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Transgenic Crops IV

Edited by
E.C. Pua and M.R. Davey

With 36 Figures, 8 in Color, and 33 Tables

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Series Editors

Professor Dr. Toshiyuki Nagata
University of Tokyo
Graduate School of Science
Department of Biological Sciences
7-3-1 Hongo, Bunkyo-ku
Tokyo 113-0033, Japan

Professor Dr. Horst Lörz
Universität Hamburg
Institut für Allgemeine Botanik
Angewandte Molekularbiologie
der Pflanzen II
Ohnhorststraße 18
22609 Hamburg, Germany

Professor Dr. Jack M. Widholm
University of Illinois
285A E.R. Madigan Laboratory
Department of Crop Sciences
1201 W. Gregory
Urbana, IL 61801, USA

Volume Editors

Professor Dr. Eng-Chong Pua
School of Arts and Sciences
Monash University Malaysia
2 Jalan Universiti, Bandar Sunway
46150 Petaling Jaya, Selangor, Malaysia

Professor Dr. Michael R. Davey
Plant Sciences Division
School of Biosciences
University of Nottingham
Sutton Bonington Campus
Loughborough LE12 5RD, UK

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Dedicated to Linda Davey
for more than thirty-five years of unfailing support

Michael R. Davey

Preface

Exciting developments in crop biotechnology in recent years have prompted the necessity to update the first series of *Transgenic Crops I*, *II* and *III*, published in 1999 and 2001. In this current endeavor, 69 chapters have been compiled, contributed by a panel of experts in crop biotechnology from 26 countries. These chapters are grouped into three volumes, namely *Transgenic Crops IV*, *V* and *VI*. This new series not only reviews recent advances in cell and tissue culture and genetic transformation methodologies, but also presents aspects of the molecular genetics of target crops and the practical applications of transgenic plants. In addition, more than 30% of crop species that were not discussed previously are included in the present series.

This new series commences with the volume *Transgenic Crops IV*, consisting of 23 chapters that focus on cereals, vegetables, root crops, herbs and spices. Section I is an introductory chapter that places into perspective the impact of plant biotechnology in agriculture. Section II focuses on cereals (rice, wheat, maize, rye, pearl millet, barley, oats), while Section III is directed to vegetable crops (tomato, cucumber, eggplant, lettuce, chickpea, common beans and cowpeas, carrot, radish). Root crops (potato, cassava, sweet potato, sugar beet) are included in Section IV, with herbs and spices (sweet and hot peppers, onion, garlic and related species, mint) in Section V.

Transgenic Crops V also consists of 23 chapters in three sections devoted to fruit (Section I), trees (Section II) and beverage crops (Section III). Fruit crops target banana, citrus, mango, papaya, pineapple, watermelon, avocado, grape, melon, apple, *Prunus* spp, strawberry and kiwifruit, while trees include rubber, eucalyptus, legumes and conifers. Section III, on beverage crops, reports studies on coffee, cacao, tea and sugarcane.

As in volumes IV and V, *Transgenic Crops VI* has 23 chapters organized in five Sections. Section I targets oil and fiber crops (soybean, rapeseed, sunflower, oil palm, peanut, cotton, flax), followed by medicinally important plants (including ginseng, opium poppy, herbane, bellandonna, *Datura*, *Duboisia*, *Taxus*) in Section II. Ornamentals (roses, carnation, chrysanthemum, orchids, gladiolus, forsythia) are discussed in Section III, while Section IV involves forages and grains (alfalfa, clovers, tall fescue, ryegrasses, lupin). Section V has one chapter that discusses aspects of the freedom to commercialize transgenic plants, together with regulatory and intellectual property issues.

The editors express their sincere thanks to Maggie Yap Lan from Monash University, Malaysia, for her excellent secretarial and editorial assistance. She

forwarded to contributors timely reminders of deadlines, where appropriate, and assisted in editing the manuscripts for typographical errors and formatting.

This series will serve as a key reference for advanced students and researchers in crop sciences, genetics, horticulture, agronomy, cell and molecular biology, biotechnology and other disciplines in life sciences.

E.C. Pua and M.R. Davey

Contents

Section IV Root Crops

List of Contributors

F. Altpeter
Agronomy Department, Genetics Institute, Plant Molecular and Cellular Biology Program, University of Florida, 2191 McCarty Hall, Gainesville, FL 32611, USA, e-mail: faltpeter@ifas.ufl.edu

V. Anggraini
Graduate School Experimental Plant Sciences, Laboratory of Plant Breeding, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands

P. Anthony
Plant Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

F.J.L. Aragão
Embrapa Recursos Genéticos e Biotecnologia, PqEB W5 Norte, Asa Norte, Brasília, DF 70770-900, Brazil, e-mail: aragao@cenargen.embrapa.br

S. Biemelt
Lehrstuhl für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Staudtstrasse 5, 91058 Erlangen, Germany

F. Börnke
Lehrstuhl für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Staudtstrasse 5, 91058 Erlangen, Germany

D.A. Brummell
Crop and Food Research, Food Industry Science Centre, Batchelar Road, Private Bag 11600, Palmerston North, 4474, New Zealand

W. Burza
Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture and Landscape Architecture, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland

F.A.P. Campos
Universidade Federal do Ceará, Departamento de Bioquímica e Biologia Molecular, Bloco 907, Campus do Pici, Fortaleza, CE 60451-970, Brazil

A. Carlson
Department of Agronomy, University of Wisconsin–Madison, 1575 Linden Drive, Madison, WI 53706, USA

M. Chen
State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

J.-J. Cheong
School of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea

Y.D. Choi
School of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea,
e-mail: choiyngd@snu.ac.kr

I.S. Curtis
Centre for Plant Sciences, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK, e-mail: curtisis2004@yahoo.co.uk

S.K. Datta
Genomics and Plant Biotechnology Laboratory, Botany Department, University of Calcutta, Kolkata 700 019, India, e-mail: swpndatta@yahoo.com

M.R. Davey
Plant Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK,
e-mail: mike.davey@nottingham.ac.uk

B. Frame
Center for Plant Transformation, Plant Science Institute, and Department of Agronomy, Iowa State University, Ames, IA 50011-1010, USA

M. Girgi
Developmental Biology and Biotechnology, University of Hamburg, Biocenter Klein Flottbek, Ohnhorststrasse 18, 22609 Hamburg, Germany,
e-mail: girgi@botanik.uni-hamburg.de

M.J. Giroux
Department of Plant Sciences and Plant Pathology, 119 Ag. BioSciences, Montana State University, P.O. Box 173150, Bozeman, MT 59717, USA,
e-mail: mgiroux@montana.edu

T.J.V. Higgins
CSIRO Plant Industry, G.P.O. Box 1600, Canberra, ACT 2601, Australia,
e-mail: TJ.Higgins@csiro.au

J. Jayaraj
Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada

M. Joersbo
Danisco Seed, Højbygårdvej 31, 4960 Holeby, Denmark,
e-mail: shmj@danisco.com

F. Jullien
Laboratoire de Biotechnologies Végétales (BVpam), Faculté des Sciences, Université Jean Monnet, 23 rue du Dr Michelon 42023 St Etienne Cedex 2, France, e-mail: jullien@univ-st-etienne.fr

H.F. Kaeppler
Department of Agronomy, University of Wisconsin–Madison, 1575 Linden Drive, Madison, WI 53706, USA, e-mail: hfkaeppl@facstaff.wisc.edu

K.J. Kasha
Department of Plant Agriculture, University of Guelph, Guelph, Ontario N1G 2W1, Canada, e-mail: kkasha@uoguelph.ca

H. Koehorst-Van Putten
Graduate School Experimental Plant Sciences, Laboratory of Plant Breeding, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands

V. Korzun
Lochow-Petkus GmbH, Bollersener Weg 5, 29303 Bergen, Germany

S.V. Kumar
Plant Polyamine and Transgenic Research Laboratory, Department of Genetics, University of Delhi–South Campus, New Delhi 110 021, India

J. Li
State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China, e-mail: jyli@genetics.ac.cn

K.C. Lowe
School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD, UK

S. Malepszy
Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture and Landscape Architecture, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland,
e-mail: stefan_malepszy@sggw.pl

F.D. Meyer
Department of Plant Sciences and Plant Pathology, 119 Ag. BioSciences, Montana State University, P.O. Box 173150, Bozeman, MT 59717, USA

M.M. O'Kennedy
CSIR, Food, Biological and Chemical Technologies (Bio/Chemtek), P.O. Box 395, Pretoria 0001, South Africa

M. Otani
Ishikawa Agricultural College, Research Institute of Agricultural Resources, Suematu, Nonoichi, Ishikawa 921-8836, Japan

R. Pathirana
Crop and Food Research, Food Industry Science Centre, Batchelar Road, Private Bag 11600, Palmerston North, 4474, New Zealand,
e-mail: brummelld@crop.cri.nz

I. Pereira
Graduate School Experimental Plant Sciences, Laboratory of Plant Breeding, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands

W. Plader
Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture and Landscape Architecture, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland

J.C. Popelka
CSIRO Plant Industry, G.P.O. Box 1600, Canberra, ACT 2601, Australia

J.B. Power
Plant Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

Z.K. Punja
Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada, e-mail: punja@sfu.ca

K. Raemakers
Graduate School Experimental Plant Sciences, Laboratory of Plant Breeding, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands (Present address: Genetwister, Nieuwe Kanaal Weg 7b, 6709 PA Wageningen, The Netherlands), e-mail: c.j.j.m.raemakers@genetwister.nl

M.V. Rajam
Plant Polyamine and Transgenic Research Laboratory, Department of Genetics, University of Delhi–South Campus, New Delhi 110 021, India, e-mail: mv_rajam@hotmail.com

M. Schreuder
Graduate School Experimental Plant Sciences, Laboratory of Plant Breeding, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands

G. Seymour
School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

T. Shimada
Ishikawa Agricultural College, Research Institute of Agricultural Resources, Suematu, Nonoichi, Ishikawa 921-8836, Japan, e-mail: shimada@ishikawa-c.ac.jp

S.I. Song
Division of Bioscience and Bioinformatics, Myongji University, Yongin 449-728, Korea

U. Sonnewald
Lehrstuhl für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Staudtstrasse 5, 91058 Erlangen, Germany, e-mail: usonne@biologie.uni-erlangen.de

F. Torney
Center for Plant Transformation, Plant Science Institute, and Department of Agronomy, Iowa State University, Ames, IA 50011-1010, USA

G. Tucker
School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK, e-mail: Gregory.tucker@nottingham.ac.uk

P. Van Hooff
Plant Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

R. Visser
Graduate School Experimental Plant Sciences, Laboratory of Plant Breeding, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands

P. Walley
Warwick HRI, University of Warwick, Wellesbourne CV35 9EF, UK

O. Wally
Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, Canada

K. Wang
Center for Plant Transformation, Plant Science Institute, and Department of Agronomy, Iowa State University, Ames, IA 50011-1010, USA,
e-mail: kanwang@iastate.edu

Y. Wang
State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Section I Plant Biotechnology in Agriculture

I.1 Impact of Plant Biotechnology in Agriculture

S.K. Datta[1]

1 Introduction

Ever since the advent of agriculture, there has been a need to improve crop plants for increased productivity, improved quality and to satisfy changing human preferences. This need is more acutely felt today and, particularly, in the developing world where the population is continuing to increase.

Genetic modification of plants probably began through selection of novel types about 10,000 years ago when human agricultural activities began and useful results were often a product of random or chance events. Through elucidation of the laws of genetics, molecular tools for understanding plant biology, plant breeding became a deliberate and predictable activity with the result that tailor-made crops are now in place (Table 1). Traditional plant-breeding methods have been very successful and have helped provide the volume of food required to allow the world population to grow to its present 6×10^9. Breeding efforts have provided remarkable diversity amongst various crop species and even some new crops, such as triticale, in addition to the introduction of new genes from wild species (Brar and Khush 1997). However, recent trends in crop productivity indicate that traditional methods alone will not be able to keep pace with the growing demands for food, fibre and fuel. The yield increases in many food crops have hit a plateau or have fallen below the rate of population increase. Farmers in South and Southeast Asia must consistently produce an extra 30% more cereals in order to maintain current nutrition levels and food security. Biotechnology offers a challenging role to reduce the gap of yield improvement (Hossain et al. 2000; Lorz et al. 2000; Miflin 2000; Phillips 2000; Khush 2001; Datta et al. 2003a, b; Vasil 2005; Mackill 2006). This task does not become any easier with diminishing land and water resources. Plant biotechnology and, in future, nanotechnology, can bolster plant-breeding efforts to meet these new challenges in a sustainable way (Helmke and Minerick 2006).

Conventional plant breeding is often limited by reproductive barriers. The developments in the area of plant biology in the past three decades, such as plant genetic transformation, have opened up new vistas in crop improvement, thereby allowing transfer of desirable gene(s) across species and genera (overruling cross-ability barriers that limit the scope of conventional breed-

[1] Genomics and Plant Biotechnology Laboratory, Botany Department, University of Calcutta, Kolkata 700 019, India, e-mail: swpndatta@yahoo.com

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Table 1. Some classic developments in plant biotechnology and transgenic research

Achievement in plant biotechnology and transgenics	Response/ transgene	System/method	References
Haploidy in *Datura*	Microspore development	Anther culture	Guha and Maheshwari (1964)
Cereal protoplast regeneration	Protoplast culture	Protoplast system	Vasil and Vasil (1980)
Protoplast fusion product	Protoplast fusion	Protoplast	Gleba and Hoffmann (1980)
First transformation event demonstrated in tobacco; bean phaseolin transferred to sunflower and tobacco	*gus*; phaseolin	*Agrobacterium* for both achievements	Bevan et al. (1983), Fraley et al. (1983), Herrea-Estrella et al. (1983), Murai et al. (1983)
First report of a bacterial gene expression in tobacco	*npt*II	*Agrobacterium*	Horsch et al. (1984)
Method for interspecific hybrids		Protoplasts	Sundberg and Glimelies (1986)
Biolistic transformation through particle gun bombardment established	*gus*	Biolistic	Sanford et al. (1987)
First stable soybean transgenics developed	Glyosphosphate tolearance	*Agrobacterium*	Hinchee et al. (1988)
First stable transgenic japonica rice	*Hph*	Protoplasts	Shimamoto et al. (1989)
First stable fertile homozygous transgenic indica rice	*Hph*	Protoplasts	Datta at al. (1990)
Transgenic insect-resistant cotton	*Bt*	Biolistic	Perlak et al. (1990)
Fertile transgenic indica rice	*Bar*, *gus*	Biolistic	Christou et al. (1991)
Herbicide-tolerant indica rice developed	*Bar*	Protoplasts (PEG)	Datta et al. (1992)
First successful stable herbicide-resistant wheat	*Bar*	Biolistic	Vasil et al. (1992)
Transgenic fertile japonica and indica rice	*Hph*	*Agrobacterium*	Hiei et al. (1994)
First stable independent barley transgenics developed	*Bar*, *gus*	Biolistic	Jahne et al. (1994), Wan and Lemaux (1994)
Transgenic fertile barley; transgenic red fescue	*Bar*, *gus*; *hph*	Protoplasts for both achievements	Spangenberg et al. (1994)
First detailed report on the comparative efficiency of different promoters driving agronomically important gene	*cry1A(b)*, *cry1A(c)*, *Cry1A(b)/ cry1A(c)*	Biolistic, protoplasts	Datta et al. (1998)

Table 1. (continued)

Achievement in plant biotechnology and transgenics	Response/ transgene	System/method	References
First iron-rich Japonica rice	*ferritin*	*Agrobacterium*	Goto et al. (1999)
First field testing of transgenic rice with agronomically important genes	*Bt*, *Xa21*	Biolistic	Tu et al. (2000a, b)
β-Carotene-rich (golden) rice	*Psy*, *lyc*, *crtI*	*Agrobacterium*	Ye et al. (2000)
Protein-improved potato	*Ama1*	*Agrobacterium*	Chakrovorty et al. (2000)
Nutrition improvement in commercial indica rice	*Ferritin*, *psy*, *crt1*	Molecular breeding	Datta et al. (2003a), Vasconcelos et al. (2003)
Molecular biopharming	Several genes	Chloroplast	Daniell et al. (2004)
Marker-free and enhanced carotenoids in rice	*Crt1*, *psy*	*Agrobacterium*	Paine et al. (2005), Parkhi et al. (2005)
QTL for plant regeneration; yield improvement	Rice QTL, *GN1*, *SD1*	Molecular breeding	Ashikari et al. (2005), Nishimura et al. (2005)
Environment-friendly transgenic crop	*Cry* genes	Molecular breeding	Chen et al. (2006)
Intragenic vectors (gene transfer without foreign DNA)	Intragenic vector	Molecular breeding	Conner et al. (2006)
Post-transgeneration enhanced targeted end-products	*Psy, crt1* genes	Molecular breeding	Datta et al. (2006)

ing) for developing transgenic plants with novel traits, such as built-in resistance/tolerance to several biotic and abiotic stresses, improving nutritional qualities and grain filling (Potrykus 1990; Goldberg 2001). Moreover, the advances in genetic transformation techniques provide plant breeders access to new and broader gene pools. Transgenic plants can be considered as the most recent development in our efforts to genetically improve crops.

2 Crops and Genomics

Genomics implies DNA sequencing, the routine use of DNA microarray technology to analyse the gene expression profile at the mRNA level, and improved information tools to organize and analyse such data. Genomics-based strategies for gene discovery, coupled with the high-throughput transformation process, will accelerate the identification of candidate genes. The recent reports on rice genome sequencing by Monsanto, the International Rice Genome Sequencing Project (IRGSP), the Beijing Genome sequencing (BGI) and Novartis, and completion of the genome sequencing of *Arabidopsis*, will accelerate gene discovery and further crop improvement (Datta 2004; Vasil 2005).

2.1 Addressing Issues/Concerns in the Post-Genomics Era

How do we reorganize crop breeding in the genomics era, particularly in using DNA chip/microarray? How does rice/*Arabidopsis*/tomato genome discovery help us in such an endeavour? How do we move forward with such knowledge-based intensive technology and obtain public confidence, particularly in solution to the working together of the public and private sectors? We must be sure to respect intellectual property rights (IPR) while farmers' plant varietal protection (PVP) rights also need to be respected. The awareness of mutual interest and respect will serve this purpose and will benefit all in society. How do we convince policy makers of national governments to take the advantage of the combined green-and-gene revolution to reach most farmers whose livelihood can be improved by such knowledge-based intensive technology? This task poses many challenges and will provide rewards for human welfare.

3 Genetic Transformation of Plants

Plant transformation was first demonstrated independently in 1983 by three research groups at Gent (Belgium), Monsanto (St Louis) and a collaborating group from Washington State University, St Louis and Cambridge University, UK (Bevan et al. 1983; Fraley et al. 1983; Herrera-Estrella et al. 1983). All three groups transferred and expressed bacterial antibiotic resistance genes, using the *Agrobacterium*-mediated method. However, plant transformation became routine in the 1990s, a decade after genetically engineered human insulin went on sale. After the first report of gene transfer with the seed protein phaseolin from bean to sunflower and tobacco (Murai et al. 1983) and a bacterial gene for neomycin phosphotransferase II (*npt*II) to tobacco a year later (Horsch et al. 1984), plants have been transformed with a range of genes from other species and genera, and with those from bacteria, viruses and animals. Following dramatic progress in the improvement of transformation technology, more than 50 different species of transgenic plants have been produced, both including those of monocotyledons and dicotyledons, and some (including rice in China) are under field assessments worldwide (James 2005). A selective description of the development of biotechnological tools and product is summarized in Table 1.

Many transformation approaches have been tested in the past for their comparative efficiency and efficacy, including *Agrobacterium tumefaciens*-mediated transformation and direct gene transfer, i. e., protoplast- and biolistic-mediated procedures (Vasil and Vasil 1980; Datta et al. 1990; Potrykus 1990; Christou et al. 1991; Datta and Datta 2001; Altpeter et al. 2005; Vasil 2005).

3.1 Methods of Gene Transfer

Amongst the methods available, *Agrobacterium* and biolistic methods are the most widely explanted.

3.1.1 Agrobacterium tumefaciens

Agrobacterium tumefaciens is a soil-borne, Gram-negative bacterium which is capable of genetically colonizing susceptible host plants. It is capable of transferring any piece of DNA inserted in its T-DNA between a pair of direct repeats called border sequences, with the help of a site-specific, strand-specific endonuclease. This feature has been extensively exploited in the genetic transformation of plants. Different strains of *Agrobacterium* have different host ranges and some crop plants, particularly monocotyledons, are considered recalcitrant to *Agrobacterium* infection. Several strategies have been implemented to overcome this recalcitrance issue. Very often T-DNA integration occurs in transcriptionally active regions of the plant genome and hence the expression of the transgene becomes a routine phenomenon. A detailed insight into the *Agrobacterium*-mediated DNA transfer process into plant cells is given in the report of Zupan and Zambryski (1995). A number of variants of the *Agrobacterium*-mediated transformation protocol have been used to transform *Arabidopsis*, the model plant. Many laboratories routinely transform *Arabidopsis* using the whole plant or the floral dip method, which is efficient and easy to practice. A recent modification of the floral dip method, called the floral spray method, might help in expanding this approach to other plants (Chung et al. 2000).

3.1.2 Biolistic Transformation

Biolistic transformation (also referred to as particle gun bombardment or microprojectile bombardment) is carried out by shooting DNA-coated tungsten or gold particles into target tissue (Sanford et al. 1987). The microprojectiles can be accelerated with gun powder, helium or an electric discharge. The advantage of the method is that any tissue can be transformed, provided that tissue can be regenerated through culture into plants. Usually, transformation using this method results in complex patterns of DNA integration as compared with T-DNA transfer that usually results in precise, low-copy integrations and simple integration patterns (Tinland 1996; Parkhi et al. 2005). Co-suppression of the transgene/endogenous gene can occur due to integration of multiple copies of the transgene (Flavell 1994). Transfer of long DNA molecules can be a challenge, since the molecules can be sheared due to the forces involved in accelerating the microprojectile, unlike the case in *Agrobacterium*-mediated gene delivery (Hamilton et al. 1996). Generally, the whole plasmid representing the clone of the transgene is bombarded into the target tissue, resulting

in the integration of vector backbone into the plant genome, as is also possible in the case of *Agrobacterium*-mediated gene delivery (Ramanathan and Veluthambi 1995). Variations of the protocol in which only the transgene is introduced as a linear fragment with bombardment of a minimal expression cassette also exist with efficient transformation (Fu et al. 2000; Datta et al., unpublished data). The biolistic method is appropriate in transforming plants that are known to be recalcitrant to *Agrobacterium*-mediated transformation. Its utility in transient expression studies is also immense. Many of the commercially available transgenic plants have been developed by biolistic transformation.

3.2 Promoters Used in Transgenic Crops

The fate of the introduced gene(s) in the transgenic plant depends largely on the promoter that drives its expression as well as its position in the genome. Promoter sequences upstream to the gene(s) of interest are very important in plant transformation for determining the levels and patterns of transgene expression. Two major categories of promoters, namely constitutive and tissue-specific, are used extensively. Constitutive promoters direct the expression of a foreign gene in all plant tissues at all stages of plant development, with some variation in expression across tissues and stages of organ development. This group of promoters include the cauliflower mosaic virus 35S (CaMV 35S) promoter, rice actin I (*Act* I) promoter, maize ubiquitin (*Ubi* 1) promoter and maize alcohol dehydrogenase I (*Adh* 1, also called *Emu*) promoter; barley hordein promoter, etc. (Cho et al. 1999; Bajaj and Mohanty 2005). A hierarchy of several constitutive promoters was shown on the basis of levels of transient expression of the *gus* transgene in rice suspension cell cultures namely: *Ubi* 1 > *Act* 1 > *Adh* 1 > CaMV 35S (Li et al. 1997). However, such a comparison would be more meaningful when data become available based on the stable transformation of at least ten events for each construct (different promoters + other elements of genetic transformation remain constant). Further, comparison would also be effective when a single transgene (one copy vs. multiple copies) in an homozygous state is compared with the event of a different transgene under similar conditions (one copy vs. multiple copies). The CaMV 35S and *Act* 1 promoters have been shown to strongly drive the constitutive expression of transgenes in rice (Datta et al. 1990, 1999; Lin et al. 1995; Tu et al. 1998a).

Tissue-specific promoters drive the spatial and temporal expression of the transgene(s). Such promoters studied and used so far in rice and other monocotyledons include the maize phosphoenol pyruvate carboxylase (PEPC) promoter driving green tissue-specific expression, pith-specific, wound-inducible, root-specific, endosperm-specific, pollen-specific and stress-inducible promoters (Bajaj and Mohanty 2005). These promoters are useful for directing the expression of the transgenes in only those tissues where and when it is required.

The expression of the introduced genes also varies depending on where they are integrated in the plant genome. In order to normalize gene expression and to reduce position effects, matrix attachment regions (MARs) have been used in the gene construct for both biolistic and *Agrobacterium*-mediated transformations (Lucca et al. 2001).

3.3 Selectable Markers Used in Development of Transgenic Crops

The selection of putative transgenic tissues following transformation, irrespective of the methods of gene delivery, is the key step for the final recovery of transgenic plants. Dominant selectable markers are an integral part of plant transformation strategies. For this purpose, a selectable marker gene is used either co-integrated in the plasmid with the gene of interest or harbored on a separate plasmid for co-transformation. A number of selective agents and suitable resistance genes have been investigated concurrently with studies on gene transfer and cell culture.

The most widely used inhibitors are kanamycin, geneticin (G418) and hygromycin. All are aminoglycoside antibiotics which interfere with the translation machinery of prokaryotic and eukaryotic cells. However, they can be inactivated by phosphorylation reactions mediated by the products of either the Tn5 neomycin phosphotransferase II (*npt*II gene, also known as *aph*II or *neo*; Herrera-Estrella et al. 1983), or the hygromycin phosphotransferase gene (*hph*, also called *hpt* or *aph*-IV) originally isolated from *Eschericia coli* (Blochinger and Diddelmann 1984). Although kanamycin has been successfully used as a selective agent in plant transformation, it has some limitations, such as its low efficiency in screening transformed calli and the inability of transformed calli of some species to regenerate green plants. These problems were circumvented by the use of G418 (Peterhans et al. 1990). Currently, the hygromycin B-resistance gene is widely used as an efficient selective agent for almost all the transformation methods in several crops, including rice, without any problems relating to albino plant regeneration or plant fertility (Datta et al. 1990; Lin et al. 1995; Tu et al. 1998a, b).

The bialaphos (also called Basta) resistance gene (*bar*), encodes phosphinothricin (PPT) acetyltransferase (PAT), and acetylated phosphinothricin is no longer inhibitory to glutamine synthase. PPT or Basta has been used as a selective agent for a number of crop plants (Datta et al. 1992; Rathore et al. 1993; Ho et al. 2006). Hence, the usefulness of a particular resistance marker depends upon the characteristics of the selection agent, the resistance gene and the plant material (Angenon et al. 1994).

The use of all these genes as selectable markers poses a cautionary risk for the environmental release of the transgenic products. A recent development is based on the use of selective genes, which give the transformed cells a metabolic advantage compared with the untransformed cells, which are starved with a concomitant slow reduction in viability. Such a strategy involves

the use of mannose as the selective agent, which after uptake is phosphorylated by a hexokinase to an unmetabolized mannose-6-phosphate that accumulates in cells, resulting in severe growth inhibition. However, the phosphomannose isomerase gene (*pmi*) allows conversion of mannose-6-phosphate to fructose-6-phosphate, which is readily metabolized. The *pmi* gene as a selectable marker gene has been and is being used for plant transformation, including rice (Joersbo et al. 1998; Datta et al. 2000, 2003a, b, 2006; Lucca et al. 2001).

Similarly, in plant transformation studies, reporter (assessable marker) genes are necessary for rapid detection of DNA introduction. They are usually fused to the plant regulatory sequences in vitro and are used to determine when, where and at what level a regulatory sequence directs gene expression in vivo. Also, they can be used for protein targeting if fused to a signal peptide coding sequence. Such reporter genes of very common use include the luciferase gene (*luc*) and β-glucuronidase (*gus*) genes (Jefferson et al. 1986). The intrinsically fluorescent proteins (IFPs), such as the green, yellow and cyan fluorescent proteins, have been used as reporter genes to monitor transcriptional regulation and protein kinase activity (Dixit et al. 2006).

4 Transgenics in Stabilizing Production

A considerable proportion of the crop produce is lost due to biotic and abiotic stresses. Conventional breeding, which has often exploited the natural variablility in a species, has produced crop varieties with built-in resistance to several of these stress agents. However, in instances where the natural variability is limited or non-existent, transgenic breeding could be a viable and an alternative solution. Transgenic plants that are tolerant to biotic agents, like insect pests, and disease agents, like viruses, fungi and bacteria, have been produced, although only insect- and virus-resistant transgenic crops have been commercialized extensively. Weeds also reduce significantly crop yields. Transgenic crops with resistance to broad-action herbicides have also been commercialized in several countries. These transgenic crops allow the spraying of the herbicide in a standing crop: the weeds are killed while the crop remains unaffected, making weed control more effective and less costly. Further, it allows "no-till" cultivation aiding in soil and water conservation. Abiotic stresses have been more difficult to tackle by transgenic approaches, but some of the recent developments hold considerable promise (Shinozaki et al. 2003; Singlas-Pareek et al. 2003; Verslues et al. 2006).

4.1 Non-Segregating Homozygous Stable Lines

Isogenic lines using marker-assisted breeding or homozygous lines using anther culture may accelerate crop breeding and stabilizing the improved traits. Since the pioneering report of anther culture published by Guha and Mahesh-

wari (1964), many researchers globally have made significant contributions to crop improvement (Datta 2005). The impact of this technology has now been well appreciated and utilized in marker-assisted population studies, gene tagging and transgenic breeding.

5 Plant Protection

5.1 Insect Resistance

Transgenic crops with built-in plant protection can be cited as one of the exemplary success stories of agricultural biotechnology. The transgenic *Bacillus thuringiensis* (*Bt*) varieties are in many ways better than using *Bt* as a spray formulation. In the *Bt*-transgenics, the protein is expressed in all tissues at all times, whereas the effectiveness of the sprays is affected by lack of uniform coverage and instability of the *Bt* protein, especially on exposure to sunlight. Considerable progress has been made in developing transgenic crops with resistance to the target insect pests during the past decade. Although there have been many approaches to incorporate insect resistance in transgenic plants, transgenic plants carrying the insecticidal protein gene from *Bt* have been the most successful by far. *Bt* is a soil bacterium that makes crystalline inclusions (*cry* proteins) during sporulation. These crystals dissolve in the alkaline environment of the insect gut and release protoxin molecules that are processed by the gut proteases to give active insecticidal proteins. These proteins interfere with the ion channel pumps and ultimately lead to the death of the insect larva that ingested the crystals. Such proteins are quite specific in their host range (determined largely by ligand–receptor interaction) and this fact has been exploited in the development of transgenic plants tolerant to specific groups of insect pests. More than 50 different *cry* proteins have been characterized which have different target insect specificity.

The first transgenic tobacco plants with *Bt* were produced in 1987 (Fischoff et al. 1987; Vaeck et al. 1987). Gene truncation, use of different promoters, enhancer sequences and fusion proteins resulted in significant improvement of the amount of insecticidal proteins in the transgenic plants (Perlak et al. 1991). NewLeaf is the brand name of the first *Bt* product to be commercialized in 1995, a transgenic potato expressing *cry*3A protein to control Colorado potato beetle. The introduction of this product reportedly reduced chemical insecticide use by 40%. This was followed by the release of pest-resistant transgenic cotton and corn. Subsequently, several *Bt* crops have been released for cultivation and, in 2000, such insect-resistant crops occupied 8.2×10^6 ha globally (James 2005). It has been estimated that *Bt*-cotton alone cut the use of chemical insecticides in the United States by over 2×10^6 lb (approx. 10^6 kg) or nearly 10^6 gal (approx. 3.75×10^6 l) from 1996 to 1998. Further, the study found that *Bt*-cotton increased yields by 85×10^6 lb and farmer profits by U.S. $\$ 92\times10^6$

in 1998, whilea significant economic benefit to small-holding farmers has been reported from India and China (James 2005). This is an example of the potential of biotechnology to provide a solution for combating a problem in a manner that is more environment-friendly. Among the cereals, maize was the first one to be transformed and field-tested with a *Bt* gene exhibiting high-level resistance to European corn borer (Koziel et al. 1993). After the first transgenic crop produced with a codon-optimized and truncated *cry* gene, several reports have accumulated in the recent past for developing transgenic rice carrying single or fused *cry* genes under different constitutive or tissue-specific promoters that showed resistance to stem borers and leaf-folder insects under glasshouse as well as field conditions (Wu et al. 1997; Alam et al. 1998, 1999; Datta et al. 1998; Tu et al. 2000a; Ye et al. 2001). Two reports from the International Rice Research Institute (IRRI), Philippines, in collaboration with Wuhan University and Jhejang Agricultural University are the first reports of transgenic hybrid rice (Shan you 63) as well as an elite indica IR72 with fused *Bt*-genes that were field-tested in China (Tu et al. 2000a; Ye et al. 2001). It is reported that some farmers at Hubei province in China found it beneficial to grow and to consume this pesticide-free GM rice (Gu 2005). The reports provided quotes such as "Zhang Qifa, conducted the mainland's largest field trials on GM rice. When interviewed by Newsweek in December last year, Professor Zhang mentioned that farmers near the GM test areas in Hubei had grown and eaten such rice without any side effects". There were many challenges in the production of the first *Bt* transgenic crops, but the infrastructure for these is now well established in some countries, including the United States, Canada, China and India. An important recent finding shows that *Bt*-rice in the field does not have any significant effect on non-targeted environment-friendly insects (Chen et al. 2006).

The insecticidal protein gene, being bacterial in origin, is expressed poorly in plants. Extensive codon optimization has been carried out with many of these native bacterial genes in order to obtain useful levels of expression in plants. Low expression levels were also addressed by directly transforming the plastids of plants with transgenes (De Cosa et al. 2001). Since the plastid has a gene expression machinery similar to prokaryotes, the genes could be introduced without extensive modification and the number of plants in a given plant cell results in very high expression.

There have been some concerns regarding the use of *Bt*-transgenic crops, the two major ones being their effect on non-target organisms and the possibility of the target insects developing resistance to the *Bt* protein. A report in *Nature* (Losey et al. 1999) indicated that monarch butterfly larvae were affected when fed with pollen from *Bt*-corn; and this was widely and incorrectly interpreted to mean that *Bt*-crops were threatening non-pest insects. Several follow-up studies showed the effect of pollen from *Bt*-crops had negligible effect on non-target insects, including butterflies under field conditions (Hodgson 1999). Though *Bt*-crops have been under wide cultivation since 1995, there has not been any instance of pests developing resistance. However,