Chittaranjan Kole Editor

Genomic Designing of Climate-Smart Pulse Crops



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ISBN 978-3-319-96931-2 ISBN 978-3-319-96932-9 (eBook) https://doi.org/10.1007/978-3-319-96932-9

Library of Congress Control Number: 2019934793

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Dedicated to (Late) Prof. Sukumar Dana Formerly Professor in the Department of Genetics and Plant Breeding of the Bidhan Chandra Krishi Viswavidyalaya (Agricultural University) who supervised my Post-Graduate Thesis work on a leading pulse crop, mungbean (green gram), and during that period inculcated in my mind the urge and habit of exploration, collection, characterization and utilization of indigenous varieties, local landraces and allied wild species, and taught me the art and science of 'talking' to the plants.



Preface

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

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

Meantime, the manual DNA sequencing methodology of 1977 was being improved with regard to speed, cost-effectiveness and automation. The first-generation sequencing technology led to the whole genome sequencing of Arabidopsis in 2000 and followed by rice in 2002. The next-generation sequencing technologies were available over time and used for sequencing of genomes of many other models and crop plants. Genomes, both nuclear and organellar, of more than 100 plants have already been sequenced by now and the information thus generated are available in public database for most of them. It must be mentioned here that bioinformatics played a remarkable role in handling the enormous data being produced in each and every minute. It can be safely told that the 'genomics' era started at the beginning of the twenty-first century itself accompanying also proteomics, metabolomics, transcriptomics, and several other 'omics' technologies.

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

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

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

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

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

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

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

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

I must confess that it has been quite a difficult task for me to study critically the different concepts, strategies, techniques and tools of plant breeding practiced over the past 12 decades that also on diverse crop plants to gain confidence to edit the

chapters authored by the scientists with expertise on the particular crops or crop groups and present them in a lucid manner with more or less uniform outline of contents and formats. However, my experience gained over the past 7 years in the capacity of the Founding Principal Coordinator of the International Climate-Resilient Crop Genomics Consortium (ICRCGC) was highly useful while editing these books. I have the opportunity to interact with a number of leading scientists from all over the world almost on regular basis. Organizing and chairing the annual workshops of ICRCGC since 2012 and representing ICRCGC in many other scientific meetings on climate change agriculture offered me a scope to learn from a large number of people from different backgrounds including academia, industries, policymaking, and funding agencies and social workers. I must acknowledge here the assistance I received from all of them to keep me as a sincere student of agriculture specifically plant breeding.

This volume entitled Genomic Designing of Climate-Smart Pulse Crops includes 9 major crops including Common Bean, Pigeonpea, Chickpea, Lentil, Mungbean, Pea, Fava Bean, Bambara Groundnut and Grass Pea. These chapters have been authored by 80 scientists from 12 countries including Australia, Argentina, Brazil, China, Egypt, India, Malaysia, Pakistan, Puerto Rico, Spain, UK and USA. I place on record my thanks for these scientists for their contributions and cooperation.

My own working experience on pulse crops dates back to late 70s in the laboratory of (Late) Prof. Sukumar Dana in the Department of Genetics and Plant Breeding in the Bidhan Chandra Krishi Viswavidyalaya (Agricultural University), West Bengal, India. While working as a postgraduate student with him on genetics of mungbean also known as green gram, I learnt for the first time the importance of collection, characterization and utilization of indigenous varieties, local landraces and wild allied species in crop improvement. It is him who inculcated in me the 'love' for the plants and the art to 'care' them and 'talk' to them and guided me to become a plant breeder one day. Hence, I have dedicated this book to (Late) Prof. Dana as a token of my respect, thanks and gratitude.

New Delhi, India

Chittaranjan Kole

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Abbreviations

β-ODAP	β-N-oxalyl-L-α,β-diaminopropionic acid
6PP	6-Pentyl-α-pyrone
AAC	1-Aminocyclopropane-1-caboylic acid
ADH	Alcohol dehydrogenase
AFLP	Amplified fragment length polymorphism
AOCC	African Orphan Crops Consortium
APX	Ascorbate peroxidase
ASAP	Allele specific amplified primer
ATP	Adenosin triphosphate
AUSPC	Area under symptom progress curve
AVRDC	Asian Vegetable Research and Development Centre
BA	6-Benzylaminopurine
BA	Benzyladenine
BAC	Bacterial artificial chromosome
BAP	Benzyl amino purine
BARI	Bangladesh Agricultural Research Institute
BC	Backcross
BCL	Blocked cotyledon lethal
BCMV	Bean common mosaic virus
BES	BAC-end sequence
BIBAC	Binary bacterial artificial chromosome
BLAST	Basic local alignment search tool
BLRV	Bean leaf roll virus
BM	Biomass
Bt	Bacillus thuringiensis
BUSCO	Benchmarking universal single-copy orthologs
BYMV	Bean yellow mosaic virus
CAPS	Cleaved amplified polymorphic sequence
CBD	Convention on Biological Diversity
CBN PMP	Conservatoire Botanique National des Pyrénées et de Midi Pyrénées

CcCDR	Cajanus cajan cold and drought regulatory
CcCYP	Cajanus cajan cyclophilin
CcHyPRP	Cajanus cajan hybrid proline-rich protein
cDNA	Complimentary DNA
CDS	Coding sequence
CFF	Crops for the Future
CGIAR	Consultative Group for International Agricultural Research
CID	Carbon isotope discrimination
CIM	Composite interval method
CLD	Crinkle leaf dwarf
ClYVV	Clover yellow vein virus
CMS	Cell membrane stability
CMS	Cytoplasmic male sterility
CNV	Copy number variation
COS	Conserved orthologous set
СР	Coat protein
CRISPR	Clustered regularly interspaced short palindromic repeats
CS	Climate-smart
CST	Climate-smart trait
CTDB	Chickpea Transcriptome Database
CTD	Canopy temperature depression
CWR	Crop wild relative
CWSI	Crop water stress index
DArT	Diversity arrays technology
DArT Seq	Diversity arrays technology Sequencing
dCAPS	Derived CAPS
DDS	Direct disease resistance
DEG	Differentially expressed gene
DH	Doubled haploid
DL	Dwarf lethal
DREB	Dehydration responsive element binding
DS	Dormant seeding
DTI	Drought tolerance index
DUS	Distinctiveness, Uniformity and Stability
EC	Electrical conductivity
EIAR	Ethiopian Institute of Agricultural Research
eIF(iso)4E	Isoform of eukaryotic translation initiation factor 4E
eIF4E	Eukaryotic translation initiation factor 4E
EMBL	European Molecular Biology Laboratory
EMS	Ethyl methane sulphonate
eQTL	Expression QTL
EST	Expressed sequence tag
EST-SSR	EST-derived SSR
ET	Ethylene
FAO	Food and Agriculture Organization

FBCM FISH FNEE	Faba bean consensus map Fluorescence in situ hybridization Food, Nutrition, Energy & Environment (Security)
Fpod	Number of filled pods per plot
FW	Fusarium wilt
GA3	Gibberelic acid
GABA	γ -Aminobutyric acid
GBS	Genotyping-by-sequencing
GC-MS	Gas chromatography–mass-spectrometry
GEBV	Genomic estimated breeding value
GEMs	Gene expression markers
GEO	Gene expression omnibus
GHMM	Generalized hidden Markov model
GLM	General linear model
GMO	Genetically modified organism
GMP	Geometric mean productivity
GP	Gene pool
GS	Genomic selection
GSI	Germination stress index
GSS	Genome survey sequences
GUS	β-Glucuronidase
GWAS	Genomewide association study
GY	Grain yield per plot
HI	Harvest index
HM	Harmonic mean
HMM	Hidden Markov model
HPL	Hydroperoxide lyase
HRM	High resolution melting
HS	Heat stress
HSP	Heat shock protein
HT	High temperature
IAA	Indolacetic acid
ICAR	Indian Council of Agricultural Research
ICARDA	International Center for Agriculture Research in the Dry Areas
ICMR	Indian Council of Medical Research
IIPR	Indian Institute of Pulses Research
IITA	International Institute of Tropical Agriculture
IL	Introgression line
InDel	Insertion/deletion
IPCC	Intergovernmental Panel on Climate Change
IRLC	Inverted repeat lacking clade
ISR	Induced systemic resistance
ISSR	Inter-simple sequence repeat
ITAP	Intron targeted amplified polymorphism

ITPGRFA	International Treaty on Plant Genetic Resources for Food and
	Agriculture
ITS	Internal transcribed spacer
JA	Jasmonic acid
KASP	Kompetitive allele specific PCR
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS	Liquid chromatography-mass-spectrometry
LD	Linkage disequilibrium
LG	Linkage group
LINE	Long interspersed element
LIS	Legume Information System
LMICs	Low and Medium Income Countries
LMP	Long mate pair
LOD	Logarithm of odds
LRR	Leucine-rich repeat
LRS	Likelihood ratio statistics
LTR	Long terminal repeat
MABC	Marker-assisted backcrossing
MABCB	Marker-assisted backcross breeding
MAB	Marker-assisted breeding
MAGIC	Multiparent advanced generation intercross
MAMP	Microbe-associated molecular pattern
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MAT	Mutually agreed term
miRNA	MicroRNA
MLO	Mildew resistance locus O
MP	Mean productivity
MS	Murashige and Skoog (medium)
MTA	Marker-trait association
NAA	1-Naphthalenenacetic acid
NADH	Nicotinamide adenine dinucleotide
NAM	Nested association mapping
NARS	National Agricultural Research Systems (India)
NBPGR	National Bureau of Plant Genetic Resources (India)
NBS-LRR	Nucleotide binding site-leucine-rich repeat
NCBI	National Center for Biotechnology Information (USA)
NDRE	Normalized difference red edge
NDVI	Normalized difference vegetation index
NGS	Next-generation sequencing
NILs	Near-isogenic lines
NMR	Nodule mass ratio
NMR	Nuclear magnetic resonance
NUE	Nutrient-use efficiency
ODAP	β -N-Oxalyl-L- α , β -diaminopropionic acid

PAL	Dhanylalanina ammonia lyasa
PAL PAMP	Phenylalanine ammonia-lyase Pathogen-associated molecular pattern
PAMP	Photosynthetically active radiation
PAV	Presence and absence variation
PBA	
	Pulse Breeding Australia
PCR	Polymerase chain reaction
PDC	Pyruvate decarboxylase
PEG	Polyethylene glycol
PEMV	Pea enation mosaic virus
PGRFA	Plant Genetic Resources for Food and Agriculture
PIC	Prior informed consent
PLANEX	Plant co-expression database
PMV	Pea mosaic virus
PSbMV	Pea seedborne mosaic virus
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
R/FR	Red/far red
RADP	Random amplified polymorphic DNA
RDp	Root depth
RFLP	Restriction fragment length polymorphism
RFS	Root frost susceptibility
RGA	Resistance gene analog
RGR	Relative growth rate
RIL	Recombinant inbred line
RNAi	RNA-interference
RNA-seq	RNA sequencing
ROS	Reactive oxygen species
RS ratio	Root-shoot ratio
RUE	Radiation use efficiency
RWC	Relative water content
SA	Salicylic acid
SAGE	Serial analysis of gene expression
SAR	Systemic acquired resistance
SCAR	Sequence characterized amplified region
SDG	Sustainable development goals
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SINE	Short interspersed nuclear element
SMD	Sterility mosaic disease
SMTA	Standard Material Transfer Agreement
SNF	Symbiotic nitrogen fixation
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SPLAT	Specific polymorphic locus amplification test
SRAPS	Sequence-related amplified polymorphism
SRR	Seed replacement rate
JILI	seed replacement fac

SSD	Single good descent
SSD	Single seed descent
	Suppression subtractive hybridization
SSI	Stress susceptibility index
SSR	Simple sequence repeat
STI	Stress tolerance index
STS	Sequence-tagged site
SuSy	Sucrose synthase
TAG	Triacylglycerol
TALEN	Transcription activator-like effector nucleases
Tc	Canopy temperature
TDZ	Thidiazuron
TE	Transposable element
TF	Transcription factor
TI	Heat tolerance index
TILLING	Targeting induced local lesions in genome
TRIPS	Trade-Related Aspects of Intellectual Property Rights
TS	Total number of seeds per plot
TUE	Transpiration-use efficiency
TUS	Tentative unique sequence
UN	United Nations
USDA	United Sates Department of Agriculture
UV	Ultraviolet
VAM	Vesicular-arbuscular mycorrhizae
VEP	Variant effect predictor
VIGS	Virus-induced gene silencing
VNTR	Variable number tandem repeats
VPD	Water pressure deficit
VS	Visual scoring
WGRS	Whole genome re-sequencing
WGS	Whole genome sequencing
WHO	World Health Organization
WUE	Water-use efficiency
YAC	Yeast active chromosome
ZFN	Zinc finger nuclease

Chapter 1 Common Bean Genetics, Breeding, and Genomics for Adaptation to Changing to New Agri-environmental Conditions



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Abstract Common bean (*Phaseolus vulgaris* L.) has become, over the last 20 years, a competitive crop in national, regional, and international markets. This situation presents a dynamic environment for producers and researchers of this crop and requires a rethinking of current strategies against research and production needs, the opportunities and challenges of the future, and adaptation to changing agri-

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© Springer Nature Switzerland AG 2019 C. Kole (ed.), *Genomic Designing of Climate-Smart Pulse Crops*, https://doi.org/10.1007/978-3-319-96932-9_1 environmental conditions. Improvement of the common bean means possessing indepth knowledge of its genetic diversity, the genome and gene functions, to enable the analysis of pathways and networks in response to fluctuating environmental conditions. An important long-term challenge is the discovery of the gene(s) that control important production traits such as pest and disease resistance, abiotic stress tolerance, and biological fixation of nitrogen. This will need to be a cooperative worldwide effort that involves breeders, geneticists, and genomic and bioinformatics experts. Currently, new technologies built around the recently released common bean genome sequence are now being developed, and various genomic resources for common bean are available and include physical maps, bacterial artificial chromosome libraries, anchored physical and genetic maps, and expressed sequence tags. However, these approaches require precise phenotypic data. Complex interactions between the common bean crop genotype, environmental factors in combination with plant population dynamics and crop management greatly affect plant phenotypes in field experiments and are the key for the expansion of the productivity of this crop in traditional and nontraditional growing areas.

Keywords Abiotic stress tolerance · Agronomy · Diseases and pest resistance · Food legumes · Genetic resources · Genetic mapping · Molecular breeding · *Phaseolus vulgaris* L.

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1.1 Challenges, Priorities, and Prospects of Recent Plant Breeding

1.1.1 Background

Understanding the effects of domestication on genetic diversity of common bean (*Phaseolus vulgaris* L.) is of great importance, not only for crop evolution but also for possible applications, such as the implementation of appropriate biodiversity conservation strategies, and the use of genetic variability in breeding programs. One of the most important and generalized features of plant domestication is the reduction in genetic diversity, not only during the initial domestication process but also during dispersion and adaptive radiation from the centers of domestication to other areas. The reduction of genetic diversity is usually more drastic in autogamous species such as common bean, which have restricted genetic recombination and presents a higher population structure as compared with allogamous species (Jarvis and Hodgkin 1999). This reduction is caused by both stochastic events (i.e., a bottleneck and genetic drift due to a reduction in the population size) and selection (i.e., adaptation to a novel agrosystem) (Vigouroux et al. 2002).

A recent hypothesis for the origin of the common bean defended a Mesoamerican origin (Bitocchi et al. 2012, 2013), based on the extensive diversity and population structure within the Mesoamerican gene pool, and the signature of pre-domestication bottlenecks in the south of the Andes detected in five gene fragments across 102 wild bean accessions. This novel structure of population not only evidences a Mesoamerican origin but also excludes an Andean origin of common bean. Additionally, these authors suggested that the wild common bean from northern Peru and Ecuador represents an old relict germplasm including a part of the genetic diversity of the ancestral common bean populations, displaying a type I phaseolin that probably was extinct in Mesoamerica. The resequencing of the genome of the common bean by Schmutz et al. (2014) recently confirmed this hypothesis.

Domestication took place after the formation of the Mesoamerican and Andean gene pools, and thus their structure is evident in both the wild and the domesticated forms (Papa and Gepts 2003; Papa et al. 2005, 2007, Rossi et al. 2009). This clear subdivision of the common bean germplasm is well documented, and it has been defined through several studies (Papa et al. 2007; Angioi et al. 2009; Bitocchi et al. 2012, 2013). However, the number of domestication events within each pool is still debated. Bitocchi et al. (2013) hypothesized a single domestication event within each gene pool and indicated the Oaxaca valley in Mesoamerica and southern Bolivia and northern Argentina as geographical areas of common bean domestication.

The exploration of The Americas by the Europeans, from the 15th century, marked the arrival into the Old World of many plant species such as common bean (*Phaseolus vulgaris* L.), peanuts (*Arachis hypogaea* L.), cocoa (*Theobroma cacao* L.), corn (*Zea mays* L.), potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), etc. The introduction of these exotic species in a new agricultural area under different environmental conditions raises relevant questions about adaptation, taking into account the requirements of tolerance to several stresses, as well as competitiveness with other indigenous crops in production and economic value (De Ron et al. 2016).

No records of common bean earlier than 1543 have been found in European herbariums; however, as reported by Zeven (1997), in 1669 it was widely grown in many areas of Europe. The dispersion of the common bean to Europe probably started from the Iberian Peninsula (Spain and Portugal), where the species was introduced mainly from Central America around 1506 and from the southern Andes after 1532, through sailors and traders who brought with them the nicely colored and easily transportable seeds as a curiosity (Brücher and Brücher 1976; Debouck and Smartt 1995). The pathways of dissemination of the crop across Europe were very complex, with several introductions from America combined with direct exchanges between European and other Mediterranean countries (Papa et al. 2007). Over time, the dissemination across Europe surely occurred through seed exchanges among farmers being facilitated by territorial contiguity and similarity of environments. The protein marker phaseolin was used as a marker in describing the worldwide dissemination of common bean (Gepts 1988). A higher frequency of Andean types (T, C, H, and A) was recorded with respect to Mesoamerican ones (S, B, M) (Lioi 1989; Santalla et al. 2002).

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

1.1.2 The Common Bean as a Food Resource

Grain legumes (pulses) are considered an essential source of nutrients and are also recognized as poor man's meat, showing their importance for people of developing countries, where the consumption of animal protein is limited by nonavailability or is self-imposed because of religious or cultural habits. Furthermore, legume seeds contain many bioactive and/or antinutritional compounds, such as phytate, oligosaccharides, phenolic compounds, nonprotein amino acids, lectins, enzyme inhibitors that play metabolic roles in humans or animals that frequently assume these seeds. These effects may be regarded as positive, negative, or both (Champ 2002).

From a nutritional point of view, the amino acid profile of legume storage proteins reveals low amounts of the essential sulfur-containing amino acids (i.e., methionine and cysteine) and tryptophan, while lysine, another essential amino acid, is quite abundant. Legume proteins complement very well those of cereals, which are normally rich in sulfur amino acids and poor in lysine and threonine. Besides the composition in essential amino acids, the nutritional quality of seed proteins is also largely determined by their digestibility. In fact, amino acids composition only represents the potential nutritional quality of a protein, being their bioavailability critical for the supply of amino acids in the diet (Sparvoli et al. 2015).

The common bean is the third most important food legume crop worldwide, surpassed only by soybean (*Glycine max* (L.) Merr.) and peanut (*Arachis hypogea* L.), and it is the first one for direct human consumption. Beans are produced and consumed mainly as a dry food legume, due to the high protein content of the grain, but the use of the fresh pod (snap bean) is common in many countries. Common bean is highly consumed in many areas of Africa and Latin America (as the most important source of plant protein), as well as in traditional diets of the Middle East and Europe (Broughton et al. 2003; Casquero et al. 2006). This legume is part of the healthy diet of the European Mediterranean basin and gaining importance in the USA where consumption has been increasing due to public interest in ethnic and healthy foods (Blair and Izquierdo 2012).

Recently the role of bean in human diet is being focused not only in its protein content but in the functional properties also and some authors have reported that its consumption could contribute to reduce the risk of obesity, diabetes, cardiovascular diseases and colon, prostate, and breast cancer (Hangen and Bennink 2003; Thompson et al. 2009). These health benefits could be due to the fiber content in the grain but also to antioxidant compounds as the phenolic ones. All the molecules present in legumes having anticancer properties are soluble in aqueous-alcohol extracts, while resistant starches, present in high amount in legumes, together with non-starch polysaccharides, are primarily insoluble residues from aqueous-alcohol extracts (Sparvoli et al. 2015). Colon carcinogenesis was induced by azoxymethane treatment in obese ob/ob mice fed with a diet containing cooked navy beans (whole beans), the insoluble or soluble fraction of aqueous-alcohol extracts, or a standard diet (Bobe et al. 2008).

1.2 Prioritizing Climate Smart (CS) Traits

1.2.1 Disease Resistance

1.2.1.1 Introduction

The abnormal functioning of diseased plants generally leads to a reduction in quantity and quality of yield. Disease is the result of an interaction among the plant and its environment and it is often affected by biotic and abiotic factors (e.g., microorganisms, humidity, temperature, etc.) that are detected as signals for the activation of plant response mechanisms (American Phytopathological Society 2005). When a plant is present in a stress situation (biotic or abiotic), it shows a minimum resistance to this situation, which will slow down their vital functions, reducing their development. This alarm phase is the one that will trigger all the mechanisms to get over it. If this situation persists, the plant will die. However, if it triggers some defense mechanisms, it will enter a resistance phase reaching a maximum level. If the stress continues, the plant will enter a phase of exhaustion. This phase may cause plant death if the stress does not disappear. Nevertheless, if the stress situation ends, plant recovers its physiological functions, being able to regenerate and to reach a new physiological state optimal for the present conditions, which corresponded to the regeneration phase (Tadeo and Gómez-Cadenas 2008).

Crops are affected by a wide diversity of fungal pathogens, for example, *Sclerotinia* spp., *Fusarium* spp., *Botrytis* spp., *Rhizoctonia* spp., etc., causing important economic losses (Mayo et al. 2017). A form of control to diseases is the application of synthetic fungicides. Its application on the seed or directly to the soil can be effective against fungi that affect the crops during or shortly after germination (Beebe and Corrales 1991) because they reduce its incidence and improve the emergence of plants (Valenciano et al. 2004). However, applications with fungicides aimed at avoiding damage caused by fungi that cause root rot or yellowing and wilting are often ineffective and usually impracticable due to the large volume of soil to which they should be directed. Actually, the number of authorized plant protection products has been reduced in order to ensure food safety and its sustainable in the long term. It is therefore proposed to prioritize nonchemical methods in integrated production, organic farming, and others (Mayo et al. 2017).

As a strategy to control plant infectious diseases, mainly those caused by fungi, the use of biocontrol agents can reduce the negative effects of plant pathogens and they also can promote positive responses in the plant (Shoresh et al. 2010). Biocontrol agents are perceived to have specific advantages over synthetic fungicides, including fewer nontarget and environmental effects, efficacy against fungicide-resistant pathogens, reduced probability of resistance development and use in organic farming situations where synthetic fungicides are restricted (Brimner and Boland 2003).

Bacterial species belonging to genera such as *Agrobacterium*, *Pseudomonas*, *Streptomyces*, and *Bacillus*, and fungal genera such as *Gliocladium*, *Trichoderma*, *Ampelomyces*, *Candida*, and *Coniothyrium*, are beneficial organisms that have shown good efficiency as biocontrol agents against pathogenic microorganisms (Vinale et al. 2008a).

1.2.1.2 Trichoderma spp.

Trichoderma spp. (Teleomorph: Hypocrea) is a fungal genus that is found in the soil, and it is a secondary fast growing opportunistic invasive (Mayo et al. 2016a, b) producer of chitinases, glucanases and proteases, and metabolites with antimicrobial activity (Lorito et al. 2010). Many *Trichoderma* species are also well known as biocontrol agents of important phytopathogenic fungi. The primary mechanisms of biocontrol used by *Trichoderma* in direct confrontation with pathogenic fungi are

mycoparasitism, antibiosis, and competition for nutrients with the pathogen (Harman et al. 2004). *Trichoderma* species colonize the root surface and cause substantial changes in plant metabolism (Shoresh et al. 2010). The physical interaction between *Trichoderma* and plants is limited to the first cell layer of the epidermis and the root bark. In addition, *Trichoderma* biocontrol strains are able to induce the expression of genes involved in defense response and also to promote plant growth, root development, and nutrient uptake (Hermosa et al. 2012).

Trichoderma spp. is recognized for their important benefits to agriculture such as its ability to protect crops against diseases (Benítez et al. 2004) and increase crop yield under field conditions (Harman et al. 2004). Most species of *Trichoderma* have been linked to biocontrol and biotechnological applications (Monte 2001), and the versatility of *Trichoderma* strains to suppress diseases caused by pathogens (Howell 2003). Since *Trichoderma* strains grow and proliferate best when there are abundant healthy roots, they have evolved numerous mechanisms of action both to attack other fungi and to enhance plant and root growth (Benítez et al. 2004).

In a symbiotic relationship with *Trichoderma*, the transport of sucrose from plants with subsequent intracellular hydrolysis by *T. virens* has been shown (Fig. 1.1). This source–sink communication may be central to the mutualistic interaction, influencing the development of *Trichoderma* in the rhizosphere and root plant (Vargas et al. 2012).

Competition and Mycoparasitism

Competition between *Trichoderma* and pathogens (Fig. 1.1) would be established with the purpose to get more nutrients, oxygen, light, etc. (Paulitz 1990). *Trichoderma* is an excellent competitor for space and nutritional resources. It appears in almost all soils and in habitats that contain high amounts of organic matter. In those niches, it would be an excellent decomposer of plant and fungal material. Moreover, some species of the genus *Trichoderma* show great metabolomic versatility that allows them to grow using a wide range of nitrogen and carbon sources. Furthermore, *Trichoderma* has the ability to colonize the rhizosphere, and this skill might be essential for being used as an excellent biological control agent (Howell 2003).

Mycoparasitism (Fig. 1.1) consists in the recognition of the fungus, attacking it, and penetrating it with the purpose to cause its death. This process involves some different phases. Firstly, *Trichoderma* locates the pathogen without previous contact, beginning to enlarge toward the pathogen by tropism (Chet et al. 1981; Lu et al. 2004). During this process, *Trichoderma* secretes some enzymes that hydrolyze the cell wall of the pathogen (Howell 2003; Woo et al. 2006). It has been studied that *Trichoderma* releases an extracellular exochitinase (Brunner et al. 2003) that might cause the liberation of some oligomers from the fungus, which could induce the expression of toxic endochitinases that would diffuse and would start to attack to the pathogen, even before the physical contact had happened. Some enzymes belonging to these fungi have been purified and used for biocontrol. When they have been

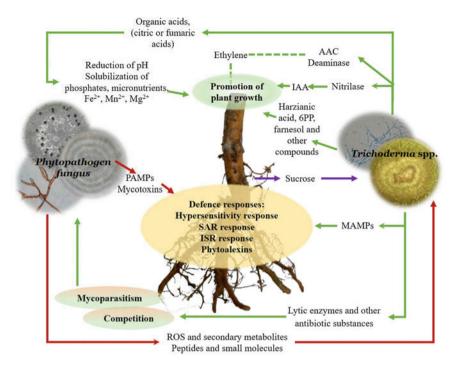


Fig. 1.1 Interactions between phytopathogen fungus, plant, and biocontrol agent *Trichoderma*. The green lines and circles are compounds and actions produced/induced by *Trichoderma*. The red lines are compounds and responses produced/caused by the phytopathogen fungus. The purple lines and circles are the compounds and plant responses produced/induced by the fungi (Altomar et al. 1999; Druzhinina et al. 2011; Howell 2003; Rubio et al. 2009; Vargas et al. 2011; Vinale et al. 2009; Vinale et al. 2008a, b) (*6PP* 6-pentyl- α -pyrone; *AAC* 1-aminocyclopropane-1-carboxylic acid; *IAA* indoleacetic acid; *ISR* induced systemic resistance; *MAMPs* microorganism-associated molecular patterns; *PAMPs* pathogen-associated molecular patterns; *ROS* reactive oxygen species; *SAR* systemic acquired resistance)

assessed, they have shown antifungal activity and have controlled a large number of pathogens, such as *Fusarium*, *Rhizoctonia*, *Alternaria*, *Ustilago*, *Venturia*, and *Colletotrichum* (Lorito et al. 1993; Lorito et al. 1994).

A major part of the *Trichoderma* antifungal system consists of a number of genes encoding an astonishing variety of secreted lytic enzymes (Sanz et al. 2004) including endochitinases, N-acetyl- β -glucosaminidases, chitin 1,4- β -chitobiosidases, proteases, glucan β -1,3-glucosidases, glucan β -1,6-glucosidases, glucan α -1,3-glucosidases, lipases, xylanases, mannanases, pectinases, pectin lyases, amylases, phospholipases, RNAses, DNAses, etc. Some of these proteins have been purified and their corresponding genes have been cloned and characterized by our group: protease PRA1 (Suarez et al. 2004), chitinases CHIT36 and CHIT37 (Viterbo