

# Genomic Designing of Climate-Smart Vegetable Crops

Chittaranjan Kole  
Editor

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*Dedicated to*

*Late Prof. Subir Sen, D.Sc.*

*Head, Department of Genetics & Plant  
Breeding, Faculty of Agriculture, and Dean,  
Post-Graduate Studies, Bidhan Chandra  
Krishi Viswavidyalaya (Agricultural  
University), West Bengal, India*

*An exceptional scientist, outstanding  
academician and a visionary*

*who lived many decades ahead of his time*

# Preface

The last one hundred and twenty 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 environment fluctuations, and hence phenotype-based breeding for these traits has hardly been effective.

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

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

other 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 in 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; genomewide 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 genetically modified crops by cisgenic crops through transfer of useful plant genes and atomically modified crops by employing nanotechnology that will hopefully be universally accepted for commercialization owing to their human-friendly and environment-friendly nature.

I wish to emphatically mention here that none of the technologies and tools of plant breeding 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 last 5 years at different platforms.

Addressing FNEE security has become more relevant today in the changing scenario of climate change and global warming. Climate change will lead to greenhouse gas emissions and extreme temperatures leading to different abiotic stresses including drought or waterlogging on 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 production from those practiced over a century aiming mostly at one or the few breeding objectives at a time such as yield, quality, resistance to biotic stresses due to disease-pests, tolerance to abiotic stresses due to drought, heat, cold, flood, salinity, acidity or improved water and nutrient use efficiency. In the changing scenario of climate change, for sustainable crop production, precise prediction of the above limiting factors by long-term survey and timely sensing through biotic agents and engineering devices and regular soil and water remediation will play a big role in agriculture. We have been discussing on ‘mitigation’ and ‘adaptation’ strategies for the last few years to reduce the chances of reduction of crop productivity and improve the genome plasticity of crop plants that could thrive and perform considerably well in a wide range of growing conditions over time and space. This is the precise reason of adopting genomic designing of crop plants to improve their adaptability by developing climate-smart or climate-resilient genotypes.

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

There are a huge number of books and reviews on traditional breeding, molecular breeding, genetic engineering, nanotechnology, genomics-aided breeding and gene editing with crop-wise and trait-wise deliberations on crop genetic improvement including over 100 books edited by me since 2006. However, I believe the present five book volumes will hopefully provide a comprehensive enumeration on the requirement, achievements and future prospects of genome designing for climate-smart crops and will be useful to students, teaching faculties and scientists in the academia and also to the related industries. Besides, public and private funding agencies, policy making bodies and the social activists will also get a clear idea on the road 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 last 12 decades that also on a diverse crop plants to gain confidence to edit the chapters authored by the scientists with expertise on the particular crops or crop groups and present them in a lucid manner with more or less uniform outline of

contents and formats. However, my experience gained over the last 7 years in the capacity of the Founding Principal Coordinator of the International Climate-Resilient Crop Genomics Consortium (ICRCGC) was highly useful while editing these books. I have the opportunity to interact with a number of leading scientists from all over the world almost on a 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 Vegetable Crops* includes eight major crops including Potato, Tomato, Brassica Vegetables, Eggplant, Capsicum, Carrot, Alliums and Garlic. These chapters have been authored by 32 scientists from 9 countries including Argentina, Bangladesh, China, France, India, Japan, Poland, UK and USA. I place on record my thanks for these scientists for their contributions and cooperation.

I have always enjoyed working on horticultural crops during my entire academic career spanning over 40 years. I worked on molecular genetics and breeding in tomato while at the Pennsylvania State University, USA; molecular genetics, breeding and genomics in peach, apricot and bitter melon while at the Clemson University, USA; molecular genetics in country bean while at the Odisha University of Agriculture & Technology, India; molecular genetics in guava while at the Sam Higginbottom University of Agriculture, technology & Sciences, India; and molecular genetics and breeding in bitter melon while at the Bidhan Chandra Krishiviswavidyalaya (Agricultural University), and ICAR-National Institute for Plant Biotechnology, both in India.

However, I started working on horticultural crops in late seventies in the laboratory of (Late) Prof. Subir Sen Head of the Department of Genetics and Plant Breeding and later on Dean of Post-Graduate Studies in the Bidhan Chandra Krishiviswavidyalaya (Agricultural University), West Bengal, India as a Ph.D. student on genetics and breeding of a medicinal and aromatic plant, citronella. It is that time, we realized the potential of medicinal and aromatic plants as 'crops' in future and importance of exploration, collection, conservation, characterization and utilization of such crops the concepts that have become important in today's world. We are coming often across the terms 'biodiversity', 'health security' and 'crops of the future' only now! Prof. Sen was not only an outstanding scientist and an excellent teacher himself but also a visionary endowed with vast knowledge on arts, music and literature who lived many decades ahead of his time. Hence, I have dedicated this book to (Late) Prof. Sen as a token of my respect, appreciation and gratitude.



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# Abbreviations

•O <sub>2</sub> –	Superoxide radical
•OH	Hydroxyl radical
5-azaC	5-Azacitidine
6-PF-2-K/Fru2,6-P <sub>2</sub> ase	6-Phosphofructo-2-kinase/fructose2,6-bisphosphatase
ABA	Abscisic acid
ABF	ABA binding factor
ABRE	ABA-responsive element
ACC	1-Aminocyclopropane-1-carboxylic acid
ACS	<i>A. chinese</i> saponins
AFLP	Amplified fragment length polymorphism
AGO	Argonaute
AIR	Anthocyanin-impaired-response
AOX	Alternative oxidase
AP2	Apetala 2
AREB	ABA-responsive element binding protein
ASE	Allele-specific expression
ASH1	Absent, small or homeotic disks 1
ASHH2	ASH1 homolog 2
ATX	ARABIDOPSIS TRITHORAX
ATXR	ARABIDOPSIS TRITHORAX-RELATED
AVRDC	World Vegetable and Development Center (Tainan, Taiwan)
BAC	Bacterial artificial chromosome
BADH	Betaine aldehyde dehydrogenase
BC	Backcross
bHLH	Basic helix-loop-helix
BiFC	Bimolecular fluorescence complement
BPH	Best-parent heterosis
BR	Black rot
BSA	Bulked-segregant analysis

BSA-seq	Bulked-segregant analysis sequencing
BSR-seq	Bulked-segregant RNA sequencing
bZIP	Basic leucine zipper
CaM	Calmodulin
CaMV	Cauliflower mosaic virus
CAPS	Cleaved amplified polymorphic sequence
Cas9	CRISPR-associated 9 protein
CCA1	CIRCADIAN CLOCK ASSOCIATED 1
CC-NB-LRR	Coiled-coil NB LRR
CDF1	CYCLING DOF FACTOR1
cDNA	Complementary DNA
CDPK	Calcium-dependent protein kinase
CGMS	Cytoplasmic genic male sterility
ChIP	Chromatin immunoprecipitation
ChIP-seq	Chromatin immunoprecipitation sequencing
CI	Cytoplasmic inclusion
circRNA	Circular RNA
CK	Cytoplasmic kinase
cM	CentiMorgan
CMT	CHROMOMETHYLASE
CMV	Cucumber mosaic virus
CO	Constans
CoIP	Co-immunoprecipitation
Col	Columbia-0
COLD AIR	COLD ASSISTED INTRONIC NONCODING RNA
COLDWRAP	COLD OF WINTER-INDUCED NONCODING RNA FROM THE PROMOTER
COOLAIR	COLD INDUCED LONG ANTISENSE INTRAGENIC RNA
CP	Coat protein
CPB	Colorado potato beetle
CPGTH	Carboxypropyl glutathione
CR	Clubroot
CR	Cold responsive
CRISPR	Clustered regularly interspaced short palindromic repeats
<i>CRTISO</i>	Carotene cis-trans isomerase gene
CS	Chilling stress
CS	Climate smart
CWR	Crop-wild relative
<i>CYP97A3</i>	Carotene hydroxylase gene
DArT	Diversity arrays technology
DAS	Days after sowing
Dc	<i>Daucus carota</i> or carrot
DcAREB3	Carrot transcription factor to ABA-responsive elements
DcHSP	Carrot heat-shock protein

DCL	DICER-LIKE
DcPSY2	Carrot phytoene synthase2 protein ( <i>gene</i> )
DDM1	Decrease in DNA methylation 1
DEG	Differentially expressed gene
DFR	Dihydroflavonol 4-reductase
DH	Doubled haploid
dpi	Days post inoculation
DREB	Dehydration responsive element binding protein
DRM	DOMAINS REARRANGED METHYLTRANSFERASE
E(z)	Enhancer of zeste
EBN	Endosperm balance number
ECD	European clubroot differential
eIF4E	Eukaryotic initiation factor 4E
EMS	Ethyl methanesulphonate
EpiRAD-seq	Epi-restriction site associated DNA sequencing
epiRILs	Epigenetic recombinant inbred lines
ER	Endoplasmic reticulum
ERF	Ethylene-responsive element binding factor
EST	Expressed sequence tag
ET	Ethylene
ET	Evapotranspiration
ETI	Effector triggered immunity
F <sub>1</sub>	First filial generation
F3',5'H	Flavonoid 3',5'-hydroxylase
FAO	Food & Agriculture Organization (of the United Nation)
FAOSTAT	FAO statistics
FD	FLOWERING LOCUS D
FDA	Food and Drug Administration (USA)
FISH	Fluorescent <i>in situ</i> hybridization
FKF1	FLAVIN KELCH F BOX 1
FLC	FLOWERING LOCUS C
FLS	Flavonol synthase
<i>Foc</i>	<i>Fusarium oxysporum</i> f.sp. <i>conglutinans</i>
FOC	<i>Fusarium oxysporum</i> f.sp. <i>cepae</i>
FRI	FRIGIDA
Fru-2,6-P <sub>2</sub>	Fructose 2,6-bisphosphate
FT	FLOWERING LOCUS T
FUL	FRUITFUL
FW	Fusarium wilt
G × E	Genotype × environment
GA	Gibberellin
gbM	Gene-body methylation
GBS	Genotyping-by-sequencing
GC-MS/MS	Gas chromatography-mass spectrometry

GD	Genetic distance
GEBV	Genome-estimated breeding value
GGT	$\gamma$ -Glutamyl transpeptidases
GI	Gigantea
GIS	Geographic information system
Gly	Glycine
GMO	Genetically modified organism
GMS	Genic male sterility
GO	Gene ontology
GP	Genomic prediction
GPF	Green fluorescent protein
GRSV	Groundnut ringspot virus
GS	Genomic selection
GSPP	Good Seed and Plant Practices
GWAS	Genomewide association study
G×E×M	Genotype × environment × management
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
H3K27me3	Tri-methylation of the 27th lysine of histone H3
H3K36me3	Tri-methylation of the 36th lysine of histone H3
H3K4me3	Tri-methylation of the 4th lysine of histone H3
H3K9me2	Di-methylation of the 9th lysine of histone H3
HDR	Homologous recombination
HIB	High-efficiency integrated breeding
HIGS	Host-induced gene silencing
HP	High parent
HRM	High-resolution melting
HSF	Heat-stress transcription factor
HSP	Heat-shock protein
HT	High temperature
HVR	Hyper variable region
InDel	Insertion/deletion
IPCC	Intergovernmental Panel on Climate Change
IPT	Isopentytransperase
IRR	Interspersed repeat region
ISSR	Inter-simple sequence repeat
JA	Jasmonic acid
KASP	Kompetitive allele-specific polymerase chain reaction
KEGG	Kyoto Encyclopedia of Genes and Genomes
KYP	KRYPTONITE
LC-MS/MS	Liquid chromatography-mass spectrometry
LC-QqQ-MS	Liquid chromatography quadruple-mass spectrometer
LD	Long day
LD	Linkage disequilibrium
LEA	Late embryogenesis abundant
LF	Least fractionated

LG	Linkage group
LHP1	LIKE HETEROCHROMATIN PROTEIN 1
LHY	LATE ELONGATED HYPOCOTYL
lncRNAs	Long noncoding RNAs
LOD	Logarithm of odds
LP	Low parent
LRR	Leucine-rich repeat
MABC	Marker-assisted backcrossing
MAGIC	Multiparent advanced generation intercross
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MAS	Marker-assisted selection
MBD-seq	Methyl-CpG-binding domain sequencing
mC	Methylated cytosine
MDB	Molecular design breeding
MeDIP-seq	Methylated DNA immunoprecipitation sequencing
MET1	METHYLTRANSFERASE I
MethylRAD	Methylation-dependent restriction site associated DNA
MF	More fractionated subgenomes
MIP	Major intrinsic protein
miRNA	Micro-RNA
MLMM	Multi-locus mixed model
MLPK	M-locus protein kinase
MPH	Mid-parent heterosis
MPV	Mid-parent value
mRNA	Messenger-RNA
MTMM	Multi-trait mixed model
MYB	Myeloblastosis oncogene
MYBR	Myeloblastosis oncogene responsive
MYC	Myelocytomatosis oncogene
MYCR	Myelocytomatosis oncogene responsive
NB	Nuclear-binding
NB-LRR	Nucleotide-binding leucine-rich repeat
NBS	Nucleotide-binding site
ncRNA	Noncoding RNA
NGS	Next-generation sequencing
NHEJ	Nonhomologous end joining
NILs	Near-isogenic lines
NIP	Nodulin-26 like intrinsic protein
NMR	Nuclear magnetic resonance
NRPD1	Nuclear RNA polymerase D1A
NRPE1	Nuclear RNA polymerase D1B
NUE	Nutrient use efficiency
OP	Open-pollinated
ORF	Open reading frame

<i>OST2</i>	OPEN STOMATA 2 gene
P5CS	Pyrroline-5-carboxylate synthetase
PAM	Protospacer adjacent motif
PAMP	Pathogen-associated molecular pattern
PAR	Photosynthetic active radiation
PAT	Phosphinothricin acetyltransferase
<i>Pb</i>	<i>Plasmodiophora brassicae</i>
PcG	Polycomb group
PCR	Polymerase chain reaction
PepMV	Pepino mosaic virus
PHD	Plant homeodomain
PIP	Plasma membrane intrinsic protein
Pol IV	Polymerase IV
Pol V	Polymerase V
PP2C-A	Protein phosphatase type 2C
PPR	Pentatricopeptide repeat
PR	Pathogenesis-related
PRC2	POLYCOMB REPRESSIVE COMPLEX 2
PRR	Pattern recognition receptor
PSII	Photosystem II
PTI	PAMPs/MAMPs triggered immunity
qPCR	quantitative PCR
QRL	Quantitative resistance loci
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
R	Resistance
RAD-seq	Restriction site associated DNA sequencing
RAPD	Random amplified polymorphic DNA
RCA	Root cortical aerenchyma
RdDM	RNA-directed DNA methylation
RDR	RNA-DEPENDENT RNA POLYMERASE
retr	Recessive turnip mosaic virus resistance
<i>Rf</i>	Restorer-of-fertility gene
RFLP	Restriction fragment length polymorphism
RFO	RESISTANCE TO FUSARIUM OXYSPORUM
RGR	Relative growth rate
RILs	Recombinant inbred lines
RLCK	Receptor-like cytoplasmic kinase
RLK	Receptor-like kinase
RLP	Receptor-like protein
RNAi	RNA-interference
RNA-Seq	Ribonucleic acid sequencing
rnt1	Resistance and necrosis to tumv 1
ROS	Reactive oxygen species
rRF	Ribosomal RNA fragment

RSA	Root system architecture
RT	Reverse transcription
SA	Salicylic acid
SAM	Shoot apical meristem
SC	Self-compatibility
SCAR	Sequence-characterized amplified region
SCR	<i>S</i> -locus cysteine rich
SD	Short day
SE	Standard error
SET	SU(VAR)3-9, E(z), TRX
SI	Self-incompatibility
SIP	Small basic intrinsic protein
siRNAs	Small interfering RNAs
SIX1	Secreted-in-xylem 1
SLG	<i>S</i> -locus glycoprotein
Smi	<i>SP11</i> -methylation inducer
SMI	<i>SP11</i> -methylation-inducing region
SMRT	Single molecule real-time
snoRF	snoRNA fragment
SNP	Single nucleotide polymorphism
snRF	Small nuclear RNA fragment
SOC1	Suppressor of Overexpression of CO 1
SOD	Super oxidase dismutase
SP11	<i>S</i> -locus protein 11
SRAP	Sequence-related amplified polymorphism
SRK	<i>S</i> receptor kinase
SS	Salinity stress
SSH	Suppression subtractive hybridization
SSR	Simple sequence repeat
STF	<i>S</i> -locus retrotransposon family
STS	Sequence tagged site
SU(VAR)3-9	SUPPRESSOR OF VARIEGATION 3-9
SUVH4	SU(VAR)3-9 HOMOLOG
SWI2/SNF2	Switch 2/sucrose non-fermentable 2
TALEN	Transcription activator like effector nuclease
TCSV	Tomato chlorotic spot virus
TDB	Transcriptome database
TE	Transposable element
TF	Transcription factor
TGRC	Tomato Genetic Resources Center (UC-Davis, USA)
TILLING	Targeting-induced local lesions in genomes
TIP	Tonoplast intrinsic protein
TIR-NB-LRR	Toll interleukin-1 receptor-NB-LRR
TLP	Thaumatococcus-like protein
TMV	Tobacco mosaic virus



ToBRFV	Tomato brown rugose fruit virus
ToMV	Tomato mosaic virus
tRF	tRNA fragment
tRNA	Transfer RNA
TRX	Trithorax
TSWV	Tomato spotted wild virus
TuMV	Turnip mosaic virus
<i>TuRB01</i>	<i>Turnip mosaic virus RESISTANCE IN BRASSICA 01</i>
TYLCV	Tomato yellow leaf curl virus
USDA	United States Department of Agriculture
VIN3	VERNALIZATION INSENSITIVE 3
VPg	Viral protein genome
VRE	Vernalization response element
WAKL22	WALL-ASSOCIATED KINASE-LIKE KINASE 22
WD	Water deficit
WGBS	Whole genome bisulfite sequencing
WGT	Whole genome triplication
WT	Wild type
WUE	Water-use efficiency
<i>Xcc</i>	<i>Xanthomonas campestris</i> pv. <i>campestris</i>
XIP	X intrinsic protein
Y2H	Yeast two hybrid
ZAT	Zinc finger of <i>Arabidopsis thaliana</i>
<i>ZEP</i>	<i>Zeaxanthin epoxidase</i> gene
ZF	Zinc finger
ZFN	Zinc finger nuclease
Zip	Zinc finger protein