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).

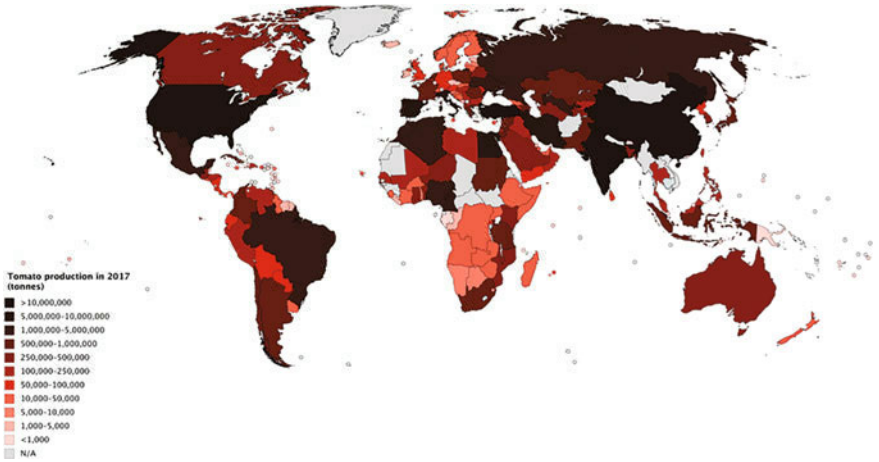

Fig. 1.1 Tomato production in tons, based on data from the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT 2017)

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

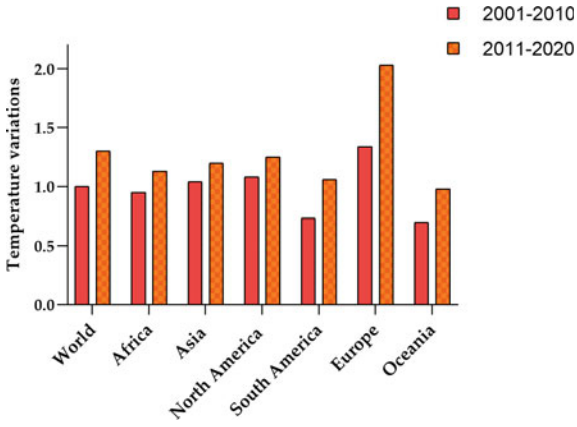

Fig. 1.2 Mean annual temperature measured globally and, in each continent in last two decades (FAOSTAT 2021)

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

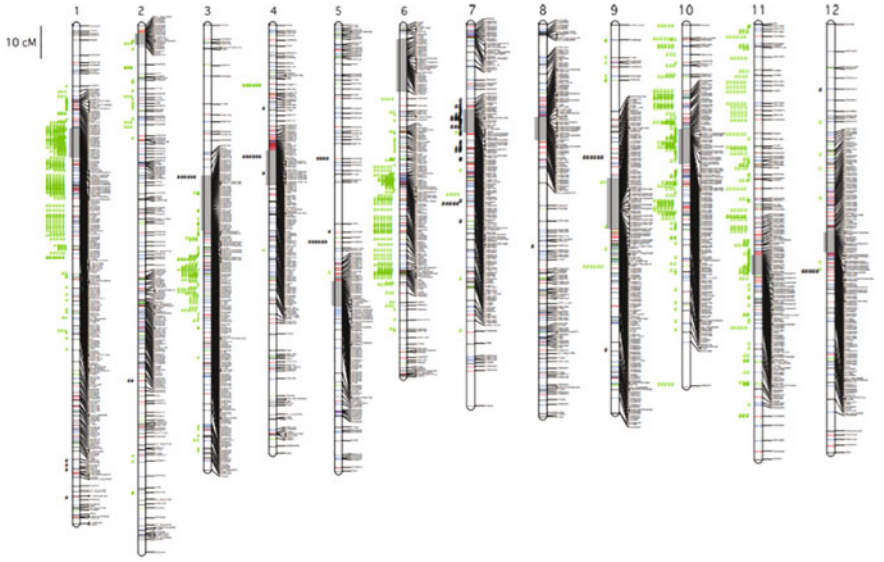

Fig. 1.3 Genetic linkage map of tomato genome derived from *S. lycopersicum* × *S. pennellii* using 2,116 molecular markers spanning 1,503.1 cM genetic distance (Shirasawa et al. 2010)

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

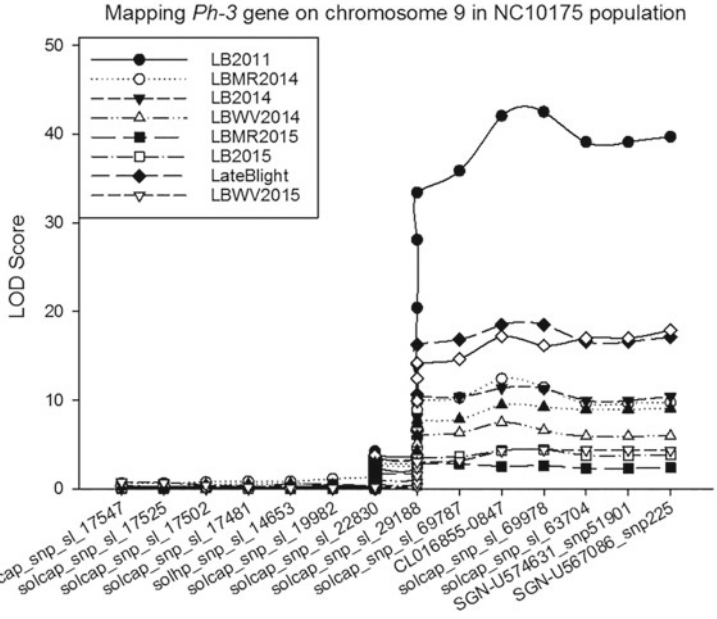

Fig. 1.4 Mapping *Ph-3* on chromosome *9* in segregating tomato population derived from an intraspecific cross (Panthee et al. 2017)

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.