I.1 Impact of Plant Biotechnology in Agriculture

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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-

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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

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