Transgenic Crop Plants

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Editors

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Volume 2: Utilization and Biosafety

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Preface

Transgenic Plants – known also as Biotech Plants, Genetically Engineered Plants, or Genetically Modified Plants – have emerged amazingly fast as a boon for science and society. They have already played and will continue to play a significant role in agriculture, medicine, ecology, and environment. The increasing demands for food, feed, fuel, fiber, furniture, perfumes, minerals, vitamins, antibiotics, narcotics, and many health-related drugs and chemicals necessitate the development and cultivation of transgenic plants with augmented or suppressed trait(s). From a single transgenic plant (Flavr Savr tomato with a longer shelf-life) introduced for commercialization in 1994, we have now 13 transgenic crops covering 800 million ha in 25 countries of six continents. Interestingly, the 13.3 million farmers growing transgenic crops globally include 12.3 million (90%) small and resource poor farmers from 12 developing countries. Increasing popularity of transgenic plants is well evidenced from an annual increase of about 10% measured in hectares but actually of 15% in "trait hectares." Considering the urgent requirement of transgenic plants and wide acceptance by the farmers, research works of transgenic plants are now being conducted on 57 crops in 63 countries. Transgenic plants have been developed in over 100 plant species and they are going to cover the fields, orchards, plantations, forests, and even the seas in the near future. These plants have been tailored with incorporation of useful alien genes for several desirable traits including many with "stacked traits" and also with silencing of genes controlling some undesirable traits.

Development, applications and socio-political implications of transgenic plants are immensely important fields now in education, research, and industries. Plant transgenics has deservedly been included in the course curricula in most, if not all, leading universities and academic institutes all over the world, and therefore reference books on transgenic plants with a class-room approach are essential for teaching, research, and extension. There are some elegant reviews on the transgenic plants or plant groups (including a 10-volume series "Compendium of Transgenic Crop Plants" edited by two of the present team of editors C. Kole and T.C. Hall published by Wiley-Blackwell in 2008) and on many individual tools and techniques of genetic transformation in plants. All these reviews could surely serve well the purpose for individual crop plants or particular methodologies. Since transgenic plant development and utilization is studied, taught, and practiced by students, teachers, and scientists of over a dozen disciplines under basic science, agriculture, medicine, and humanities at public and private sectors, introductory reference books with lucid deliberations on the concepts, tools, and strategies to develop and utilize transgenic plants and their global impacts could be highly useful for a broad section of readers.

Deployment of transgenic crop plants are discussed, debated, regulated, and sponsored by people of diverse layers of the society, including social activists, policy makers, and staff of regulatory and funding agencies. They also require lucid deliberations on the deployment, regulations, and legal implications of practicing plant transgenics. More importantly, depiction of the positive and realistic picture of the transgenic plants should and could facilitate mitigation of the negative propaganda against transgenic plants and thereby reinforce moral and financial support from all individuals and platforms of the society. Global population is increasing annually by 70 millions and is estimated to grow to eight billion by 2025. This huge populace, particularly its large section from the developing countries, will suffer due to hunger, malnutrition, and chemical pollution unless we produce more and more transgenic plants, particularly with stacked traits. Compulsion to meet the requirements of this growing population on earth and the proven innocuous nature of transgenic plants tested and testified for the last 13 years could substantiate the imperative necessity of embracing transgenics.

Traditional and molecular breeding practiced over the last century has provided enormous number of improved varieties in economic crops and trees including wheat and rice varieties that fostered the "green revolution." However, these crop improvement tools depend solely on the desirable genes available naturally, creatable by mutation in a particular economic species, or their shuffling for desired recombinations. Transgenic breeding has opened a novel avenue to incorporate useful alien genes from not only other cross-incompatible species and genera of the plant kingdom, but also from members of the prokaryotes including bacteria, fungi, and viruses, and even from higher animals including mice and humans. An array of plant genetic engineering achievements starting from the development of insect resistance cotton by transforming the *cry* genes from the bacteria *Bacillus thuringiensis* to the present-day molecular pharming that enables the expression of *interferon*- gene from human in tobacco evidence for this pan-specific gene transfer.

Human and animal safety is another general concern related to transgenic food or feed. However, there is no reliable scientific documentation of these health hazards even after 13 years of cultivation of transgenic plants and consumption of about 1 trillion meals containing transgenic ingredients. Utilization of transgenic plants has reduced the pesticide applications by 359,000 tons that would otherwise affect human and animal health besides causing air, water, and soil pollution and also mitigated the chance of consumption of dead microbes and insects along with foods or feeds.

Gene flow from transgenic crop species to their cross-compatible wild relatives is a genuine concern and therefore required testing of a transgenic crop plant before deployment followed by comprehensive survey of the area for presence of inter-fertile wild and weedy plants before introduction of a transgenic crop are being seriously conducted.

Addition of novel genotypes with transsgenes in the germplasms is increasing the biological diversity rather than depleting it. Using the genetically engineered plants has also eliminated greenhouse gas emission of 10 million metric tons through fuel savings. In fact, 1.8 billion liters of diesel have been saved because of reduced tillage and plowing owing only to herbicide-resistant transgenic crops. Many transgenics are now being used for soil reclamation. Above all, cultivation of transgenic crops has returned $44 billion of net income to the farmers. Perhaps, these are the reasons that 25 Nobel Laureates and 3,000-plus eminent scientists appreciated the merits and safety and also endorsed transgenic crops as a powerful and safe way to improve agriculture and environment besides the safety of genetically modified foods. Many international and national organizations have also endorsed health and environmental safety of transgenic plants; these include Royal Society (UK), National Academy of Sciences (USA), World Health Organization, Food and Agriculture Organization (UN), European Commission, French Academy of Medicine and American medical Association, to name a few.

Production, contributions, and socio-political implications of biotech plants are naturally important disciplines now in education, research, and industries and therefore introductory reference books are required for students, scientists, industries, and also for social activists and policy makers. The two book volumes on "Transgenic Crop Plants" will hopefully fill this gap. These two book volumes have several unique features that deserve mention. The outlines of the chapters for these two books are formulated to address the requirements of a broad section of readers. Students and scholars of all levels will obtain a lot of valuable reading material required for their courses and researches. Scientists will get information on concepts, strategies, and clues useful for their researches. Seed companies and industries will get information on potential resources of plant materials and expertise for their own R&D activities. In brief, the contents of this series have been designed to fulfill the demands of students, teachers, scientists, and industry people, for small to large libraries. Students, faculties, or scientists involved in various subjects will be benefited from this series; biotechnology, bioinformatics, molecular biology, molecular genetics, plant breeding, biochemistry, ecology, environmental science, bioengineering, chemical engineering, genetic engineering, biomedical engineering, pharmaceutical science, agronomy, horticulture, forestry, entomology, pathology, nematology, virology, just to name a few.

It had been our proud privilege to edit the 23 chapters of these two books those were contributed by 71 scientists from 14 countries and the list of authors include one of the pioneers of plant transgenics, Prof. Timothy C. Hall (one of the editors also); some senior scientists who have themselves edited books on plant transgenics; and many scientists who have written elegant reviews on invitation for quality books and leading journals. We believe these two books will hopefully serve the purposes of the broad audience who are studying, teaching, practicing, supporting, funding, and also those who are debating for or against plant transgenics. The first volume dedicated to “Principles and Development” elucidates the basic concepts, tools, strategies, and methodologies of genetic engineering, while the second volume on “Applications and Safety” enumerates the utilization of transgenic crop plants for various purposes of agriculture, industry, ecology, and environment, and also genomics research. This volume also deliberates comprehensively on the legal and regulatory aspects; complies to the major concerns; and finally justifies the compulsion of practicing plant transgenics.

Glimpses on the contents of this volume (Volume 2: Transgenic Crop Plants: Applications and Safety) will perhaps substantiate its usefulness. This volume enumerates the application of transgenic technologies in crop plants for particular objectives in the first ten chapters. Biotic stress resistant, specifically insect resistant, transgenics have been developed and commercialized in several crops. An example with *Bt*-expressing cotton and maize alone, with current market share of about $3.26 billion substantiates their success and popularity (Chap. 1). Abiotic stresses, particularly drought, salinity, and temperature extremes, have always been difficult to manipulate. Still success stories are pouring in recently from works mainly in cereals and vegetables (Chap. 2). Herbicide-resistant transgenic plants (in cotton and canola) were first deregulated in 1995 and in 2008 more than 80% of the transgenic plants grown globally possess a transgenic trait for herbicide resistance. Chapter 3 details the present and emerging herbicide-resistant transgenic plants. Although the first transgenic trait was developmental, shelf-life in tomato to be precise, transgenics research for these traits are yet to make significant commercial headway but started producing encouraging results (Chaps. 4 and 5). Deployment of transgenic plants for biofuel, pharmaceuticals, and other bioproducts has been enunciated in three chapters (Chaps. 6, 7, and 9). Transgenic plants have been labeled as a culprit for potential threats to ecology and environment by a few groups of social activists. Chapter 8 addresses these weird concerns with suitable examples of utilization of transgenic plants for phytoremediation, biomonitoring, and the production of bioplastics and biopolymers for amelioration of ecology and environment. Plant genomics has emerged fast within the last three decades and facilitated fine-scale view of the plant genes and genomes. Transgenic plants have provided enormous resources for functional genomics studies and expected to play their roles as more plants systems and genes are targeted (Chap. 10). Scientists practicing transgenics are no less aware of the potential risks of genetic engineering than the few people with antagonistic views. Neither are the regulatory agencies at institutional, state, national, and international level regulatory agencies unaware of the steps to be involved for inspection, monitoring, and approval of transgenic plants for commercial use. Chapter 11 delineates all these aspects with examples from US and other continents and countries. Any original innovation or effort deserves recognition and also an incentive. The scope of patenting and intellectual property rights for materials owned and generated and methodologies implemented have been appreciated and enforced legally. These aspects related to transgenic crop plants have been discussed in Chap. 12.

The concluding chapter (Chap. 13) briefs the contributions and concerns with the compliances and compulsion of practicing plant transgenics for science and society.

We thank all the 41 scientists from nine countries for their elegant and lucid contributions to this volume and also for their sustained support through revision, updating and fine-tuning their chapters. We also acknowledge for the recent statistics that have been accessed from the web sites of Monsanto Company on "Conversations about Plant Biotechnology" and "International Service for the Acquisition of Agri-Biotech Applications on ISAAA Brief 39-2008: Executive Summary" and used them in this preface and elsewhere in the volume.

We enjoyed a lot of our Clemson–Purdue–Texas A&M triangular interaction, constant consultations, and dialogs while editing this book, and also our working with the editorial staff of Springer, particularly Dr. Sabine Schwarz who had been supportive since inception till publication of this book.

We will look forward to suggestions from all corners for future improvement of the content and approach of this book volume.

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Abbreviations

1-FFT	Fructan:fructan 1-fructosyltransferase
1-MCP	1-Methylcyclopropene
1-SST	Sucrose:sucrose 1-fructosyltransferase
2,4-D	2,4-Dichlorophenoxyacetic acid
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
4C3H	4-Coumarate 3-hydroxylase
4CL	4-Hydroxycinnamoyl CoA ligase
6G-FFT	Fructan:fructan 6G-fructosyltransferase
6-SFT	Sucrose:fructan 6-fructosyltransferase
AAFC	Agriculture and Agri-Food Canada
ABA	Abscisic acid
ABRE	ABA responsive element
Ac	*Activator* gene
ACC	1-Aminocyclopropane-1-carboxylate
AChE	Acetylcholinesterase
ACP	Acyl-carrier protein
ADP	Adenosine di-phosphate
ae1	*amylose extender* gene
AHK2/3	*Arabidopsis* histidine kinase
AL-PCD	Apoptotic-like programmed cell death
ALS	Acetolactate synthase
AMGT	*Agrobacterium*-mediated gene transfer
AMPA	Aminomethylphosphonic acid
ANVISA	National Agency for Health and Surveillance of the Ministry of Health
AOS	Allene oxide synthase
AOX	Altenative oxidase
ap	*apetalla* gene
AP1	*APETALA1* gene
AP2	*APETALA2*/ *Apetela2* gene

APHIS	Animal and Plant Health Inspection Service
APX	Ascorbate peroxidase
ARF	Auxin response factor
arsC	Arsenate reductase
Asc	Ascorbate
at	*antherless* gene
AtCKX1	*Arabidopsis thaliana cytokinin oxidase* gene
Avr	Avirulence
AZ	Abscission zone
BA	Benzyladenine
BADH	Betaine aldehyde dehydrogenase
BAP	6-Benzylaminopurine
BAR	Bialaphos resistance gene
BBC	British Broadcasting Corporation
BC	Biotech crop
BGI-RIS	Beijing Genomics Institute- Rice Information System
bla	Beta-lactamase
BMR	Brown midrib
BRS	Biotechnology Regulatory Service
Bt	*Bacillus thuriengensis*
bZIP	Basic leucine zipper
CAD	Cinnamoyl alcohol dehydrogenase
CAL	*CAULIFLOWER* gene
CAMBIA	Center for the Application of Molecular Biology to International Agriculture
CaMV	Cauliflower mosaic virus
CAT	Chloramphenicol acetyltransferase/catalase
CBER	Centre for Biologics Evaluation and Research
CBF	CRT binding factor
cbLCV	Cabbage leaf curl virus
CBS	Columbia Broadcasting System
CCR	Cinnamoyl CoA reductase
CDC	Centers for Disease Control
CDF	Cycling DOF Factor
cDNA	Complementary-DNA
CEL	Cellulase
CEPA	Canadian Environmental Protection Act
CFIA	Canadian Food Inspection Agency
CFSAN	Centre for Food Safety and Applied Nutrition
CGH-1	Cardenolide 16’-*O*-glucohydrolase
CGIAR	Consultative Group on International Agricultural Research
cGMP	Current GMP
CHO	Chinese hamster ovary
CHO	Choline dehydrogenase

chs	*chalcone synthase* gene
CIGB	Cuban Centre for Biotechnology and Genetic Engineering
CMO	Choline monooxygenase
CMS	Cytoplasmic male sterility
CMS	Cellular membrane stability
CNN	Cable News Network
CNR	*Colorless non-ripening* gene
CO	*Constans* gene
ConA	Concanavalin A
CONABIA	National Advisory Commission on Agricultural Biotechnology
conz1	*constans of Zea mays1* gene
COR	Cold responsive
COR	Cold responsive gene
CP	Chloroplast
CpTI	Cowpea trypsin inhibitor
CRE	Cytokinin response
CRIIGEN	Comité de Recherche et d'Information Indépendantes sur le Génie
CRT	C-Repeat
Cry	Crystal
CTNBio	National Technical Commission on Biosafety
CVM	Centre for Vetinary Medicine
CV-N	Cyanovirin
D2GT2A	Diacylglycerol acyltransferase 2A
dab	*delayed abscission* gene
DDB	Damaged DNA binding protein
DDT	Dichlorodiphenyltrichloroethane
DEFRA	Department of Environment, Food and Rural Affairs
DET	*Detiolated* gene
DGDG	Digalactosyldiacylglycerol
DHAsc	Dehydroascorbate
dlf1	*delayed flowering1* gene
DNMA	Directorate of Agricultural Markets
DRE	Dehydration responsive element
DREB	DRE binding protein
driPTGS	Direct repeat-induced PTGS
Ds	*Dissociation* gene
DsE	Enhancer trap *Ds*
DsG	Gene trap *Ds*
DSL	Domestic Substance List
dzr1	*delta zein regulator1* gene
EA	Environmental assessment
EBV	Epstein-barr virus
EC	European Commission
EDB	Ethylene dibromide

EFSA	European Food Safety Authority
EFSA	European Food Standards Agency
EIN	*Ethylene-insensitive* gene
EIS	Environmental Impact Statement
EMEA	European Agency for Evaluation of Medicinal Products
EMS	Ethylmethane sulfonate
En	*Enhancer* transposon
EPA	Eicosapentaenoic
EPA	Environmental Protection Agency
EPSP	Enolpyruvyl-shikimate-3-phosphate
EPSPS	5-Enolpyruvyl-shikimate-3-phosphate synthase
ER	Endoplasmic reticulum
ERE	Ethylene responsive element
ERS	Economic Research Service
EST	Expressed sequence tag
ETC	Electron transport chain
ETH	Ethylene
EU	European Union
F	Florigenic signal
F1	Fraction 1 anti-phagocytic capsular envelope protein
FAD1	Flavin adenine dinucleotide
FAD3	Omega-3 fatty acid desaturase
FAO	Food and Agriculture Organization of the United Nations
FD	*FLOWERING LOCUS D* gene
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
fl2	*floury-2* gene
FONSI	Finding of no significant impact
FPPA	Federal Plant Pest Act
Fr	*Fertility restorer* gene
FST	Flanking sequence tag
FT	*Flowering Locus T/Flowering Transition* gene
FT-ICR-MS	Fourier-transform ion cyclotron mass spectrometry
Fuc	Fucose
FucT	Fucosyltransferase
FUL	*FRUITFUL* gene
Fx	Fucoxantine
G3P	Glycerol-3-phosphate
GA/ GA_3	Gibberellic acid
Gal	Galactose
GalNAc	*N*-Acetylgalactosamine
GalT	Galactosyltransferase
GAT	Glyphosate *N*-acetyltransferase

GC	Glutathione synthetase
GCase	Glucosyl-*N*-acylspingosineglycohydrolase
GCS	γ-Glutamylcysteine synthase
GDP	Gross domestic product
GE	Genetic engineering/Genetically engineered
GFLV	Grapevine fanleaf virus
GFP	Green fluorescent protein
GI	*Gigantea* gene
gigz1	*gigantea of Zea mays1* gene
GlcNAc	*N*-Acetylglucosamine
GlyBet	Glycine betaine
GM	Genetically modified
GMHT	Genetically modified herbicide tolerant
GMO	Genetically modified organism
GMP	Genetically modified plant
GMP	Good manufacturing practise
GMPO	Genetically modified plant organism
GMS	Genic male sterility
GNA	*Galanthus nivalis* agglutinin
GOI	Gene of interest
GOX	Glyphosate oxidoreductase
GPAT	Glycerol-3-phosphate acyltransferase
GPX	Glutathione peroxidase
GR	Glutathione reductase/ Glyphosate resistant
GRC	Glyphosate resistant Crop
GS1	*Glutamine synthase* gene 1
GSH	Glutathione/ Glutamate synthase
GSSG	Glutathione disulfide
GST	Glutathione S-transferase
GTN	Glycerol trinitrate
GUS	ß-Glucuronidase
HBcAg	Hepatitis B core antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCMV	Human cytomegalovirus
hEPO	Human erythropoietin
hGM-CSF	Human granulocyte-macrophage colony-stimulating factor
HIV	Human immunodeficiency virus
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HR	Hypersensitive response/ Herbicide resistant
HRC	Herbicide resistant crop
HS	Heat shock
HSP	Heat shock protein
HSV	Herpes simplex virus

HT/Ht	Herbicide tolerant/tolerance
I	*Inhibitor* transposon
i.p.	Intraperitoneal
iAc	*Immobile Ac* transposon
IBA	Indole-3-butyric acid
ICTSD	International Center for Trade and Sustainable Development
id1	*indeterminate1* gene
ida	*inflorescence deficient in abscission* gene
IDD	ID-domain
IFN	Interferon
IgA	Immunoglobulin A
IL-12	Interleukin-12
IMI	Imidazolinone
IPM	Integrated pest management
IPP	Isopentyl diphosphate
IPR	Intellectual Property Rights
IPT	Isopentenyl transferase/ Isopentyl transferase
IR	Insect resistant/resistance
IRGSP	International Rice Genome Sequencing Project
ISAAA	International Service of AgriBiotech Applications
ISIS	Institute for Science and International Security
ISR	Induced systemic resistance
JA	Jasmonic acid
LB	Left border of T-DNA
LD	Long-day
LEA	Late embryogenesis abundant
Leu	Leucine
LFY	*Leafy* gene
LOG	*Lonely guy* gene
LOX1/2/3	*Lipoxygenase* gene
lpa1	Lysophosphatidic acid receptor
LPS	Lipopolysaccharide
LRR	Leucine rich repeats
LTB	Heat-labile enterotoxin, subunit B
Lys	Lysine
MAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MGDG	Monogalactosyldiacylglycerol
MHBsAg	Middle HBsAg
Mi	*Meloidogyne incognita* resistance gene
MIP	Major intrinsic protein
miRNA	Micro-RNA
MPSS	Massively parallel signature sequencing
mRNA	Messenger-RNA

MS	Mass spectrometry
MS	Murashige and Skoog (medium)
MST	Members of the Landless Rural Workers Movement
MT	Metallothionein
MTP	Metal-tolerance protein
MTT	Multi-tasking transgenics
Mu	*Mutator* transposon
MuIL-12	Murine IL-12
MV	Methyl viologen
MVL	Microcystis viridis lectin
NAA	α-Napthalene acetic acid
NAM	Napthaleneacetamide
NAS	National Academy of Sciences
NAS	Nicotinamine synthase
NBS	Nucleotide binding site
NCBI	National Center for Biotechnology Information
NDV	Newcastle disease virus
NEPA	National Environmental Policy Act
Neu5Ac	5-*N*-Acetyl-D-neuraminic acid
NIH	National Institute of Health
NIL	Near-isogenic line
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOI	Notice of Intent
NoV	Norovirus
NR	Nitrate reductase
NST	Nac secondary wall thickening promoter factor
NUE	Nitrogen use efficiency
o2	*opaque-2* gene
OECD	Organization for Economic Cooperation and Development
OFB	Office of Food Biotechnology
OMT	O-Methyl transferase
ORF	Open reading frame
Ori	Origin of replication
OSTP	Office of Science and Technology Policy
PAHs	Polycyclic aromatic hydrocarbon
PAL	Phenyalanine ammonia lyase
PAMPs	Pathogen associated molecular patterns
PAT	Phosphinothricin-acetyltransferance
PC	Phytochelatin
PCB	Polychlorinated biphenyl
PCD	Programmed cell death
PCR	Polymerase chain reaction
PCS	Phytochlelatin synthase

PEG	Polyethylene glycol
PETN	Pentaerythritol tetranitrate
PG	Polygalacturonase
PG	Phosphatidylglycerol
PHB	Polyhydroxybutyrate
pi	*pistillata* gene
PiP	Plant Incorporated Protectant
PIP	Plasma membrane intrinsic protein
PL	Pectate lyase
PLC	Phospholipase C
PLD	Phospholipase D
PLE	Phospholipid cleaving enzyme
PME	Pectin methylesterase
PMP	Plant-made pharmaceutical
PNT	Plant with novel trait
PPO	Polyphenol oxidase
PR	Pathogenesis-related
Pro	Proline
PS	Phytosiderophores
PSI	Photosystem 1
PSII	Photosystem 2
PTGS	Post-transcriptional gene silencing
Put	Putrescine
PVP	Plant Variety Protection
PVX	Potato virus X
PyMSP4/5	Murine *P. yoelii* merozoite surface protein 4/5
QPM	Quality protein maize
QTL	Quantitative trait loci
RAP-DB	Rice Annotation Project-Database
rasiRNA	Repeat-associated siRNA
RB	Non-toxin B-chain from ricin
RB	Right border of T-DNA
rDNA	Recombinant-DNA
RDX	Hexahydro-1,3,5-trinitro-1,3,5 triazine
Rf	*Restorer of fertility* gene
RFLP	Restriction fragment length polymorphism
R-gene	Resistance-gene
rGSII	Recombinant *Griffonia simplicifolia* lectin II
rhCVFVIII	Recombinant human clotting factor VIII
rhEPO	Recombinant human erythropoietin
rhIF	Recombinant human intrinsic factor
RHS	Royal Horticultural Society
RID1	Rice *Indeterminate1* gene
RIL	Recombinant inbred line

rin	*ripening inhibitor* gene
Rip	Ribosome inactivating protein
RNAi	RNA-interference
ROIs	Reactive oxygen intermediates
ROS	Reactive oxygen species
RT-PCR	Reverse transcriptase-PCR
RWC	Relative water content
s.c.	Subcutaneously
SA	Salicylic acid
SA	Splice acceptor
SAGE	Serial analyses of gene expression
SAGPyA	Secretariat of Agriculture, Livestock, Fisheries and Food
SAM	S-Adenosylmethionine
SAM	Shoot apical meristem
SAR	Systemic acquired resistance
scFv	Single chain variable fragment
scN	Soyacystatin N
SD	Short-day
SE	Substantial equivalence/ equivalent
SENASA	National Agri-food Health and Quality Service
Ser	Serine
SFI1	*Segestria florentina* venom peptide
sh2	*shrunken2* gene
siRNA	Short/Small interfering RNA
SIV	Simian immunodeficiency virus
SL	Selenocysteine lyase
SMT	Seleno-cysteine methyl transferase
soc	*suppessor of overexpression of constans* gene
SOC1	*Suppressor of Overexpression of Constans1* gene
SOD	Superoxide dismutase
SOliD	Supported Oligo Ligation Detection
Spd	Spermidine
Spm	Spermine
Spm	*Suppressor-Mutator* transposon
SQDG	Sulfoquinovosyldiacylglycerol
ssRNA	Single-stranded RNA
STP	Signal transduction pathway
su1	*sugary1* gene
SVN	Scytovirin
TA	Transcriptional activator
TAC	*Tiller angle control* gene
TAGI	The Arabidopsis Genome Initiative
tasiRNA	Transacting siRNA
tb1	*teosinte branched1* gene

TCE	2.4,6-Trichloroethylene
TCOH	Chloral and trichoethanol
TCP	2,4,6-Tricholorophenol
T-DNA	Transferred-DNA
TDZ	Thidiazuron
TET	Transiently expressed transposase
TETRYL	*N*-Methyl-*N*, 2, 4, 6-tetranitroaniline
TF	Transcription factor
TFL	*Terminal Flower* gene
Thr	Threonine
ti	Trypsin inhibitor allele
TILLING	Targeting induced local lesions in genomes
TIP	Tonoplast intrinsic protein
TMV	Tobacco mosaic virus
TNT	Trinitrotoluene
Trp	Tryptophan
TRV	Tobacco rattle virus
TSCA	Toxic Substances Control Act
UAS	Upstream activator sequence
uf	*uniflora* gene
UN	United Nations
UNCTAD	United Nations Conference on Trade and Development
US	United States
USAID	US Agency for International Development
USDA	United States Department of Agriculture
USPTO	United States Patent and Trademark Office
UV	Ultraviolet
VB	Vector backbone
Vgt1	*Vegetative to generative transition1* gene
Vgt2	*Vegetative to generative transition2* gene
VIGS	Virus-induced gene silencing
VIP	Vegetative Insecticidal Protein
VLP	Virus-like particle
VRO	Variety Registration Office
WHO	World Health Organisation
WT	Wild type
WUE	Water use efficiency
XTH	Xyloglucan endotransglucosylase/hydrolase
Xyl	Xylose
XylT	Xylosyltransferase
YCF1	Yeast vacuolar glutathione Cd transporter
YFP	Yellow fluorescent protein
ZCN	*Zea CENTRORADIALIS* gene
zfl1	*Zea FLO/LFY1* gene

zfl2	*Zea FLO/LFY2* gene
ZFN	Zinc-finger nuclease
ZMM4/5	*Zea mays FULL1*-like gene
ZmRap2	*Zea mays related to AP2* gene
γ-GCS	γ-Glutamyl cysteine synthetase
ω3	Omega 3

Chapter 1
Transgenic Crop Plants for Resistance to Biotic Stress

N. Ferry and A.M.R. Gatehouse

1.1 Introduction

> We couldn't feed today's world with yesterday's agriculture and we won't be able to feed tomorrow's world with today's. – Lord Robert May, President of the Royal Society, March 2002

The human population is everexpanding; conservative estimates predict that the population will reach ten billion by 2050 (United Nations Population Division), and the ability to provide enough food is becoming increasingly difficult (Chrispeels and Sadava 2003). The planet has a finite quantity of land available to agriculture and the need for increasing global food production has led to increasing exploitation of previously uncultivated land for agriculture; as a result wilderness, wetland, forest and other pristine environments have been, and are being, encroached upon (Ferry and Gatehouse 2009). The minimization of losses to biotic stress caused by agricultural pests would go some way to optimizing the yield on land currently under cultivation. For nearly 50 years, mainstream science has told us that this would be impossible without chemical pesticides (Pimental 1997). The global pesticide market is in excess of $30 billion per year (Levine 2007); despite this, approximately 40% of all crops are lost directly to pest damage (Fig. 1.1).

These figures are simplified rough estimates; in reality crop losses to biotic stress are extremely difficult to quantify and vary by crop, year, and region.

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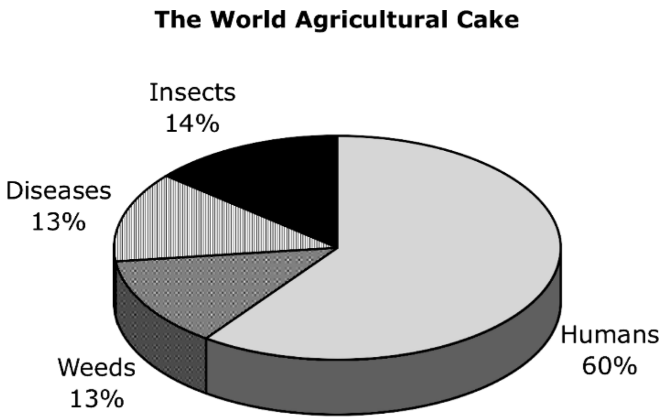

Fig. 1.1 The world agricultural cake

1.2 Biotic Stress due to Insect Pests

1.2.1 Crop Losses due to Insect Pests

> [A]nimals annually consume an amount of produce that sets calculation at defiance; and, indeed, if an approximation could be made to the quantity thus destroyed, the world would remain skeptical of the result obtained, considering it too marvelous to be received as truth.
> – John Curtis, 1860

Arthropods are the most widespread and diverse group of animals, with an estimated 4–6 million species worldwide (Novotny et al. 2002). While only a small percentage of arthropods are classified as pests, they cause major devastation of crops, destroying around 14% of the world annual crop production, contributing to 20% of losses of stored grains and causing around US$100 billion of damage each year (Nicholson 2007).

Herbivorous insects and mites are a major threat to food production for human consumption. Larval forms of lepidopterans are considered the most destructive insects, with about 40% of all insecticides directed against heliothine species (Brooks and Hines 1999). However, many species within the orders Acrina, Coleoptera, Diptera, Hemiptera, Orthoptera and Thysanoptera are also considered agricultural pests with significant economic impact (Fig. 1.2).

Insect pests may cause direct damage by feeding on crop plants in the field or by infesting stored products and so competing with humans for plants as a food resource. Some cause indirect damage, especially the sap-feeding (sucking) insects by transmitting viral diseases or secondary microbial infections of crop plants.

Fig. 1.2 Phylloxera, a sap-sucking pest of grape, almost devastated the European wine industry in the nineteenth century

1.2.1.1 The Phylloxera Plague

In the late nineteenth century, a phylloxera epidemic destroyed most of the vineyards for wine grapes in Europe, most notably in France. Grape phylloxera (*Daktulosphaira vitifoliae*, family Phylloxeridae) is a pest of commercial grapevines worldwide, originally native to eastern North America. These minute, pale yellow sap-sucking insects feed on the roots of grapevines. In *Vitis vinifera*, the resulting deformations and secondary fungal infections can damage roots, gradually cutting off the flow of nutrients and water to the vine. *Phylloxera* was inadvertently introduced to Europe in the 1860s. The European wine grape *V. vinifera* was highly susceptible to the pest and the epidemic devastated most of the European wine-growing industry. Some estimates hold that between two-thirds and nine-tenths of all European vineyards were destroyed. Native American grapes *Vitis labrusca* are naturally *Phylloxera*-resistant. The grafting of European grape vines onto resistant grape rootstock is the preferred method to cope with the pest problem even today (http://www.calwineries.com). Thus, phylloxera provides a clear example of how a single insect pest can nearly devastate a whole industry.

Innumerable examples exist of insect pests that are highly injurious to agricultural production. The most notable for their destructive capacity being the

Fig. 1.3 Monument to the cotton boll weevil *Source*: Wikimedia Commons

migratory locust (*Locusta migratoria*), Colorado potato beetle (*Leptinotarsa decemlineata*), boll weevil (*Anthonomus grandis*), Japanese beetle (*Popillia japonica*), and aphids, which are among the most destructive pests on earth as vectors of plant viruses (many species in ten families of the Aphidoidea), and the western corn rootworm (*Diabrotica virgifera virgifera*), also called the billion dollar bug because of its economic impact in the US alone.

Curiously, one of these pests, the cotton boll weevil, responsible for near-destruction of the cotton industry in North America, is also ultimately responsible for subsequent diversification of agriculture in many regions, thus warranting a monument in the town of Enterprise, Alabama, in profound appreciation of its role in bringing to an end the state's dependence on a poverty crop (Fig. 1.3).

The global challenge facing agriculture is to secure large and high-quality crop yields and to make agricultural production environmentally sustainable. Control of insect pests would go some way towards achieving this goal.

1.2.2 Insecticides

Insecticides have been, and still are, a highly effective method to control pests quickly when they threaten to destroy crops. The chemical nature of the insecticides used has evolved over time. In early farming practices, inorganic chemicals were used for insect control; however, with the advances in synthetic organic chemistry that followed the two world wars the synthetic insecticides were born. In the 1940s, the neurotoxic organochlorine, DDT, was the pesticide of choice, but following its indiscriminate use it was reported to bio-accumulate in the food chain where it affected the fertility of higher organisms – such as birds. Rachel Carson first highlighted this in the book *Silent Spring* published in 1962; while her presumptions have since been proven to be wrong, the book was nevertheless an important signature event in the birth of the environmental movement. This pesticide was subsequently replaced by the comparatively safer organophosphate and carbamate-based pesticides (both acetylcholinesterase inhibitors) and many of these were replaced in turn by the even safer pyrethroid-based pesticides (axonic poisons). Synthetic pyrethroids continue to be used today despite the fact that they are broad-spectrum pesticides.

The major limiting factor on the insecticide strategy is the occurrence of resistance in insect populations. In fact, resistance to insecticides has now been reported in more than 500 species (Nicholson 2007). Furthermore, resistance has evolved to every major class of chemical. The underlying causes of insecticide resistance are manyfold. Owing to wide usage and narrow target range, arthropods have been put under a high degree of selection pressure (Feyereisen 1995). Insecticide resistance may be characterized by:

(a) Metabolic detoxification (upregulation of esterases, glutathione-*S*-transferases, and monoxygenases)
(b) Decreased target site sensitivity (via mutation of the target receptor)
(c) Sequestration or lowered insecticide availability

In addition, cross-resistance to different classes of chemicals has occurred because of the fact that many insecticides target a limited number of sites in the insect nervous system (Raymond-Delpech et al. 2005). The five target sites in insects comprise: nicotinic acetylcholine receptors (e.g., imidacloprid), voltage-gated sodium channels (e.g., DDT, pyrethroids), γ-aminobutyric acid receptors (e.g., fipronil), glutamate receptors (e.g., avermectins), and acetylcholinesterase (AChE) (e.g., organophosphates and carbamates). The world insecticide market is dominated by compounds that inhibit the enzyme AChE. Together, AChE inhibitors and insecticides acting on the voltage-gated sodium channel, in particular the pyrethroids, account for approximately 70% of the world market (Nauen et al. 2001).

Unfortunately, as insecticide target sites are conserved between invertebrates and vertebrates, insecticides have undesirable nontarget effects and unacceptable ecological impacts. Insecticides are implicated in the poisoning of nontarget insects, other arthropods, marine life, birds, and humans (Fletcher et al. 2000). The poisoning of nontarget organisms has obvious implications for biodiversity.