilton et al. 1999). An increase in thrips tolerance by selection was reported by Singh and Cramer (2019), though no progress in associated resistance to IYSV was achieved.

Genetic and agronomic factors affect the susceptibility of onion cultivars (Martin and Workman 2006). Various morphological traits viz., leaf arrangement (Jones et al. 1934), round or flat sized leaves, open plant architecture (Coudriet et al. 1979), wider contact angle between leaves (Patil et al. 1988), pH of the plant (Monzen 1926), waxiness (Molenaar 1984; Khosa et al. 2020) and bulb color (Verma 1996) have been associated with thrips resistance. Jones et al. (1934) identified 'White Persian' as the resistant variety with wide angled circular leaves that provide less protection to thrips. Alimousavi et al. (2007) evaluated Iranian onion accessions and identified 'Meshkan', 'Sefid-e-Kurdistan', 'Sefid-e-Qom' and 'Eghlid' as resistant accessions. Diaz-Montano et al. (2010) suggested that resistant cultivars had yellow-green foliage whereas susceptible one had blue-green foliage.

The role of morphological traits towards thrips resistance has been evaluated by various researchers. In cultivar Alfa São Francisco RT, a wider central angle (16.4°), a thinner cuticle, a larger amount of epicuticular waxes, and stomata on the surface of leaves accounted for resistance. In contrast, in cultivars BR 29 and Sirius, the presence of resistance-conferring substances or high amounts of some component in the chemical composition inferred resistance (Silva et al. 2015). Ferreira et al. (2017) observed negative correlations between bulb yield and central angle of the plant, indicating that plants with lower angle of central leaves yield higher under thrips pressure. Njau et al. (2017) screened onion accessions under Tanzanian conditions. A significant negative correlation between leaf angle, leaf toughness and thrips damage were observed. Total epicuticular waxes were weak and non-significantly related with thrips damage. Significant negative correlation between total phenol content and non-significant and inverse correlation between total foliar amino acids or total sugars and thrips damage was reported.

6.3.9.1 Onion Maggot

Onion maggot [*Delia antiqua* (Meigen)] is a major pest in temperate climates. Onion maggot has a limited host range within Alliums crops only. Nevertheless, this pest can destroy more than 50% seedlings in absence of proper control measures (Eckenrode and Nyrop 1995). In some fields where onions are grown continuously for several years, this pest becomes problematic.

Preliminary reports have shown little variation in resistance to onion maggot among onion accessions (Munger and Page 1974; Ellis et al. 1979), but *A. fistulosum* was reported to sustain low maggot damage (Ellis et al. 1979). Screening of onion and related species (McFerson et al. 1996) against this pest showed that no resistance existed in onion and seedlings, whereas mature plants of *A. ampeloprasum* sustained low injury. Hence, *A. ampeloprasum* holds mechanisms of resistance that need to be examined. This knowledge can be a tool for resistance against fly maggots in onion breeding.

6.4 Prospect for Genomic Breeding Against *Allium* Biotic Stresses

This chapter summarized *Allium* progress in breeding for resistance against diseases and pests, with emphasis in onion, and reflects a rather limited picture for the breeders in relation to marker assisted selection. The picture is even more limited for diseases relevant in tropical regions. Breeders rely on phenotypic differences among onion germplasm as resistance sources, phenotypic evaluation of breeding lines and recurrent selection towards enhanced resistance. Although molecular markers and QTLs discovery were tools available from the nineties, their use was scant in crop breeding, as reflected in the query from Lindhout (1995): to what extent, mapping disease resistance genes (in tomato, at that time) was 'a toy for the geneticist or a joy for the breeders?'.

Only recently, the availability of automation and the availability of large numbers of SNP markers, with 'a plus or minus' result and automatic marker reading for hundreds of breeding lines, or just to confirm the hybrid nature of a seed lot, have become regular processes in breeding companies in the last decades.

More emphasis to include genomic studies for identification of genes involved in resistance, their mode of action and how to use those genes for development of resistant varieties should be the focus in future. A recent compilation of genomic resources in *Allium* by Shigyo et al. (2018) gives a comprehensive coverage on genomic tools and their utilization in Alliums. Classical genetics and cytogenetic tools are enriched in the last decade with genomic markers from next generation sequencing (NGS) available tools (Duangjit et al. 2013; Scholten et al. 2016), analysis of transcriptome profiling and metabolomic profiles (Abdelrahman et al. 2017). Publication of chromosome level assembly of garlic genome (Sun et al. 2020) and first genome

assembly of onion (Finkers et al. 2021) supplemented with the transcriptomics and metabolomic atlas in both crops will serve as a guiding force to facilitate genomic breakthroughs in breeding for disease resistance in Alliums.

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Chapter 7
Genomics-Assisted Design of Biotic Stress Resistant Vegetable Amaranths

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Abstract The *Amaranthus* genus contains various species showing differential economic importance based on the use of various plants parts, from leafy vegetables, to biomass fodder to high protein grain. The genus has ~75 species across the world with most being wild or weedy and a few are edible plants. Among the species those used for vegetable purpose are *A. hybridus*, *A. tricolor*, *A. dubius*, *A. blitum*, *A. lividus*, *A. viridis*, *A. spinosus*, *A. graecizans* and some others; while those that are primarily grain crops are *A. caudatus*, *A. cruentus*, and *A. hypochondriacus*.The latter species are from the New World while the former species are mostly of Asian origin with worldwide spread due to them having been consumed in many different ways. Amaranth plants contain multiple nutritional components with high nutraceutical value that provide several health benefits. Climate change and associated disease and pest outbreaks are projected to have extensive impacts on agricultural production in the future. Several diseases and insect pests have been reported to have adverse effect on yield and quality of vegetable and grain amaranths. A small number of diseases including leaf blight, *Choanephora* rot, white rust, and damping-off are found on various amaranths. Meanwhile, a large number of pests including leaf beetles, leaf miners, stem weevils, and lepidopteran caterpillars are of

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major concern in reducing yield and marketability of vegetable amaranths in particular. Although, some efforts have been made towards the improvement of amaranth through conventional breeding approaches, an efficient and comprehensive breeding work for the species has yet to be adopted using modern breeding approaches such as molecular breeding. The advanced approaches like genomics assisted breeding, transgenics, or genome editing could be useful in amaranth improvement for biotic stress resistance.

Keywords Amaranth · Diseases · Insect pests · Biotic stress · Genomics assisted breeding

7.1 Introduction

7.1.1 Amaranths: Crop of the Future

The *Amaranthus* genus belongs to the Amaranthaceae family and includes monoecious and dioecious herbs of various heights and ecological adaptations with a total of 75 species across six continents (Stetter and Schmid 2017). Species of *Amaranthus* are commonly known as amaranths. They are mostly wild species of drylands, forests and swamps or annual weeds growing in disturbed soils (Das 2016). However, a few have been domesticated as vegetables, pseudo cereals, and ornamentals hence; they have been categorized based on their uses (Sauer 1967; Singh et al. 2019).

Among the vegetable types are *A. hybridus* (worldwide) and *A. blitum*, *A. dubius*, *A. graecizans*, *A. lividus*, *A. spinosus*, *A. tricolor*, and *A. viridis* whose leaves are excellent sources of dietary fibers, protein, certain vitamins (pro-vitamin A carotenoid), and essential minerals (e.g. Ca, Fe, Mn, Mg, Cu, P, and K) (Peter and Gandhi 2017). Amaranth leaves are also an outstanding source of some antioxidant leaf pigments: including betalain, β-xanthin, β-cyanin, and a source of amaranthine, carotenoids, anthocyanin, and other bioactive, nutraceutical compounds (Rashad and Sarker 2020; Riggins et al. 2021).

Amaranth is known as "a crop of the future" due to of its incredible nutritional quality (Tiwari et al. 2021). The seeds of the pseudocereal types (*A. caudatus*, *A. cruentus* and *A. hypochondriacus*) have tremendous nutritional value because their grains are rich in lysine and tryptophan amino acids, in overall proteins (18%), and complex starches (75–80%). The grains also contain health enhancing oils, vitamin A, vitamin C, vitamin E, some vitamin B, cholesterol-reducing soluble fibers, and some minerals (e.g. Fe, Ca, Zn, Mn) (Peter and Gandhi 2017).

Meanwhile, *A. tricolor* and sometimes *A. caudatus* are considered as ornamental species given their attractively colored leaves and flower panicles, respectively (Das 2016). Other *Amaranthus* spp. namely, *A. retroflexus*, *A. gracilis*, *A. paniculatus*, *A. gangeticus*, and *A. hybridus* have been considered as weedy amaranths and/or wild relatives of some domesticated or cultivated amaranths. Some weedy amaranths have been considered as weedy but can also be used as vegetable amaranths viz., *A.*

graecizans, *A. hybridus*, and *A. viridis.* Some of weedy amaranths are monoecious (*A. albus*, *A. blitoides*, *A. hybridus*, *A. powellii*, *A. retroflexus*, and *A. spinosus*) and some are dioecious (*A. arenicola*, *A. palmeri*, *A. rudis*, and *A. tuberculatus*) (Sauer 1967; Das 2016; Singh et al. 2019).

7.1.2 Economic Importance of Amaranths for Healthy Diets and Increasing Human Populations

Humans in the modern world have tended to modify their diets to stay fit and healthy. This has involved shifting food habits, increased vegetarianism, and avoiding consumption of highly processed foods or ready to eat market and street foods that pose health risks from contamination (Alimi 2016). The United Nation launched the Zero Hunger campaign through the World Food Program (WFP) to meet enough, safe and nutritional food and human dietary requirements for people to lead healthy and active lives (Li and Siddique 2020).

Consumption of amaranth is good for healthy lifestyles because of its high nutritional quality which are helpful against diabetes, heart issues, and osteoporosis. Amaranths can be immunity boosters for various gastrointestinal issues, and treatment of diarrhea, excessive menstruation, internal bleeding, and even nosebleeds, snake bites, stomach disorders, ulcerated mouths, vaginal discharges, and wound healing (Sarker et al. 2020).

The leaves, shoots, and tender stems of several cultivated vegetable amaranth and young leaves of grain amaranth are used as a food and as an animal feed (Dharajiya et al. 2021). Some amaranth species possess potential phytoremediation ability to uptake of heavy metals in soil (Ziarati and Alaedini 2014). Amaranth species are used to extract natural nutritive pigments (red-violet betacyanins) and oil for the food industry (Cai et al. 1998). Amaranth grains are used in making custards, pastes, and salad dressings; grain flour in bread, cookies, and bakery products; and starch in thickening of sauces, soups, and gravies (Jimoh et al. 2018). Amaranth seeds are an underexploited plant source of squalene, a very important compound in the cosmetic, food, and pharmaceutical industries (Krulj et al. 2016).

By 2050, the Food and Agriculture Organization (FAO) estimates that the food demand will be increased over 60% to feed the 10 billion people on the Earth (Miladinovic et al. 2021). It can reduce poverty, malnutrition, and food insecurity by diversifying our food supplies and reducing the risks associated with our reliance on a few basic crops. The adaptation of many amaranths to reduced water supply compared to other leafy vegetables or even most grain crops, means that this genus if very important in the face of growing worldwide droughts and rainfall variability due to climate change effects. Many amaranths are also adapted to marginal soils and are very successful at capturing nutrients before these are leached from soils. Furthermore, they are deep rooted and capable of growing in a variety of soil types from sandy to silt-loams to heavy clays.

7.1.3 Limitations of Traditional Breeding and Rational of Genome Designing

Some efforts have been made towards the improvement of amaranth over the past few decades but the achievements have not met consumer demand. The result has mostly occurred because most yield- or quality-related characters are quantitative. Conventional breeding alone is insufficient in simultaneous improvement of multiple quantitative complex characters due to low heritability, genotype–environment (G × E) interaction and linkage drag (Zhang 2007). Implementation of advanced breeding approaches along with novel selection approaches can accelerate gains from crop improvement programs. Marker assisted breeding or genomics assisted breeding have not been much employed yet due to unavailability of genomic information for vegetable amaranths, although some advances for grain amaranths have been made (Maughan et al. 2011; Lightfoot et al. 2017; Tiwari et al. 2021).

In the last decade, some molecular markers have been made available in different amaranth species; however, most have been deployed only for improvement or understanding of abiotic stress tolerance (Jamalluddin 2020; Kreiner et al. 2021; Murphy et al. 2021). The study of the genetics of biotic stress resistance has been limited in all kinds of amaranths. Resistances and tagged genes for both types of resistances are needed for rational genomic design of new varieties, the subject of this chapter. Genomic design requires abroad collection of molecular tools and genetic data about important agronomic traits and biotic stress resistance in various amaranth species of interest; and has been recommended to enhance the precision and efficacy of selection and to shorten the duration required for trait improvement and pyramiding multiple desirable traits in crop plants (Qian et al. 2016).

Conventional breeding has facilitated to improve food security and crops with improved yield and resistance/tolerance to biotic and abiotic stresses along with increased quality characters (Miladinovic et al. 2021). However, the changing climate and greater consumer demands in recent time resulted in increased challenges for plant breeders expected to be overcome. Climate changes (increasing temperatures, droughts or floods in a certain geographical area) are projected to have extensive adverse impacts on agricultural production, disturbing food production in future. Furthermore, climate changes could result in damaging effects which might be associated with diseases and pests' outbreaks resulting in reduced crop production and quality of the harvested products (Raza et al. 2019). These conditions will have adverse effects on plants and demand new improved varieties and altered production systems in different geographic regions. Although seemingly efficient, it is not resilient to sudden changes in yield shocks posed by environmental changes or changed trading due to changed demands or changed financial market balance.

Genomics assisted breeding and genome editing approaches provide new tools for the designing of crops with improved characters (e.g. disease/pest resistance). These approaches will enable rapid development of new crop varieties with better adaptability to any biotic or abiotic changes through precision breeding. Although few examples of marker assisted selection of the priority biotic stresses exist for

vegetable amaranths, we discuss in this chapter which diseases and pest challenges and resistances would be amenable to this approach. Hence, we review the biotic stresses of amaranth below and then describe the genomic resources developed for certain grain and weed amaranths and the implications these will have combined with plant breeding and genetic engineering on the vegetable species. We hope to touch on the state-of-the-art for amaranth improvement as it currently exists even if it is in initial stages of development.

7.2 Biotic Stresses

7.2.1 Diseases and Pests of Amaranth

Amaranth is relatively less susceptible to pathogens and insect pests than most comparable vegetable and agronomic crops. However, several diseases can be of major importance (including leaf blight, wet rot/Choanephora rot, white rust, and damping-off) and all amaranths, even weeds but especially leaf and grain species, host numerous insect pests (including flea beetles, leaf miner, stem weevil, and lepidopteran caterpillars among others). Here we concentrate on those biotic stresses of greatest concern for reducing yield and marketability of the amaranth crop (Mureithi et al. 2017).

The main fungal diseases include anthracnose, damping-off, wet rot/Choanephora rot, white rust, leaf spot, Alternaria leaf spot, root rot, and white blister rust of amaranth. Bacterial diseases of amaranth have been poorly studied but seem to be mostly irrelevant. Meanwhile amaranth has a few well-characterized viruses such as *Amaranthus leaf mottle virus* (*AmLMV*) and *Amaranthus mosaic virus* (*AMoV*) but seems to host many others as well especially from other vegetables that grow in similar conditions. Details of the diseases of grain amaranths, their causal organisms and symptoms have been annotated in Table 7.1.

The major insect groups causing losses to amaranth belong to the orders Lepidoptera, Coleoptera, Hemiptera, and Diptera (Mureithi et al. 2017). These affect various plant parts, but mostly leaves which being broad and single petiole can be rapidly consumed. Amaranth leaves of domesticated species are characteristically large compared to those of weedy species which have small leaves, less susceptible to attack. Insect pests can also be troublesome to stem tissues at the base of the plant or near the panicle, resulting in plant collapse or failure of seed formations.

The major insects on vegetable amaranth crops include stem weevil, pigweed weevil (Coleoptera), leaf miner (Diptera), cutworms/leaf worms, fall armyworm, leaf webber, lepidopteran defoliator, and Amaranthus caterpillar (Lepidoptera). Leaf beetles cause more damage on grain amaranth but are considered minor pests of vegetable types, although they cause cosmetic damage that may influence consumer purchases of leaves. These include flea beetle, leaf twisting weevil, and tortoise beetle (Coleoptera). Meanwhile, other pests include mealy bugs, aphids

Table 7.1 Diseases of amaranth

Disease	Causal organism	Symptoms	References
Fungal diseases			
Alternaria leaf spot	*Alternaria* spp., *A. tenuissima*	Brown to black, circular to oval, necrotic lesions on leaves, may cause complete crop loss	Blodgett and Swart (2002)
Anthracnose	*Colletotrichum gloeosporioides*, *Glomerella cingulata*	Necrotic lesions on leaves, dieback of leaves and branches	Kwon and Park (2003)
Cercospora leaf spot	*Cercospora* spp.	Leaf spots are amphigenous, circular or irregular, 2–5 mm in diameter, coalescent, necrotic, light brown, with dark brown margin, sometimes with chlorotichalo	Vieira et al. (2019)
Damping-off	*Pythium* spp., *P. aphanidermatum*, *P. myriotylum*,	Poor germination, seedling collapse, brown-black lesions girdling stem close to soil line	Lopez et al. (2018)
Leaf spot	*Cercospora* spp., *C. brachiata*	Brown spots and necrosis on leaves	Vieira et al. (2019)
Root rot	*Fusarium* spp., *F. oxysporum*, *F. sambucinum*, *Rhizoctonia* spp.	Severe stunting of plants with chlorotic and wilted foliage, amber to brown discoloration of taproot and secondary roots, white mycelium on diseased tissue	Chen and Swart (2000)
Wet rot (choanephora rot)	*Choanephora cucurbitarum*	Water-soaked lesions on stems, lesions have hairy appearance based on fungal spores, may have leaf loss	Awurum and Uchegbu (2013)
White rust	*Albugo candida*, *A. bliti*, *A. occidentalis*, *A. amaranthi*	Defoliation and withering of whole plant	Talukder et al. (2012); Islam (2019)
White blister rust disease	*Wilsoniana amaranthi*, *W. bliti*	Yellow spots on the upper surface of leaves and typical white rust pustules on the lower surface of leaves	Kim et al. (2019); Lee et al. (2020)

(continued)

Table 7.1 (continued)

Disease	Causal organism	Symptoms	References
Viral diseases			
Amaranthus leaf mottle virus (AmLMV)	*AmLMV* (Potyviridae)	Leaf mottling, blistered mosaic, and growth reduction	Casetta et al., (1986); Sastry et al. (2019); Segundo et al. (2007)
Amaranthus mosaic virus (AMV)	*AMoV*	Severe mosaic, mottling, and curling of leaves with stunting	Kareem et al. (2011); Sastry et al. (2019)
Capsicum chlorosis virus (CaCV)	*CaCV*	Characteristic symptoms of tospoviruses	Sharma and Kulshrestha (2014)
Chili leaf curl virus (ChiLCV)	*ChiLCV* begomovirus (Geminiviridae)	Plants displaying leaf curling, leaf distortion, leaf crinkling and yellow leaf margins	George et al. (2014)
Cucumber mosaic virus (CMV)	*CMV*	One isolate causing leaf crinkle and severe mosaic	Raj et al. (1997)
Iris yellow spot virus (IYSV)	*IYSV tospovirus* (Bunyaviridae)	Thrips damage on leaves indicate overwintering host of onion disease	Karavina and Gubba (2017)
Telfairia mosaic virus (TeMV)	*TeMV*	Photosynthetic pigments of *A. viridis* were decreased by *TeMV* infection	Mofunanya et al. (2021)

(Hemiptera), grasshopper (Orthoptera), thrips (Thysanoptera), and root-knot nematode (Tylenchida). The details of insect pests damaging amaranth, their common names, species identification and damage characteristics are provided in Table 7.2.

7.2.2 Reduction in Yield and Quality Due to Biotic Stresses

Some of the diseases and insect pests listed above cause considerable reduction in yield and quality of amaranth. For instance, yield losses of 20–100% by major arthropod pests have been reported in Kenya (Sithanantham et al. 2003; Mureithi et al. 2017). Massive yield losses to Amaranthus caterpillar (*Spoladea recurvalis*) have been reported in Nigeria (Aderoluet al. 2013). Extensive yield losses to amaranths by another lepidopteran, *Spodoptera littoralis* (Lepidoptera; Noctuidae), have been reported in both Nigeria and Mexico (Aragón et al. 1997; Aderolu et al. 2013). This same species of insect is widely distributed in most parts of sub-Saharan Africa and

Table 7.2 Insect pests of amaranths

Order	Family	Common name	Scientific name	Damage	References
Major insect pests					
Coleoptera	Chrysomelidae	Flea beetles Cucumber beetles	*Disonycha melanocephala* *D. bicolor*, *Diabrotica balteata*	Amaranth bulging flea beetle, leaf beetles	Aragón-García et al. (2011)
	Curculionidae	Pigweed weevil	*Hypolixus haerens*, *H. nubilosus*	Withering plants, stems bending and collapsing	Kagali et al. (2013); Anil (2017)
		Stem weevil	*Hypolixus truncatulus*	Scratching on stem and branches, eat up tender margin of leaves	Tara et al. (2009)
Diptera	Agromyzidae	Leaf miner	*Liriomyza* spp., *L. huidobrensis*	Creates tunnels inside leaves resulting in leaf yellowing and shed. Death of seedlings follows in severe infestation	Mureithi et al. (2017)
Lepidoptera	Noctuidae	Cutworms or leaf worms	*Spodoptera* spp.	Cuts through the stem of young plants just above/below ground level causing plant wilt and death	Mureithi et al. (2017)
		Fall armyworm	*Spodoptera frugiperda*	Skeletonizing upper epidermis, spaces on leaves, and faecal pellets in the whorls	Maruthadurai and Ramesh (2020)
	Pyraustidae	Leaf webber	*Hymenia recurvalis*, *Psara basalis*	Attack on stem and leaves, larvae fold/web leaves and feed within leaves	Kagali et al. (2013); Aragón-García et al. (2011); Mureithi et al. (2017); Oliveira et al. (2012); Anil (2017)

(continued)

Table 7.2 (continued)

Order	Family	Common name	Scientific name	Damage	References
	Crambidae	Lepidopteran defoliator (white grubs)	*Herpetogramma bipunctalis*	Defoliation of plants	
		Amaranthus caterpillar	*Spoladea recurvalis*	Feed within leaves, cause yield loss	
	Heliodinidae	Lepidopteran defoliator	*Eretmocera impactella*	Leaf feeding	
Minor insect pests					
Coleoptera	Curculionidae	Leaf twisting weevil	*Apoderus tranquebaricus*	Leaf rolling	Anil (2017)
	Cassididae	Tortoise beetle	*Aspidiomorpha exilis*	Feed by scrapping outer tissues of leaves, defoliation	Sultan et al. (2008)
Hemiptera	Coreidae	Bugs	*Cletus* spp., *Cletomorpha* spp.	Insects damage flowering head, feed on seeds, cause discoloration, shrivelling, and premature drying of seeds, reduce seed yield and viability	Oke and Ofuya (2011)
	Pseudococcidae	Mealy bugs	*Ferrisia virgata*	Sap-feeding, reduced plant growth, sticky exudate which favours fungal growth, leaf discoloration and drop	McCorquodale and Hodges (2017)
	Aphididae	Aphids	*Aphis craccivora*, *Myzus persicae*	Curling, wrinkling, and discolouring of leaves, stunting of plants, seed deformation, plants may dry out	Yarou et al. (2020)

(continued)

Table 7.2 (continued)

Order	Family	Common name	Scientific name	Damage	References
Orthoptera	Acrididae	Grasshopper	*Atractomorpha crenulata*	Feed by scrapping outer tissues of leaves, defoliation	Seni (2018)
Thysanoptera	Thripidae	Thrips	*Haplothrips ceylonicus*	Infest inflorescence	Iftikhar et al. (2016)
	Phlaeothripidae		*Euryaplothrips crassus*		
Tylenchida	Heteroderidae	Root-knot nematode	*Meloidogyne* spp. (*M. javanica*, *M. incognita*, *M. arenaria*)	Galls formation on roots, reduced branching of roots, reductions in shoot height, leaf area and shoot and root dry weight	Vaingankar et al. (2018)

affects leaf production and income generation as amaranths are the most important leafy vegetable of this region (Mureithi et al. 2017). Another caterpillar, the fall armyworm (*Spodoptera frugiperda*) (Lepidoptera: Noctuidae) damages amaranth in India and causing c. 13% yield loss (Mureithi et al. 2017).

Amaranth stem weevils (*Hypolixus* spp.) are among the most serious coleopteran pests of amaranth. Infestation by stem weevil of 81% has been reported in India for vegetable amaranths (Mureithi et al. 2017). The leaf miner (*Liriomyza huidobrensis* Blanchard) (Diptera; Agromyzidae) is widespread in the Mediterranean and reduces production of any type of amaranth. This species also has colonized other areas of the world from Asia to America. Even if scanty data on geographical distribution, host range, virus transmission, and economic importance of some of these pests are available, they are of concern in amaranth production due to the capacity for long distance migration, temporal spread, quarantine issues and moderate to severe damages they cause.

7.2.3 Control of Diseases and Pests

The use of chemicals to control diseases and pests has not been recommended due to their excessive cost, residue, and environmental issues. Therefore, research has focused on implementing non-chemical methods of pest control, which are cheap, safe, easy to use, and available to farmers. Botanicals from various plants have shown considerable potential for pest control (Yarou et al. 2020).

Integrated pest management (IPM) combines host plant resistance and cultural methods of control as options for pest and disease management (Vaingankar et al. 2018). Many plant species contain biocidal components which can be utilized in controlling insect pests, leading to reduced use of synthetic pesticides and to increase the quality of vegetable crops (Yarou et al. 2020). For example, *Ocimum* spp. (*O. gratissimum* L. and *O. basilicum* L.) can be used as an alternate method to control aphids (*Aphis craccivora* Koch, *A. fabae* Scopoli, and *Myzuspersicae* Sulzer) in *A. hybridus* and can help to avoid the use of synthetic pesticides (Yarou et al. 2020). Plants of *Ocimum* spp. have an ability to repel pests and they can also be harvested, providing a direct economic return to the farmer (Yarou et al. 2020).

Vegetable oil-based extracts of *Xylopia aethiopica*, *Eucalyptus globolus*, and *Alium sativum* can reduce the infestation of nine pests (major, minor or occasional) belonging to three orders namely, Orthoptera, Coleoptera, and Lepidoptera in *A. hybridus* (Borisade et al. 2019). African marigold (*Tageteserecta* L.) has been reported to destroy nematodes as an intercrop (Hooks et al. 2010; Vaingankar et al. 2018). Biorational insecticides from different plants (e.g. *Jatropha curcas*, *Azardirachta indica*, *Ocimum gratisimum*, *Vernonia amygdalina*, and *Chrysanthemum* spp.) and microorganisms (e.g. *Bacillus thuringiensis* and *Saccharopolyspora spinosa*) are effective in pest management of leafy vegetables including amaranth (Iwuagwu et al. 2019; Muralikrishna et al. 2019; Vorsah et al. 2020).

Trap cropping has been proven to be an effective strategy to control nematodes (Vaingankar et al. 2018). Nematodes enter and grow in the susceptible host plant of an intercrop which is consequently detached before the completion of nematode's life cycle (Vaingankar et al. 2018). Plant color (green or red) has been associated with the preference of insect in feeding and oviposition. Host preference differences can also be exploited for pests. For example, many insects prefer green plants and tend to avoid red plants because it indicates that red plants are defended by phytochemicals or that red compounds are accompanied by colorless phenolics (Niveyro et al. 2013). The development and use of red plants/varieties might decrease the incidence of insects.

7.3 Glimpses on Classical Genetics and Traditional Breeding

7.3.1 Breeding Objectives for Vegetable Amaranth

Efficient and comprehensive breeding programs for grain or vegetable amaranth improvement have yet to be established, except in a few locations mostly within universities and non-profit organizations (Das 2016). Experimental approaches and breeding objectives are very important for continuous genetic improvement and they are quite different in grain and vegetable amaranths. The breeding objectives for vegetable amaranth are tolerance to heat, improved seedling establishment, improved nutritional profile, improved seedling vigor, increased leaf size, reduced length of petiole, improved leaf/stem ratio (should be >1), attractive leaf color (dark green is preferable), reduced antinutritional compounds (e.g. nitrates, oxalates etc.), more days to 50% bolting (late bolting lines are preferable), increased yield, increased tolerance to drought, and increased resistance to biotic stresses. Breeding objectives for grain amaranths have to do with seed yield and ease of threshing. Across both types, drought tolerance and adaptation to marginal soils has been important.

7.3.2 Classical Breeding Achievements

Conventional (or classical) breeding has played a key role in the genetic improvement of grain and vegetable amaranths. Commercial amaranths have been selected from field studies in many developing countries eager for new crop alternatives and heat tolerant vegetables such as in China, Peru, Kenya, India, Mexico, and Thailand (Das 2016). A collection of world germplasm and breeding lines was established in Taiwan at WorldVeg Center (previously known as Asian Vegetable Research and Development Center or AVRDC). Among developed countries, only the United States has had an interest in amaranth cultivar selection and mainly for hot or dry areas in

the South and West. In India, various research organizations and universities are actively involved in amaranth improvement for promising varieties (Dua et al. 2009; TNAU 2017; KAU 2020). All the varieties have been developed through conventional breeding methods e.g. selection and hybridization. Some varieties are resistant to one or more disease(s)/pest(s) e.g. varieties 'Kashi Suhaavani' (VRAM-42), 'Arka Arunima', and 'Arka Suguana' are tolerant, highly resistant and moderately resistant to white rust, respectively. CO-1 is resistant to Rhizoctonia leaf blight, and PLR-1 is moderately resistant to several pests and diseases. These resistant varieties can be exploited in developing new varieties resistant to various diseases or pests.

Field evaluation in Tanzania has been important for the identification of amaranth genotypes resistant to biotic stresses in other parts of the world as well. Two accessions of *A. cruentus* (TZ51 and TZ53), one of *A. dubius* (TZ34), and one of unknown *Amaranthus* spp. (TZ39) have moderate resistance against Lepidopteran insects [*Spoladea recurvalis* (Crambidae), *Spodoptera exigua* (Noctuidae), and *Spodoptera littoralis* (Noctuidae)] (Smith et al. 2018). In the same study, *A. cruentus* (TZ06 and TZ27) had moderate resistance against stem weevils [*Neocleonussannio* Herbst, *Gasteroclisus* pr. *rhomboidalis* Boheman, *Hypolixus* pr. *haerens* Boheman, and *Baradine* spp. (Curculionidae)]. Furthermore, Othim et al. (2018) also working in Tanzania reported that breeding lines VI036227 (*A. blitoides*), RVI00027 (unknown *Amaranthus* sp.), VI054569 (*A. gracilis* Desf.), VI033487 (*A. cruentus*), VI044432 (*A. viridis*), VI048076 (*A. tricolor*), VI049639 (*A. viridis*), VI049530 (unknown *Amaranthus* spp.), and VI049698 (*A. viridis*) were highly resistant against Lepidopteran (leaf-webbers and leaf-worms). Three accessions namely, VI047517-B (*A. tricolor*), VI036227 (*A. blitoides*), and VI056563 (*Amaranthus* spp.) have been reported for resistance against stem weevil while VI048076 (*A. tricolor*), VI056563 (*Amaranthus* sp.) and VI047555-B (*A. tricolor*) shown moderate resistance against *Spoladea recurvalis*.

Breeding in the United States has produced one main grain amaranth variety, 'Plainsman', with good plant architecture for row crop production. However, a number of dual-purpose amaranth selections are mass marketed for sale such as 'Burgundy' and 'Hopi Red' by seed companies selling to the ornamental and home gardener. The details of vegetable amaranth varieties released in India and Unites States are given in Table 7.3.

Mutation breeding has been used in amaranth for development of new cultivars and generation of variability. These include, 'New Asutake' for early maturity in Japan, 'Centenario' for improved grain yield in Peru, 'Sterk' for tolerance to moisture and heat stress in Russia, and 'Pribina' and 'Zobor' in Slovakia (Gómez-Pando et al. 2009; Das 2016). Promising mutant lines of *A. cruentus* namely, lines C26 and C82 with enhanced 1000-seed weight have been developed through gamma irradiation (Gajdošová et al. 2008; Hricova et al. 2016). Putative mutant lines of *A. cruentus* and *A. hypochondriacus* with higher protein have been developed through gamma irradiation (Kečkešová et al. 2021). Two mutant varieties 'Pribina' and 'Zobor' belonging to *A. cruentus* and *A. hypochondriacus* × *A. hybridus*, respectively have been developed by gamma irradiation in Slovakia. They showed changes in quantitative traits

Table 7.3 Some varieties of vegetable and grain amaranth released in India and the United States

Name of variety	Species	Pedigree and breeding method	Year of release	Green yield (t/ha)	Resistant to biotic stress	Developed by
CO-1	*A. dubius*	Selection from local germplasm introduced from Tirunelveli	1968	8	Resistant to leaf blight and white rust	HCRI, TNAU, Coimbatore
CO-2	*A. tricolor*	Selection from local germplasm introduced from Thanjavur	1979	10.78	–	
CO-3	*A. tristis*	Selection from local germplasm	1988	30.72	–	
CO-4	*A. hypochondriacus*	Selection from local germplasm	1989	8.2	–	
CO-5	*A. tricolor*	–	1998	40.7	–	
PLR 1	–	Selection from Tiruvannamalai	2013	8–9	Moderately resistant to pests and diseases	VRS, Palur, TNAU
Pusa Chhoti Chaulai	*A. blitum*	Selection at IARI	–	–	–	ICAR-IARI, New Delhi
Pusa Badi Chaulai	*A. tricolor*	Selection at IARI	–	–	–	
Pusa Kirti	*A. blitum*	–	1991	55	–	
Pusa Kiran	*A. tricolor*	Hybridization between *A. tricolor* and *A. tristis*	1991	35	–	
Pusa Lal Chaulai	*A. tricolor*	–	1991	45–49	–	
Arka Suguana	*A. tricolor*	Pure line selection from IIHR Acc. No. 13560, an exotic introduction from Taiwan	–	25–30	Moderately resistant to white rust	ICAR-IIHR, Bangalore

(continued)

Table 7.3 (continued)

Name of variety	Species	Pedigree and breeding method	Year of release	Green yield (t/ha)	Resistant to biotic stress	Developed by
Arka Arunima	*A. tricolor*	Pure line selection from IIHR Acc. No. 18384	–	26–28	Resistant to white rust	
Arka Samraksha (IlHR-1-21) (green stem)	–	Modified bulk method of selection from F_6 population of IIHR-4× IIHR-70	2018	10–12	–	
Arka Varna (pink stem)	–	Modified bulk method of selection from F_6 population of IIHR-7× IIHR-30	2018	10–12	–	
Arun	–	Palapoor local (mass selection)	1992	–	–	KAU, Kerala
Renusree (green)	–	Selection	2006	15.5	–	
Krishnasree (red)	–	Selection	2006	14.8	–	
KAU Vaika	–	Local collection from Vellarada	2019	–	–	
Kashi Suhaavani (VRAM-42)	–	–	2019	30–33	Tolerant to white rust	ICAR-IIVR, Uttar Pradesh
Plainsman	*A. cruentus*	Breeding line for Nebraska ADAP	1992		Architecture, drought	Rodale

Abbreviations: DBSKKV: Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth; HCRI: Horticultural College and Research Institute; IARI: Indian Agricultural Research Institute; ICAR: Indian Council of Agricultural Research; IIHR: Indian Institute of Horticultural Research; IIVR: Indian Institute of Vegetable Research; KAU: Kerala Agricultural University; VRS: Vegetable Research Station

of seed along with higher oil and squalene content compared to commercial cultivars. Additionally, 'Zobor' also showed significantly higher linoleic acid content (Szabóová et al. 2020).

7.3.3 Global Collection of Amaranth Germplasm

The maximum number of amaranth germplasm accessions have been collected and conserved by the Indian Council of Agricultural Research within the National Bureau of Plant Genetic Resources (ICAR-NBPGR). This National Gene Bank of India has 6,309 amaranth accessions (ICAR-NBPGR 2020). The next largest Gene Bank is held by the United States Department of Agriculture Agricultural Research Services (USDA-ARS) at its North Central Regional Plant Introduction Station (NCRPIS) location in Ames, Iowa, USA with 3,502 accessions. Other smaller collections are held at institutes in Bolivia, Ecuador, Mexico, Peru and Taiwan or within University programs in India and the United States, primarily (Fig. 7.1) (Jacobsen and Mujica 2003; AVGRIS 2020; GENESYS 2020; ICAR-NBPGR 2020; TNAU 2021).

These germplasm collections are useful for finding sources of resistance against different biotic (disease pathogens and insects), soil (low nitrogen and phosphorus) or weather related (heat, drought and cold climates) stresses. These sources of resistance can be further utilized to develop new variety or population resistance against particular disease or insect through conventional breeding or advanced biotechnological approaches. Some of the Gene Banks emphasize on a few species of *Amaranthus*, such as those in Latin America while others particularly those of South Asia and North America emphasize on multiple *Amaranthus* species.

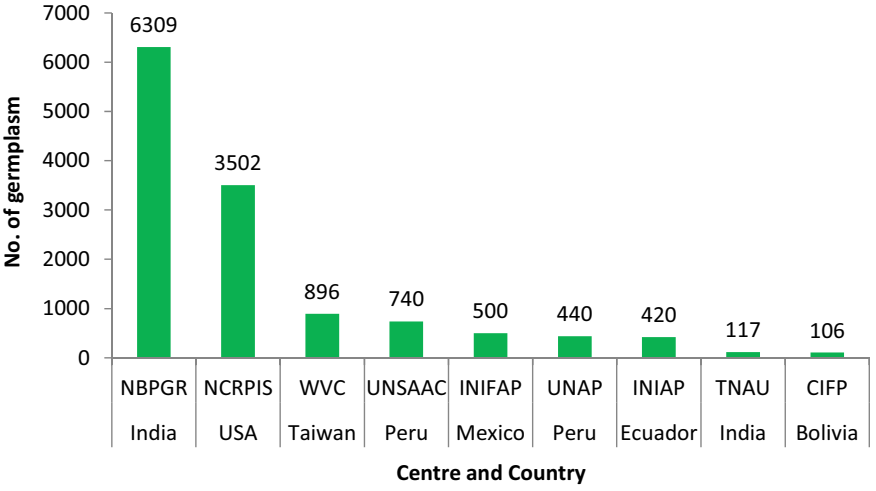

Fig. 7.1 Amaranth germplasm collections found around the world. Abbreviations: NBPGR: National Bureau of Plant Genetic Resources, New Delhi, India; NCRPIS: North Central Regional Plant Introduction Station, USDA-ARS, Ames, Iowa, USA; WVC: World Vegetable Centre, Taiwan; UNSAAC: Universidad Nacional de San Antonio Abad del Cusco, Peru; INIFAP: Instituto Nacional de Investigaciones Forestales, Agrrícolas y Pecuarias, Mexico; UNAP: Universidad Nacional del Altiplano, Escuela de Peru; INIAP: Instituto de Investigaciones Agropecuarias EE. Santa Ecuador; TNAU: Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India; CIFP: Centro de Investigaciones Fitoecogenéticas de Pairumani, Cochabamba, Bolivia

7.4 Genetic Diversity in Amaranths and Their Wild Relatives

Amaranth species have great genetic variability in morphological characteristics, particularly in relation to growth habit, inflorescence type and color, leaf shape and color, stem color, as well as resistance to diseases and pests (Nyonje et al. 2021). Elucidation of genetic diversity is very advantageous to a plant breeder to ascertain diverse parents in creating segregating populations with genetic variability. It also enables introgression of desirable genes from a diverse germplasm into the prevailing population (Thompson et al. 1998). Although, vegetable amaranth is used as an inexpensive source of antioxidants, minerals, other nutrients, and the main food crop in many countries of the world, not many efforts have been made towards its genetic improvement (Shukla et al. 2006).

Genetic variability can be evaluated by collecting information on morphological, cytological, biochemical, or molecular markers (Dharajiya et al. 2021). The phylogenetic relationships to study extents of variation among different species of amaranths have been studied (Das 2016). The extensive genotypic diversity in *Amaranthus* spp. could be due to frequent interspecific and intervarietal hybridizations or introgression events (Suresh et al. 2014). Two different major groups of amaranths: namely the grain and vegetables types have evolved from their specific wild relatives through individual domestication events in different parts of the world. There is much confusion in evolutionary relations of amaranth species which can be resolved by assessing genetic diversity (Dharajiya et al. 2021).

7.4.1 Morphological Diversity in Amaranth

Characterization and morphological diversity assessment of plant genetic resources of crop species provides essential information for breeding programs of crops (Gerrano et al. 2017). Morphological characterization of amaranth can play an important role in resolving taxonomic obscurities in *Amaranthus* spp. Morphological characters of different plant parts like inflorescence, flowers, seed, leaves, stem, pollen, and phyllotaxy are considered as an important part in distinguishing taxa (Das 2016). On the bases of morphological characters, intra-specific and interspecific genetic diversity have been assessed in some *Amaranthus* species (Table 7.4). A wide range of intraspecific diversity in vegetable *Amaranthus* spp. viz. *A. tricolor* (Shukla et al. 2010; Ahammed et al. 2013), *A. hybridus* (Oboh 2007), and *A. lividus* (Rashad and Sarker 2020) has been evaluated. Interspecies genetic diversity among *A. tricolor* var. *tristis*, *A. tricolor*, *A. blitum*, and *A. dubius* has been assessed and resulted in heterogeneous clusters concerning species and geographical origin (Anuja and Mohideen 2007). Accessions belonging *A. hybridus*, *A. dubius*, *A. tricolor*, and *A. cruentus* have been evaluated for the assessment of genetic diversity resulted in formation of clusters on the basis of morphological characters and their geographical

Table 7.4 Diversity analysis in amaranth species based on morphological and biochemical characters

Sr. no.	Species	No. of genotypes	Characters		References
			Type	No.	
1	*A. hybridus*	16	Quantitative	14	Oboh (2007)
2	*A. tricolor*	39	Quantitative	16	Shukla et al. (2010)
3	*A. hypochondriacus*, and *A. tricolor*	13	Quantitative	11	Erum et al. (2012)
			Qualitative	4	
4	*A. cruentus*, *A. tricolor*, *A. dubius*, and *A. hybridus*	28	Quantitative	22	Shankar et al. (2012)
5	*A. tricolor*	22	Quantitative	12	Ahammed et al. (2013)
6	*A. blitum*, *A. caudatus*, *A. dubius*, *A. hybridus*, *A. spinosus*, *A. tricolor*, and *A. viridis*	53	Quantitative	9	Andini et al. (2013)
			Qualitative	3	
7	*A. caudatus*, *A. viridus*, *A. graecizans*, *A. tricolor*, and *Amaranthus* sp. (unknown)	32	Quantitative	14	Gerrano et al. (2015)
8	*A. spinosus*, *A. gracilis*, *A. hybridus*, and *A. tricolor*	18	Quantitative	12	Gueco et al. (2016)
			Qualitative	8	
9	*A. caudatus*, *A. viridus*, *A. graecizans*, *A. cruentus*, *A. tricolor*, and *Amaranthus* sp. (unknown)	32	Qualitative	16	Gerrano et al. (2017)
10	*A. cruentus*, *A. hypochondriacus*, *A. caudatus*, *A. hybridus*, *A. quitensis*, *A. powellii*, *A. retroflexus*, *A. palmeri*, and *Amaranthus* sp. (Unknown)	293	Qualitative	9	Thapa and Blair (2018)
11	*A. spinosus*, *A. atropurpureus*, *A. cruentus*, *A. viridis*, *A. thunbergii*, *A. caudatus*, *A. graecizans*, *A. mantegazzianus*, *A. hypochondriacus*, *A. blitum*, *A. lecuocarpus*, *A. dubius*, *A. retroflexus*, *A. gracilis*, *A. tricolor*, *A. hybridus*, and *A. palmeri*	50	Quantitative	8	Kiruthika et al. (2019)
			Qualitative	14	
12	*A. lividus*	20	Quantitative	9	Rashad and Sarker (2020)
13	*A. albus*, *A. blitiodes*, *A. caudatus*, *A. graecizans*, *A. hybridus*, *A. lividus*, *A. retroflexus*, *A. spinosus*, *A. tricolor*, and *A. viridis*	10	Quantitative	4	Taia et al. (2021)
			Qualitative	22	

origin along with the co-existence of accessions native to different geographic regions (Shankar et al. 2012). Evaluation of morphological diversity can be helpful in identifying superior genotype for particular character. Amaranth genotypes belonging to *A. viridis*, *A. tricolor*, *A. dubius*, *A. blitum*, *A. spinosus*, *A. hybridus*, and *A. caudates* have been assessed for morphological diversity which indicated that *A. dubius* and *A. viridis* genotypes could be used as valuable parental lines in breeding programs for yield improvement and protein content, respectively (Andini et al. 2013). The clustering of the accessions can be useful in the recognition and selection of genetically diverse parents having the greatest inter-cluster distance which may give high levels of heterosis for the desired traits in breeding programs (Anuja and Mohideen 2007).

7.4.2 Molecular Diversity in Amaranth

Molecular markers are powerful tools to identify, characterize, and elucidate origin and diversity of genotypes (Dharajiya et al. 2020). Various molecular markers viz., random amplified polymorphic DNA (RAPD) (Ray and Roy 2009; Sammour et al. 2020), internal transcribed spacer (ITS) (Xu and Sun 2001), amplified fragment length polymorphism (AFLP) (Chandi et al. 2013), inter-simple sequence repeat (ISSR) (Gelotar et al. 2019), simple sequence repeat (SSR) (Oo and Park, 2013; Nguyen et al. 2019), and single nucleotide polymorphism (SNP) (Xu et al. 2020) have been utilized in the diversity analysis among and/or within *Amaranthus* spp. Work done on inter- and/or intra-specific diversity analysis in vegetable amaranth have been shown in Table 7.5.

Suresh et al. (2014) grouped 348 amaranth accessions from 33 vegetable species into seven groups collected from different geographical locations in order to investigate relationship among 33 weedy, grain and vegetable *Amaranthus* using 11 SSR markers. The cluster analysis showed that weedy type appeared to be more diverse based on expected heterozygosity (H_E) and polymorphic information content (PIC) followed by vegetable type having and then grain types. In that study, simple grouping did not strictly follow geographic affiliations (Suresh et al. 2014) as was the case for Thapa and Blair (2018). By characterizing the genetic diversity using a combination of morphological, biochemical, physiological, and molecular data, accessions with superior stress resistance traits can be interpreted.

7.5 'Omics' Assisted Breeding

The advent of molecular biology applied to higher plants especially crops in agronomy and horticulture has led to a revolution in plant breeding based on more accurate genotyping of breeding lines. "Omics" are bodies of knowledge about genes (genomics), messenger RNAs (mRNAs) and expression (transcriptomics), proteins

Table 7.5 Genetic diversity among grain and vegetable amaranth species using molecular markers

Sr. no	Species	Type	No. of accessions	Molecular marker	Coefficient used and range	References
1	*A. caudatus*, *A. cruentus*, *A. hypochondriacus*, *A. hybridus*, *A. powellii*, *A. retroflexus*	Grain, Wild	179	SSR	H: 0.14 to 0.83	Mallory et al. (2008)
2	*A. gangeticus* (syn. *tricolor*), *A. paniculatus*, *A. viridis*, *A. hypochondriacus*, *A. caudatus*, and *A. cruentus*	Grain, Wild	30	RAPD	J: 0.16–0.97	Ray and Roy (2009)
3	*A. caudatus*, *A. cruentus*, *A. hypochondriacus*, *A. hybridus*, *A. powellii*, *A. retroflexus*, *A. tuberculatus*	Grain, Wild	480	SNP-Kasp	MAF: 0.05–0.5	Maughan et al. (2011)
4	*A. blitum*, *A. Deflexus*, *A. graecizans* subsp. *sylvestris*, *A. mantegazzianus*, *A. standleyanus*, *A. viridis*, *A. quitensis*, *A. caudatus*, *A. hybridus*, *A. tricolor*, and *Amaranthus* spp. (unknown)	Wild, Vegetable, Ornamental	75	SSR	N: 0.03–0.89	Oo and Park (2013)

(continued)

Table 7.5 (continued)

Sr. no	Species	Type	No. of accessions	Molecular marker	Coefficient used and range	References
5	*A. acutilobus*, *A. albus*, *A. arenicola*, *A. australis*, *A. blitoides*, *A. blitum*, *A. blitum*var. *oleraceus*, *A. bouchonii*, *A. caudatus*, *A. caudatus*var. *albiflorus*, *A. crassipes*, *A. crispus*, *A. cruentus*, *A. deflexus*, *A. dubius*, *A. fimbriatus*, *A. floridanus*, *A. gangeticus* var. *melancholicus*, *A. hybridus*, *A. hypochondriacus*, *A. lividus*, *A. mangostanus*, *A. mantegazzianus*, *A. palmeri*, *A. powellii*, *A. powellii* subsp. *bouchonii*, *A. quitensis*, *A. retroflexus*, *A. spinosus*, *A. standleyanus*, *A. tricolor*, *A. tuberculatus*, *A. viridis*, and *Amaranthus* sp. (unknown)	Grain, Wild, Vegetable	348	SSR	CS: 0.48–0.91	Suresh et al. (2014)
6	*A. caudatus*, *A. cruentus*, *A. hypochondriacus*, *A. powellii*, *A. quitensis*, *A. retroflexus*	Grain, Wild	10,668	SNP-GBS	n/a	Wu and Blair (2017)

(continued)

Table 7.5 (continued)

Sr. no	Species	Type	No. of accessions	Molecular marker	Coefficient used and range	References
7	*A. tricolor* and *A. hypochondriacus*	Vegetable, Grain	300	SSR	–	Nguyen et al. (2019)
8	*A. hypochondricus*, *A. cruentus*, *A. caudatus*, *A. hybridus*, *A. palmeri*, *A. retroflexus*, *A. quitensis*, *A. powellii*, *A. tricolor*, and *A. spinosus*	Grain, Wild, Vegetable	25	RAPD	J: 0.11–0.88	Sammour et al. (2020)
9	*A. albus*, *A. arenicola*, *A. blitoides*, *A. blitum*, *A. bouchonii*, *A. capensis*, *A. caudatus*, *A. crispus*, *A. cruentus*, *A. deflexus*, *A. dubius*, *A. fimbriatus*, *A. graecizans* ssp. *sylvestris*, *A. hybridus*, *A. hypochondriacus*, *A. palmeri*, *A. polygonoides*, *A. powellii*, *A. retroflexus*, *A. spinosus*, *A. standleyansus*, *A. tennuifolius*, *A. tricolor*, *A. tuberculatus*, *A. tuberculatus* var. *tuberculatus*, *A. viridis*	Grain, Wild, Vegetable	26	SNP	–	Xu et al. (2020)

Abbreviations: CS: Chord distance; D: Dice similarity coefficients; J: Jaccard's similarity coefficient; H: Heterozygosity; MAF: Minor allele frequency; N: Nei's genetic distance matrix; NL: Coefficient

(proteomics) and metabolites (metabolomics) which assist in both basic and applied sciences.

Each area of omics has many of its own technologies, under-laid with the central dogma of transcription and translation as well as modifications in gene regulation, protein modification and metabolic pathways. The characterization of these pools of biological molecules provide a context for functional genomics which allows for a deeper understanding of each gene and its manipulation via selection and targeted modifications such as clustered regularly interspaced short palindromic repeat (CRISPR). Within the scope of functional genomics are studies for gene discovery through marker tagging by association or other statistical means all the way to the creation of genetically modified organisms (GMOs). The initial part of the spectrum of science, especially gene tagging, is the one most useful in the immediate future of amaranth breeding, as gene modification, transgenesis and protein/metabolite study are still in its infancy for the genus.

7.5.1 Association Mapping Studies

Genome-wide association study (GWAS) and population genomic methods have been employed not surprisingly in the weedy species of the *Amaranthus* genome, where economic losses are high for major industrial agriculture. A case in point is the study of the genetic architecture of glyphosate resistance in waterhemp, *A. tuberculatus*, an important weed species in the United States. GWAS enabled appropriate recognition of the gene targeted by glyphosate and additional 250 genes related to non-target site resistance (NTSR) (Kreiner et al. 2021). Genome-wide SNPs showed a remarkable variation in glyphosate resistance to monogenic mechanisms and under-appreciated polygenic contribution to the evolution of herbicide resistance in *A. tuberculatus* (Kreiner et al. 2021). In one of the first studies in a vegetable amaranth, GWAS in *A. tricolor* was used to discover 25 marker trait associations (MTAs) associated with branching index, inflorescence color, petiole pigmentation, and terminal inflorescence shape and attitude (Jamalluddin 2020). The markers associated with specific characteristics can then be used for marker-assisted selection (MAS) for the respective traits under stress or non-stress conditions.

7.5.2 Molecular Mapping of Resistance Genes and Quantitative Trait Loci (QTLs)

Gene tagging is often assisted by molecular maps or whole genomes. To this end, Maughan et al. (2011) characterized the first complete genetic linkage map in the *Amaranthus* genus using SNP markers. This study followed up the partial sequencing of the *A. caudatus* genome through 454 pyro-sequencing (Maughan et al. 2009).

For the genetic mapping study, PI 481125 (female parent; *A. hypochondriacus*) and PI 642741 (male parent; *A. caudatus*) were crossed to develop an interspecific F_2 population (Maughan et al. 2011). Pairwise linkage analysis clustered all 411 SNP markers into 16 linkage groups (LGs) at a minimum logarithm of the odds (LOD) score of 5. The number of markers within the linkage groups varied from 9 to 47 SNPs/LG. The total map contained 411 SNP loci and covered 1288 cm. This map was a preliminary first step in the genetic dissection of agronomically important traits in cultivated grain amaranths.

In a similar study, but with weedy amaranths, two large-effect QTLs were recognized governing 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor resistance in *A. tuberculatus* which was the first QTL mapping study to characterize herbicide resistance in a weedy amaranth species (Murphy et al. 2021).

7.5.3 Transcriptomics and Metabolomics

Although less well studied, certainly than the genes of *Amaranthus*, some mRNA, and metabolite studies have been conducted. For example, transcriptomics and metabolomics of edible amaranth cultivars (*A. mangostanus*) under salinity stress helped in acquiring a comprehensive view of the expression of key enzymes and alterations in metabolites, respectively (Guo et al. 2018, 2020).

Similarly, transcriptomics and metabolomics approaches can provide better understanding of plant's response to biotic stresses and the underlying mechanisms of resistance at the metabolite level. The expression levels of two candidate genes viz., *Ah-2880* and *Ah-HFR* were assessed through qRT-PCR and their high expression levels was observed under several stress conditions. The expression of the *Ah-HFR* gene was increased in response to herbivory, defoliation, and salinity whereas the expression of *Ah-2880* was increased at high salinity stress and infection by *Pseudomonas syringae* pv. *syringae* (avirulent bacteria) (Álvarez et al. 2017; Cabrales Orona 2017).

7.5.4 Genome Research in Amaranth

In recent times, advanced research towards understanding the amaranth genome and modern genetic marker systems have been conducted (Maughan et al. 2009, 2011; Clouse et al. 2016; Lightfoot et al. 2017; Stetter and Schmid 2017). Such information and resources can be of great use in the development of markers for the improvement of breeding methodologies for various amaranths (Joshi et al. 2018).

A well-assembled reference genome is one of the most important resources for genome assisted breeding. Genome sequencing of *Amaranthus* spp. was first attempted on a weed amaranth, waterhemp (*A. tuberculatus*) by Lee et al. (2009)

and on a grain amaranth (*A. caudatus*) by Maughan et al. (2009). Recently, a more complete *A. tuberculatus* genome has been published (Kreiner et al. 2019).

Within the grain amaranths, physical mapping of *A. hypochondriacus* and *A. caudatus* genomes provided chromosome scale scaffolds (Maughan et al. 2008; Lightfoot et al. 2017). Along with these high-quality assemblies, two draft genomes of *A. hypochondriacus* (Sunil et al. 2014; Clouse et al. 2016), chloroplast genomes of *A. hypochondriacus*, *A. cruentus*, *A. caudatus*, and *A. hybridus* (Chaney et al. 2016), a transcriptome (Delano-Frier et al. 2011; Clouse et al. 2016), and a genetic map (Maughan et al. 2011) were made available. The details of various genome sequences of various amaranth species have been provided in Table 7.6.

Genome-wide SNPs (Maughanet al. 2011) and SSRs (Tiwari et al. 2021) have been developed for *A. caudatus* and *A. hypochondriacus*, respectively and their cross-species transferability has been evaluated. These multiple reference and draft genomes of various amaranth species, along with the reported SSRs, SNPs, and InDel, is an important genetic resource will boost up genomic studies in amaranth to understand evolution and diversity within *Amaranthus*. Sources of resistance to biotic and abiotic sources can be identified and tracked through new genetic markers and available genomic information in amaranth. Various molecular marker systems can be applied at different stages in breeding programs. The study of diversity in ex situ collections and use of this genetic diversity to map QTLs by GWAS will impressively increase our knowledge of the genetic architecture of traits and provide targets for MAS. Genomic selection has not been investigated in amaranth so far, although the use of genomic prediction could generally increase the speed of the genetic gain for nutritional traits per generation via early selection and possesses great potential for biofortification breeding in amaranth (Joshi et al. 2018).

Table 7.6 Details of genome sequences of amaranth species

	A. hypochondriacus			*A. tuberculatus*			*A. palmeri*	*A. hybridus*
Quality of genome assembly	Draft	Draft	Reference	Draft	Reference	Draft	Draft	Draft
Cultivar	Domesticated cultivar from farmers in northern Karnataka	Plainsman[a]	Plainsman	Local collection	Female plant	–	-	-
Sequencing platform	Illumina GAIIx (Illumina)	Illumina HiSeq (Illumina)	Illumina paired-end (Illumina)	454-pyrosequencing Genome Sequencer FLX system (Roche)	HiSeq 3000 instrument (Illumina)	HISEQ 3000 instrument (Illumina)	HISEQ 3000 instrument (Illumina)	HISEQ 3000 instrument (Illumina)
Estimated genome size (Mb)	431.8	431.8	431.8	675.6	675.6	675.6	421.8	503.8
The assembled genome size (Mb)	318.8	376.4	403.9	42.8	663.7	572.9	408.1	403.0
Total no. of contigs	491,569	17,366	1589	19,925	2,514	841	638	640
N_{50} contig length (Mb)	0.0019	0.0445	1.254	–	1.74	2.58	2.54	2.26
Total no. of scaffolds	367,441	3,518	908	–	16	16	303	16
N_{50} scaffold length (Mb)	0.035	0.37	24.36	–	43.1	34.7	20.11	24.5
Annotated genes	24,829	23,059	23,847	10,620	56,936	26,784	29,758	24,325

(continued)

Table 7.6 (continued)

	A. hypochondriacus			*A. tuberculatus*			*A. palmeri*	*A. hybridus*
Quality of genome assembly	Draft	Draft	Reference	Draft	Reference	Draft	Draft	Draft
References	Sunil et al. (2014)	Clouse et al. (2016)	Lightfoot et al. (2017)	Lee et al. (2009)	Kreiner et al. (2019)	Montgomery et al. (2020)	Montgomery et al. (2020)	Montgomery et al. (2020)

[a] Plainsman is a release variety from Baltensperger et al. (1992)

7.6 Genetic Engineering in Amaranth

Transformation methods in amaranth are still undeveloped. Only few reports are available on the development of transgenic amaranth plants. Munusamy et al. (2013) developed the protocol for *Agrobacterium*-mediated transformation in female reproductive system of amaranth. A standard floral dip protocol for amaranth floral transformation was developed by introduction of *p5b5*, *p5d9*, and *p5f7* individually in pDRB6b vector for *Agrobacterium*-mediated transformation using *A. tumefaciens* strain AGL1 which resulted in more than 95% seed productivity. It was reported that transgenic amaranth (*A. retroflexus*) plants obtained by floral dip transformation method containing *ARGOS-LIKE* gene (derived from *A. thaliana*) along with the dahlia mosaic virus promoter showed increased (190%) fresh weight due to increased length of stem and leaf (Kuluev et al. 2017).

A different plant regeneration protocol via somatic embryos of transgenic *A. hypochondriacus* (grain) and *A. hybridus* (vegetable) produced from hairy roots was established by Castellanos-Arévalo et al. (2020) by using *A. rhizogenes*. Castellanos-Arévalo et al. (2020) also proposed that genetic factors were affecting the transformation as only *A. hypochondriacus* among grain amaranth species, was efficiently transformable in the generation of transgenic hairy roots, while *A. caudatus* (grain) and *A. cruentus* (grain) remained recalcitrant. *A. hybridus* (vegetable) considered to be a common ancestor of all three grain amaranths (Stetter and Schmid 2017), was also acquiescent to *A. rhizogenes*-mediated transformation.

In whole plant transformation, transgenic plants of *A. caudatus* cv. "Kremoviyrannii" and "Karmin" resistant to herbicide—phospinotricin (PPT) were obtained after treatment with *A. tumefaciens* using the floral-dip method (Yaroshko et al. 2018). Few other successful transformation protocols have been developed in *Amaranthus* spp., including *A. hypochondriacus* (Jofre-Garfias et al. 1997), *A. tricolor* (Swain et al. 2010; Pal et al. 2013a), *A. spinosus* (Pal et al. 2013b), *A. cruentus* (Taipova et al. 2020), and *A. caudatus* (Yaroshko et al. 2020; Mani et al. 2021).

Experimental uses of transgenesis with amaranth sequences have proven valuable for study of gene function. For example, transformation of *Ah24* gene of *A. hypochondriacus* into *Nicotina tabacum* and *A. thaliana* has confirmed its role in defense against mechanical damage and herbivory due to higher jasmonic acid expressed in young or developing tissues (Massange-Sanchez et al. 2015).

In another example, the gene*AhDGR2* from *A. hypochondriacus* showed expression of abiotic stress-induced DUF642 protein in transgenic *A. thaliana* which modified cell wall structure and composition and caused salt and ABA hypersensibility (Palmeros-Suárez et al. 2017). It has been reported that overexpression of *A. hypochondriacus* transcription factors namely, *AhDOF* and *AhERF* in *A. thaliana* increased salt stress and water deficit tolerance, respectively (Massange-Sanchez et al. 2016). Some developed methods of transformation in few amaranth species can be applicable to develop faster and efficient genetic transformation methods in different vegetable amaranth species.

7.7 Role of Bioinformatics

Bioinformatics of various amaranth species is incipient. Apart from sequences deposited in Phytozome by authors of sequencing papers, Amaranth GDB (https://amaranthgdb.org/) is a resource combining amaranth genomics and population genetics (Gonçalves-Dias and Stetter 2021). According to the authors, popAmaranth is an intuitive and user-friendly population genetic genome browser for grain *Amaranthus* and their wild relatives, including three grain amaranth species (*A. hypochondriacus*, *A. cruentus*, and *A. caudatus*) and two wild relatives (*A. hybridus* and *A. quitensis*) providing statistical analysis of genes from all five species through whole genome sequencing data. Total twelve tracks in the database are grouped in five categories of gene annotation, differentiation, diversity, selection, and variant call. Annotation provides sub-features including coding sequence (CDS), mRNAs, and untranslated region (UTRs). Differentiation provides statistical summary of fixation index, average pairwise differences, estimator of genetic diversity population observed and expected heterozygosity for SNP genotype, inbreeding coefficient (F) for each variant and Nei's nucleotide diversity (Gonçalves-Dias and Stetter 2021).

7.8 Recent Concepts and Strategies Developed

The progress in genomics and transformation described above can lead to expanded progress in other areas of research and applied biotechnology such as those listed below, including gene editing as a more directed method of mutagenesis and nanotechnology as a way to develop biocontrol methods for many plants. This will aid in the development of new varieties, as to date plant breeders have utilized only existing natural mutations combined with some chemical and physical mutagens enabling fast 'genebanking' of large sets of genetic variation. However, as a consequence of the evolutionarily slow generation of random mutations, the recognition of desired mutations is a long and laborious procedure.

7.8.1 Gene Editing

The development of sequence-specific engineered endonucleases, the homing endonucleases (HENs) or mega-nucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9), has improved the techniques of targeted gene editing in plant genomes (Vats et al. 2019). These engineered nucleases enable the generation of double-stranded DNA breaks (DSBs) at specific target sites. The induced DSBs can be repaired either end-joining

pathway or via the homology-directed repair (HdR) pathway which can be responsible for the introduction of gene modifications at the target loci (Gaj et al. 2016). In the past few years, highly versatile genome-technology, CRISPR–Cas9 has transformed genome engineering by providing investigators with the ability to introduce sequence-specific alterations into the genomes of a broad range of cell types and organisms (Gaj et al. 2016). These methods of gene editing can be used in amaranth for the trait improvement for biotic stress resistance.

7.8.2 Nanotechnology

Nanotechnology combines biological elements with engineered molecules to deploy for various purposes. An area of research that is having nanotechnology success is that of nanoparticles (NPs) for combating disease organisms, especially fungi. Resistant fungal strains emerge constantly and to combat them green NPs biosynthesized by plants have found useful. For example, silver nanoparticles (AgNPs) synthesized with leaf extract of *A. retroflexus* possessed antifungal activity against plant pathogenic fungi namely, *Alternaria alternata*, *Macrophomina phaseolina,* and *Fusarium oxysporum* (Bahrami-Teimoori et al. 2017). Some other species have also been utilized in the synthesis of NPs. These include *A. cruentus*, *A. gangeticus*, *A. dubius*, and *A. tricolor* leaf extracts for synthesis of AgNPs, *A. spinosus* for gold nanoparticles (AuNPs), and *A. caudatus* for zinc oxide nanoparticles (ZnONPs) all showing antimicrobial activity (Das et al. 2012; Kolya et al. 2015; Sigamoney et al. 2016; Jeyabharathi et al. 2017; Baghani and Es-haghi 2019; Fatimah and Aftrid 2019). NPs developed by various plant extracts can be screened against pathogens and insects of vegetable amaranth to identify suitable NP-based control of biotic stress.

7.9 Future Perspectives

Various modern approaches are now available for crop improvement but limited efforts have been made in amaranth breeding. Combining conventional and advanced approaches can speed up crop improvement with more accuracy. The breeding of any crop starts with the genetic resources available. World-wide collections of germplasm and their conservation in gene banks include natural populations, wild relatives, landraces, varieties, and breeding lines. They contain both beneficial and harmful alleles which can be utilized as breeding lines or as parents in the development of mapping populations. The germplasm must be evaluated for diversity to analyze the extent of variation among different populations or genotypes. The diversity at morphological, biochemical, cytological, and molecular levels provides a collection of information for selecting parents or lines as a source of resistance against particular biotic stress. The diversity estimation aids in identification and selection

of diverse lines with superior alleles/haplotypes (heterotic group). High-throughput phenotyping at multiple locations with diverse environments helps in recognizing the superior plants/genotypes. Moreover, study G × E interaction by GGE biplot or related models can aid in determining response of genotype in specific environment (Pagi et al. 2017). Next-generation sequencing (NGS) including whole genome sequencing (WGS), whole exome sequencing (WES), and transcriptome sequencing (RNA-seq) provides genome annotation and other genetic information.

The sequencing data have been used in the discovery and development of reliable and co-dominant genetic markers (e.g. SSRs, SNPs, and InDels) which can be employed in genotyping, linkage mapping, and other genomics application. The identified heterotic groups are used in the development of mapping populations (e.g. F_2, recombinant inbred lines (RILs), multi-parent advanced generation inter-cross (MAGIC), nested association mapping (NAM) etc.) to identify QTLs, genes, or linked markers for the trait of interest. The data collected from the phenotyping of population are combined with the data of genotyping of the population for GWAS. Combination of multi-omics approaches and phenotyping under field condition offers a great way to associate genomic variations with the important phenotypes. After identification of a gene-trait association, functional validation leads to identification of a causative gene. Information of genes responsible for key characters/traits of plant creates the way for haplotype-based breeding/genomic assisted breeding or de novo domestication.

Simultaneously, genome-wide genotyping information leads to genomic prediction approach which can also be used in breeding programs. Identified functional markers or genes can be used in MAS and marker assisted backcrossing (MAB). Genetic transformation can be utilized to insert specific gene of interest into the plant or gene editing methods (e.g. CRISPR/Cas9) can be used to eliminate undesired gene or to modify of the targeted gene.

A proposed work flow of genome design of amaranths resistant to biotic stress is shown in Fig. 7.2. Execution of these new breeding methods and tools will help in accumulation of desired alleles or deletion of undesired alleles in plant population leading to improvement in genetic gains of breeding programs for designing future crops.

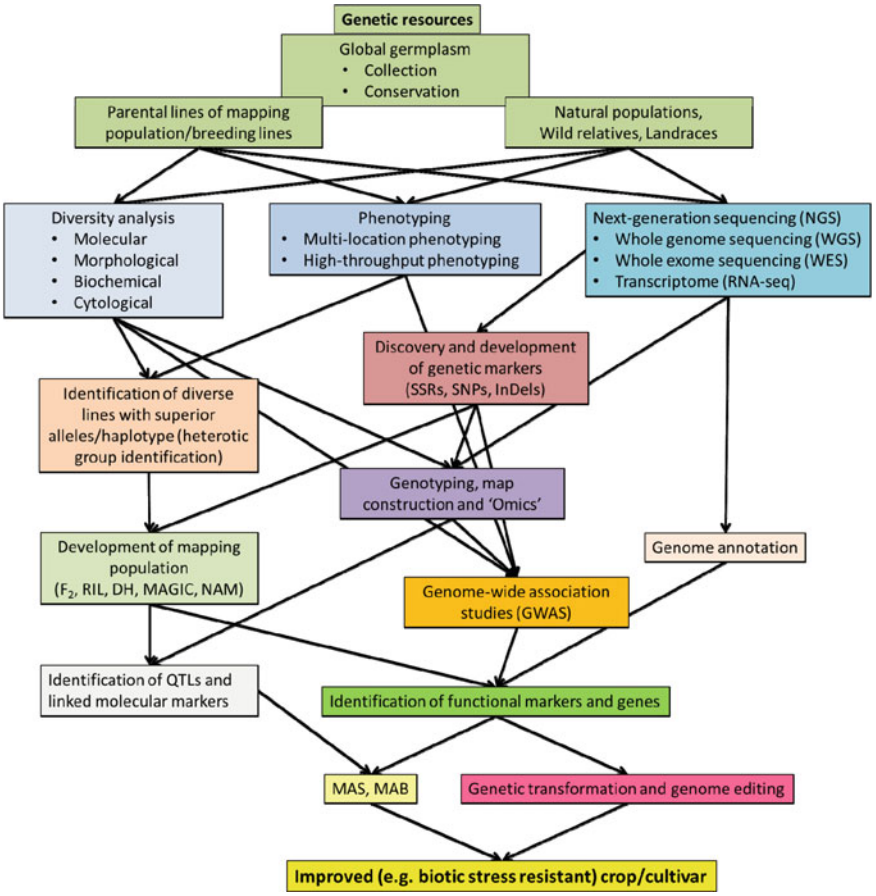

Fig. 7.2 Approaches to accumulate desired alleles or eliminate harmful alleles in the plant genomes for designing future crops resistant to biotic stress

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Chapter 8
Genomic Designing for Biotic Stress Resistance in Carrot (*Daucus carota* L.)

Raman Selvakumar and Pritam Kalia

Abstract Carrot productivity may be impacted by an array of insect pests and diseases. Carrots are affected by at least 36 fungal and oomycete pathogens, five bacterial pathogens, 13 viruses, two phytoplasmas and, in addition to seven nematode species and two parasitic plant taxa. Additionally, a number of insect pest and mite infestations may result in loss. There have been significant efforts to identify wild species that are resistant to certain biotic stresses for introduce into breeding populations and viable varieties, as well as to choose carrot varieties that are partially or completely resistant to a variety of these diseases and insect pests. Significant advances have been made in identifying resistance to a range of diseases and insect pests, as well as mapping that resistance to the carrot nuclear and mitochondrial genome. However, progress in understanding the inheritance of resistance and building extremely efficient resistance to the majority of these many stresses has been slow. Due to the myriad of stresses and relations among insect pests and diseases, it may be challenging to develop hybrids or varieties that are resistant to all of the carrot growing region's key biotic stresses while still fulfilling market and consumer expectations. Novel strategies for detecting resistant varieties and speeding up conventional breeding are being developed using molecular breeding tools like as marker development and deep-coverage carrot genome libraries. These critical genetic techniques will aid researchers in identifying and developing disease, insect, and virus-resistant carrot varieties.

Keywords Carrot · Fungi · Resistance · Insect · Pest · Virus

8.1 Introduction

Diseases and insect pest or mite infestations reduce carrot production considerably in the majority of carrot-growing regions around the world (Rubatzky et al. 1999). Powdery mildew Cercospora leaf spot, Alternaria leaf blight, and bacterial blight are

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the most common foliar diseases (Davis and Raid 2002). Carrot cavity spot, white mould and root-knot nematodes are the most common soil-borne root diseases (Davis and Raid 2002).

Pests such as the carrot willow aphids, carrot rust fly, and the two-spotted spider mite pose a risk to carrot growers (Simon et al. 2008). Fusarium dry rot, Violet root rots and bacterial soft rots are other carrot diseases that cause regional losses (Davis and Raid 2002). Breeders have emphasized on primary infection in places where the disease or insect pest is quite well established in order to discover the most appropriate for genetic resistance to the majority of these biotic stresses. Biotic stress is exacerbated by interseeding the extremely sensitive cultivars or breeding lines with carrot entrants. Plants are injected with diseases or infected with insect pests in some of these insect pest and disease screening and breeding approaches. Due to the difficulties involved in establishing relatively homogeneous soil-borne disease stresses, testing for resistance to diseases and soil-borne insect pests can be difficult, even more so when screening a large number of characters and for stresses derived from multiple pathogen species or races or a pathogen-host interaction. This chapter discusses attempts to create resistance to certain carrot insect pests and diseases, as well as phenotypic screening approaches and current understanding of the genetic component of susceptibility, including inheritance and resistance gene annotation on the carrot nuclear and mitrochrondrial genome. Regrettably, the majority of biotic stresses affecting carrots are unknown, as is the recognised genetic foundation of resistance. As previously stated in this chapter, there are significant gaps in our knowledge of carrot germplasm response to a number of biotic stresses, emphasising the need for more study. This chapter is not intended to be a thorough review of all current information on carrot illnesses and insect pest resistance. The chapter gives insight into the genetic basis and genetics of tolerance for a few of the most prevalent diseases and caused by pests for which scientists have endeavored to screening for resistance. Several illnesses and insect pests that were previously mentioned in this chapter have been updated. The need of using current scientific language was emphasised. Several insect pests and diseases have acquired nicknames in recent years. This chapter differentiates between foliar pathogen and soil pathogen-caused carrot diseases, and it finishes with a discussion of insect pests and nematode.

8.2 Foliar Diseases

8.2.1 Powdery Mildew

Carrots are sensitive to *Leveillula lanuginosa* and *L. taurica* caused by *oidiopsis* and *oidiodium*, as well as *Erysiphe heraclei* caused *oidiodium* (syn. *E. polygoni* and *E. umbelliferarum*) (Aegerter 2002). *Erysiphe heraclei* is found all throughout the globe, although it is most common in warm, semi-arid climates. Powdery mildew

severity is weather dependent, crop development stage dependent, production technique dependant, and cultivar dependent (du Toit and Derie 2008; Aegerter 2002; Abercrombie and Finch 1976; Palti 1975). Powdery mildew is especially damaging to delicate cultivars and parent lines cultivated in hot, semi-arid environments with drip or furrow irrigation. Infections on the leaves may prevent mechanical harvesters from extracting roots from the ground. Disease has the potential to be lethal in greenhouses (Geary and Wall 1976). Carrots in the India, Kazakhstan, Armenia, Middle East, and other Central Asian republics, as well as the Mediterranean areas of Europe and Africa, are infested with *Leveillula* spp (Palti 1975). It is established that *Toxoplasma heraclei* produces haustoria during ectotypic development on carrot, which invade the carrot epidermal cells, resulting in infection. The fungus produces milky white mycelial growth on the petioles, leaves, bracts, umbels, flower stalks and roots of the plants (Aegerter 2002). Foliage that has been significantly affected may develop chlorotic and die prematurely. *L. lanuginosa* and *L. taurica*, on the other hand, produce the endophytic and ectotopic mycelium. During the development of conidiophores that emerge from stomata, *L. lanuginosa* and *L. taurica* generate conidia at the tips of their long conidiophores. Powdery mildew (*Leveillula spp.*) is a fungus that causes light yellow lesions on the surface of the leaves as well as white sporulation (Aegerter 2002). Infections that are contained within leaf veins cause angular lesions. Spores may form on the upper surface of the leaf, and chlorotic regions may necrotize. *Erysiphe heraclei* produces a white fungal bloom that is less noticeable. Powdery mildew fungus conidia are distributed by the air (Aegerter 2002). Unlike other fungal plant diseases, the spores survive and infect plants under conditions of high humidity and moderate temperature. Because sunlight destroys conidia and mycelium, powdery mildews flourish in shaded locations. Powdery mildew occurs on older leaves and spreads to younger plants as a result of increasing humidity and shadow levels in the canopy. Powdery mildew causes havoc on mature carrot plants (Aegerter 2002). Symptoms appear 7–14 days after infection, and sporulation occurs 7–14 days later. The disease may be more severe due to the dense cover of carrot seed fields (du Toit et al. 2009). While powdery mildew growth does not seem to infect carrot seeds, it is possible that cleistothecia (sexual fruiting structures) are affected. At least 86 distinct species of plants belonging to the genus Apiaceae have been found to be infected by the *Erysiphe heraclei* (Hammarlund 1925; Marras 1962; Braun 1987; Aegerter 2002; Glawe et al. 2005; Cunnington et al. 2008). One host species' inoculum may be incompatible with another host species' inoculum. While certain isolates may infect a broad range of plant species and genera, Apiaceae genera and species vary in their virulence (Koike and Saenz 1994, 1997; Cunnington et al. 2008). Similarly, *L. lanuginosa* has been shown to infect a wide variety of Apiaceae genera and species, with isolate specificity varying greatly (Cirulli 1975).

As a consequence, *Leveillula taurica* has a far wider host range and is much more host specific than *Leveillula taurica* (Palti 1975; Braun 1987; Aegerter 2002).

Four *Daucus* subspecies have been chosen for resistant breeding (Umiel et al. 1975; Bonnet 1977). Bonnet (1983a, b) revealed a single powdery mildew resistance gene in *D. c.* subsp. *dentatus* that conferred resistance to powdery mildew. Backcrossing with the susceptible 'Touchon' revealed that resistance is governed by a

monogenic dominant, *Eh*, which was identified during the research process. Orange roots were used to identify resistant lineages. Bonnet (1983a, b) proposed *Daucus siculus* and *Daucus carota* 'Bauers Kieler Rot' as powdery mildew resistant plants, while Lebeda and Coufal (1987) tested the resistance of 111 *D. c.* subsp. *sativus* cultivars to E. heraclei in the Czechoslovak wild. '*Gavrilovskaya*,' one cultivar, was entirely free of powdery mildew, whilst the other thirteen had considerable powdery mildew. Almost half of the 111 cultivars tested demonstrated "possible partial dominance and quantitative resistance to powdery mildew". The enzyme, as previously noted, is lytic against pathogenic fungus and bacteria. Resistance to Alternaria leaf blight was found in one of the transgenic 'Nantes Scarlet' plants (Table 8.1). Human lysozyme production increased in these lines in response to resistance. When Wally et al. (2009a) employed the *Arabidopsis thaliana* (*At*) *NPR1* gene to develop transgenic 'Nantes Coreless' carrot lines; they were the first to report on this technique (non-expressor of PR genes). A study of two transformants, *NPR1-1* and *NPR1-XI*, found that when treated with isolated *Sclerotiorum* cell membrane segments or 2, 6-dichloroisonicotinic acid, the *DcPR-1*, *DcPR-2*, and *DcPR-5* genes were expressed at higher levels than when exposed with a control. When these lines were infected with *E. heraclei*, they experienced a 90% decrease in powdery mildew relative to non-transgenic cultivar lines. *NPR1*, a master switch for systemic acquired resistance (SAR), has been shown to be overexpressed in plants, conferring resistance to powdery mildew, *X. hortorum* pv. *carotae* and necrotrophic diseases. In Czechoslovakia in 1987, Lebeda and Coufal employed spontaneous infections to test for resistant cultivars, but only one out of every three fields had adequate disease pressure. Powdery mildew pressure may be easily induced in the field or greenhouse by using highly susceptible variety as "*spreader*" plants under warm, dry conditions. In a greenhouse, inoculum may be maintained by regularly growing healthy plants alongside infected ones. Powdery mildew grows on close-up pictures of plants. du Toit et al. (2009) studied the impact of extremely high powdery mildew pressure on carrot seed rates.

8.2.2 Alternaria Leaf Blight

Alternaria leaf blight (*Alternaria dauci*) is the most common foliar disease in the majority of carrot-growing countries. *A. dauci* was found in Germany in 1855 and is a major carrot crop pest in areas with considerable precipitation and high temperatures (Farrar et al. 2004). Every day of the growth season, massive amounts of saprophytic spores are generated and disseminated aerially throughout a broad temperature and moisture range (8–28 °C) (Maude, 1966). According to Langenberg et al. (1977), little green–brown lesions appear 8–10 days following the infection. During the progression of the lesion, the sick tissue darkens to the point of being completely black, and a chlorotic haze is seen (Farrar et al. 2004). As well as infecting developing florets and seeds inside inflorescences, *A. dauci* may cause symptoms on the leaves

Table 8.1 Genetics of disease and pest in carrot

Disease/Pest	Scientific name	Resistance gene/QTL	Resistance variety	References
Alternaria leaf blight	*A. dauci*	Three QTL		Le Clerc et al. (2009)
	A. dauci	Elevan QTLs		Le Clerc et al. (2015a, b)
Cercospora leaf spot	*C. carota*	*Ce*	Wisconsin Inbred 1 (WCR-1)	Angell and Gabelman (1968)
Aster yellows	Mycoplasma like organism		Scarlet Nantes, Royal Chantenay, Gold King	Gableman et al. (1994)
Motley dwarf	Virus		CVC-14	Watson and Falk (1994)
			Autumn	Dunn (1970)
			Kurnella Strongtop, Western Red	Tomlison (1965)
Cavity spot	*Pythium* sp.		Redca, Nandor	Bonnet (1983a, b), Cofal (1987)
			Amsterdam Forcing, Nantes, Chantenay, Berlicum, Autumn King	Bonnet (1983a, b), Cofal (1987)
Powdery mildew	*Erysiphe heraceli*	*Eh*	*Daucus siculus, Bauers Kieler Rote, Gavriloskaya*	Bonnet (1983a, b), Cofal (1987)
			Daucus carota ssp. dentatus	Bonnet (1983a, b)
Lygus bug	*Lygus hesperus, Lygus elisus*		Imperida	Scott (1977)
Carrot fly	*Psila rosae*		Gelbe Rheinische St. Valery Clause's Sytan Original, Royal Chantenay Elite (Rota) No.275, Vertou LD, Long Chantenay,, and Danvers Half Long 126, Clause's Jaune Obtuse de Doubs	Ellis and Hardman (1981)
Root knot nematode	*M javanica* and *M. incognita*	*Mj-1*	Brasilia and Tropical	Ali et al. (2014), Simon et al. (2000)

(continued)

Table 8.1 (continued)

Disease/Pest	Scientific name	Resistance gene/QTL	Resistance variety	References
		Brasillia × B6274		Simon et al. (2000)
		1 or few		Yunhee et al. (2014)
			BRS Planalto	Pinheiro et al. (2011)
Root knot nematode	*M.incognita*	7 QTLs		Parsons et al. (2015)
			DR-333	Siddiqui et al. (2011)
		Mj-2	PI652188	
Root knot nematode	*M.e hapla*	*Mh-1, Mh-2*		Wang and Goldman (1996), Bridge and Starr (2007)
	M.chitwoodi, M. fallax		Berlanda, Bolero, Chantenay, Nantucket, Parmex	Wesemael and Moens (2008)
			Ingot	
			Daucus capillifolius	Ellis et al. (1991)
			Flyaway	Simon et al. (2013)
Aphids				
Carrot-willow aphid	*C. aegopodii*		Osborne Park, Autumn King	

of plants as well. Damping-off is an inoculum-induced disease that spreads via the seeds or seedlings of infected plants (Maude 1966; Farrar et al. 2004).

Certain carrot types have exhibited resistance to *A. dauci*. Despite the fact that only three cultivars are completely immune to Alternaria leaf blight, additional study is needed. A total of 90 carrot inbred lines and 241 PI lines from 31 regions were studied by Strandberg et al. (1972). After a natural infection emerged in Brazil less than a week afterwards, the variant designated 'Brasilia' was shown to be the most resistant. (Boiteux et al. 1993). Resistance stability data is useful to breeders since it displays the frequency with which a trait appears in a variety of situations. Rogers and Stevenson (2010) identified three commercial carrot cultivars that reacted differently to *A. dauci* isolates. When 11 A. dauci isolates from across the world were employed, Le Clerc et al. (2015a) discovered no significant interaction between isolates, inbred lines, and a segregating population. Certain data might be explained by genome polymorphisms, fungal isolates, or other environmental factors. Different kinds of resistance components, according to Le Clerc et al. (2015b), may impact resistance effectiveness in a variety of settings. While Rogers and Stevenson (2010) collected

samples eight and sixteen days after infection, Le Clerc et al. (2015b) collected samples twenty and thirty-five days later, with further samples collected every fifteen days. Due to the fact that various defence mechanisms are triggered at different stages after infection, disease development in carrot cultivars may vary considerably.

Simon and Strandberg (1998) identified a relationship between *A. dauci* resistance in the experiment and greenhouse resistance ratings. Although field testing is often employed, it is inefficient, costly, and difficult to maintain. To solve these challenges, experiments like as growth chambers, tunnels, and greenhouses are utilised. The bulk of field experiments focus on plant penetration. Fewer plants are utilised in controlled settings, sometimes just one specimen of a specific species or unconnected plant components. Baranski et al. studied transgenic plant resistance by inoculating detached leaves and petioles with a fungal pathogen (2007). Pathogen-treated greenhouse plants, according to Pawelec et al. (2006), are capable of effectively grading carrot varieties. Experiments with the excised leaf and hypocotyl, on the other hand, were a failure. To accelerate screening, utilise less plant material, and reduce environmental impact, a drop inoculation method was devised (Boedo et al. 2010). In addition, we investigated the sensitivity of carrot lines to A. dauci in vitro (Dugdale et al. 2000; Lecomte et al. 2014). To determine disease resistance, the chlorophyll content of damaged and excised leaves was measured in seedling hypocotyls from regenerant somaclone plants. Courtial et al. (2018) investigated A. dauci resistance in carrot embryogenic cell cultures. Because of the necessity for automated testing, these tests will help in high-throughput characterisation.

Breeders must understand the inheritance and combining capabilities of resistance sources in order to develop resistant hybrid carrot varieties. In the open-pollinated cultivar 'Brasilia,' resistance to *A. dauci* was shown to be 40% narrow-sense heritable (h^2) (Adults and their consorts.) A F_2 population of the carrot cultivars 'Kuroda' and 'Nantes,' used to investigate foliar leaf blight resistance, was reported by Vieira et al. (1991), who did not identify the most likely causal agent(s) as *X. hortorum* pv. *carotae*, but did report increased genetic variation. According to Simon and Strandberg (1998), a high amount of positive diversity, in combination with dominant genetic alterations and epistasis, may result in resistance to *A. dauci* in a plant population. Le Clerc et al. (2009) found three QTLs in an $F_{2:3}$ progeny population, demonstrating that disease resistance is polygenic. Each QTL explained between 10 and 23% of the phenotypic variation. The identification of particular QTLs in a tunnel or field experiment shows that they are environment-dependent and display expression delay after infection. Over a two-year period, two additional genetically distinct populations were studied in the field, yielding 11 QTLs. Because the advantageous alleles at each QTL are mutually exclusive, breeders may be able to raise resistance levels by mixing resistance alleles into a single genotype. In the case of carrots, certain QTLs may prevent pathogen entry into the epidermal tissue, whereas others may prevent pathogen invasion after the leaf has been pierced (Le Clerc et al. 2015b).

Understanding the processes of carrot foliar disease resistance is crucial for developing strong, highly resistant cultivars with a range of resistance mechanisms. Boedo et al. (2008) used resistant and sensitive carrot cultivars to test *A. dauci* resistance

and susceptibility in carrot leaves. Following inoculation 21 days later, SEM analysis indicated that the two cultivars grew differently (dpi). The fungus, for its part, quickly infiltrated the weak cultivar's leaf tissues. At 15 days post-infection, a quantitative real-time PCR assay established that the susceptible cultivar's leaves contained significantly more fungal biomass than the resistant cultivar's leaves, whereas by using a susceptible cultivar as a comparison, Boedo et al. (2010) revealed that two partly resistant varieties had considerably less fungal infection than one partially resistant variety. It was discovered that the two partly resistant cultivars of *A. dauci* had up to 3.42 ± 0.35% more germ tubes per conidium than the susceptible cultivar when *A. dauci* conidia were planted on carrot leaves in a laboratory setting (1.26 ± 0.18). The fungus is very infectious and spreads quickly via the skin. The spores of the resistant cultivar included several germ tubes per conidium, indicating that the fungus tried to enter the epidermis on multiple times.

Lecomte et al. (2012) studied *A. niger* resistance to falcarindiol and 6-methoxymellein (6-MM) in breeding lines infected with *A. dauci*. A statistically significant difference in 6-MM production between resistant and susceptible cultivars (Bolero vs. Presto) demonstrated that this phytoalexin helped to resistance by delaying disease transmission. In vitro, falcarindiol suppressed fungal growth and permeabilized *A. daucii* better than 6-MM. It is found in greater abundance in 'Bolero' leaves than in 'Presto' leaves, suggesting that it aids in fungal resistance. According to Lecomte et al. (2014) carrots are resistant to *A. niger* toxins. Dauci's involvement in the small resistance seems conceivable. To evaluate embryogenic cellular cultures derived from resistant carrot genotypes, fungi extracts were used. Overall plant resistance and cellular resistance to fungal exudates have a substantial association, showing that resistant and susceptible cultivars respond differently. More research is needed to determine the presence of phytotoxic chemicals in exudates. Fungal extracts were equally efficient as fungal extracts on carrot embryogenic cell cultures, but presented a reduced danger, according to Courtial et al. (2018). The fungus may produce aldaulactone, a very toxic chemical. It is necessary to identify its cellular targets. Koutouan et al. (2018) used bulk segregant analysis to examine the leaf metabolomes of four different carrot accessions with varying levels of resistance to *A. daucii*, as well as resistant and susceptible progenies. Bulk populations sensitive and resistant to camphene, caryophyllene, bisabolene, luteolin 4′-O-glucoside, and apigenin 4′-O-glucoside produced and accumulated feruloylquinic acid and luteolin 7-O-glucuronide in different ways. The relevance of those secondary metabolites in A. dauci resistance, as well as their relationship to previously identified QTLs, are being studied using metabolite QTL approaches and microarray testing to analyse gene expression in metabolic pathways.

Arbizu et al. (2017) proposed employing prediction algorithms based on the relationship between *Daucus* clades and *Alternaria* leaf blight severity ratings rather than screening wild and farmed carrot accessions for novel sources of resistance. A phylogenetic linear regression model using 106 wild and farmed *Daucus spp.* and related taxa revealed that plant height was the most important explanatory variable for disease resistance prediction. *Daucus carota* subspecies *capillifolius*, *maximus*,

and *crinitus* may have additional resistance sources. Carrots have been found to exhibit hybridization potential.

The approach was studied in order to create transgenic carrot plants that are resistant to fungal and bacterial foliar infections. Plant-derived lysozymes prevent and defend against bacterial and fungal infections. Both bacterial peptidoglycan and fungal chitin are cleaved by human lysozyme. *Agrobacterium tumefaciens* and the human lysozyme gene were used to create carrots resistant to *A. daucii* (Takaichi and Oeda 2000). Punja (2005) developed two thaumatin-like genetically modified carrot lines using *A. radiobacter*. In both lines, Sclerotiorum and A. dauci decreased sickness. In carrot transgenic plants, the *MF3* gene was studied. *Pseudomonas fluorescence i*s a plant-growth-stimulating rhizobacterium (Baranski et al. 2007, 2008). *MF3* is thought to be involved in the signalling cascade that results in induced systemic resistance due to its interaction with FKB. When compared to non-transformed plants, transgenic plants have a 20–40% boost in disease resistance. The polyethylene glycol transformation of carrot protoplast chitinase genes yielded less impressive results. Two of the clones were more resistant to *A. dauci*, whereas a third was more sensitive to the pathogen. According to researchers Wally et al. (2009a), monitoring a higher amount of induced genes was more effective than modulating gene expression in the creation of disease resistant transgenic lines. To change systemic developed tolerance, we upregulated the *NPR1* gene in a carrot cultivar. When *B. cinerea*, *A. radicina*, and *S. sclerotiorum* were used as pathogens, the transgenic lines significantly reduced disease severity by 80%, and when *X. hortorum* pv. *carotae* was used as a pathogen, the transgenic lines greatly reduced disease severity by 35–50%. Klimek-Chodacka et al. (2018) disclose the first effective site-directed mutation in the carrot genetic code, conferring resistance against foliar fungal and bacterial infection.

8.2.3 Cercospora Leaf Spot

Unlike Alternaria leaf blight, Cercospora leaf spot produces circular lesions on the leaves and petioles, and the leaves lack dark-edged borders and a lighter centre (Milosavljevic et al. 2014; Gugino et al. 2007; Raid 2002; Carisse and Kushalappa 1990; Bourgeois et al. 1998). The fungus only affects the aerial parts of carrots, not the root portions. Conditions for infection include temperatures ranging from 20 to 28 °C, followed by six hours of leaf wetness and 100% relative humidity (Carisse and Kushalappa 1992).

There is a scarcity of data on screening *C. carotae* for resistance. Lebeda et al. (1988) investigated the resistance of 142 carrot cultivars from throughout the globe to *C. carotae*. Resistance was found in just 30% of the cultivars tested in the field. Outdoor testing were carried out by Gugino et al. (2007). Resistance varied greatly amongst cultivars, although it was not constant. Cercospora leaf spot resistance is poorly understood, and little effort has been made to develop resistant plants (Table 8.9.1).

Both *X. hortorum* pv. *carotae* and *C. carotae* may infect carrot breeding lines.

Resistance to *C. carotae* may be mediated by a single gene with a range of morphological variations, according to Lebeda et al. (1988). Angel and Gabelman (1968) demonstrated that an inbred line's resistance was produced by a dominant gene using glasshouse research.

By infecting carrots with *Cercospora carotae*, Mercier and Kuć (1996) acquired systemic resistance. *C. carotae*-infected carrot leaves exhibited much fewer lesions than control leaves, showing that the foliar pathogen strengthened carrot leaf defence systems.

8.2.4 Bacterial Leaf Blight

Bacterial leaf blight is caused by *Xanthomonas hortorum* pv. *carotae*, a seed-borne pathogen. The foliar symptoms of *A. dauci* and *C. carotae* infections are identical to those of this fungus. Bacterial leaf blight creates a slimy, sticky discharge of bacteria. Petioles, umbels, and seed stalks have all been harmed (du Toit et al. 2005). In 1934, scientists in California found bacterial leaf blight. Any carrot field is at risk of being poisoned. It has been shown that the pathogen infects plant components such as stems, leaves, umbels, and seeds. According to the inquiry, tainted roots are a possibility. This is due to the fact that infection occurs exclusively at the crown, where the petioles contact the root. Certain seeds may be infected or diseased. If the pathogen is present, the seeds must be washed in hot water to kill it or dramatically reduce the degree of infection (du Toit et al. 2005; Pfleger et al. 1974).

As a consequence, little or no public study on the pathogen's genetic resistance has been conducted under these settings. There is no commercial cultivar that is blight resistant (Christianson et al. 2015). Pfleger et al. (1974) observed that the reactions of six cultivars and breeding lines to bacterial blight differed considerably from one another. They evaluated 66 carrot inbred lines, two public sector inbred lines, 17 marketable hybrids, wild or putative ancestors and land races for *X. hortorum* pv. *carotae* in a greenhouse, and they found that they were positive for the pathogen. There were eight putatively resistant PI lines found (two varieties and two carrot inbred lines), as well as five highly sensitive PI lines. To help in the production of more robust cultivars, one line from each of the three PI numbers 418967, 432,905, and 432,906 has been recognised as blight resistant. Each access point provides insufficient resistance. Only Ames 7674 and SS10 OR were identified to be bacterial blight susceptible. Infections of leaves with *X. hortorum* pv. *carotae* differed greatly amongst accessions. Visual estimates of foliar disease severity, according to Christianson and colleagues, are beneficial, but only if enough replications are performed (2015). Both investigations found a small positive association ($r = 0.52–0.62$) between sickness severity ratings and quantification of *X. hortorum* pv. *carotae*. This research highlights the significance of USDA's National Plant Germplasm System (NPGS) Daucus germplasm to plant breeders. Christianson et al. (2015)

investigated the resistance inheritance of *X. hortorum* pv. *carotae* using carrot PI lines.

C. carotae plants showed much fewer lesions than control leaves, showing that the foliar pathogen increased carrot leaf defence systems.

8.3 Soil Borne Diseases

8.3.1 Cavity Spot

A hollow patch has been noticed in almost every region where carrots are cultivated (McDonald 2002). *Pythium sulcatum* and *Pythium violae*, two species with a modest growth rate that feed on carrot roots, are the most common inhabitants in the United States (McDonald 2002). P. *intermedium, P. ultimum, P. sylvaticum* and *P. irregular* are likewise related with Cavity Spot. Surface lesions on roots make them undesirable for both fresh and processed markets (McDonald 2002). During the first four to six weeks after planting, carrot roots are more likely to remain infected with *Pythium spp* (McDonald 1994b). The hollow space at the base of the roots will be kept for storage purposes (Vivoda et al. 1991). Secondary microorganisms such as bacteria invading root lesions generate the colour surrounding the cavities. The hollow location deteriorates due to a lack of root development (Montfort and Rouxel 1988).

Despite the fact that no carrot cultivar is completely devoid of cavity spots at the time (Soroker et al. 1984; Groom and Perry 1985; Sweet et al. 1986; White 1988; Vivoda et al. 1991; McDonald 1994b, 2002). Some cultivars, according to Guba et al. (1961) are susceptible to cavity spot. 'Hutchinson' roots exhibited fewer hollow portions than 'Waltham Hicolor' roots, despite the larger diversity found across lines of 'Waltham Hicolor.' The National Institute of Agricultural Botany in the United Kingdom revealed differences in susceptibility among carrot varieties. Redca was a rougher Chantenay variety, whereas Nandor was a stronger Nantes cultivar. Furthermore, late-maturing plants were more prone to cavernous spot. Autumn King Vita Long is more resistant to delay harvesting than early harvesting (Sweet et al. 1989).

Six California Imperator cultivars were cultivated in growth chambers at 20 °C with *P. ultimum* and *P. violae* injected into the potting mix. In all six cultivars, both species were capable of causing cavity spot, with *P. violae* isolates being more virulent (Vivoda et al. 1991). 'Topak' was especially vulnerable to attacks from both species. P. violae was more resistant to the other five cultivars, although *P. ultimum* was very sensitive. The most vulnerable varieties were 'Pakmor,' and 'Caropak' followed by 'Dominator.' and 'Sierra'. Vivoda et al. (1991) found that the lack of diversity in reaction to *P. ultimum* and *P. violae* might be attributed to the cultivars' ancestors.

White et al. (1987) examined 19 commercial carrot varieties for resistance to the hollow spot lesions *P. violae, P. sulcatum* and *P. intermedium*. They found that the

varieties were resistant to all three pathogens *Pythium* species were colonised on roots produced in a greenhouse using agar plugs that had been cleaned and colonised. *P. violae* was found in 19 different carrot cultivars and in all five types of carrot. Cavity spot variations in *P. sulcatum* have been discovered in different carrot kinds, but not in different cultivars of carrot. White et al. (1987) found significant variation in *P. intermedium* only between cultivars and in one of three cavity spot tests, and only in one of three cavity spot assays. Any of the three Pythium species that were evaluated showed no signs of having a genetic advantage in terms of resistance.

According to White et al. (1988) *Pythium spp.* was found in the periderm of asymptomatic carrots from the cultivars 'Sweetheart,' 'Chantenay New Supreme, and 'Fingo,' as well as in the periderm of symptomatic carrots from the cultivars 'Sweetheart' 'Chantenay New Supreme,' and 'Fingo,'. Following infection with mycelial plugs from the pathogens *P. sulcatum*, *P. intermedium* and *P. violae*, they discovered that genetic resistance was absent in 19 carrot cultivars from five distinct groups. Mycelial plug inoculation, according to Vivoda et al. (1991) did not give a credible measure of cultivar resistance variation. They discovered that infecting 36 carrot cultivars in the lab with *P. violae* resulted in susceptibility variations that were similar with their field findings. Using a combination of field nurseries, greenhouse screening, and laboratory root injection studies, a large number of individual breeding programmes have made significant progress in creating hybrids with improved resistance to hollow spot in recent years.

McDonald (1994b) demonstrated that the partially resistant 'Six Pak' varieties was effective for cavity spot elimination in the province of Ontario. The Chanton and Huron were the most susceptible species, with Red Core Chantenay, Eagle, and SR-481, showing intermediate resistance to mortality. Six Pak elicited a greater number of negative reactions than either 'Cellobunch' or 'Chancellor.' In non-irrigated regions, 'Eagle' was as resistant to blight as 'Six Pak,' but was more susceptible in irrigated plots. The susceptibility of the cultivars to cavity spot changed only as the roots grew in size. For the first time, this study demonstrated that stored carrots are not always more susceptible to hollow spot lesion than freshly harvested carrots, as previously thought. Towards the end of the season, fewer hollow patches were seen (McDonald 1994b).

Benard and Punja (1995) used in vitro mature root inoculation to assess cavity spot reactivity in 37 carrot cultivars. The most resistant strains were E0792, Fannia, Caropride, Panther, and Navajo. "Six Pak," "Imperator," and "XPH 3507" were shown to be resistant to the pathogen despite only having been tested once. "Eagle," one of 18 cultivars evaluated in 1991 and 1992, was found to be resistant in 1991, but susceptible in 1992, despite the fact that the results for the other cultivars were equal in both years. They hypothesised that year-to-year differences in cultivars were caused by rootstock or growing conditions. They assessed the vulnerability of commercial carrot varieties Narbonne, Bolero, Bertan, and Eastern carrot gene bank variation 'Purple Turkey,' to *P. violae* inoculation under the greenhouse phenotype screening and field experiments. In compared to other commercial cultivars, 'Purple Turkey' outperformed them in terms of quality and yield. Resistance to cavity spot is suggested to be a result of the 'Purple Turkey's' tiny cell size and enhanced enzyme

levels in the tap and adventitious roots. The study examined commercial cultivars like as 'Bolero,' 'Narbonne,' and 'Bertan.'

Cooper et al. (2006) studied cavity spot resistance in carrot seedlings from 19 somoclonal derived lines as well as commercial control varieties such 'Vita Longa', 'Nando,' 'Bolero,' and 'Bertran,' Although hollow spot susceptibility differed genetically amongst somaclones, there was no association between greenhouse and outdoor data. For many years, scientists at the University of Guelph's Muck Crops Research Station in Ontario's Holland Marsh have compared USDA experimental carrot breeding lines to commercial carrot cultivars. Infection of a cavity disease by a naturally existing pathogen in the area. Hollow spots emerge in variable degrees in breeding lines and cultivars from year to year. Orange parent lines CS736 and CS732 outperform USDA parent lines viz., 5367, 6526, and 1137 in terms of cavity spot resistance (1137B-F2M5). 2205B, 2205, 5494, and CS 724, as well as additional crosses with those lines, have all shown consistent responsiveness. Despite a very consistent disease burden in this nursery, determining cavity spot resistance was difficult (McDonald et al. 2017). In the muck nursery experiments, there was no link between carrot root forking and the existence or severity of cavity regions (McDonald et al. 2017).

Screening for cavity spot resistance is challenging due to the unequal distribution of field inoculum and the intermittent character of the diseases. Because carrot roots from the same cultivar respond so differently, a significant number of marketable roots from each carrot inbred line must be tested in duplicated and randomised plots over different seasons to allow for meaningful different responses. A variety of factors (including soil microbiology) may impact the presence and severity of hollow spot when phenotypic screening procedures are applied (McDonald 1994b, 2002; Benard and Punja 1995). It grows well in wet soil (especially after a flood) and at cold temperatures (*15 °C). Extensive roots in the soil exacerbate the hollow region (Montfort and Rouxel 1988). This might be due to increasing root sensitivity, the accumulation of seasonal lesions, root diameter expansion, or an infection change (Wagenvoort et al. 1989; Vivoda et al. 1991). Despite comparable spore concentrations and environmental conditions, symptom severity and frequency differed amongst genotypes later in the season, despite same inoculum and environmental conditions (McDonald 1994b). She discovered that increasing the severity of hollow spots did not always mean that roots were more sensitive with age, but rather that the illness advanced. Carrot age (1–3 months) has no effect on the formation of cavity spots, according to Benard and Punja (1995). The number of lesions per root rose three to five months after planting, according to Vivoda et al. (1991). McDonald (1994b) found that seasonal oscillations in hollow spot were caused by climatic variables rather than plant age, implying that the timing of cavity spot exams may influence disease resistance screening activities. Several breeding programmes have used mature carrot roots injected with *Pythium spp.* agar plugs to test cavity spot resistance. Root inoculation lesions, in contrast to lesions caused by roots growing in contaminated soil or planting medium, are often shorter, discoloured, and lack distinct boundaries (Vivoda et al. 1991). Screening for cavity spot resistance using colonised agar plugs, according to Vivoda et al. (1991), may not adequately represent

cultivar or breeding line response in soil. Because of quick epidermal suberization, which precludes root infection, carrot roots may be infected with *P. violae* colonised agar plugs 24 h after harvest. Root inoculation with P. sulcatum-infected plugs, on the other hand, may be done up to a week after harvest provided the roots are maintained cold to minimise root suberization. To overcome these challenges, several root inoculation bioassays involved cutting the tips of the roots prior to collection and immersing the roots in water until infected (Cooper et al. 2004). Other methods for improving root inoculation uniformity include culturing roots in the dark for 7–10 days at low temperatures (15–20 °C) and high RH. A significant number of roots must be afflicted and examined in order to accurately determine the extent of the lesion at various inoculation locations on the same root and throughout different backgrounds of the same plant. Their value in carrot breeding is restricted because to the time required to inoculate root agar plugs. Others have grown sick roots in high relative humidity settings to assess the size of the hollow patches. According to Suffert and Montfort (2007), introducing an inoculated and diseased carrot root to the same environment as healthy carrot roots may result in the growth of hollow spot lesions in the carrots. This method resulted in a greater number of cavity spot lesions than *P. violae* inoculated soil.

Several publications have been written about cavity spot analysis methods. Each lesion's severity is determined by its amount of lesions per root, its horizontal and/or vertical lengths, as well as any combination of these two lesion parameters (McDonald 1994b), and classification of lesions as small, medium, or large have all been used to analyse large numbers of roots. When a variety of evaluation procedures are utilised, comparing outcomes may be challenging. Because the frequency and severity of cavity spots vary seasonally, evaluating incidence or severity at a certain harvest date may provide different findings. In Canadian field study, McDonald (1994b) discovered that *AUDPC* was more successful than incidence ratings in identifying treatment effects. Several exams are required to establish the *AUDPC*. The slopes and altitudes of disease emergence curves in the *insitu* may be used to estimate cultivar resistance to cavity spot (McDonald 1994b).

According to research, Cavity Hole Growths are induced by a hypersensitive reaction of carrot root core tissues to *Pythium* infections (Klisiewicz 1968; Endo and Colt 1974). According to other researchers, there was practically little variation in quantitative resistance amongst cultivars of the same species (White 1991; Johnston and Palmer 1985). In terms of published (open-access) research, there does not seem to be any on the inheritance of cavity spot resistance available. Cavity spot lesions are caused by the enzyme cellulose and the pectate lyase of *Pythium spp.* (Cooper et al. 2004). Degrading enzymes of cells are triggered during hyphal penetration of root tissue (Guérin et al. 1994; Campion et al. 1988; Campion et al. 1988). *Pythium spp.* isolates that are extremely pathogenic, according to Benard and Punja (1995), generate more pectolytic enzymes than isolates that are moderately hazardous. As the infected zone killed host cells and hyphae developed under the epidermis, a hollow was formed. When carrot roots get infected, they produce oxidised phenolics and phenylalanine-ammonia lyase, which are subsequently deposited around the site of infection. Furthermore, it is thought that the lignin that forms surrounding the

lesion functions as a physical barrier against infection. The internal distribution of pyrthium has been connected to hollow spot resistance (Endo and Colt 1974). These results reveal that in order to battle infection, root defence mechanisms are engaged in response to cell disintegration (Soroker et al. 1984; Perry and Harrison 1979). The amount of phenol in cavity lesions tissue increased with the severity of the hollow spot lesions. Lignin and suberin were found in the periderm cell membrane, and in parenchyma cells at the infected outer region of the carrot (Perry and Harrison 1979). Chemical accumulation of antifungal drugs was more essential in *Pythium* resistance than structural barriers. Falcarindiol and phytoalexin 6-methoxymellein have been isolated from healthy root tissue, while phytoalexin 6-methoxymellein has been extracted from diseased root tissue (Garrod et al. 1978). Kurosaki et al. (1985). Guérin et al. (1998) showed that more resistant cultivars had thicker cell walls, which they hypothesised was due to higher synthesis of phenolic fungitoxic compounds as a result of the infection responses. According to Cooper et al. (2004), 'Purple Turkey' has a smaller root cell width and greater levels of constitutive enzymes than commercial cultivars, which explains for its cavity spot resistance. The pace at which a carrot root reacts to infection, according to White et al., may be connected to its sensitivity to cavity spot (1988). Pythium spp. were found in juvenile tissue more often than in mature tissue eight weeks following planting. As a result, either the carrot's defence mechanisms protect it from infection by these fast growing organisms (McDonald 1994b). Slow-growing plants, such as *P. sulcatum* and *P. violae*, prpdice the cavity reactions. Slow-growing organisms entered carrot core tissue for 3–4 days, liberate minute levels of degrading enzymes of cell wall before eliciting a host reaction, according to White et al. (1988) and Zamski and Peretz (1995). Patches of carrot root cavity degrade often during cold storage (McDonald 1994b). Bolting, a physiological change from vegetative to reproductive growth caused by vernalization, might be linked to enhanced storage vulnerability. Furthermore, storage may increase the number of lesions per root, indicating that latent disorders may resurface during storage. Minor cavity spot lesions may cure on their own if treated properly (McDonald 1994b).

8.3.2 White Mold

While *Sclerotinia* soft rot, often known as white mould, does minimal damage in the field, it is harmful to cold storage and long-distance shipment. Sclerotia are black animals with a melanized surface that colonise open root zones quickly and change into mycelium, a white flocculent mycelium. Sclerotia may live in the soil for up to ten years. Three *Sclerotinia* species have been linked to the pandemic (Leyronas et al. 2018). *Sclerotium rolfsii*, a basidiomycete unrelated to white mould, causes carrot southern blight. Ascomycetes are pathogens that cause white mould. White mould may be found on roughly 500 different species, including weeds, all across the globe (Rubatzky et al. 1999; Kora et al. 2003). A phenotyping test was employed *Sclerotiorum sclerotiorum* on different carrot accessions (Ojaghian et al. 2016). After

three minutes in 2% sodium hypochlorite, the carrot roots were washed with sterile tap water and dried on sterile filter paper. Fungi grown on carrot dextrose agar were used to inoculate the roots. An agar plug with a diameter of 5 mm was constructed from the tip of a 3-day-old culture and was used to insert the root in the core of the agar plug. A damp chamber was made using 12 plastic boxes (12 carrots each). The roots were kept in humidified cotton wool trays at 21–23 °C. Lesions Serious disease was defined as 1–4 cm in length without sclerotium development, 4–8 cm in length with 1–4 mature or immature scales, and 8 cm in length with more than 4 mature or immature scales six days after inoculation. The illness index was computed as [(1.25) + (2.53) + (3.75y4)/total carrots 1/0.05, where 0.05 is a constant (Ojaghian et al. 2016).

Using detached petioles and leaflets, Punja and Chen (2004) revealed that transformants of carrot plants encoding a rice thaumatin-like protein exhibited considerably enhanced understanding the consequences when *Sclerotiorum sclerotiorum* was injected. According to Wally et al. (2009b) carrot breeding lines upregulating the peroxidase enzyme *OsPrx114* were shown to be particularly resistant to *Sclerotiorum sclerotiorum.* Pathogenesis-related (*PR*) gene transcript levels rose in tissues treated with *S. sclerotiorum* cell wall fragments (Wally and Punja 2010).

8.3.3 Gray Mold

Botrytis cinerea, sometimes known as grey mould, has the potential to devastate temperate Asia, Europe, and North America (Rubatzky et al. 1999). Spores are the principal disease vectors in crops. The development of symptoms is accelerated by cold storage. Carrot roots are often attacked by the fungus at the petiole base or crown. Watery brown lesions develop into dark brown lesions with grey mycelium and minute sclerotia as they grow. The root inoculation resistance experiments were meant to see whether carrot varieties are sensitive to *B. cinerea* during cold storage and to look into artificial resistance (Goodliffe and Heale 1975; Bowen and Heale 1987). To measure the vulnerability of carrot leaves to grey mould, Baranski et al. (2006) designed a foliar test employing colonised agar plugs. Heat-killed *B. cinerea* conidia in carrot slices, according to Mercier et al. (2000) conferred systemic resistance to *B. cinerea.* There is considerable disagreement over whether a 24-kilodalton chitinase plays a role in induced resistance. Transgenic carrot plants expressing *CHIT36*, a chitinase lytic enzyme produced by the biocontrol agent *Trichoderma harzianum*, to study the effect of chitinase on grey mould. *B. cinerea*'s assault on transgenic plants has been decreased by up to 50% (Baranski et al. 2008).

8.3.4 Fusarium Dry Rot

Fusarium dry rot has been detected in the China, United States, Canada, Japan, and France, (Zhang et al. 2014; Villeneuve 2014; Sherf and MacNab 1986; Rubatzky et al. 1999). There is a chance that some businesses may incur major financial losses (Zhang et al. 2014; Villeneuve 2014; MacNab 1986; Rubatzky et al. 1999). Losses in China's Tuo Ke Tuo County topped 80% in 2014 (Zhang et al. 2014). A dark circular lesion with a diameter of 3–4 cm covers the root surfaces. Soft rot disease is caused by lesions, rendering the roots unmarketable. Nutrient transmission between roots and leaves, on the other hand, may be influenced by root quality and production. Disease might result in significant loss during storage. The four species that cause this disease, according to the CDC, are *avenaceum*, *culmorum*, and, most recently, *Fusarium caeruleum.* In order to examine variance in variety, Zhang et al. (2014) proposed two ways for replicating frequent symptoms. To begin, 5 mm diameter plugs were carved into potato dextrose agar plates. On this side, a mycelial plug was inserted into the root. Infected roots were incubated in a humidified atmosphere at a temperature of 25 °C (90% relative humidity). White mycelium covered the root surface, forming black bruises after four days of incubation. The second step was to fill each container with 15 carrot seeds (30 cm 25 cm). The soil contained 1104 CFU/g of spore suspension. Plants grown in uninfested soil were used as the control treatment. Each risky factory was assigned a field. Dried red emerged after 13 weeks. In the absence of known resistance sources or published varietal testing, Sidorova and Miroshnichenko (2013) confirmed genetic change. When coupled with 'Nantskaya 4,' this gene was demonstrated to be resistant to *F. avenaceum* infection.

8.3.5 Black Rot

The bacteria *Alternaria radicina* is responsible for black carrot rot (formerly *Stemphylium radicinum*). Black rot was often reported as a post-harvest disease, infection of plantation seedlings, and contamination of carrot seed harvests. Radicine induces leaf, petiole, and umbel blackening (Meier et al. 1922). The first black red record was made in New York. Planting or concealing disease-related problems Radicine may remain in the soil for up to eight years, causing carrot crops to become ill (Maude 1966; Scott and Wenham 1972; Pryor et al. 1998; Farrar et al. 2004). The black red taproot and crown are divided by dark, deep necrotic lesions. When harvesters remove reproductive tips from the ground with their heads in moist settings, a coronary infection may cause petiole rot and bladder symptoms similar to *Alternaria dauci*, culminating in catastrophic plant loss (Pryor et al. 1998; Grogan and Snyder 1952; Farrar et al. 2004). The pathogen quickly spreads throughout the root system after root infection. Seed production and germination may be hampered if the umbel becomes sick. Fungicides such as azoxyestrobin, fludioxonil, Iprodon, or thiram, as well as disinfectants such as hot water or sodium chlorite, may be used to reduce

seed-borne inoculum (Pryor et al. 1994; Biniek and Tylkowska 1987; Soteros 1979; Maude 1966). Chen and Wu (1999) revealed that 229 *Burkholderia cepacia* and 224 *Bacillus amyloliquefasciens* had a substantial affect on A. radicina. Prior to *A. radicina* infection, *Candida melibiosica* yeast was shown to suppress the development of black rot (Kordowska-Wiater et al. 2012). Pryor and his colleagues After sterilisation, the teeth were cultured for five days at 28 °C with 2 ml A. radicina conidia (1 t/104 conidia/ml). The colonised toothpick tip was put into the shoulder of a ten to twelve-week-stored carrot root after nine to ten weeks. Grzebelus et al. (2013) created a protoculum for selecting plants that outperform *A. radicina* in vitro. In protoplastic cells attacked by fungus, somaclonal alterations were seen, leading in disease-resistant plants. Cwalina-Ambroziak et al. (2014) used agar discs to inoculate *A. radicina* petioles and seedlings (every 5 mm in diameter).

In 46 field-grown carrot crops, Pryor et al. (2000) observed substantial diversity in cultivar lesion frequency. While Panther and Caropak were resistant, Royal Chantenay and Nogales were quite suceptible. While cultivars were resistant to *A. radicina*, lesions occurred faster in cold storage than in the field conditions. A black-red experimental investigation with production in 2008–2009 and achieved a wide range of findings (Karkleliene et al. 2012). Magi was the most sensitive to *A. radicina* of the 13 varieties tested. According to Cwalina-Ambroziak et al. (2014) Koral exhibited more suceptible than Bolero.

Baranski et al. (2008) used transgenic *CHIT36* plants to confirm the positive effect of chitinase on *A. radina* in vitro, which had previously been documented for the grey form produced by Botrytis cinerea. The number of those infected with *A. radicina* was cut in half. The gravity of the *A. radicina* taproot (width of injuries lowered by 50%) and the quantity of necrotic foliar patches (approximately 33% reduction in the measure of severity of foliar disease) were dramatically reduced when transgenic plants expressing the *NPR1* gene were infected. *P23*, a cationic peroxidase inhibitor in rice that improved resistance to necrotrophic foliaire infections, was studied by Wally and Punja (2010). Overexpression of *OsPrx114* increased the lignin synthesis in the outer peridermal tissues and pathogenesis-related (*PR*) genes according to Wally and Punja (2010).

8.3.6 Bacterial Soft Rot

Bacteria such as *Pectobacterium carotovorum* subsp. *carotovorum*, *Dickeya dadantii*, *Pectobacterium atrosepticum* subsp. *atrosepticum*, Bacterial soft rot of carrots is a critical concern during storage because secondary invaders of damaged or diseased roots may cause significant losses. Soft rot symptoms are more common in low-lying locations and other saturated places (e.g., near broken irrigation pipes). These bacteria, as thermophilic facultative anaerobes, have been linked to major outbreaks in fields with extended wet soil conditions and high temperatures (Farrar 2002). Irrigation water and the water used to wash carrot roots after harvesting both have the potential to contain pathogens (Segall and Dow 1973). Small, water-soaked blisters

appear on carrot roots as a result of a highly contagious bacterial disease. The squishy roots of *D. dadantii* and the infected roots of *P. carotovorum* subsp. *carotovorum* become mushy and squishy when the temperature is high (30–35 °C for *D. dadantii* squishy) and 30–35 °C for *P. carotovorum* subsp. *carotovorum* and infected roots of *D. dadantii* become mushy and squishy (Phillips and Kelman 1982). If infected roots have been macerated, internal tissue may flow through cracks in the root surface, resulting in an infection (McDonald 1994a). For determining carrot resistance to soft rot, a number of different approaches are available (Michalik and Ślęczek 1997; Michalik et al. 1992; Lebeda 1985; Bedlan 1984; Skadow 1978). It was shown that if the roots were kept at 21 °C for four days after being exposed to 2 °C for three days, they would experience more soft rot than if they were exposed to 2 °C for three days followed by four days at 21 °C. According to the findings of the research, phenolic or similar compounds generated during chilling may result in less severe soft rot in infected carrots. Carrot roots harvested immediately after harvesting contained 3 methyl-6 methoxy-8 hydroxy-3, 4-dihydroxoisocoumarin, but carrot roots stored at 0 °C for 4–8 weeks did not. According to Segall and Dow (1973), this may aid in the prevention of bacterial soft rot in carrots when in cold storage.

Michalik et al. (1992) assessed the resistance of carrot germplasm collections to soft rot caused by *P. atrosepticum* and *P. carotovorum* subsp. *carotovorum* using four root inoculation procedures. The roots were cleansed in sterile water and air dried after being stored at 0–4 °C for 1–3 weeks (Michalik et al. 1992). A fungicide was applied to soil samples and they were held at 22 °C for 48–96 h. Both bacteria elevated the severity of soft rot in response to increasing inoculum concentration, although *P. carotovorum* subsp. *carotovorum* colonised more significantly than the isolate of *P. atrosepticum.* Amount of the bacterial strain carrot line had no impact. In compared to treatments with larger root pieces, both carrots cut root inoculation strategies resulted in increased rot severity and a decreased response variance. Using bacterial-soaked filter discs, the inoculum was not dried by evaporation. Individual root slices also allowed for repeated seed creation and screening. The amount of time carrot roots were stored after harvest had no influence on soft rot (2, 6, or 12 weeks). The findings were same whether the roots were utilised whole or cut; however, the root tip was more responsive. The diversity of carrot lines revealed that breeding for resistance to soft rot may be advantageous (Michalik et al. 1992).

Michalik and Ślęczek (1997) investigated resistance to *P. carotovorum* subsp. *carotovorum* in progeny by crossing carrot orange cultivars, four Uzbek Mirzoe varieties and five wild *Daucus carota* subspecies. They detected genetic variation in orange carrot cultivars susceptible to soft rot, although it was insufficient for breeding purposes. They infected carrot root discs using filter discs that had been immersed for 30 min in a bacterial solution (5 × 106 CFU/ml). Despite an increase in the severity of soft rot in the F_2 generation, one indigenous Mirzoe cultivars shown promise as a source resistance. Carrot inred lines, open-pollinated varieties and F_1 hybrids all exhibit considerable differences in their susceptibility to bacterial soft rot, according to a group of German researchers. For the purpose of avoiding misunderstandings, any laboratory screening technique must be reinforced by field evaluations during phenotypical and storage stage. It is an imperative to use roots that have been planted,

harvested, and kept in the same location (Michalik and Ślęczek 1997; Michalik et al. 1992; Lebeda 1985; Skadow 1978).

8.3.7 Crown Rot

Carrot infections have been linked to *Rhizoctonia carotae* and *Rhizoctonia crocorum* (Davis and Raid 2002). They were buried alive in their entirety. *R. solani* may be found in practically every soil type. *Rhizoctonia solani* infections cause seedling damping-off and crown rot in adult carrots (Nuñez and Westphal 2002). The most common anastomosis groups for carrot damping-off pathogen isolates are AG-2, AG-1, and AG-4 (Nuñez and Westphal 2002; Grisham and Anderson 1983). Damping-off thrives in cold, moist soils where seeds have a difficult time germinating and emerging. Damping-off results in root dieback, seed rot due to apical meristem loss, seedling mortality before to or during emergence, and stunted seedlings (Nuñez and Westphal 2002). Crown rot is a problem in muck soils with a high organic matter content, and it often manifests itself just before to harvest (Punja 2002b; Howard and Williams 1976). The disease appears late in the season, when the leaves begin to age quickly, sometimes in patches. Toxic fungus causes dark brown lesions in the crown of the plant and, in rare cases, beneath the root of the plant (Punja 2002b). Crown rot lesions and cavity spot lesions are quite similar in appearance. Lesions in the crown or taproot reduce the marketability of the roots, and bacterial penetration may result in soft rot. In wet wounds, mycelium that resembles a web may grow. Lesions form when roots are stored. Howard and Williams (1976) reported that based on varietal responses in *in-situ* with different levels of treated pathogen and disease-friendly circumstances, it has been hypothesised that certain cultivars are somewhat resistant to crown rot.

Violet root rot (*R. crocorum*), which damages a broad range of plants, including carrots, parsley, parsnips, celery, and fennel, as well as table beets and potatoes (Punja and McDonald 2002; McDonald 1994e; Cheah and Page 1999). Violet root rot affects carrots all across the globe, although it has been particularly severe in Europe, New Zealand, and Australia. The first signs of this disease are usually dead or wilting plants with dirt sticking to their roots. The roots create substantial dark purple-brown lesions that are coated in a thick mat of fungal mycelium with a leathery look that ranges in colour from violet to dark brown. Between the plants, a thick brown mycelial mat may form (McDonald 1994b). The root decomposes gradually underneath the lesions. Violet root rot symptoms occur later in the season and may last into winter. Carrot roots may get infected at temperatures ranging from 5 to 30 °C, with a predilection for temperatures between 20 and 30 °C, according to the USDA. But in places with high soil moisture content, low pH, and nitrogen scarcity, the problem is more severe than in other locations. (Garrett 1949; Cheah and Page 1999). Dalton et al. 1981 proposed that in three naturally infected *R. crocorum* sites in the United Kingdom, susceptibility testing found no change in sensitivity to violet

root rot. In New Zealand, violet root rot was reported in all commercial carrot varieties that were examined (Cheah and Page 1999).

R. carotae is a postharvest fungus that causes crater rot in carrots that have been stored for a long time (Punja 2002a). There have been no reports of other plant species being affected. Crater rot is a mould disease that infest North America and Northern Europe, causing up to 70% damage in Denmark (McDonald 1994a). With the influence of milky white mycelial lining and adhering to the root surface as well as dark brown colored sclerotia, roots form dry, deep craters or pits under humid, cold storage conditions (Punja 2002a; McDonald 1994c). When exposed to moisture, the storage fungus spreads very fast. In the case of Crater Rot, the root system has been contaminated by bacteria. In the field, latent root infections may occur, and roots with senescent foliage retain a greater amount of inoculum than healthy roots. The fungus thrives when a layer of water accumulates on the roots or when the relative humidity is high (Punja 2002a). *R. carotae* may grow at temperatures as low as 1 °C (Punja 1987). Carrot harvesting is postponed until late October, exposing the crop to disease.

Sowing carrot seeds in cool, moist, poorly drained soils, or overwatering immediately after planting, may result in damping-off, which require additional screening (Nuñez and Westphal 2002). Screening experiments have indicated that raised beds improve soil drainage and damping-off. The ability to discern between carrot varietal responses to various damping-off organisms, such as *Pythium spp*., may be difficult to determine unless carrots are tested in sterilized or pasteurised soil or the other sowing media containing specific organism, or unless seed is treated with a mefenoxam fungicide.

After four weeks of treatment with *R. solani*-infected maize kernels, Howard and Williams (1976) counted the number of atypical and normal roots at 16–20 weeks. A highly virulent *R. solani* strain was introduced to flasks containing sterilized maize grains after two weeks at 20–24 °C and spun every 2–3 days to achieve homogeneous fungal colonisation of the corn kernels. They did, however, advise that each test be carried out with "fresh" inoculum. The most successful technique, as Mildenhall and Williams (1970) had discovered, was to cut carrots three weeks after sowing and then inject pathogene inoculum 7 days later. Howard and Williams (1976) advocated growing carrots at temperatures of 20, 24, or 28 °C to minimise crown rot and maintaining a soil moisture level of 0.1 bar. Growing carrots close together to create a humid microclimate, as well as confronting the crown and petioles with filthy soil or carrot detritus, may increase the risk of crown rot (Punja 2002b; Gurkin and Jenkins 1985). In resistance screening trials, adding inoculum into colonised grain kernels and to the soil or other potting media may increase disease stresses (Breton et al. 2003).

Because high soil humidity and low soil pH promote violet rot, using acidic soils or an acidifying medium may help with screen resistance, as disease frequency and severity increase when infected soil roots remain in the soil for a long length of time (Garrett 1949; Punja and McDonald 1994e; Punja and McDonald 1994e; Cheah and Page 1999; McDonald 2002). In three naturally infected *R. crocorum* field sites in the United Kingdom, three Berlicum species, six Feonia or Imperator species, and one

unknown type) were studied. Commercial carrot varieties, according to Cheah and Page, were similarly sensitive (1999). A lack of disease pressure in one site excludes cultivar variations in violet root rot response, while a high amount of disease burden in another place prohibits cultivar differences in violet root rot response. According to Dalton et al. (1981) Western carrots were evolved by selection or intercrossing of closely related varieties such as Early Half Long, Early Scarlet Horn, and Late Half Long. Resistance should be found in anthocyanin and yellow cultivars, which are the forerunners of western cultivars. Resistance testing for violet root rot is still required.

Carrot root hyphae may quickly blanket a carrot root in the absence of appressoria or other infection structures, penetrating the root surface and causing root cell injury (McDonald 1994a). Roots may become unmarketable after three weeks. Despite the fact that crater rot is a postharvest disease, root screening should be beneficial due to the pathogen's aggressive tendency when kept in cold, moist settings. To include wounding into a screening procedure, roots are wounded, causing crater rot to form. Adopting a soil inoculation strategy may be difficult due to latent field infections.

8.3.8 Rubbery Brown Rot

In damp soils, carrot root rot (*Phytophthora* root rot) is a common disease that may be devastating. It often emerges after a period of heavy rain or irrigation (Browne 2002). All of these species, including *P. porri, P. megasperma, P. cryptogea*, and, *P. cactorum*, have been connected to disease in the past. The fungus *Phytophthora* root rot has been found in the United States, Norway, Australia, Canada, and France among other places (White 1945; Rader 1952; Stelfox and Henry 1978; Ho 1983; Browne 2002; Saude et al. 2007). During storage, the roots become black to dark brown and become rubbery. On the other hand, the symptoms usually appear after a long time of root storage. These solid lesions cause harm to the root's centre and crown (Saude et al. 2007). France has sustained severe agricultural losses this winter. On root lesions, a white mycelium may form. Soft rot develops when bacteria and fungi infiltrate wounds. Soaking carrots in water for an extended period of time during cultivation, storage or processing steps increase the zoospores production and its invasion. Cool to moderate temperatures aid in the formation of inoculum and the spread of disease.

There is a scarcity of information about testing carrots for *Phytophthora* root rot resistance. According to Stelfox and Henry (1978), the pathogen was detected in cold storage carrot variety 'Imperator II' in Alberta, Canada, in 1969–1970, and it has since spread around the world. Aside from the fact that they were gathered and washed, there was no detrimental influence on them. Saude et al. (2007) detected this disease on carrot processing farms in Michigan, however they did not include any information on specific varieties or changes in disease severity between cultivars in their findings. It should be possible to evaluate breeding lines or carrot varieties for resistance against rubbery root rot using a procedure similar to that used to screen for cavities.

Inoculation of agar plugs on the spot root (Stelfox and Henry 1978; Saude et al. 2007). The pathogen was cultivated on cleaned carrot roots for up to seven days in conditions ranging from high relative humidity to low to moderate temperature. Infected roots were examined at between 20 and 25 °C in many investigations on *Phytophthora spp.*; however, the optimal temperature varied depending on the *Phytophthora spp.*, studied. After a week at 20 °C, symptoms began to manifest, but not until seven weeks after the temperature was lowered to 0 °C (McDonald 1994d). They discovered that no damage was necessary for this kind of inoculation to cause rubbery root rot symptoms. Wounds caused a wide range of symptoms. One method for phenotypic resistance screening is to keep carrots at 20 °C with a high RH (>95%) to imitate the saturated soil conditions required for the formation of *Phytophthora* spores.

8.3.9 Common Scab

Infections with the fungal pathogen *Streptomyces scabies* are the cause of carrot scab. Although it may be found around the world, it is most widespread in Europe and Canada, notably the Netherlands and France (Villeneuve 2014; Janse 1988). Viruses and bacteria spread via lateral secondary roots or wounds, causing the death of latent epidermal cells to occur. After a few months, a corky protrusion appears on the root surface, with the most prominent protrusion appearing at the top. As a saprophyte, *Streptomyces scabies* may persist in soil for years at a time. Schoneveld (1994) observed that the most sensitive period for *S. scabies* infection was 4–5 weeks after spring planting, which corresponded to the period following spring planting. A 60-mL volume of bacterial culture (107 spores/mL) was treated with 20 L of sterilized loamy soil with a pH of 5.9. The plants were cultivated at 18 °C, 10,000 lx light, 80% relative humidity, and 50% soil saturation. Four months after seeding, roots were collected and checked for symptoms. The germplasm of carrots is sensitive to common scab.

8.4 Virus Diseases

Carrot viruses have infected around 14 individuals (Moran et al. 2002; Nuñez and Davis 2016). The economic repercussions of various ailments varied. Several viruses, such as AMV, CTLV, and TSWV, have little economic consequences (Lebeda and Coufal 1985; Stein and Nothnagel 1995; Nuñez and Davis 2016). The most common and persistent carrot virus is mottled dwarf (CRLV and CMoV) (Watson and Sarjeant 1964; Waterhouse 1985). There has been a paucity of extremely efficient viral and/or vector resistance, according to attempts to categorize it (Elnagar and Murant 1978; Van Dijk and Bos 1985). There are differences in virus susceptibility across carrot breeding lines, which may help explain why commercial cultivars are so resilient.

8.4.1 Motley Dwarf

Carrot cultivars respond to motley dwarf in various ways, and some are resistant (Koike et al. 2002). Danvers, a delicate California cultivar, was determined to be CVC-14 resistant (Watson and Falk 1994). It's difficult to tell the difference between the two cultivars when it comes to resistance to the willow aphids (Dunn 1970). Autumn was susceptible to aphids but resistant to motley dwarfs, according to Dunn (1970). Nantes, on the other hand, was more suceptible to motley dwarf and had a lower tolerance for aphids. 'Kurnella Strongtop' and 'Western Red' are motley dwarf tolerant, according to Tomlinson (1965), while only 'Western Red' is motley dwarf tolerant, according to Kinsella (1966). 'Early Market', 'rootless Cluseed Stump,' and 'Nantes,' to name a few. He saw a broad variety of dwarf symptoms. Dunn (1970) reported that both cultivars were resistant to *Aegopodii*, with 'Berlikum' being the most resistant.

8.4.2 Carrot Virus Y *(*CarVY*)*

Carrot virus Y (*CarVY*) has been found in every common carrot type in Australia, producing a range of symptoms (Latham and Jones 2004). Green peach aphids (*M. persicae*) attacked 22 Apiaceae plants in a glasshouse. Aphids were raised in canola cages at temperatures ranging from 15 to 20 °C. Rotenone, an insecticide, was applied to the aphids for two hours. The aphids were fed tainted carrot leaves after a 10-min fast and then sprayed onto healthy carrot plants. Aphids were fed for an hour before being killed. Carrot, five Apiaceae herbs (anise), and two Apiaceae native plants (native parsnip, *D. glochidiatus* and Australian carrot, *D. glochidiatus*) and were found to be *CarVY*-infected (Jones 2005). *Trachymene pilosa* is a species of Trachymene. *Trachymene pilosa* is a species of Trachymene. In the field, infection was found in seven of the 22 host plant species, with significant variation in host plant type and disease severity. In a greenhouse, the severity of symptoms differed substantially across Daucus spp. and other wild ancestors samples fed aggressive green peach aphids. A Polish collection of 21 wild germplamic accessions (seven wild carrots, six *D. muricatus*, two *D. bicolor*, and six unidentified *Daucus* species) and a UK collection of 29 wild germplamic accessions viz., seven wild carrots, two *D. bicolor*, six unidentified Daucus species six and *D. muricatus* were used to obtain systemic *CarVY*-infected plants (27 wild carrots, one *D. littoralis*, one *D. hispidifolius*,). When more lines were introduced to the collection, some were infected many times, indicating infection, while others remained infection-free, indicating *CarVY* resistance. Finally, accessions from Australian field trials were tested for a larger spectrum of symptoms than accessions from greenhouse trials.

8.4.3 Parsnips Yellow Fleck Virus (PYFV)

PYFV resistance in carrots has not been tested. The genetic resistance of the carrot line to viruses such as motley dwarf and *CarVY* is unclear. Molecular screening approaches, as shown by the wide range of symptoms seen in virus-treated carrot lines, may be helpful for discovering viral resistance genes, including QTLs.

Carrot diseases are caused by a variety of mollicutes (phytoplasmas and spiroplasmas) that are restricted to the phloem of the crop. Infections caused by Phytoplasma affect a diverse range of cultivated and wild species, including carrots and over 300 other crops, ornamental crops, and weeds (Blomquist 2002). Leafhoppers are the vectors that carry them. However, despite the fact that phytoplasma losses in carrots are rare, aster yellows have been discovered in all major carrot-producing countries, while BLTVA yellows have only been discovered in the western United States. Yellows derived from the BLTVA Phytoplasma are categorized as subgroup A of the *16SrVI* clover proliferative group, while yellows derived from asters are classified as subgroup B. Yellows derived from asters are designated as subgroup A of the *16SrVI* clover proliferative group. Phytoplasma is considered a member of subgroup I of the *16SrI* clover proliferation group, according to the *16SrI* trefoil proliferation group (Lee et al. 2006) Phytoplasmas produce symptoms related to those of infections. It is potential for leaf veins to become chlorotic, which will ultimately result in the chlorosis of the whole leaf. The leaves of infected plants are much thinner than the leaves of healthy plants. Dormant crown buds give rise to adventurous shoots. Hand gathering is required due to the fragility of golden, crimson, or purple leaves (Blomquist 2002). Infected plants have a short main root and a taproot from which numerous branch roots grow. After bolting, carrot seed harvests develop phyllody (leaf-like petals on blossoms) and virescence (flower greening). BLTVA assists in the relief of pain. Plants infected with Phytoplasma look like aster yellows, but they bloom early and have weak, woody taproots with secondary root development. "Dormant umbels" are ones that lack virescence and phyllody.

Spiroplasma citri was reported in carrot plants in Washington State by Lee et al. (2006). It was observed that the leaves of symptomatic plants had yellowing leaves, purpling, and reddening, as well as the creation of a crown arrangement, shortening of roots and shoots, fibrous secondary root growth, and an abundance of adventitious roots. It was discovered during the carrot harvesting operation in central Washington. Yellow pigments produced from *Synechocystis citri* and *BLTVA* phytoplasma has been isolated from several plant species. Citrus greening is caused by the bacterium *S. citri* in Florida and California. A class of prokaryotes known as Phytoplasmas and Spiroplasmas colonise and reproduce inside the sieve cells of plant phloem (Blomquist 2002). In addition, their leafhopper vectors are flourishing. Given the inability of these obligate organism to be grown on agar, invasion is verified by using a polymerase chain reaction (*PCR*) or an enzyme-linked immunosorbent assay (*ELISA*) using primers specific for Phytoplasma or Spiroplasma. Golden aster yellows are disseminated by the aster leafhopper (*Macrosteles fascifrons*), which is the most common vector of Aster yellows (Boivon 1994; Blomquist 2002). The

beet leafhopper (*Circulifer tennellus*) obtains and spreads Phytoplasma and Staphylococcus citri. Leafhoppers infected with Phytoplasmas and Spiroplasmas propagate the pathogens till they die. Anise aster leafhoppers disseminate aster phytoplasma throughout the Midwest each spring as they migrate from the south on infected weeds and other crops. Aster leafhoppers do not migrate throughout the winter, except in the west and east. During the summer dry season in the western United States, insect leafhoppers gather *BLTVA* yellows Phytoplasma from infected wild plants and disseminate it to irrigated regions. In carrots, Phytoplasmas and *S. citri* do not transmit seed. Female leafhoppers are incapable of infecting their offspring (Blomquist 2002).

In 1982, Gabelman et al. (1994) started breeding carrots to improve resistance to aster yellows. They were able to produce an *AYSYN* breed with four open-pollinated carrot varieties and five lines of inbreds by evaluating 200 accessions in the field. Carrot rows were interspersed with lettuce rows to keep aster leafhoppers away from each four-row bed of carrot lines. Leafhoppers infected with phytoplasma were cultured in a greenhouse in June and July and then spread evenly over the field. In order to estimate infection rates, they performed a search for aster yellows symptoms in October. For pollination, 189 roots from the top 10% of the 200 lines were verbalised and planted in a greenhouse. Five inbred lines and four open pollinated cultivars were developed from the roots of twenty flowering plants using an unknown Russian line F_1 and W33 (Nanco, Scarlet Nantes, Gold King and Royal Chantenay). The *AYSYN* population was established via crossing seed, and inbred lines were isolated using a number of approaches. Using Gabelman et al. (1994) third technique, carrot inbred lines were derived from the Wisconsin carrot breeding programme (WBP). Four WBP roots were combined and inbred over eight generations to generate the inbred W1-1. To develop inbred lines for this population, three ways were used: they were mixed with the population's inbred selections, the population was mixed with high-color inbred lines, and the population was combined with elevated inbred lines. *AYSYN* lines were utilised to generate *AYSYN* hybrids after five generations of inbreeding. Field studies were conducted in 1990, 1991, and 1993 to test the resistance of 26 chosen lines to aster yellows on six commercial carrot cultivars. According to Gabelman et al. (1994) resistant lines showed infection rates ranging from 2.5 to 35.3% per plot, while regular cultivars had infection rates ranging from 12 to 43%. A large number of resistant lines were chosen based on their lower incidence of aster yellows. The least infected plants were 'Scarlet Nantes,' 'Royal Chantenay,' and 'Gold King,' with an infection rate of 15.3% on average. In 33.3% of instances, leafhopper populations were similar across genotypes, demonstrating that resistance had minimal effect on vector feeding. Feeding preferences for carrot genotypes were not detected. Using a synthetic population in conjunction with pre-existing inbred lines seems to have been very beneficial in generating the most successful resistance breeding approach. Inbreeding, according to Gabelman et al. (1994) led in the creation of resistance-causing recessive alleles. The resistance of naturally infected and contaminated crops was tested by exposing them to high selection pressure. The data imply that aster yellow resistance is empirical, based

on morphological heterogeneity and the relevance of various exposures in disease responses.

8.5 Carrot Fly (*Psila Rosae*)

The carrot fly, sometimes known as the carrot rust fly, is a pest of carrots and other Apiaceae crops that may cause severe damage (Hardman and Ellis 1982). Carrot plants are preferred by females for egg laying. Carrot roots are unsaleable due to the damage of carrot fly larva (Ellis 1999). In the vast majority of cases, quality losses exceed yield losses (Dufault and Coaker 1987). It has been shown that antixenosis reduces early fly infestation and leads more to resistance than antibiosis towards larvae in Umbelliferae species; however, it has been found that the opposite is true in carrot cultivars (Degen et al. 1999a). Pesticide-resistant varieties in carrot have been tested (Degen et al. 1999b, c). Several carrot fly resistance trials, according to Ellis et al. (1978) have shown negative findings. As part of their investigation into the efficiency of pesticides against the carrot rust fly, the results revealed that Speed's Norfolk Giant and Royal Chantenay were at different extremities of a susceptibility resistance curve. The damage index, which was computed using root weights and quantities in four damage categories, showed good discriminating between cultivars, even when carrot rust fly infestation was dreadful. Michalik and Wiech (2000) screened carrot varieties and developed five resistant breeding lines. *P. rosae* damage was decreased by half in cultivated carrots with the highest level of resistance. Several Daucus species have been tested for carrot fly resistance, and they may hybridise with cultivated carrots to produce resistant cultivars (Ellis 1999). Ellis and Hardman were among the first to generate resistant F_3 and F_4 carrot cultivars from hybrid *D. capillifolius* (1981). In order to develop new varieties with low resistance to the carrot fly, nine carrot inbred lines were developed in 1991 from a hybrid of two carrot varieties namely Long Chantenay and Sytan (Ellis et al. 1991). Varieties, wild ancestors, putative land rances and wild accessions were used to develop resistance, giving in the partly resistant variety Flyaway, as well as lines with much higher resistance than Sytan (Simlat et al. 2013; Ellis 1999). Identifying the physiological, pharmacological, and genetic factors of carrot fly resistance may aid breeders make better accurate cross selection decisions in their breeding programmes. Guerin et al. (1983) and Städler and Buser (1984) revealed that the chemical composition of the leaf surface is complicated. Carrot leaves, on the other hand, contain a range of oviposition stimulants that are very effective in attracting the carrot fly. According to Städler and Buser (1984), propenylbenzene, coumarins, and polyacetylene are effective antibacterial and antifungal compounds. Several experiments have been carried out in order to get a better knowledge of the processes behind carrot fly resistance. Oviposition, according to one point of view, is undesirable. Guerin and Stadler (1984) looked at how foliar chemostimulants affected this variable in four cultivars. The colour of the leaves, as well as their morphological characteristics, influenced host selection and oviposition. While some plants were resistant to antixenosis, resulting in lower egg

production, the roots were the predominant source of resistance (Guerin and Ryan 1983). Carrot roots containing chlorogenic acid have been linked to an increased risk of carrot fly larval damage (Cole 1985). When this chemical was evaluated in selected lines of 'Sytan,' there was no consistent sign of resistance, showing that this was not resistance chemistry (Ellis 1999). Simlat et al. (2013) found a link between carrot resistance phenotypes and phenolic component concentrations. The expression of *PAL1* and *PAL3* was found to be higher in resistant carrot lines. As a consequence, numerous sources of carrot fly resistance in both wild and cultivated plants have been found. Unlike Ellis (1999), few researches have looked into the genetics of carrot fly resistance. This knowledge might help in the improvement of carrot genotypes that are resistant or partially resistant or immunity to carrot fly.

8.6 Aphids

Aphid saliva may induce plant disease in addition to mechanical harm (Rubatzky et al. 1999). When beetles feed on plant leaves, they produce honeydew. Honeydew is a pleasant substance that creates a protective covering on the photosynthetic surfaces of plants. Furthermore, it spreads viruses that are harmful to *C. moestum*, including as *CMoV* and *CRLV*, which cause the plant to become motley dwarf (Carrot-Willow Aphids). Carrots are a host plant for *M. persicae*, a green peach aphid. The peach green aphid (*M. persicae*) prefers carrots as a host plant. Other popular names for these insects are melons aphids (*Aphis gossypii*), purple pea aphids (*Acyrothosiphon pisum*), bean aphids (*Aphis fabae*), potato aphids (*Macrosteles fascifrons*) and carrot-willow aphids (*Myzus ornatus*),

Because of its vigour and rapid growth, Lamb (1953) hypothesised that Osborne Park, an Australian carrot cultivar, would be resistant to the insect willow aphid. The carrot cultivar 'Autumn King' was aphid resistant in the United Kingdom because to its less severe motley dwarf symptoms as compared to lesser varieties. Dunn (1970) conducted a study on the Autumn King and the tolerance of aphids. Three Australian cultivars, Berlikum, Nantes, Autumn King and Chantenay were investigated for aphatic susceptibility in cages and field testing over a three-year period at different temperatures. Aphid levels, on the other hand, were consistently high throughout the board, with very little reproductive variation. 'Osborne Park' was more susceptible to carrot-willow aphid infection than Lamb (1953). The 'Autumn King,' on the other hand, was especially susceptible. Berlikum is more sensitive to aphids and viruses than Nantes. Dunn (1970) claims that cultivar fertility is not as temperature-dependent as aphid fertility. Antibiosis, as well as preference or non-preference and tolerance, were proposed by Painter (1951) as components of aphid resistance. Dunn (1970) proposed Berlikum in the outdoors. Aphids prefer between 20 and 30% less aggressive aphids than the host. The short cultivar tested, 'Berlikum,' may have signalled aphid escape rather than resistance.

Painter (1951) distinguished three forms of aphid resistance: antibiosis, antixenosis, and tolerance. Prior to the invention of the phrase, only minor genetic

changes in plants and insects occurred. Smith and Chuang (2014) conducted a comprehensive evaluation of the known research on plant aphid defence. They examined the genes and sequencing of aphid-resistant cultivars created for a diverse variety of plant species, as well as their host selection behaviour. They investigated the pathogenicity of aphids as well as the utility of aphid resistance genes in agricultural pest and disease management. This resistance is dominant, although it might also be polygenic, recessive, or partly dominant. Despite this, at least 17 aphid species have been identified as being harmful to plant aphid resistance genes, emphasising the crucial need for the development of novel and diversified sources of protection. Using linkage maps and fluorescence in situ hybridization, researchers discovered viral resistance genes in plants that were aphid and aphid-vectored. Aphid resistance is not bred into carrot varieties.

8.7 Thrips

Damage to carrot leaves and petioles is caused by thrips' rasping mouthparts, which induce silvering and injury (Rubatzky et al. 1999). Carrots may be attacked by thrips such as *Thrips tabaci*, *Frankliniella tritici*, and *Frankliniella occidentalis*. The tomato spotted wilt virus (*TSWV*) is disseminated by the carrot-feeding western flower thrips, which is a vector for the virus. Wild, cultivated, and biofortified carrots enhanced with the antioxidant chlorogenic acid were used in a study by Leiss et al. (2013) to investigate non-specific durable resistance to the western flower thrips (*F. occidentalis*). A total of six commercial carrot varieties (Ingot, Sugarsnax, Nantes, Paris Market, and Chantenay) and four wild accessions (D3, D2, D1 and S1) were tested (four biofortified genotypes (two germplasms with high chlorogenic acid, 309-2 inbred line (purple-yellow) and B7262 inbred line (purple-orange) from the WBP as well as a purple and an orange accession from a seed source). Silvering (feeding damage) severity varies by a factor of two most resistant and sensitive carrot inbred lines. Nuclear magnetic resonance microscopy was used to analyse the three most resistant and sensitive carrots (NMR). According to the investigation, thrips were found on wild carrots. The carrot fly (*P. rosae*), was the most resistant to Ingot. There was no thrips resistance found in biofortified carrots. Three biofortified carrots were found to have thrips. Despite having the leaf area, leaf hair content and same size, the metabolic profiles of susceptible and resistant carrot cultivars differed. The leaves of resistant cultivars contained much more sinapic acid, alanine and luteolin than the leaves of susceptible cultivars. In vitro, these compounds limit thrips growth. The natural variety of these chemicals observed in growing carrots, according to Leiss et al. (2013), may be utilised to boost thrips resistance. The compounds improve the advantages of thrips resistance breeding due to their antioxidant characteristics. More sensitive metabolomics, they reasoned, would signal an increase in the number of host resistance chemicals that may infect them.

8.8 Nematodes

Root knot nematodes that feed on carrots include *Meloidogyne javanica*, *Meloidogyne incognita* and *Meloidogyne chitwoodi*. Crop output and morphological flaws such taproot forking and galling may result in 100% losses, rendering carrots unsaleable roots (Roberts and Mullens 2002). Most common nematode in the temperate areas of the countries is *M. hapla*, while *M. incognita* and *M. javanica* are also common in these zones (Parsons et al. 2015; Bridge and Starr 2007). *M. chitwoodi* and *M. fallax* are less common, although they do cause substantial infestation to carrot taproots. *M. chitwoodi* severely galls the lenticels, resulting in a tenacious taproot (Wesemael and Moens 2008). Soil nematicides, crop rotation, and floods are used to control root knot nematode (RKN). On the other hand, genetic tolerance seems to be the most efficacious and ecologically friendly approach of minimising RKN destruction. Carrot germplasm has a high level of genetic diversity, which is linked to nematode resistance. Yarger and Baker (1981) investigated the susceptibility of 21 cultivars and breeding lines to *M. hapla* in a controlled greenhouse and in the field. Nantes and Long Chantenay rootstocks were resistant in general, although Danvers rootstocks were more vulnerable. Certain cultivars indicate tolerance by parasitizing the roots but not reproducing, whilst others demonstrate tolerance by parasitizing the roots but reproducing (Wang and Goldman 1996).

Using primary root galling on carrot seedlings, Huang et al. (1986) presented a stability study for testing *M. javanica* resistance in the greenhouse. The severity of symptoms was larger in the Nantes and Kuroda groups, indicating that these two worm species have different resistance mechanisms than *M. hapla*. The cultivar Brasilia has a low worm population density because to its resistance to worm penetration, development, and egg production delays (Huang 1986). *M. incognita* race 1 resistance was assessed in 170 Korean carrot lines by Yunhee et al. (2014). As genetic resources for breeders, they have 61 resistant lines accessible. Susceptible root tissues created huge changed cells surrounding the nematodes seven weeks after infection with *M. incognita*, while resistant root tissues developed tiny modified cells (Yunhee et al. 2014). The presence of necrotic layers around altered cells may be caused by the expression of the RKN resistance gene in resistant carrot root tissues. The northern Indian cultivar DR-333 has been shown to be resistant to southern root knot nematode (Siddiqui et al. 2011). When discussing resistance, it is an important to look at the different types of nematodes. There are three races of Columbia root-knot nematode in the United States (Wesemael and Moens 2008). The sensitivity of fifteen carrot cultivars to *M. chitwoodi* varied according to the racial group that infected the seedlings in the experiment (Santo et al. 1988). There are thirteen of fifteen *M. chitwoodi* race 1 cultivars, with quality ranging from medium to excellent. Aside from Orlando Gold, none of the *M. chitwoodi* race 2 hosts were present or performed badly (moderate host). Wesemael and Moens (2008) reported *M. chitwoodi* egg masses in 19 carrot varieties produced in glasshouses. Charchar et al. (2009) identified a novel RKN race capable of parasitizing two important vegetable

crops farmed in Brazil. In the battle against RKN, finding resistant carrot cultivars to include into crop rotations is crucial.

Prior to establishing RKN-resistant carrot cultivars, scientists must conduct resistance genetics research. The species *M. javanica* and *M. incognita* were extensively used in this investigation. According to Huang et al. (1986) *M. javanica* exhibited a high degree of narrow-sense heredity when it came to root distressing and egg mass production. Resistance to *M. incognita* was also shown in field testing using a Brasilia carrot cultivar (*Mj-1*, one or two dominant genes duplicated at a single locus). RAPD markers associated with the *Mj-1* gene that might be used in conjunction with marker-aided selection to generate hybrids resistant to *M. javanica* (Boiteux et al. 2004). According to Boiteux et al. (2004), the *Mj-1* locus dosage has an effect on phenotypic resistance, and the *Mj-1* locus may be a quantitative resistance locus. Ali et al. (2014), for example, discovered a segregating population resistant to *M. javanica* and *M. incognita*, which they attribute to *Mj-2*, a single dominant gene on the same chromosome as *Mj-1*. Using three segregated populations, Parsons et al. (2015) identified five *M. incognita* resistance QTLs. QTLs have been discovered on carrot chromosomes 1, 2, 4, 8, and 9. *Mj-1* is a chromosome 8 quantitative trait locus (QTL) that is shared by all three populations. The cross of three resistance sources from Europe, South America, and Syria resulted in two carrot populations with broad-sense heritabilities of 0.33 and 0.25 against *M. incognita* (Parsons et al. 2015). In *M. hapla*, Wang and Goldman (1996) found two homozygous recessive resistance genes. Nematode infection, on the other hand, has been linked to quantitative and qualitative resistance. According to Yunhee et al., resistance to *M. incognita* is controlled by a single or a few genes. A commercial variety with *Meloidogyne* resistance genes was found in a populations generated from the resistant variety Brasilia (Vieira et al. 2003). Brasilia germplasm remains one of the most promising sources of RKN-resistant carrots on a long-term, broad-spectrum basis (Vieira et al. 2003). The BRS Planalto cultivar was developed by Embrapa Vegetables in Brazil in 2009 to be resistant to RKN (Pinheiro et al. 2011). Standard RKN resistance breeding strategies, according to Ali et al. (2014), required labor-intensive greenhouse and field phenotyping experiments. Certain kinds of nematode resistance may be produced by using RNA interference (RNAi) to target and silence nematode genes in host plants that produce *dsRNA* and *siRNA* (Roderick et al. 2018). *Pratylenchus thornei* and *Pratylenchus zeae* were subjected to dsRNA treatment in order to inhibit the expression of two genes that are important in structural stability and muscle function, respectively (Tan et al. 2013).

Singh et al. (2019) reported RKN, *Meloidogyne spp*., in carrot genotypes in vitro. To examine carrot genotypes for RKN resistance, we inserted about 20 larvae J_2 of *M. incognita* per root tip onto pluronic gel media. The larvae pierced the roots in large numbers, with 12.5 larvae per root in black carrot Pusa Asita and 1.0 J2s per root in '6526B Sun2000'. *Mj-1* resistant carrot lines ("6526 B Sun2000" and "8542B Vilmorin") have been shown to confer RKN resistance to susceptible carrot cultivars. RKN resistance breeding will be aided by the STS-SQ1 marker and in vitro screening.

8.9 Minor Pests

Pest insects and mites attack on carrot roots and leaves, preventing seed germination and root growth (Rubatzky et al. 1999). Pests include carrot leaf miners (*Lisonotus latiusculas*, *Napomyza carotae*), leafhoppers (aster and beet leafhoppers), carrot psyllids (*Trioza apicalis*), red spider mites (*Tetranychus urticae*) and carrot weevils (*L. latiusculas* and *Lisonotus oregonensis*). With the exception of anecdotal data from breeders and producers, nothing is known about the resistant origins and genetic paths of the majority of these pests. In carrot pests that transmit viruses, phytoplasmas, and spiroplasmas, it is difficult to separate vector resistance from pathogen resistance. Lygus bugs, a microscopic root crop pest, have the ability to devastate seed yield. Insects favour seed and blossom development, causing carrot seed embryos to die and become non-viable. Scott (1970) reports that 'Nantes,' 'Imperator,' and 'Royal Chantenay' have variable degrees of resistance to lygus bug feeding. The purpose of this research was to determine how immune lygus bugs are to insect attack on flowering carrot inflorescences. In Idaho, Scott (1977) used a similar technique in order to choose for lygus insect resistance. In none of his studies, Scott (1970, 1977) seems to have tested umbels for lygus insect damage to developing seeds. It's conceivable that the insects didn't die because of pest resistance-related dietary changes. For a number of reasons, comparing cultivar sensitivity to lygus bugs proved difficult. Certain carrot infloresences may be deficient in lygus insect feeding, impairing seed development and expansion. He reported that the mortality of lygus insects varied across cultivars and among cultivars. The persistent impact of lygus insect losses on several aspects of carrot seed production raises doubt on the findings.

A diversified feeding approach, according to Kainulainen et al., promotes the acceptability of sucking insect oviposition. *T. anthrisci*, an Apiaceae psyllid, was investigated in the laboratory, greenhouse, and field. The lygus was dissected. In Northern Europe, these pests induce root stunting and leaf bending. On the other hand, Lygus bugs bite off salmon seeds to feed the growing egg, resulting in seeds that are unsustainable (Scott 1977). Leaf oil was present in variable amounts in Nantes 3 Express, Splendid, Panther, Napoli, Nantura, Parano, and Flakkeer 2 (Kainulainen et al.). Egg production, on the other hand, varies greatly across varieties. Despite their proximity to the hosts, females on Nantes Express 3 lay more eggs than Panther. The fragrance test found no indication of this preference, suggesting that physical touch is more essential than usage in the selection of lygus bug hosts. The egg-laying choice of the insect lygus was shown to be unrelated to essential oil concentration in the research. Cauliflower psyllids are drawn to high concentrations of limonene oil. This carrot psyllid was particularly fond of sabinene. Previous study indicates that the carrot psyllid favours plants with high-pinene-sabinene concentrations (Valterova et al. 1997; Nehlin et al. 1996). *T. anthrisci*, an Apiaceae psyllid, was shown to have a positive relationship between egg number and myrcene. It is a chervil scavenger, a European plant (*Anthriscus sylvestris*). According to this study, compounds in the psyllid diet, but not carrot leaves or essential oils, may influence egg-laying behaviour. According to our findings, the essential oil content of carrot cultivars

seems to be more important for *T. anthrisci* than for lygus bugs. Psyllid resistance may be enhanced in cultivars with a high limonene concentration.

8.10 Conclusion

This chapter extensively discusses the disease and insect pest resistance and susceptibility of carrot germplasm. Cercospora leaf spot and powdery mildew both exhibit monogenic resistance (Bonnet 1983a, b; Angell and Gabelman 1968). Carrot leaf blight is the most prevalent disease worldwide. Numerous studies have examined the genetics of resistance, with two identifying three and eleven QTL, respectively (Le Clerc et al. 2015a, b; Le Clerc et al. 2009). Due to the critical nature of ALB resistance, breeders are searching for markers that may be used to select for it. Root knots, or RKN, wreak havoc on carrot roots worldwide. Three RKN species are resistant genetically. Genetic resistance to *Meloidogyne hapla* is determined by two genes (Wang and Goldman 1996). On chromosome 8, a single dominant gene is involved for conferring *Mj-1* resistance. The resistance gene was selected using marker-assisted selection (Boiteux et al. 2000, 2004). *Mj-2* is also present on chromosome 8, conferring further resistance on M. javanica (Ali et al. 2014). *Mj-1*, when paired with six additional QTL on chromosomes 1, 2, 4, and 9, provides resistance to *M. incognita* (Parsons et al. 2015), a prevalent RKN species found worldwide in temperate carrot-growing areas (Parsons et al. 2015). Selective markers for *Mj-1* have been found (Boiteux et al. 2004). Numerous biotic stressors have been systematically investigated for potential phenotypic resistance, candidate genes identified, and resistance introduced into commercial cultivars. Others are unaware of potential resistance sources and lack screening tools for phenotypic resistance. The scope of this research should be widened to include a broad spectrum of other carrot diseases and pests found in regional and worldwide regions. Resistance to biotic stresses has significantly benefited in the reduction of disease and insect pests when accompanied with biological, chemical and cultural management strategies (Ben-Noon et al. 2003). Because there are no interspecific barriers between wild and cultivated carrot species, resistance genes may be transferred more easily between them. The identification of resistance genes and the breeding of resistant crops have been aided by molecular markers and other technologies (Stein and Nothnagel 1995). A proprietary array of three hundred microsatellite markers was used along with a distributed, in depth coverage carrot nuclear genome library that had > 17X coverage, as reported by (Cavagnaro et al. 2009, 2011). It was discovered that the carrot nuclear genome has a structure after a recent analysis of BAC-end sequences totaling 1.74 Mb. Iorizzo et al. estimate that it accounts for *90% of the anticipated carrot genome (Iorizzo et al. 2016). Researchers will be able to identify genes associated with biotic and abiotic stress, as well as other critical characteristics. Wang et al. (2018) decoded the sequencing of the carrot cultivar 'Kurodagosun,' which was previously uncharacterized (473 Mb). These genetic resources will benefit basic and applied carrot research,

particularly in the area of insect pest and disease resistance development. Klimek-Chodacka et al. (2018) established the very first successful site-directed mutagenesis system utilising the carrot genome, opening the possibility for disease and insect resistance. Resistance to insect pests and diseases is essential for all aspects of seed formation, carrot root growth, storage, nutritional quality, flavour, and processing.

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Chapter 9
Biotic Stresses in Cucurbits: Status, Challenges, Breeding and Genetic Tools to Enhance Resistance

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Abstract Cucurbits are a major vegetable crop that contributes significantly to world vegetable output and nutritional security. In many circumstances, biotic stressors result in significant crop losses or full crop failure. In light of this, the chapter discusses the importance of cucurbits in global nutritional security, major biotic stresses, genetic resources of resistant/tolerant genes, conventional and genomic assisted breeding strategies, biotic stress management through transgenic approaches, and the development of multiple biotic stress resistant varieties. Furthermore, the chapter stresses efforts such as genetic engineering, Genome editing technology in cucurbits crop improvement, Gene stacking, Gene Silencing, cisgenic approaches and vegetable grafting technology for improvement for biotic stresses.

Keywords Cucurbits · Biotic stresses · Genetic resources · Transgenic

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9.1 Introduction

Vegetables are the key components of a balanced human diet and also the main constituent in achieving world wide nutritional security by providing nutrients, vitamins, minerals and several nutraceticals. Vegetables provide all the nutrients ingredients viz., vitamins, minerals and protein that are essential for balanced diet. The presence of good number of vitamins and minerals in vegetables makes them protective food. Many vegetables carry good amount of nutraceutical properties and having capabilities to ensure good health.

Cucurbits are the most numerous of the veggies. For cultivated members of the plant family Cucurbitaceae, Liberty Hyde Bailey developed the term "cucurbits" (Robinson and Decker-Walters 1997). There are two well-defined sub-families, eight tribes, 118 genera, and 825 species in all. Approximately 20 species from nine genera are currently being cultivated (Jeffery 1990). Cucurbit species may adapt to a broad range of settings, including tropical and subtropical climates, arid deserts, and temperate temperatures, due to genetic heterogeneity within the family. Cucurbits are used in salads (cucumber, gherkins, long melon), desserts, and other dishes (ash gourd, pointed gourd), Gherkins (pickles), melons (desserts), and cooking Bitter gourd/bitter melon (Momordica charantia) and other Momordica sp. are well-known for their therapeutic benefits. Vegetable yield must be increased in order to provide balanced nourishment to a growing population with limited resources. Due to their high yield potential, early maturity, superior quality, disease and insect resistance features, hybrid cultivars can play a critical role in increasing vegetable crop production and average productivity per unit of area. The use of high-quality seeds with built-in inbred and hybrid vigour, along with contemporary vegetable technology and appropriate government policies, can result in a constant increase in output. Like other vegetables, cucrbits are also prone to several biotic stresses like disease, insect-pest, nematodes and various weed spsecies. The primary purpose of cucurbitaceous vegetable research is to increase productivity on a long-term basis by generating biotic and abiotic resistant varieties/hybrids with high quality features.

Breeding biotic stress-resistant/tolerant cultivars through traditional breeding procedures is difficult and time-consuming since strains, races, and pathotypes change and mutate quickly to overcome resistance (Zhou et al. 2007). Linkage drag problems, resulting from the combination of multiple undesired genes with the desired genes, make it difficult to achieve yield potential and stress tolerance in conventional breeding (Wang et al. 2015). Despite these drawbacks, traditional breeding methods are critical for the conservation of wild germplasm, cross-pollination between different parents, and the discovery of novel genetic variants and mutations (Werner et al. 2005). Recent advances in molecular biology and genomics have led to the identification of important resistant genes and quantitative trait loci (QTLs) for significant biotic stress, and subsequent advances in marker technologies have paved the way for biotic stress tolerant breeding to be completed more quickly.

For biotic stress tolerance and productivity and quality implement in last two decades extensive applications of techniques related with genetic engineering were

employed globally. In the last two decades a big number of cucurbits crops has been developed by employing genetic engineering techniques and large number are still underway (Parmar et al. 2017). To overcome biotic stresses, a large number of related and unrelated species genes were incorporated to get the traits of significance and ultimately enhanced the productivity. To get the resistance and protection from different bacterial and fungal diseaeses, a number of genes like defensin, glucanase, chitinase, and genes linked with pathogenesis were transferred by group of researchers to various cucurbits crops over the world. In several cucurbit crops, precise genome editing techniques, particularly CRISPR/Cas9, have been successfully used for gene mutation, repression, activation, and epigenome editing. (Wang et al. 2019).

Genetic engineering technology now a day's accepted as the rapidly elevated techniques in agriculture (ISAAA 2017). Using these techniques researchers are able to transfer gene of interests from different source, it may be microorganisms, animals, plants, or genes synthesized artificially in the laboratory) crossing the taxonomic limits into a plants of interest by employing non-conventional techniques. As opposed to regular breeding practices which includes the arbitrary blending of a huge number of genes present both in the susceptible and tolerant plants, recombinant DNA techniques permits the exchange of just the alluring genes to the susceptible plants and the conservation of important traits of significance. Also, the hereditary hotspots for tolerance are not restricted distinctly to firmly related species of plants (DeSalle and Yudell 2020). Resisting against different sorts of biotic stresses is the establishment and essence of futuristic agricultural practices. The significant benefits of transgenic innovation depend on that the genes responsible for different agronomically significant attributes can be sourced from any living being—plants or microorganisms, and so on and can be utilized for plant change. In this way, novel attributes from any foundation can be introduces in the selected plant easily. Although, for one gene shift into elite foundations, the turn of events and normalization of a high recurrence, proficient plant recovery and hereditary change convention is the most extreme preessential. The principle safe characteristics brought into green plants and right now marketed are insect and pest resistance (Bt. toxin gene) and herbicide resilience while other significant research concern tolerance against viruses, male sterility, and so on. The utilizations of this innovation cover wide reach from insect tolerance, viral and infection resistance (Marco et al. 2015).

9.2 Description of Different Biotic Stresses of Cucurbits

9.2.1 Diseases

Diseases in vegetables are caused by biotic agents such as fungi, bacteria, virus, viroid, phytoplasma, and nematode, which result in a reduction in yield and quality. The main restrictions in total vegetable output are disease pressure in standing crops from seedling through harvest, as well as spoiling caused by microorganisms during

transit, storage, and marketing. It is very prone to pathogen attack due to its high proportion of water and relatively quick metabolic activity. Fungi are the diseases that cause the most damage to potential yield in vegetable crops, particularly in tropical and subtropical developing countries. Diseases caused by fungus, bacteria, and viruses, as well as a few diseases caused by mycoplasma, are the most common biotic stressors in cucurbits. The causal organism of 83 diseases of cucurbits is presented in Table 9.1. The brief description of some important diseases is presented here.

9.2.1.1 Anthracnose

Colletotrichum orbiculare is the cause of this condition (Berk. and Mont.). In most cucurbits, especially bottle gourd, anthracnose is a prevalent disease. Disease symptoms can be detected on all sections of the plant above ground, from the cotyledon leaf to the fruits. Water-soaked little yellow dots on leaves expand and turn brown are the first signs of the disease. The necrotic part dries out and breaks. On the stem, elongated water-soaked sunken lesions form. Because of the extensive sporulation, the stem lesions turn pale yellow to brown. On young fruits, small, sunken, light brown, and broken dots emerge in high numbers.

9.2.1.2 Downy Mildew

Pseudoperonospora cogenesis is the cause of the symptom. The disease affects the majority of cucurbits. Cucumber, bitter gourd, bottle gourd, sponge gourd, ridge gourd, pointed gourd, and muskmelon, on the other hand, are badly affected. Symptoms emerge as a scattering of uneven, tiny yellow patches surrounded by green tissues on the leaf lamina. The veins run through the yellow patches, which are angular. Light brown symptoms appear on bottle gourd leaves, and in hot, humid weather, a faint white downy fungus growth can be seen on the lower side of the leaves.

9.2.1.3 Powdery Mildew

The disease is particularly severe in bottle gourd, bitter gourd, and pumpkin. This is especially pronounced in the winter and in greenhouse crops. Spaerothea fuligena and Erysiphe cichoracearum are two pathogens linked to this condition. Symptoms show as white to dull white, powdery growth on all foliar parts, resulting in a significant loss in photosynthetic area. It's possible that the plant will wilt and die. The plant's growth and fruits have been halted. Powdery mildew sporulation can only be seen on the underside of leaves and does not spread across leaf veins, whereas downy mildew sporulation can be seen on the tops or bottoms of leaves and easily spreads over leaf veins.

Table 9.1 Diseases of cucurbits caused by fungus, bacteria and virus

Name of the disease	Causal organism
1. Alternaria fruit rot of pointed gourd	*Alternaria alternata* (Fr.) Kiessler
2. Alternaria leaf blight	*Alternaria cucumerina* (Ell. and Ev.) Elliot
3. Alternaria leaf spot	*Alternaria alternata* f.sp. *cucurbitae* Vakal
4. Anthracnose	*Colletotrichum orbiculare* (Berk. and Mont.) Arx. (¼*C. lagenarium* (Pass.) Ellis and Halsted
5. Belly rot	*Rhizoctonia solani* Kuhn
6. Black root rot	*Thielaviopsis basicola* (Berk. and Br.) Ferraris
7. Blue mould rot	*Penicillium* spp., *P. digitatum* (Pers.:Fr.) Sacc
8. Cephalosporium root and hypocotyl rot, stem streak and dieback	*Acremonium* spp. (¼ *Cephalosporium* spp.)
9. Cercospora leaf spot	*Cercospora* spp., *Cercospora citrullina* Cooke
10. Charcoal rot of fruits	*Macrophomina phaseolina* (Tassi) Goid. [¼*Macrophomina phaseoli* (Maubl.) Ashby]
11. Choanephora fruit rot	*Choanephora cucurbitarum* (Berk. and Ravenel) Thaxt
12. Choanephora fruit rot	*Choanephora cucurbitarum* (Berk. and Ravenel) Thaxt.,
13. Collapse of melon	*Monosporascus eutypoides* (Petr.) Arx (¼ *Bitrimonospora indica* Sivan., Talde and Tilak)
14. Corynespora blight/target spot	*Corynespora cassiicola* (Berk. and Curtis) Wei
15. Crater rot (fruit) or black canker	*Myrothecium roridum* Tode:Fr
16. Downy mildew	*Pseudoperonospora cubensis* (Berk. and Curt.) Rostow
17. Fruit and vine rot of pointed gourd	*Phytophthora melonis* Katsura
18. Fruit rot caused by other pathogens	*Diplodia natalensis* Pole-Evans, *Diplodia gossypina* Ellis and Everh., *Mycosphaerella melonis* (Pass.) Chiu and Walker, *Fusarium solani* (Mart.) Appel and Wr., *F. moniliforme* Sheld., *F. oxysporum* Schlecht. emend. Snyder and Hansen., *Fusarium equiseti* (Corda) Sacc., *F. gibbosum* W.C. Snyder and H.N. Hans., *F. graminearum* Schwabe, *Myrothecium roridum*, *Colletotrichum capsici* (Syd.) Butler and Bisby, *Helminthosporium hawaiiense* Bugnic., *Curvularia pallescens* Boedijn, *Alternaria tenuis* Nees, *Myrothecium roridum* Tode:Fr., *Sclerotium rolfsii* Sacc., *Rhizopus* sp., *Phoma* sp., *Cladosporium* *tenuissimum* Cooke

(continued)

Table 9.1 (continued)

Name of the disease	Causal organism
19. Fusarium fruit rot	*Fusarium equiseti* (¼ *Fusarium roseum* f.sp. *gibbosum*), *Fusarium graminearum*, *Fusarium semitectum*, *Fusarium solani* f. sp. *cucurbitae*, *Fusarium* spp.
20. Fusarium root rot Crown and foot rot)	*Fusarium solani* f.sp. *cucurbitae* Snyder and Hansen
21. Fusarium wilt	*Fusarium oxysporum* Schlecht. emend. Snyder and Hansen, *F. o.* f.sp. *benincasae* Gerlagh and Ester, *F. o.* f.sp. *cucumerinum* Owen, *F. o.* f.sp. *lagenariae* Matuo and Yamamota, *F. o.* f.sp. *luffae* Kawai et al. *F. o.* f.sp. *melonis* Snyd. and Hansen, *F. o.* f.sp. *momordicae* Sun and Huang, *F. o.* f.sp. *niveum* (Smith) Snyder and Hansen
22. Grey mould	*Botrytis cinerea* Pers
23. Gummy stem blight (vine decline)	*Didymella bryoniae* (Fuckel) Rehm (¼ *Mycosphaerella melonis* (Pass.) Chiu and Walker), *Phoma cucurbitacearum (Fr.: Fr.) Sacc*
24. Helminthosporium Leaf spots	*Helminthosporium rostratum* Drechsler, *Phyllosticta cucurbitacearum* Sacc
25. Lasiodiplodia vine decline/fruit rot	*Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. (¼ *Diplodia natalensis* Pole-Evans)
26. Marginal leaf blight	*Exserohilum rostratum* (Drechsler) Leonard and Suggs
27. Monosporascus root rot	*Monosporascus cannonballus* Pollack and Uecker
28. Myrothecium canker (black canker)	*Myrothecium roridum* Tode
29. Net blight/web blight/leaf blight/belly rot	*Rhizoctonia solani* Kuhn
30. Net spot	*Leandria momordicae* Rangel
31. Phoma blight	*Phoma exigua* var. *exigua* Sacc. (¼ *Ascochyta phaseolorum* Sacc.)
32. Phomopsis black stem	*Phomopsis sclerotioides* Van Kesteren
33. Phyllosticta Leaf spots	*Phyllosticta cucurbitacearum* Sacc
34. Phytophthora root rot	*Phytophthora* spp., *Phytophthora capsici* Leonian
35. Pink mould rot	*Trichothecium roseum* (Pers.) Link
36. Plectosporium blight	*Plectosporium tabacinum* (Beyma) Palm, Gams and Nirenberg

(continued)

Table 9.1 (continued)

Name of the disease	Causal organism
37. Powdery mildew	*Erysiphe cichoracearum* DC., *Sphaerotheca fuliginea* (Schl.) Salmon
38. Purple stem	*Diaporthe melonis* Beraha and O'Brien
39. Pythium fruit rot (cottony leak)	*Pythium* spp., *Pythium butleri* Subramaniam, *P. aphanidermatum* (Edson) Fitzp
40. Rhizoctonia fruit rot	*Rhizoctonia bataticola* (Taub.) Butler
41. Rhizoctonia root rot	*Rhizoctonia bataticola* (Taub.) Butler
42. Rhizopus soft rot (fruit)	*Rhizopus stolonifera* Vuillemin (¼ *Rhizopus nigricans* Ehrenberg)
43. Scab/gummosis	*Cladosporium cucumerinum* Ellis and Arthur
44. Seed rot and damping-off	*Pythium aphanidermatum* (Edson) Fitzp., *P. debaryanum* Hesse, *P. myriotylum* Drechsler, *P. butleri* Subram., *Rhizoctonia solani* Kühn, *R. bataticola* (Taubenh.) Butler, *Phytophthora parasitica* Dastur, *Fusarium* spp., *Fusarium equiseti* (Corda) Sacc., *Acremonium* spp., *Thielaviopsis basicola* (Berk. and Br.) Ferraris and some other fungi
45. Septoria leaf blight	*Septoria cucurbitacearum* Sacc
46. Stem rot/collar rot/southern blight	*Sclerotium rolfsii* Sacc
47. Sudden wilt	*Pythium aphanidermatum* (Edson) Fitzp
48. Ulocladium leaf spot	*Ulocladium consortiale* (Thüm.) Simmons
49. Verticillium wilt	*Verticillium albo-atrum* Reinke and Berthold, *V. dahliae* Kleb
50. White mould	*Sclerotinia sclerotiorum* (Lib.) de Bary
51. Angular leaf spot	*Pseudomonas syringae* pv. *lachrymans* (Smith & Bryan) Young et al
52. Bacterial wilt	*Erwinia tracheiphila* (Smith) Bergey et al *Ralstonia solanacearum* (Smith) Yabuuchi et al
53. Bacterial leaf spot	*Xanthomonas campestris* pv. *cucurbitae* (Bryan) Dye
54. Bacterial soft rot	*Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al., *E. aroideae* (Townsend) Holland, *Pectobacterium carotovorum* subsp. *brasiliense* Nabhan et al
55. Brown spot	*Erwinia ananas* Serrano
56. Bacterial rind necrosis	*Erwinia* spp.
57. Bacterial fruit blotch/seedling blight	*Acidovorax avenae* subsp. *citrulli* (Schaad et al.) Willems et al. (¼ *Pseudomonas pseudoalcaligenes* subsp. *citrulli*)
58. Phyllody	Phytoplasma

(continued)

Table 9.1 (continued)

Name of the disease	Causal organism
59. Witches' broom	Phytoplasma
60. Little leaf	Phytoplasma
61. Cucumber green mottle	Cucumber green mottle mosaic virus (CGMMV)
62. Cucumber mosaic	Cucumber mosaic virus (CMV)
63. Watermelon mosaic	Watermelon mosaic virus (WMV, WMV 1 & 2)
64. Zucchini yellows	Zucchini yellows mosaic virus (ZYMV)
65. Chlorotic leaf spot	Bean yellow mosaic virus (BYMV)
66. Mosaic	Potato virus Y (PVY)
67. Mosaic	Papaya ring spot virus (PRSV-W)
68. Cucumber latent	Cucumber latent virus (CLV)
69. Tobacco ring spot	Tobacco ringspot virus
70. Curly top	Beet curly top virus (BCTV)
71. Cucumber vein yellowing	Cucumber vein yellowing virus (CVYV)
72. Lettuce infectious yellows	Lettuce infectious yellows virus (LIYVV)
73. Melon leaf curl	Melon leaf curl virus (MLCV)
74. Melon necrotic spot	Melon necrotic spot virus (MNSV)
75. Muskmelon vein necrosis	Muskmelon vein necrosis virus (MkVNV)
76. Squash leaf curl	Squash leaf curl virus (SqLCV)
77. Squash mosaic	Squash mosaic virus (SqMV)
78. Tomato spotted wilt	Tomato spotted wilt virus (TSWV)
79. Cucurbit aphid-borne yellows	Cucurbit aphid-borne yellow virus (CABYV)
80. Zucchini yellow flecks	Zucchini yellow fleck virus (ZYFV)
81. Pumpkin yellow vein mosaic	Pumpkin yellow vein mosaic virus (PYVMV)
82. Squash vein yellowing	Squash vein yellowing virus (SqVYV)
83. Melon yellow spot	Melon yellow spot virus (MYSV)
(Modified from Monadal et al. 2020)	

9.2.1.4 Gummy Stem Blight

Didymella bryoniae/Phoma cucurbitacearum causes the disease. In muskmelon, bottle gourd, ridge gourd, and cucumber, the disease is becoming more severe. In hybrids, the sickness is more severe. Water-soaked patches on the stem near the soil line are first noticed. Later, translucent gum-like exudates from the damaged area are deposited on top of it. Typically, a silvery grey to dark brown lesion near the stem base, which causes girdling of the stem as the disease progresses and eventually kills the plant. The damaged bark also has a black dot that looks like pycnidia. Occasionally, rapid drops and withering are observed, especially in bottle gourd and cucumber.

9.2.1.5 Cercospora Leaf Spot

Cercospora citrulline causes this leaf spot, which appears as little patches with light to tan brown centres on older leaves. Lesions grow to cover significant sections of the leaf surface as the disease proceeds; lesions may have a black border and be bordered by a chlorotic area. The lesions' centres can grow brittle and fracture. Plant waste is where fungus thrives. Wind and water splashes spread the disease. This disease is mostly found in tropical and subtropical growth areas.

9.2.1.6 Scab

Cladosporium cucumerinum causes scab disease in cucumbers. Small veins limit angular dark blemishes on leaves. Lesions that are pale green and have been drenched in water. Lesions that have dried out have left holes in the leaves. Petioles, stems, and fruit may also have lesions. Fungus lives on agricultural detritus in the soil. Wet conditions and temperatures below 21 °C encourage disease onset.

9.2.1.7 Verticillium Wilt

Verticillium wilt, caused by Verticillium dahlia, usually manifests themselves after fruit set. Leaves that have become chlorotic and have developed necrotic regions have collapsed. The symptom only arises on one side of the vine. There is also vascular tissue discoloration in the roots. Fungus may live in soil for a long time. In the spring, chilly or mild weather favours disease onset.

9.2.1.8 Cucumber Mosaic virus (CMV)

CMV is one of the most dangerous viral diseases that affects cucumbers. Aphids spread the virus; more than 80 species of aphids, including Myzus persicae and Aphis gossypii, are capable of doing so. CMVD symptoms include significantly stunted growth. A striking yellow mosaic covers the foliage. The plant's leaves curl downwards, and the leaves are smaller than typical. Infected plants' flowers may be malformed, having green petals. Fruits grow deformed and tiny, and they are frequently discoloured. This virus is unable to survive in highly dry environments. The virus can infect a wide variety of hosts.

9.2.1.9 Cucumber Green Mottle Mosaic Virus

This virus was first discovered in the United States in a California melon seed production farm. Cucurbit species such as watermelon, melon, cucumber, pumpkin, squash, gourds, and others are also hosts. Mottling and mosaic on leaves, as well as fruit

mottling and deformation, are all signs. Vein clearing and crumpling on young leaves are early indications, while mature leaves become bleached and chlorotic. Cotyledons may turn yellow in severe infections, but symptoms are usually not visible until the first or second leaf stage.

9.2.1.10 Zucchini Yellow Mosaic Virus (ZYMV)

It's a virus disease that affects cucurbits that was originally discovered in Europe in 1981. It has now been documented in most southern and southwestern states, as well as New York State, where it was discovered in 1983. ZYMV belongs to the Potyvirus genus. A noticeable yellow mosaic, necrosis, green vein-banding, chlorotic patches, blistering distortion, leaf deformation, and stunting are all foliar symptoms. Fruits are still little, misshapen, and speckled green. ZYMV is spread by aphids of various types. Aphis gossypii and Aphis craccivora are the most common aphid vectors, with Aphis gossypii and Aphis craccivora being the most important. It can also be spread through contaminated seeds and plant fluids harbouring the virus.

9.2.1.11 Chlorotic Curly Stunt

Plants that have been impacted are severely stunted, with little chlorotic and weakly curled leaves. The illness can be spread easily by the whitefly, Bemisia tabaci, but not via sap. Cucumis sativus, Luffa acutangula, Luffa cylndrica, and other cucurbits are also susceptible to the virus. It was first discovered in India in 2003–2006 as chlorotic curly stunt disease (CCSD) in the vegetable-growing areas of Delhi and the neighbouring state of Haryana.

9.2.2 Insect Pest

Insect pests are also a key biotic barrier to the production of cucurbits. Many of them, in addition to causing direct damage, serve as vectors for a variety of viral infections. The following are insect pests that are very important to vegetable crops and cause production loss (Table 9.2). A shift in pest status has been noted in recent years, due to changes in cropping techniques and climate, as well as the introduction of highly input intensive high yielding varieties/hybrids. Many pests have evolved new hosts, developed pesticide tolerance, and secondary outbreaks are common. Here's a quick rundown of some of the most common diseases.

Table 9.2 Yield losses due to major insect pests in cucurbits in India

Crop/Pest	Yield loss (%)
Bitter gourd	60–80
Cucumber	20–39
Ivy gourd	63
Musk melon	76–100
Snake gourd	63
Sponge gourd	50
(Rai et al. 2016)	

9.2.2.1 *Red Pumpkin Beetle* (*Aulacophora Foveicollis, A. Cincta, A. Intermedia)*

The crop is harmed by both grubs and beetles. Beetles, on the other hand, are more damaging. They feast on flowers and eat holes in leaves. Beetles eat holes in the foliage, flowers, and cotyledons, causing damage. Almost all cucurbits are infested. Cucurbits that were planted too early are badly destroyed, necessitating resowing. Beetle damage causes a lot of holes on the leaves. Grubs feed on plant roots below the soil surface after hatching. Grubs eat fruits that come into contact with the soil as they bore through vines.

9.2.2.2 *Epilachna Beetle/Hadda Beetle* (*Epilachna Vigintioctopuncata*)

From East Asia to South Asia and Australia, spotted beetles can be found. Cucurbits and solanaceous vegetables are their primary sources of nutrition. Chewing mouthparts are present in both the grub and the adult. As a result, they scrape the chlorophyll from the leaves' epidermal layers. A classic ladder-like window appears as a result of the feeding. The leaves will be pierced by the windows as they dry and fall off. Several windows consolidate together in severe infestations, resulting in skeletonization, or the creation of a papery structure on the leaf.

9.2.2.3 *Fruit Fly* (*Bactrocera Cucurbitae* (Coquillet)

Only maggots cause damage to mature fruits by feasting on them, riddling them, and fouling the pulp. Maggots burrow into the flesh of the fruit and feed on the pulp, causing sores. This pest's maggots produce leaking of brown, resinous fluid from the fruits, which causes the fruits to become warped and misshapen. Fruits rot as a result of bacterial infestation. Melons and bitter gourds take the worst of the damage. Fruits that are still in the early stages of development are also attacked. These kind of fruits do not grow. Fruits drop prematurely due to infestation.

Besides these insect pests, some minor insect which affects cucurbits are: Stem gall fly (*Neolasioptera falcata*), stem borer/Clear winged moth (*Melittia eurytion*), Stem boring grey beetle (*Apomecyna saltator*), Plume moth (*Sphenarches caffer*), Stink bug (*Aspongopus janus*) and Flower feeder (*Mylabris pustulata*).

9.3 Genetic Resources of Resistance Genes

The wild relatives serve as a great store of huge genetic variability and a valuable resource of genes for the biotic stress's resistance in cucurbits. For several reasons, the evolution of genes relevant to disease resistance in plants has been the subject of significant research. These genes are most likely associated to plant adaptability to varied habitats where diseases are emerging. The collection of biotic stress tolerant genetic resources available in different gene banks as well as their collection from biotic stress hot spot of the country would be an important step towards developing biotic stess tolerant/resistant varieties. Proper evaluation of such germplasm in hot spot areas, documentation and utilization in breeding programmes is likely to accelerate development of stress tolerant varieties in cucurbits. Besides, these valuable germplasm resources can also be used for pre-breeding, developing mapping populations, allele mining, association mapping for QTLs and identification and isolation of useful genes against abiotic stresses. Genetic resources of different cucurbits resistant/tolerant to different diseases and insect pests as listed by Naik et al. (2013) has been presented in Table 9.3.

9.4 Biotic Stress Management Through Transgenic Approach

9.4.1 Insect-Pest Resistance

Presently, resistance against insect and pest is missing commonly in crop plants. The utilization of chemicals to protect the plants from insect-pest is found hazardous to the users and furthermore not ecologically maintainable. From a producer's point of view, any hereditary improvement that could lessen the expense of chemical implementation to protect from would be of critical advantage. Fuchs et al. (2004) studied about the transgenic squash wellness cost encoding coat protein genes for three potyviruses, Watermelon mosaic infection (WMV), Zucchini yellow mosaic infection (ZYMV) and Cucumber mosaic infection (CMV), (Tricoli et al. 1995). Plants with introduced genes showed tolerance against all the three aphid borne viruses. Along the all-field trails severely affected by diseases, the transgenic squash and its hybrid progeny exhibited enhanced tolerance against the diseases caused by viruses,

Table 9.3 Genetic resources of different cucurbits resistant/tolerant to different diseases and insect pests

Crop	Disease/Insect pests	Resistance source	References
Powdery mildew	Cucumber	PI 197,087, Poinestee, Yomaki, Sparton Salad, PI 197,088, *Cucumis ficifolia*, *C anguria*, *C dinteri* and *C sagittatus, C ficifolia* accessions IVf 1801 and PI 280,231, *C anguria* PI 147,065, *C anguria* var. *anguria*, *C dinteri* PI 374,209 and *C sagittatus* PI 282,441	Barnes (1966); Imam and Morkes (1975); Omara (1979); Munger et al. (1979); Lebeda (1984)
	Musk melon	Edisto, PMR-45 and PMR-450; Georgia-47 and C-68; Campo and PMR-6); Arka Rajhans, RM-43 and Pusa Sharbati Campo, Jacumba, Levlita, PM-5 and PMR-6, PI 164,323 and PI 180,283	Copeland (1957); Bohn and Whitaker (1964); Takada et al. (1975); Norton and Cosper (1985); Choudhury and Sivakami (1972); Khan (1973)
	Watermelon	Arka Manik (IIHR-India)	Nath et al. (1973)
	Pumpkin & squash	*C. moschata*	Sowell and Corley (1973)
	Bottle gourd	India-IC0319838, IC337078, IC296733, EC800995, EC750696	Ranjan et al. (2021) (Unpublished)
Downy mildew	Cucumber	Chinese Long and Poinsette	Imam and Morkes (1975); Seshadri (1986)
	Musk melon	Edisto, Seminole; Buduma Type-1, 2 and 3, Phoontee, Goomuk, Nakkadosa, Ex-2, Annamalai, Edisto and Harvest Queen; *Cucumis callosus,* WMR-29, MR-l, Punjab Rasila, Cinco, DMDR-l and DMDR-2; Punjab Rasila; EC 163,888; Snapmelon collections like SP-l, SP-2, SP-3, KP-2, KP-7 and KP-9	Copeland (1957); Whitner (1960); Sambandam et al. (1979); Zink et al. (1983); Nandpuri et al. (1993); Singh. (1996)
	Watermelon	Arka Manik (IIHR-India)	Nath et al. (1973)
Anthracnose	Cucumber	PI 197,087 and PI 175,111	Barnes and Epps (1952); Hayja and Peterson (1978)

(continued)

Table 9.3 (continued)

Crop	Disease/Insect pests	Resistance source	References
	Watermelon	Arka Manik (IIHR-India), Black Stone, Charleston Gray and Cargo	Nath et al. (1973), Robinson and Shail (1975) & Suvanjrakorn and Norton (1980)
Fusarium wilt	Musk melon	Delicious-51 and *C. melo* var. *reticulatus*, *indorus*, *chito* and *flexuosus*	Munger (1954) and Zink et al. (1983)
	Watermelon	Citron, Calhoun Gray, Sornkylee and Summit, Dixielle, All Sweet, Crimson Sweet, Charleston Gray and Lousiana Queen	Orton (1911); Elmstrom and Hopkins (1981)
Gummy stem blight	Musk melon	Line PI 140,471	Norton (1982)
	Bottle Gourd	Arka Nutan, Arka Shryas	IIHR, India
	Watermelon	PI 482,283 and PI 526,233 PI 279,461, PI 254,744, PI 482,379, PI 244,019, PI 526,233, PI 482,276, PI 164,248, PI 482,284, PI 296,332, PI 490,383, PI 271,771, and PI 379,243	Gusmini et al. (2017); Song et al. (2002)
Cecospora leaf spot	Bottle gourd	IC546185, EC800998, IC362403, IC385814, IC398534, IC426990, IC536594, IC550741, IC567545, IC541223, IC548580, IC277094, IC279634, IC279731, IC297489	Ranjan et al. (2021) (Unpublished)
Cucumber mosaic virus	Cucumber	TMG-1, Tokyo Long Green, Chinese Long, Wisconsim and Table Green	Provvidenti (1985)
	Musk melon	Freeman	Karchi (1975)
	Pumpkin & squash	*C ecuadorensis* and *C. foetidissima* against	Provvidenti et al. (1978)
Cucumber green mottle mosaic virus	Cucumber	*Cucumis anguria*	Den-Nij (1982)
Watermelon mosaic virus	Cucumber	Table Green and Sarinam	Takeda and Gilbert (1975) & Provvidenti (1985)

(continued)

Table 9.3 (continued)

Crop	Disease/Insect pests	Resistance source	References
	Musk melon	PI 414,723, B 66-5 and *C. metuliferus*	Webb and Bdhn (1962); Webb (1969); Provvidenti and Robinson (1977)
	Pumpkin & squash	*C ecuadorensis* and *C. foetidissima* against	Provvidenti et al. (1978)
Zuchini yellow mosaic virus	Musk melon	PI 161,375	Lecoq and Pitrat (1985)
Squash mosaic virus	Pumpkin & squash	*C. pepo*, *C. maxima* and *C. moschata*	Salama and Sill (1968)
Bacterial wilt	Pumpkin & squash	*C. pepo*, *C. maxima*, *C. andreana* and *C. lundellina*	Watterson et al. (1971)

(Modified from Naik et al. 2013)

and grow extra energetically producing increased quantity of mature fruits compared to the non-transgenic and wild types hybrid segregants.

Due to lesser potential of transformation in watermelon, very few transgenic watermelons were reported which showed viral resistance (Yu et al. 2008). Virus coat protein manipulation employing through RNA silencing method, mediated by siRNA, is the main efforts of this technique. Synthetic microRNAs, those developed implementing miRNA, make possible to silence gene efficiently. Antiviral plants generated through transgenic techniques were become possible due to such effective technique (Zhang et al. 2015; Duan et al. 2009; Parmar et al. 2017). Earlier finding demonstrated that amiRNA expressing transgenic tomato which aims CMV 2a/2b genes or the highly conserved 3-untranslated region also exhibit effective resistance to CMV infection (Zhang et al. 2011). Additionally transgenic watermelon expressing amiRNA targeting CMV 2a/2b gene also displayed CMV resistance.

9.4.2 Diseases Resistance

The significant imperative restricting the productivity of cucurbits crops is various infections brought about by fungi, bacteria and viruses (Chandrasekaran et al. 2016). Traditional breeding appears to have restricted application because of non-accessibility of tolerant gene(s) in genetic system of a cucurbits crop. One of the primary focuses of genetic change is to enhance resilience or to develop resistance in plants against various infectious organisms. Alteration at gene level for tolerance against various diseases in cucurbits has gotten mainstream and significant regarding cost and viability (Kumar et al. 2012a, b). For conferring tolerance against bacterial and fungal infections, different genes including chitinase, defensin, glucanase,

osmotin, and so on are being introduced in different crops including cucurbits globally. Among various techniques utilized for transgenic development for tolerance against diseases, the implementation of systemic acquired resistance (SAR)-linked gene is of principal significance. SAR is durable and frequently linked with confined and general accumulation of salicylic acid (SA) and induced expression of many genes (Ryals et al. 1996).

Disease management is a vital segment of maintained and enhanced productivity of melons, cucumbers, squashes, pumpkins, and many different cucurbit crops. The existing wide list of 200 diseases of cucurbit has extended in recent times which includes Cucurbit leaf curl virus Cucurbit leaf crumple virus, Acremonium collapse, cucumber root mat, bacterial blight, Rhizopycnis root rot, Cucurbit yellow stunting disorder virus, and cucurbit yellow vine disease (Vasudevan et al. 2007). Powdery mildew on watermelon, vine declines, Phytophthora blight, bacterial wilt, illnesses caused by Fusarium species, and various diseases caused by viruses, including Melon necrotic spot carmovirus and several members of the crinivirus genus (Clough and Hamm, 1995). Rotation, fumigation, minimising injury during harvest, chlorine spray or hot water treatment after harvest, sanitation, drip irrigation, culling symptomatic fruit before storage, pathogen-free seed, plastic mulch or other soil barrier, deep ploughing, adjusting soil pH, host controlling weeds and insects are some of the techniques used to manage various diseases, Plant resistance, fungicides, solarization, greenhouse climate manipulation, improved soil drainage, treated seed, planting when soil is not too cold, roguing sick plants, and correct storage conditions, including refrigeration A variety of transgenic cucurbit lines have been created and field tested by a number of commercial enterprises (Chandrasekaran et al. 2016; Azadi et al. 2011).

9.4.3 Virus Resistance

Zuchine yellow mosaic virus (ZYMV), watermelon mosaic virus II (WMVII), papaya ringspot virus (PRSV), and poty viruses are among the most common viral disease that affect cucumbers (Ling et al. 1991). Virus resistance has been generated utilising genetic engineering techniques for coat protein, and the resistance granted is typically confined to viruses belonging to the same group or are closely related. Resistance ranges from a reduction in the severity of symptoms to a delay in the emergence of symptoms to susceptibility, in which there are no side effects and viral infections can be detected in the host for longer periods of time. Fang and Gromet (1993) used an Agrobacterium-mediated transformation approach in muskmelon to insert a conserved core region and three different sized ZYMV coat protein genes; antisense version and full-length gene. Western and northern blotting techniques were used to confirm the gene's expression. The T1 descendants of transgenic plants communicating the full length CP gene were immune to ZMY infection, with no side effects and infection titres visible for at least three months, whereas the indications were delayed by a few days and infection titres were reduced in transgenic

plants expressing more limited and antisense variants of the CP gene. This study shows that plants can be given irrefutable levels of protection against viral infections. Kottearachchi et al. (2000) used Agrobacterium-mediated transformation to confer resistance to the pathogen PRSV in muskmelon cotyledon explants, with NPTII and GUS serving as reporter and selection marker genes, respectively. The integration of the PRSV replicase gene (NIb) into muskmelon was confirmed by PCR, but the resistance of transgenic muskmelon plants bearing the replicase gene to PRSV was not demonstrated. Ribozymes are employed to offer protection from potyviruses, which are a major disease of crops all over the world, using a novel approach. In contrast to the Pathogen Derived Resistance approaches used to generate viral infection-free plants, the use of genes linked to ribozyme to protect plants against viral infection provides an alternative. There may also be some legitimate worries expressed by some plant virologists concerning the use of viral genes in transgenic plants, but it's possible that the ribozyme gene will find multiple applications in agri biotechnology. Melon plants are protected against two potyviruses: WMV2 and ZYMV, using ribozyme genes. Unique polyribozyme genes were designed, manufactured, and introduced into melons plants. The offspring of transgenic melon plants expressing tolerance genes were examined by confronting them with suitable viruses. In the glasshouse investigation, the enormous number of genes tested resulted in some measure of virus resistance. Melon plants containing a gene that inhibits WMV2 were also tested in the field on smaller plots under natural virus exposure and shown to be WMV2-resistant. In melon, researchers hope to add multiple copies of resistance genes, in accordance with European laws on transgenic plant construct design (Huttner et al. 2001). Viruses are the most major danger to watermelon production and productivity. ZYMV (zucchini yellow mosaic virus), WMV-1 (watermelon mosaic virus), SqMV (squash mosaic virus), CMV (cucumber mosaic virus), and WMV-2 (watermelon mosaic virus 2) are the major viruses (WMV-2). Except for SqMV, which is transmitted by beetles and seedborne in nature and is predominantly found in melon, few other major viruses are disseminated in a nonpersistent manner by aphid species. Cucumber mosaic virus (CMV), Watermelon mosaic virus (WMV), and Zucchini yellow mosaic virus disease resistance transgenic watermelons were created by Sheng-Niao et al. (2005). (ZYMV). Agrobacterium-mediated transformation was carried out, with a transformation efficiency of 1.7 percent. Protection against viral infection was tested in the field and in a controlled glasshouse setting. Transgenic watermelon showed diverse phenotypes against virus infection throughout the late growth phase, including resistant, sensitive, and immune. T3 plants from the BH1-7 line showed a high level of tolerance. This finding suggests that transgenic technology could be used to create new varieties of watermelon that are resistant to viral illness. Cucurbitacea is primarily affected by viruses belonging to the families cucumoviredae, potyviridae, tombusviridae, geminiviridae, tobomoviridae, and comoviridae. These viruses are spread by insects, primarily whitefly and aphids. Whitefly transmits Zuccini yellow mosaic virus (ZYMV), watermelon virus (WMV), watermelon chlorotic stunt begomo Cucumber mosaic virus (CMV), papaya ring spot virus (PRSV), cucumber green motile mosaic virus (CGMMV), yellow stunting

condition crinivirus, and cucumber fruit motile mosaic virus (CFMMV) are all transmitted by aphids. By means of biotechnological tools implementation, virus coat protein gene was transferred to confer PRSV resistance. Three distinct transgenic lines, earlier found tolerant against CMVP1 virus, additionally found tolerant to CMVP0 virus. The watermelon (*Citrullus lanatus*) productivity was impacted significantly by papaya ring spot virus type W (PRSV) and Zucchini yellow mosaic virus (ZYMV) worldwide. Yu et al. (2011) transformed three cultivars of watermelon to get rid of PRSV and ZYMV altogether. RNAi technique was established to be effective in imparting protection from different diseases coused by viruses in cucurbits. Transgenic study performed in different cucurbits crop showing resistance against various biotic stress has been summarized in Tables 9.1 and 9.2.

9.5 Genome Editing Technology in Cucurbits Crop Improvement

Due to the presence of a sequence-specific DNA-binding domain, most modern technologies rely on specially developed endonucleases (EEN), which assist in slicing the DNA at specified places. These endonucleases recognise specific DNA sequences and cleave the target genes from the DNA template with pinpoint accuracy. Furthermore, DNA double-strand breaks (DSBs) trigger cellular DNA repair systems such homology-directed repair (HDR) and non-homologous end joining breaks (NHEJ), which result in gene changes at target loci in the plant genome. Several genome-editing technologies have recently emerged, including Zinc finger nucleases (ZFNs), Transcription activator–like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR/Cas), and CRISPR-associated protein 9 (CRISPR/Cas). Through the attachment of endonuclease catalytic domains to modular DNA-binding proteins, both ZFN and TALEN generate targeted DNA DSBs at specific genomic loci. Cas9, on the other hand, is led by a short RNA that pairs with target DNA via Watson–Crick base pairing (Garneau et al. 2010; Jinek et al. 2012 and Gasiunas et al. 2012).

Among these targeted nucleases, CRISPR/Cas9 system is adapted from a naturally occurring genome editing system in bacteria and *Archaea* (Wiedenheft et al. 2012). CRISPR/Cas9 system is a low cost, simple, versatile and highly efficient genome editing system (Cardi and Stewart 2016). It is also widely used as it is easy to set up, comparatively affordable and better upscaled than ZFNs and TALEN. All these features make it a powerful tool for mediating genome alteration with high precision. On the other hand, high technical complexity and low efficiency are some major drawback for ZFNs and TALENs mediated genome editing system. DSBs induced through CRISPR/Cas9 system in the plant genome are repaired by NHEJ method (Li et al. 2013). In the process of DNA repairing, small insertions/deletions or inclusion of stop codon might cause disturbance to the open reading frame of a protein (Belhaj et al. 2013). The utility of the CRIPSR/Cas9 technology has tremendously increased

due to the rapid breakdown of the Cas9 protein-guide RNA complex within the regenerating cell cultures. Thereby provides greater global acceptance to CRIPSR/Cas9 technology than transgenics. Usually a gene-edited crop does not necessarily contain any transgene; therefore avoids the current stringent GM regulations mechanisms and these crops have wider acceptance among consumers (Jones 2015).

Precise genome editing is a fantastic method for deciphering plant gene functions and crop plant enhancement. Genome editing in plants has been revolutionised thanks to the CRISPR/Cas9 technology. It has been utilised in horticultural crops for targeted gene mutation, suppression, activation, and epigenome editing (Song et al. 2016). This method has recently been used to build resistance to a variety of viruses (Baltes et al. 2015). By interrupting the activity of the recessive eIF4E (eukaryotic translation initiation factor 4E) gene, a virus-resistant cucumber was created using CRISPR-Cas9 (Chandrasekaran et al. 2016).

Ipomovirus, cucumber vein yellowing virus (CuVYV), potyviruses, Papaya ring spot mosaic virus-w (PRSMV-W), and Zucchini yellow mosaic virus (ZYMV) infection were all resistant in transgenic cucumber T3 plants designed for both eIF4E sites. Malnoy et al. (2016) used directly delivered pure ribonucleoproteins (RNPs) targeting CRISPR/Cas9 to induce mutations in the MLO-7 gene in grapes to improve resistance to powdery mildew, and in the DIPM-1, DIPM-2, and DIPM-4 genes in apple delicious to improve resistance to fire blight disease. Tian et al. employed CRIPSR/Cas9 to create targeted CIPDS (phytoene desaturase) gene mutations in watermelon in order to develop albino phenotype (2017). These transgenic watermelon plants with CIPDS mutations create a clear or mosaic albino phenotype, showing that the CRIPSR/Cas9 system is capable of 100 percent genome editing in transgenic watermelon. For industrial objectives and eating quality, parthenocarpy is usually desired in horticultural crop plants. Ueta et al. generated parthenocarpic tomato plants by introducing 100 percent somatic mutations into the SlIAA9 gene using a CRISPR/Cas9 system-based breeding technique (2017). The leaf morphology of these regenerated Tomato mutants was changed, and the fruits were seedless. Using the CRISPR/Cas9 technology, Kishi-Kaboshi et al. (2017) created transgenic chrysanthemum plants that expressed the yellowish-green fluorescent protein (CpYGFP) gene from *Chiridius poppei*.

9.6 Gene Stacking

By modifying plant genomes to produce greater nutritional value and tolerance to abiotic and biotic challenges, better raw materials for industrial purposes, and novel compounds with pharmacological promise, transgenic crops have changed agriculture, industry, nutrition, and even medicine. Several publications have been published demonstrating the enormous potential of genetically modified crops, but the technology is still in its infancy, therefore the results have been modest. Several critics and pressure groups have labelled the technology a failure since the crude first-generation 'input trait' GM crops have been misconstrued to benefit primarily seed firms and

farmers. Progress toward second-generation 'output trait' goods with nutritional, environmental, or other direct benefits for consumers has been modest, and will remain so until the bottleneck of creating technologies for the coordinated manipulation of several genes or traits is overcome (Halpin et al. 2005). Perhaps, the above mentioned constraints is not accepted as large amount of literature describes gene silencing through manipulation of single gene; meanwhile publications depicting manipulation of multiple gene is scares. However, 'stacking' or 'pyramiding' of more than one gene in the exsiting GM crop offers durable multitoxin resistance to particular pest or multiple resistance to different types of pathogen in herbicide tolerant GM crop. The potential for developing metabolically engineered plants with enhanced nutritional value or improved quality of raw materials for industrial purpose is enormous. As most metabolic processes targets for manipulation of numerous genes and flux through competing biochemical pathways; therefore, development of metabolically engineered plants is only possible through controlling multiple genes or interconnected, pathways. For example, 'Golden rice' is one such metabolically engineered plant developed through manipulation of three carotenoid biosynthesis genes to produce provitamin A (Ye et al. 2000). However, efficient absorption of provitamin A may require the resorbable iron content, which might require introduction of another three genes in to the same. Similarly, biodegradable plastic [a copolymer of polyhydroxybutyrate (PHB) and polyhydroxyvalerate] in plants necessitates the introduction of four to six genes that control multiple metabolic pathways (Slater et al. 1999).

9.7 Iterative Strategies

Conventional iterative procedures provide subsequent introgression of two or more transgenes into a single plant. For example: A plant that has one transgenic is crossed with individuals that have other transgenes, or it is re-transformed with new genes. At least at the research level, these techniques are used for either combining or reinforcing the existing transgenic traits. Transgenic broccoli pyramided with cry1Ac and cry1C *Bt* genes exhibits delayed the evolution of *Bt*-resistance diamondback moths (Zhao et al. 2003). Plants expressing the Xa21 gene (responsible for bacterial blight resistance) are crossed with plants expressing both a Bt fusion gene and a chitinase gene (responsible for yellow stem borer resistance and tolerance to sheath blight, respectively) to create transgenic rice resistant to disease and pests (Datta et al. 2002). This method has also been employed in plants to introduce new metabolic pathways. In Arabidopsis, the genes for the bacterial organic mercury detoxification system (mercuric reductase, merA, and organomercurial lyase, merB) were restored, and offspring carrying both genes have 50-fold greater methylmercury contents than wild-type counterparts. Similarly, biodegradable polymer PHB producing plants were developed by crossing series of *Arabidopsis* expressing single Alcaligenes genes from the bacterium *Alcaligenes eutrophus*. Further addition of the pea chloroplast transit peptides sequences to the bacterial genes had resulted in its

over-expression, which is manifested as PHB granules accumulated in the plastids up to 14% of plant dry weight. Furthermore, sequential cross-fertilizations in tobacco resulted in a functional secretary IgA (SigA) antibody in plants by combining four genes producing distinct immunoglobulin polypeptides (Halpin et al. 2005). Loss-of-function transgenes (e.g., co-suppressing or antisense genes) are sometimes used in conjunction with other transgenes to regulate processes like lignin production and fruit ripening (Powell et al. 2003). The feasibility of re-transformation as a research strategy has also been demonstrated. The genes for dihydroflavonol 4-reductase from Antirrhinum majus (AmDFR) and anthocyanidin synthase from Matthiola incana (MiANS) were sequentially transformed into the forsythia plant, resulting in anthocyanin synthesis and changed blossom colour. The double transgenics have showed an unique bronze-orange petal colour due to de novo deposition of cyanidin-derived anthocyanins over the carotenoid yellow background of the wild-type (Rosati et al. 2003). Similarly, double transgenic tobacco plants with two glyoxalase pathway genes outperform single transgenic tobacco plants under salinity stress. Retransforming previously transformed potato plants containing SSII and SSII genes with an antisense gene for granule-bound starch synthase produces a very freeze–thaw-stable starch. The generation of amylose free with short-chain amylopectin (freeze–thawstable) was achieved by antisense silencing of three starch synthase genes in potato. Unlike chemical alterations, contemporary technology has a wider acceptability in the food business for the production of freeze–thaw-stable starch due to environmental and consumer benefits. Recently, the successive introduction of three genes into Arabidopsis resulted in transgenic plants that synthesise health-promoting omega-3 and omega-6 fatty acids commonly acquired from fish oils, resulting in the synthesis of long-chain polyunsaturated fatty acids. Retransformation has been used to alter two genes involved in lignin biosynthesis in tree species. This is accomplished by inserting a caffeate/5-hydroxyferulate O-methyltransferase antisense gene into transgenic plants that already had an antisense cinnamyl alcohol dehydrogenase gene. Such lignin manipulation has significant impact on reducing environmental hazards otherwise caused by paper industry (Halpin et al. 2005).

9.8 Gene Silencing

Gene silencing is the technique that regulates expression/activity of the genes in a cell either during the **transcription** or **translation** process. As the term indicates, gene silencing is used to turn down or switch off the activity of genes. Usually it prevents the gene from producing the targeted protein by neutralizing targeted **mRNA** molecules. Furthermore, certain DNA elements (transposons) can also disrupts the functioning and disables the genes. Gene silencing is also known as RNA interference (RNAi) as it inhibits the gene from translation and cause degradation of the homologous RNA transcript. Consequently, post transcriptional homology dependent Mrna degradation of the corresponding gene occurs which is manifested with substantial reduction in the gene expression. Nevertheless, gene silencing has

greater potential in functional genomics (Watson et al. 2005). This technique is based on transgenically expressed proteins or RNA. Usually during the production of transgenics, *Agrobacterium*-mediated transformation is done to transfer the desirable transgene to the targeted plants through T-DNA (transfer DNA). Single T-DNA transfer from *Agrobacterium* Head to head, tail to tail, or head to tail arrays are integrated into the host genome. By offering protection against viruses, this process operates as a hereditary immune system. It could possibly play a role in genomics, as evidenced by transgenic RNA guided methylation.Gene silencing is achieved through different mechanisms:

(1) Post translational gene silencing (PTGS) or RNA interference (RNAi)
(2) Transcriptional gene silencing (TGS)
(3) Virus induce gene silencing (VIGS)
(4) MicroRNA gene silencing (miRNA).

9.9 Applications of Gene Silencing Technologies

Crop yield is hampered by biotic stressors produced by diseases, insects, and nematodes. By manipulating the expression of metabolic pathway genes in a variety of model and crop plant species, RNA silencing technologies, particularly the hpRNA transgene technology and amiRNA technology, are widely used to decipher biochemical pathways and gene function, develop pathogen and pest resistance, and improve other agronomical traits. Gene silencing has changed the industrial and medical sectors, in addition to agriculture.

9.10 Cisgenics

Foreign genes are literally introduced into the host genomic backdrop in genetically modified plants. Cisgenesis is a genetic alteration technique that uses recombinant DNA technology or a native gene from the same host and/or sexually compatible plant species. As a result, it is not a novel technique. This word clearly distinguishes GM plants or other DNA-containing organisms from unrelated ones. Schouten et al. (2006) coined the term cisgenesis to describe the genetic modification of the recipient host with a naturally generated gene from a cross compatible species, complete with introns, native promoter, and terminator flanked in the normal sense orientation. Because cisgenes share a gene pool, the final cisgenic plant should be free of extraneous DNA, such as selection markers and vector-backbone sequences. After transformation, the T-DNA from the vector (Agrobacterium tumefaciens) plasmid is injected into the recipient organism, which is referred to as Agrobacterium-mediated gene transfer (EFSA 2012). Transgenesis, on the other hand, is defined as the introduction of one or more genes from any non-plant organism, or from a donor plant that is sexually incompatible with the recipient plant, into a recipient plant.

At recent times, genetic modification is the need of the hour to develop GM crops capable to produce surplus yield from limited land area to feed the burgeoning population and economic renaissance. However, commercial cultivation of GM crops as well as their derived foods and products has always sparked fierce debate about its safety among the public. Most particularly, the controversy related to possible unpredictable hazards due to accumulation of undesired substances within crop confers toxicity, allergy and genetic threats in the human nutrition. Hence, Cisgenic approach is generally considered safer than transgenic technology and is also highly advanced than conventional breeding as it avoids linkage drag. In cisgenesis, only the desired genes are introduced into the genomic background of recipient plant. Moreover, the cisgenic approach doesn't pose hazardous reaction from unidentified hitch-hiking genes as compared to the induced translocation or mutation breeding (Schouten et al. 2008) (Table 9.4).

9.11 Grafting Techniques to Impart Biotic Stress Tolerance in Cucurbits

Grafting is a technology which involves joining together two living plant parts to produce a single, living plant. The upper plant parts which produce fruits is called scion and the lower root parts is known as root stock. The rootstock contributes vigour and disease resistance while the scion is chosen for fruit and/or its quality. It is a century old propagation technique for fruits crops/woody plants. In vegetable crops, it has become popular in past few decades for commercial vegetable industry. Watermelon seedlings were grafted onto squash rootstock in Korea and Japan around the end of the 1920s, and the production of grafted vegetable plants began (Kubota et al 2008). It has now spread across Asia and Europe. Currently, grafting technologies are used in 81 percent of Korean vegetable cultivation and 54 percent of Japanese vegetable cultivation (Rivero and Ruiz, 2003). Lower production and poor quality of vegetables are caused by a variety of biotic and abiotic stressors. Further to control the biotic stresses, farmers use to spray pesticides indiscriminately, which is major concern amongst the health-conscious people. Among the biotic stresses vegetable industry suffer a lot from soil borne diseases (Lee et al. 2010) such as increased yield, improved shoot growth, disease tolerance, nematode tolerance/resistance, low temperature tolerance, high temperature tolerance, enhanced nutrient uptake, enhanced water uptake, high salt tolerance, wet soil tolerance, heavy metal and organic pollutant tolerance, quality changes, extended harvest period, multiple and/or successive cropping allowed, convenient production of organic wastes, and ornamental values for exhibition and education For various reasons, a substantial number of root stacks have been reported. As a result, rootstock selection is critical to the viability of the vegetable graft industry. Table 9.5 lists the characteristics of various root stocks for cucurbitaceous crops.

Table 9.4 Transgenic study performed in different cucurbits crop showing resistance against various biotic stress

Crop	Transformation method	Gene	Gene source	Gene function	References
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	PR-2d promoter	Tobacco	Resistance to fungal pathogen Erysiphe polyphage and cold stress tolerance	Yin et al. (2004)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	SK_3-type DHN24 dehydrin	Solanum sogarandinum	low temperature tolerance in cold-sensitive species such as cucumber	Yin et al. (2006)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium Tumefaciens*	*ICE1*	*Arabidops*	Enhances chilling stress	Liu et al. (2010)
Cucumber (*Cucumis sativus* L.)	Pollen-tube pathway method	MAPK antisense gene	Cucumis sativus	Suppression of MAPK gene in cucumber leads salt tress susceptibility	Xu et al. (2011)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Cbf1 gene	Arabidopsis	Conferred protection against chilling stress	Gupta et al. (2012)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Transketolase	*Cucumber*	Tolerance to low temperature and low light	Bi et al. (2015)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium Tumefaciens*	*CsCER1*	Cucumis sativus	Biosynthesis of very long chain alkanes and drought stress tolerance	Wang et al. (2015)

(continued)

Table 9.4 (continued)

Crop	Transformation method	Gene	Gene source	Gene function	References
Cucumber (*Cucumis sativus* L.)	*Agrobacterium Tumefaciens*	*CsCaM3*	Cucumis sativus	Overexpression of CsCaM3 has the potential to improve their heat tolerance and protect against oxidative damage and photosynthesis system damage	Yu et al. (2018)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Rice chitinase cDNA (RCC2)	Rice	Resistant aginst cucumber gray mold (*Botrytis cinerea)*	Tabei et al. (1998)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	CMV-O coat-protein gene	Cucumber mosaic virus	Cucumber mosaic virus resistance	Nishibayashi et al. (1996)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Chitinase	*Petunia*	Resistance against *lternaria radicina*, *B. cinerea*, *R. solani*, *Sclerotium rolfsii* and *Thielaviopsis basicola*	Punja et al. (1996)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Chitinase	Tobacco	Resistance against *lternaria radicina*, *B. cinerea*, *R. solani*, *Sclerotium rolfsii* and *Thielaviopsis basicola*	Punja et al. (1996)

(continued)

Table 9.4 (continued)

Crop	Transformation method	Gene	Gene source	Gene function	References
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Chitinase	*Phaseolus vulgaris*	Resistance against *lternaria radicina*, *B. cinerea*, *R. solani*, *Sclerotium rolfsii* and *Thielaviopsis basicola*	Punja et al. (1996)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Class I chitinase cDNA (RCC2)	*Rice*	Resistant aginst cucumber gray mold caused by *Botrytis cinerea*	Kishimoto et al. (2002)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	PR-2d promoter	Tobacco	Resistance to fungal pathogen Erysiphe polyphage and cold stress tolerance	Yin et al. (2004)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Class III chitinase gene (*CHI2*)	*Cucumber*	Enhanced gray mold disease resistance	Kishimoto et al. (2004)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium tumefaciens*	Pokeweed antiviral protein gene (*PAP*)	*Phytolacca acinosa*	Resistance in different degree to CMV	Cao et al. (2011)
Cucumis melo L	*Agrobacterium tumefaciens*	CMV–WL coat protein (CP)	Mosaic Virus–White Leaf Strain	Resistance against Cucumber mosaic virus New York Strain	Gonsalves et al. (1994)

(continued)

Table 9.4 (continued)

Crop	Transformation method	Gene	Gene source	Gene function	References
Watermelon	*Agrobacterium Tumefaciens* and Injection	CGMMV coat protein gene (*CGMMV-CP*)	Cucumber green mottle mosaic virus (CGMMV)	Resistance to cucumber green mottle mosaic virus (CGMMV)	Park et al. (2005)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium Tumefaciens*	Class I chitinase gene	Rice	Resistance to Phytophthora rot (*Phytophthora nicotianae* var *parasitica*)	Kishimoto et al. (2003)
Squash	*Agrobacterium Tumefaciens*	Cucumber mosaic virus (CMV), watermelon mosaic virus 2 (WMV 2) or zucchini yellow mosaic virus (ZYMV) coat protein (CP) genes	Cucumber mosaic virus (CMV), watermelon mosaic virus 2 (WMV 2) or zucchini yellow mosaic virus (ZYMV)	Resistance to infection by CMV, WMV 2 or ZYMV	Tricoll et al. (1995)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium Tumefaciens*	Putative 54-kDa replicase gene	Cucumber fruit mottle mosaic tobamovirus (CFMMV)	A substantial delay of symptom appearance was observed following infection by three additional cucurbit-infecting tobamoviruses	Gal-On et al. (2005)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium Tumefaciens*	ZYMV-CP gene	Zucchini yellow mosaic virus	Resistance against Zucchini yellow mosaic virus	Wako et al. (2001)

(continued)

Table 9.4 (continued)

Crop	Transformation method	Gene	Gene source	Gene function	References
Cucumber (*Cucumis sativus* L.)	*Agrobacterium Tumefaciens*	*ICE1*	*Arabidops*	Enhances chilling stress	Liu et al. (2010)
Watermelon	*Agrobacterium Tumefaciens*	Artificial microRNAs	Artificial microRNAs that target *Cucumber mosaic virus* (CMV) 2a/2b	Resistance in different degree to CMV	Liu et al. (2016)
Cucumber (*Cucumis sativus* L.)	*Agrobacterium Tumefaciens*	RNA-dependent RNA polymerase 1 (RDR1)	Cucumis sativus	Broad spectrum virus resistance in cucumber	Leibman et al. (2018)

Table 9.5 Rootstocks for cucurbitaceous crops and their tolerance to soil borne pathogens

Rootstock	Cultivar	Major characteristics	Possible disadvantage
Watermelon			
Bottle gourd (*Lagenaria siceraria* L.)	Dongjanggoon, Bulrojangsaeng, Sinhwachangjo (Korea), FR Dantos, Renshi, Friend, Super FR Power (Japan),	Vigorous root stock, *Fusarium* tolerance	New *Fusarium* race, susceptible to anthracnose
Squash (*Cucurbita moschata* Duch.)	Chinkyo, No. 8, Keumkang (Korea)	Vigorous root stock, *Fusarium* tolerance	Inferior fruit shape and quality
Interspecific hybrid squash (*Cucurbita maxima* Duch. × C. moschata Duch.)	Shintozwa, Shintozwa #1, Shintozwa #2, Chulgap, (Japan, China, Taiwan, Korea)	Vigorous root stock, *Fusarium* tolerance	Reduced fertilizers required. Some quality reduction may result
Pumpkins (*Cucurbita pepo* L.)	Keumsakwa, Unyong, Super Unyong	Vigorous root stock,	Mostly for cucumbers
Wintermelon (*Benincasa hispida* Thunb.)	Lion, Best, Donga	Good disease resistance	Incompatibility
Watermelon [*Citrullus lanatus* (Thunb.) Matsum. et Nakai	Kanggang, Res. #1, Tuffnes (Japan), Ojakkyo(Syngenta)	*Fusarium* tolerance	Not enough vigor and disease resistance
African horned (AH) cucumber (*Cucumis metuliferus* E. Mey. ex	NHRI-1	*Fusarium* tolerance, Nematode tolerance	Medium to poor graft Compatibility
Cucumber			
Figleaf gourd (*Cucurbita ficifolia* Bouché)	Heukjong (black seeded figleaf gourd)	Good disease resistance	Narrow graft compatibility
Squash (*Cucurbita moschata* Duch.)	Butternut, Unyong #1, Super, Unyong	*Fusarium* tolerance,	Affected by Phytophthora
nterspecific hybrid squash (*Cucurbita maxima* Duch. × *C moschata* Duch.)	Shintozwa, Keumtozwa, Ferro RZ, 64–05 RZ, Gangryuk Shinwha	*Fusarium* tolerance,	Slight quality reduction expected
Bur cucumber (*Sicyos angulatus* L.)	Andong	*Fusarium* tolerance, Nematode tolerance	Reduced yield
AH cucumber (*Cucumis metuliferus* E. Mey. ex Naud)	NHRI-1	*Fusarium* tolerance, Nematode tolerance	Weak temperature tolerance

(continued)

Table 9.5 (continued)

Rootstock	Cultivar	Major characteristics	Possible disadvantage
Melon			
Squash (*Cucurbita moschata* Duch.)	Baekkukzwa, No. 8, Keumkang, Hongtozwa	*Fusarium* tolerance	Phytophthora infection
Interspecific hybrid squash (*Cucurbita maxima* Duch. × *C. moschata* Duch	Shintozwa, Shintozwa #1, Shintozwa #2	*Fusarium* tolerance	Phytophthora infection, poor fruit quality
Pumpkin (*Cucurbita pepo* L.)	Keumsakwa, Unyong, Super Unyong	*Fusarium* tolerance	Phytophthora infection
Melon (*Cucumis melo* L.)	Rootstock #1, Kangyoung, Keonkak, Keumgang	*Fusarium* tolerance	FQ Phytophthora problem
(Lee et al. 2010)			

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