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Bioengineered Rice for Plant Protection

SWAPAN K. DATTA*

Plant Breeding, Genetics, and Biochemistry Division, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines

Introduction

The phenotyping of the rice genome and the isolation and characterization of genes in conjunction with genetic engineering have been envisaged as routes for improving the protection of rice crops against losses. Rice is the world's most important food crop. It feeds nearly 2.5 billion people. The current world population of 6 billion is likely to reach 7.5 billion by 2020 and the rice-producing sector will have to produce 40% more rice to satisfy the growing demand of the ever-increasing population. Transgenic rice developed by genetic engineering has enormous potential to help produce affordable food for the increasing world population, particularly in developing countries. Tools for gene isolation and characterization and gene technology, such as optimization of vector construction and transformation, are available. Microarray biochip technology will accelerate the unravelling of functional genomics and facilitate the movement towards the new era of biotech-based plant breeding. Attacks by insect pests, sheath blight and bacterial blight, and abiotic stresses can cause yield losses in rice equivalent to 200 million tons per annum (Herdt, 1991, 1996). Crop protection thus plays a vital and integral role in sustainable rice production.

Pesticide applications worldwide are now estimated to cost approximately US$8.1 billion per annum and Japan tops the list of pesticide users (Krattiger, 1998). This tremendous use of pesticides has reduced the effective life span of some compounds. It has also led to serious environmental consequences and concerns for human health (Dirham, 1993). In addition to integrated pest management (IPM), and crop rotation, resistant rice varieties produced either by genetic engineering or conventional breeding would appear to be the best option for an environmentally friendly sustainable agriculture (Datta, 2000a). Transgenic rice with the *Bt* gene conferring resistance to stem borer (Alam *et al.*, 1998, 1999; Cheng *et al.*, 1998; Datta *et al.*, 1998; Tu *et al.*,

*To whom correspondence may be addressed (sdatta@cgiar.org)

Abbreviations: *Bt*, *Bacillus thuringiensis*; PR, pathogenesis related proteins; Ti, tumour inducing; nptII, neomycin phosphotransferase II; ppt, phosphinothricin acetyltransferase; pmi, phosphomannose isomerase; PEPcP, pepcarboxylase promoter; CMS, cytoplasmic male sterile; DWR, deep water rice; ML, maintainer line.

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1998a), bacterial blight resistance (Tu *et al.*, 1998b), sheath blight resistance (Lin *et al.*, 1995; Datta *et al.*, 1999a,b, 2000), blast resistance (Nishizawa *et al.*, 1999) and stripe virus resistance (Hayakawa *et al.*, 1992) has already been developed. Transgenic resistant plants would produce more yield while using fewer agrochemicals, particularly pesticides.

Gene technology

Major crops such as rice, wheat, maize, cotton, soybean, sunflower, barley, and canola have been transformed using various gene transfer technologies. Methods of transformation that have been used are as follows: microinjection, macroinjection, laser beam techniques, pollen tube pathway, DNA imbibition by dry seed, and cell/tissue electroporation (Potrykus, 1991). However, the most reproducible and most unambiguous results have been obtained from the protoplast, biolistic, and *Agrobacterium* methods (*Figure 13.1*). Comparative studies with these systems are also available (see Datta *et al.*, 1997a; Baisakh *et al.*, 1999).

THE PROTOPLAST SYSTEM

The first report by Vasil and Vasil (1980) of plant regeneration from protoplasts of **graminaceous** species opened new opportunities for genetic transformation in cereals such as in japonica rice (Toriyama *et al.*, 1988; Zhang and Wu, 1988; Shimamoto *et al.*, 1989) and indica rice (Datta *et al.*, 1990, 1992; Peng *et al.*, 1992). The single-cell origin, non-chimeric nature, and genetic fidelity of plants derived from somatic embryos subsequently proved very attractive features for any transformation system. Early success was based on the following:

i. Establishment of embryogenic cell suspension (ECS), the key source of regenerable protoplasts (Vasil, 1994).
ii. Use of suitable plant transformation vectors with selectable marker genes.
iii. Continuous efforts to improve tissue culture protocols by different laboratories, e.g. use of 2,4-D, maltose, nurse culture, PEG-MW6000, osmotic adjustment, etc. (Datta, 1999).

BIOLISTIC SYSTEM

Microprojectile bombardment of immature embryos with high-velocity metal particles to deliver the biologically-active DNA into plant cells and recovery of the whole plants from the transformed cells through selection is becoming almost a routine. Genotype-independent transformation may be carried out with the method as there is no biological limitation to DNA delivery. Japonica and indica rice cultivars have been transformed with this system (Cao *et al.*, 1990; Christou *et al.*, 1991; Datta *et al.*, 1997b, 1998; reviews: Datta, 1999; Tyagi *et al.*, 1999).

THE *AGROBACTERIUM* SYSTEM

In nature, the Gram-negative soil bacterium *Agrobacterium tumefaciens* causes crown gall formation in dicots by a multi-step transformation process. To date, *Agro-*

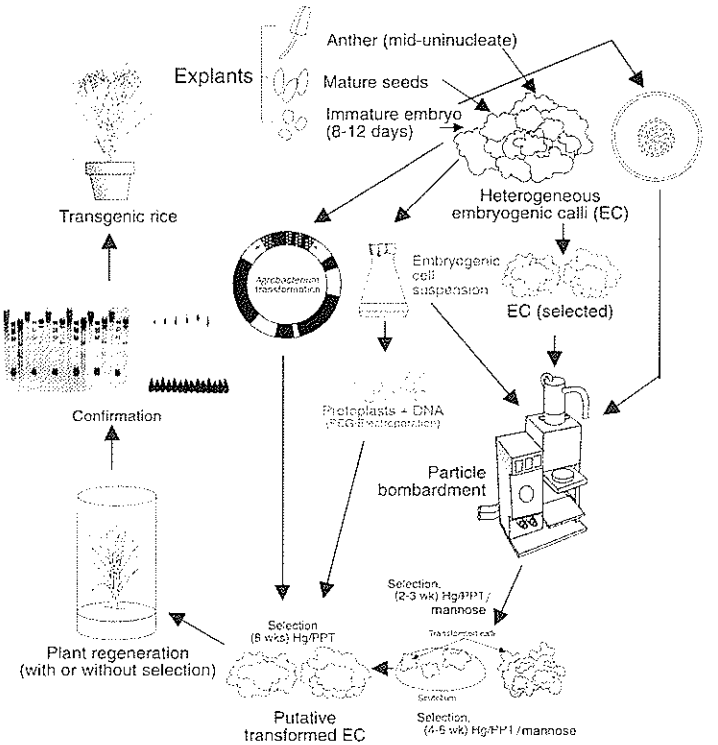

Figure 13.1. A schematic protocol for the production of transgenic rice plants using biolistic, protoplast, and *Agrobacterium* systems.

transformation has been extensively used for both dicot and monocot species (Datta and Datta, 2002). During *Agro*-infection, a small segment of one extra chromosomal plasmid Ti (tumour inducing), the T-DNA, surrounded by a 24-bp border repeat, is transferred to the plant cell and integrated into the nucleus of the host chromosome. The T-DNA transfer system is determined by the *vir* genes, whereas the flanking 24-bp direct repeats are essential as recognition signals for the transfer apparatus. For plant transformation, wild-type T-DNA was removed to create 'disarmed strains' such as LBA4404 (Hoekema *et al.*, 1983) and C58C1 (Zambryski *et al.*, 1983). Various genes, including selectable marker genes and genes of interest, can be placed in the vector and introduced into the 'disarmed strains'. The binary vector system consists of a plasmid providing the virulence functions needed for transfer and a small vector carrying an artificial T-DNA. The binary vectors replicate in *Escherichia coli* as well as in *A. tumefaciens* and allow easy cloning of genes of interest between the T-DNA borders. Besides the vectors pBin19 (Bevan, 1984), pGA482 (An *et al.*, 1988), and pBI121 (Jefferson, 1987) that are usually used in dicots, a new binary vector, pGA1611, for rice has been constructed (Lee *et al.*, 1999). This vector contains the unique *Hin*dIII, *Sac*I, *Hpa*I, and *Kpn*I for cloning of a gene between the maize *Ubi* promoter and *nos* terminator.

Chloroplast transformation

In some crops such as strawberry and sunflower, gene flow or 'outcrossing' is very common and causes serious concern to environmentalists. Maternal inheritance of foreign genes through chloroplast genetic engineering is highly desirable for those crops in which potential outcrossing is possible. This technology is emerging as an alternative for overcoming concerns regarding nuclear genetic engineering (Daniel, 1999; Bogorad, 2000; Staub *et al.*, 2000). However, whether this system works for cereals remains to be seen.

Selectable marker genes, *nptII, hph, ppt, pmi*

Several selectable marker genes have been used in cereal and rice transformation (Datta, 1999). There are several ways in which an effective selective regime for rice transformation can be established. The effective concentration of the selective agent (kanamycin, hygromycin or mannose) in a transformation system has to be determined for each crop and often at cultivar levels. Usually, a concentration is chosen that inhibits growth of untransformed tissue, resulting in the regeneration of transformed cells (Klee *et al.*, 1987). Though there is no evidence of any toxic or adverse effect of the use of antibiotic resistance genes in plant transformation, a negative perception exists for using such selectable marker genes in crops. Alternatively, a new selection strategy can be employed involving the use of mannose, a substance which cannot be metabolized by many plant species. The schematic presentation of *Figure 13.2* shows the conversion of mannose to mannose-6-phosphate (M-6-P) by a hexokinase, resulting in severe growth inhibition. The transgene product phosphomannose isomerase (PMI) converts M-6-P to fructose 6-phosphate, which can be readily metabolized, thus inferring on the transformed cells a metabolic advantage (Datta, 2000a; Negrotto *et al.*, 2000). Mannose-6-phosphate is toxic to non-transgenic cells/tissues, and this supports the mechanism of mannose selection. We are working on this 'Positech™' selection in rice, aiming at a greater degree of acceptability by the public as an environmentally friendly system (*pmi* was available from Novartis). It seems that Positech™ selection is an efficient and direct system as an alternative to complex systems such as *Cre/lox* (Dale and Ow, 1991) and MAT vectors (Sugita *et al.*, 1999).

Defence genes and plant protection

A large number of pathogenesis-related protein (PR) genes have been reported (Datta and Muthukrishnan, 1999) along with a few resistance genes (Hammond-Kosack and Jones, 1997; Richter and Ronald, 2000). It is still a significant challenge to be able to engineer rice against fungal pathogens, particularly sheath blight and blast. Considerable progress has been made in recent years in understanding host–pathogen interactions and the coordinated functioning of plant defence mechanisms against pathogenic fungi (Sakamoto *et al.*, 1999; Purkayastha, 1998). Transgenic rice with several PR-genes (*Chi11*, *RC7*, and *tlp5*) has been developed, and enhanced resistance against the fungi *Rhizoctonia solani* and *Pyricularia oryzae* that cause sheath blight and blast disease, respectively, has been reported (Lin *et al.*, 1995; Datta *et al.*, 1998, 1999a,b, 2001; Nishizawa *et al.*, 1999; Baisakh *et al.*, 2001).

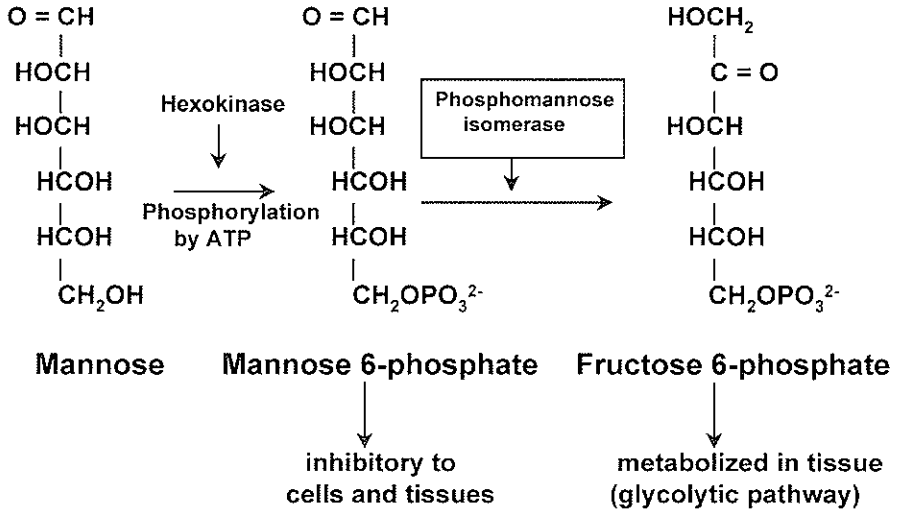

Figure 13.2. Selection system using the *phosphomannose isomerase* gene and mannose as a selectable agent (Datta, 2000a).

Most transgenic rice are grown on a laboratory scale but some that have been engineered with insect, fungal, bacterial, and herbicide resistance have been available for field testing (*Table 13.1*). *Bt* rice has shown great promise and potential as environmentally friendly plants that can be grown in the field without the application of costly pesticides. *Bt* maize and cotton have now been extensively planted in many countries, including the USA, Europe, and Asia (Carrozi and Koziel, 1997; James, 2001). A hybrid commercial *Bt* rice, 'Shan You 63', has now been grown in China on a large scale. It has shown resistance to two insect pests, leaf-folder and stem borer (Tu *et al.*, 2000b) and based on a concept published earlier (Alam *et al.*, 1999).

Case study of transgenic rice

TRANSGENIC RICE WITH *XA21* CONFERRING RESISTANCE TO BACTERIAL BLIGHT

Bacterial blight (BB) caused by *Xanthomonas* pv. *oryzae* (*Xoo*) is one of the most destructive diseases of rice worldwide. Rice yield losses caused by BB in some areas of Asia can reach 50%. The use of resistant cultivars is the most economical and effective method for controlling this disease (Ogawa, 1993).

A dominant gene for resistance to BB was transferred through conventional methods from a wild species, *Oryza longistaminata*, to the cultivated variety IR24 (Khush *et al.*, 1990). The resulting line with *Xa21* is called IRBB21. *Xa21* confers resistance to all the known races of *Xoo* in India and the Philippines (Ikeda *et al.*, 1990). The molecular structure of *Xa21* represents an uncharacteristic class of plant disease resistance genes. From studying its amino acid sequence, the gene was found to be translated into a receptor kinase-like protein carrying leucine-rich repeats in the putative extracellular domain, a single-pass transmembrane domain, and a serine threonine kinase intracellular domain. Further, *Xa21* supports a role for cellular signalling in plant disease resistance (Song *et al.*, 1995).

Table 13.1. The development and use of transgenic rice for plant protection.

Rice	Method used	Genes transferred	Traits	Reference
Indica (IR72)	Protoplast (PEG)	*bar*	Resistance to herbicide	Datta *et al.*, 1992
Japonica	Protoplast (electroporation)	cp-stripe virus	Resistance to stripe virus	Hayakawa *et al.*, 1992
Japonica	Protoplast (electroporation)	*Bt*	Resistance to insects	Fujimoto *et al.*, 1993
Japonica	*Agrobacterium*	*Bt*	Resistance to YSB & SSB	Cheng *et al.*, 1998
Indica	Protoplast (PEG)	*chi11*	Resistance to sheath blight	Lin *et al.*, 1995
Japonica	Protoplast	cc	Resistance to insects	Irie *et al.*, 1996
Japonica	Biolistic	*Xa21*	Resistance to bacterial blight	Wang *et al.*, 1996
Indica	Protoplast and biolistic	*Bt/(DWR)*	Resistance to stem borer	Alam *et al.*, 1998
Japonica	Biolistic and protoplast	*pin*II	Resistance to insects	Duan *et al.*, 1996
Indica	Biolistic	*Bt*	Resistance to stem borer	Nayak *et al.*, 1997
Indica	Biolistic	*Bt*	Resistance to stem borer	Tu *et al.*, 1998a
Indica/ Japonica	Biolistic and protoplast	*Bt* (tissue-specific)	Resistance to stem borer	Datta *et al.*, 1998
Indica (IR72)	Biolistic	*Xa21*	Resistance to bacterial blight	Tu *et al.*, 1998b, 2000a
Indica	Biolistic	*Bt* ML for hybrid rice	Resistance to stem borer	Alam *et al.*, 1999
Indica	Biolistic	*Bt* restorer for hybrid rice	Resistance to stem borer field evaluated	Tu *et al.*, 2000b
Indica	Biolistic	PR genes	Resistance to sheath blight	Datta *et al.*, 1999b
Indica	Biolistic	viral replicase CP1, CP2, CP3	Resistance to RTSV and RTBV	Ang *et al.*, 1999
Indica	Biolistic	PR-genes	Resistance to sheath blight	Baisakh *et al.*, 2001
Indica	*Agrobacterium*	PR-genes	Resistance to sheath blight	Datta *et al.*, 2000a
Indica	Biolistic	PR-genes	Resistance to sheath blight	Datta *et al.*, 2000b
Indica	Biolistic	hybrid *Bt*	Resistance to pests	Ye *et al.*, 2001
Indica	Biolistic	*Xa21*	Resistance to blast and bacterial blight	Narayanan *et al.*, 2002a
Indica	Biolistic	*Xa21*	Resistance to blast and bacterial blight	Narayanan *et al.*, 2002b
Indica	Biolistic	*Xa21, PR, Bt*	Resistance to sheath blight, stem borer, and bacterial blight	Datta *et al.*, 2002

ML = maintainer line; DWR = deep water rice; YSB = yellow stem borer; SSB = striped stem borer

Xa21 has been transferred to susceptible japonica rice T309, which has shown resistance to BB (Wang *et al.*, 1996). As T309 is not a commercial variety, we introduced the gene in elite breeding cultivars, such as IR72, MH63, IR51500, etc. (*Figure 13.3*). Molecular analysis of transgenic plants revealed the presence of a 3.8 kb *Eco*RV-digested DNA fragment corresponding to most of the *Xa21* coding region and its complete intron sequence and indicating the integration of *Xa21* in the genome of rice. Transgenic plants were challenged with two prevalent races (4 and 6) of *Xanthomonas oryzae*. T_0 and T_1 plants positive for the transgene were resistant to bacterial blight (Tu *et al.*, 1998b). We also observed that the level of resistance to race 4 of *Xoo* was higher due to pyramiding of *Xa21*, in addition to the *Xa4* already present in IR72. Transgenic BB-rice (cv. IR72) has been evaluated repeatedly under field conditions in Wuhan, China in the period from 1998 to 2000. The particular isolates

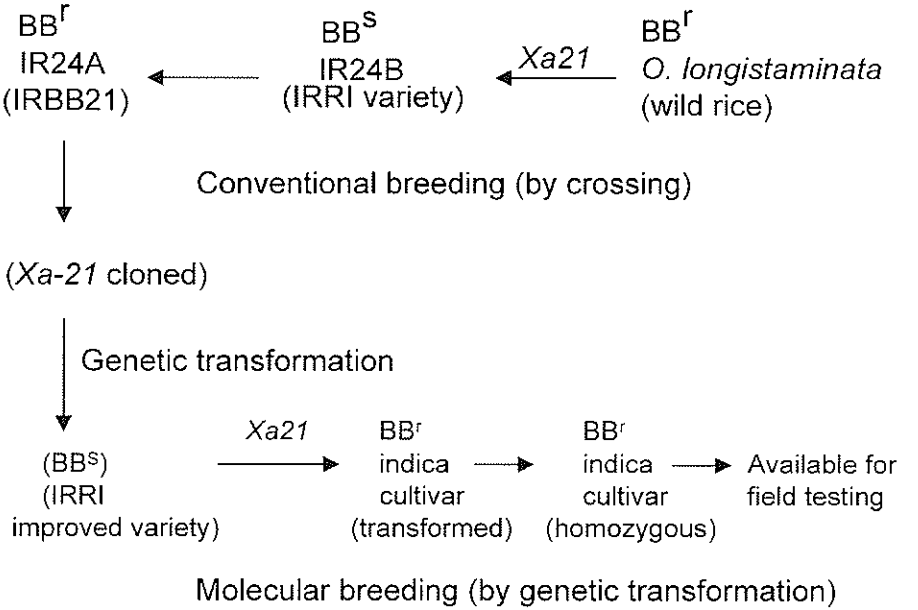

Figure 13.3. Schematic demonstration of the transfer of *Xa21* from original source to cultivated rice by conventional and molecular breeding.

of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) used in these experiments were PXO61, PXO79, PXO99, together with PXO112 isolated from the Philippines, T2 isolated from Japan, and Zhe173 isolated from China. The results demonstrated that the transgenic homozygous line expressed the same resistance spectrum, but with a shorter lesion length to each inoculated isolate, as compared to the lesion length of the *Xa21* donor line IRBB21. The non-transformed control IR72 carrying *Xa4* was resistant to PXO61, PXO112, Zhe173 and T2, but susceptible to PXO99 and PXO79. The negative control variety IR24 was susceptible to all isolates under field conditions. The results clearly demonstrated that the *Xa21* transgene led to an excellent field performance due to the introduced bacterial blight resistance trait on the recipient plants. The yield performance of this transgenic homozygous line, T103-10, is comparable with that of the resistant control under field conditions.

We also noticed that an increased level of resistance to the BB pathogen persisted in transgenic plants through generations, indicating its stable inheritance. The heritable increased level of resistance to the BB pathogen can, in turn, provide an advantage for genetic engineering over classical breeding in cases where the highest levels of resistance are desirable and can be achieved in a short time. It is also noteworthy that various national agricultural systems in Asia are making efforts to incorporate the other *Xa* genes into popular cultivars through marker-aided selection. The availability of various cultivars with different resistance genes could significantly decrease the yield loss due to *Xoo*. Assuming a minimum yield loss of 0.75% due to this disease, around $715.5 million could be saved over 132.5 million ha with an average yield of 3.6 t/ha in Asia (Tu *et al.*, 2000a).

The study described in the previous paragraph demonstrates that conventional and molecular breeding techniques could be a powerful combination in rice breeding. Genetic transformation is a one-step process of introducing novel genes into a desirable genetic background of important crops. Because it is a fast and efficient gene-integration tool, it could well be the answer to catching up with the pathogen's ability to mutate fast and render once-resistant plants susceptible. For instance, rice cultivars carrying the *Xa4* gene for resistance, which were widely deployed in the Philippines from the early 1970s, became susceptible to the predominant race of *Xoo* within five years (Mew *et al.*, 1992). Transformation techniques could help to develop transgenic plants in less than two years to minimize the effects of the breakdown of resistance in the host plant. With the availability of resistance genes from other sources, the strategic deployment of transgenic rice with gene pyramiding may provide desirable resistance in rice breeding.

TRANSGENIC *BT* RICE RESISTANT TO STEM BORER

Stem borer damage is a serious problem in rice, causing estimated losses of 3–30% of the total yield. *Scirpophaga incertulas* (yellow stem borer) and *Chilo suppressalis* (striped stem borer) are the major stem borers and are widely distributed from Japan to India. Stem borer larvae start their attack by boring through the inner portion of the leaf sheath. The subsequent boring through the stem by caterpillars causes considerable damage, resulting in 'deadheart' symptoms, and the affected tillers do not bear panicles. Panicles often emerge with empty grains, called 'whiteheads'. *Bacillus thuringiensis* (*Bt*), the common soil bacterium, produces crystals containing insecticidal proteins. These toxins kill insects by binding to and creating pores in the midgut membranes. *Bt* toxins are highly specific and therefore are not toxic to beneficial insects, birds, and mammals, including humans (Koziel *et al.*, 1993). Tissue-specific promoters, particularly the green-tissue specific promoter (PEPcP) used in *Bt* gene expression in rice, were allowed to express preferentially in green tissue, and significantly reduced expression in grain. Thus, *Bt* rice plants with PEPcP or the pith promoter, either alone or in combination, should provide a better strategy for providing rice plants with protection against insect pests, thus minimizing the expression of the CryIA(b) protein in seeds and other tissues (Datta *et al.*, 1998).

Hybrid rice was first developed and commercialized in China in 1976. It became popular because it had a 20% yield advantage over inbred varieties. Rice hybrids are now cultivated in about 55% of the rice-growing areas in China and contribute 66% of China's total rice production. During the past 4 years, India, Vietnam, Bangladesh, and the Philippines have been using this technology successfully. The production of hybrid rice involves three-line systems: cytoplasmic male sterile (CMS) lines, and maintainer and restorer lines. Incorporation of resistance gene(s) in a CMS line makes this technology widely applicable to the development of resistant rice hybrids. Since a CMS line is maintained by backcrossing to its isogenic maintainer line, the presence of resistance gene(s) in the latter will lead to the development of a CMS line possessing these genes (*Figure 13.4*). Alternatively, the gene(s) can be incorporated into a restorer line and homozygous restorer line carrying the gene, which, when hybridized with the CMS line, will produce a desirable hybrid plant with a gene of interest (*Figure 13.4*).

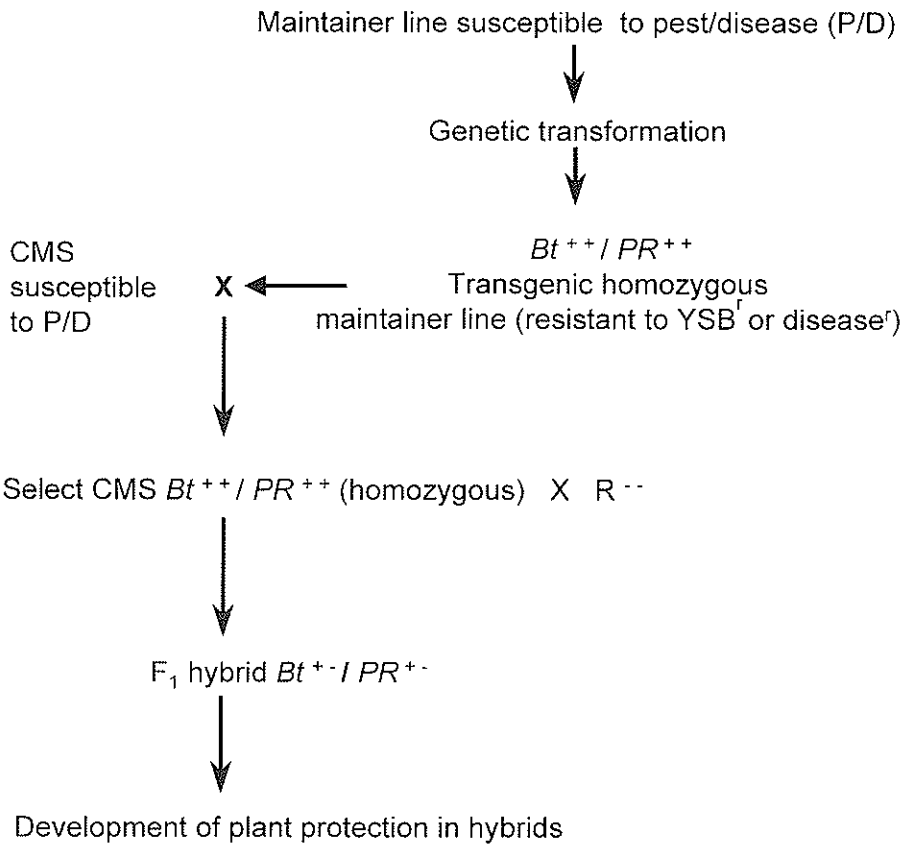

Figure 13.4. Schematic demonstration of the use of a transgenic breeding approach for developing plant protection in rice hybrids.

A restorer line, Minghui 63, was transformed with a fusion of two genes *cryIA(b)* and *cryIA(c)*, driven by the actin1 promoter. A selected homozygous MH63 *Bt* line was hybridized with CMS line Zhenshan 97A to produce a hybrid Shanyou 63 *Bt* rice. Shanyou 63 has been the most widely used popular hybrid in rice production in China for the past 15 years. Transgenic cultivars were selected based on co-transformation using *hph* (selectable antibiotic marker gene, hygromycin phosphotransferase). However, during the subsequent progeny selection, careful molecular analysis helped us select a MH63-CMS-*Bt* line without the marker gene. Finally, our hybrid rice was selected and used in the field carrying only the hybrid *Bt* genes without the selectable marker gene. This was possible because of the integration of the transgenes (*Bt* and *hph*) in two different loci (Tu *et al.*, 2002).

Hybrid *Bt* rice (Shanyou 63) was field tested in Wuhan, China, between the period 1999 to 2000. Transgenic plants were field tested in natural and repeated heavy manual infestation of two lepidopteran insects, leaf-folder and yellow stem borer. The transgenic hybrid plants showed high protection against both insect pests. The yield of the hybrid *Bt* rice was 28.9% more than that of the non-*Bt* hybrid (*Table 13.2*). Considering that the field trial was conducted without the use of chemicals after

Table 13.2. Agronomic traits of the *Bt* and non-*Bt* hybrids under field conditions (Huazhong Agricultural University, Wuhan, China, 1999; Tu *et al.*, 2000b).

Hybrid	Sowing–heading duration (days)	Plant height (cm)	Panicles per plant	Filled-grains per panicle	Non-filled-grains per panicle	Total grains per panicle	Seed-setting rate (%)	1000 grain weight (g)	Expected yield weight (g)	Observed yield[a] (t/ha)+/– (%)
Bt Shanyou 63	95	108.6	9.98	140.0	21.9	161.7	87.6	28.00	7.44	8.69+28.9[b]
Shanyou 63	95	106.4	9.60	143.7	28.8	172.5	83.3	28.76	7.44	6.74

[a]The observed yield was measured based on the average production per unit of four subplots after harvesting and then converted into tons per hectare.
[b]Significantly different from control at $P < 0.01$.

transplantation, these results demonstrate that expression of the *Bt* fusion protein in the genome of the transgenic hybrid rice provided season-long protection against the natural outbreak and heavy manual infestation of the two lepidopteran insects.

Rice grows in different ecosystems. Adaptive cultivars already developed by plant breeders should be used for incorporation of the *Bt* gene. For example, we have developed deepwater rice (DWR) with the *Bt* gene. DWR is grown in areas usually flooded deeper than 50 cm (sometimes up to 400 cm) for 1 month or longer during the growing season. Consequently, any traditional tall and elongating rice cultivar can be grown in these areas. Yield is generally low (1–2 tons per hectare) and very often diminished further by insect attack. The application of insecticides to DWR causes many problems. Ordinary ground-level applications are limited to the pre-flood period and spraying is not possible when the water is deeper than 50 cm. Moreover, pesticides could affect beneficial natural predators and cause fish mortality. Fish harvested from DWR are a major source of income and protein for the people living in these areas. The development of DWR varieties with resistance to yellow stem borer will significantly help farmers in flood-prone ecosystems. We have successfully introduced a *cryIA(b)* gene (provided by Novartis) into an elite DWR *Vaidehi* variety, and homozygous material is now ready to transfer to India for field testing and further use.

Bt management will play an important role subject to social, economic, and ecological values and several adaptive varieties should be used for each cropping season. A system well adapted and optimized for the larger land area of the United States farms may not be applicable for a country such as India, having a large number of small farms. A suitable system needs to be developed for specific crops and countries and this can only be achieved by having more field testing, evaluation, and data collection. *Bt* toxins are insecticidal and, as with conventional chemical insecticides, insects may quickly adapt to them unless *Bt* plants are carefully designed and deployed to counter this. A greater guarantee of a durable resistance can be achieved if a *Bt* toxin is combined with a second type of toxin (Cohen *et al.*, 1998).

It is critical that *Bt* crops remain a viable option for agriculture. After 30 years of successful use, *Bt* is considered one of the safest pesticides available. It is biodegradable and has no adverse effects on beneficial insects, other wildlife, and farm workers. Successful *Bt* crops, including the first commercial *Bt* hybrid rice and *Bt* IR72, have now been well evaluated in fields. In 1999, we developed a promising tool for the use of *Bt* technology to improve hybrid rice. Consequently, we developed commercial

hybrid rice with a fusion hybrid *Bt* gene. The advancement of this product will help to considerably reduce pesticide use. In the future, the use of *Bt* crops within an adaptable Integrated Pest Management strategy may lead to durable and environmentally friendly plant protection (see Datta, 2000b).

Herbicide resistance technology and relevance to rice

Herbicide resistance genes that originate from various organisms, including bacteria and plants, are frequently used for the selection of transformants (Datta *et al.*, 1992; Wilmink and Dons, 1993). This is now commercially very important for use against weeds in crops such as cotton, soybean, and maize.

Resistance to herbicide is conferred by phosphinothricin-N-acetyltransferase (PAT), which inactivates PPT by acetylation using acetyl co-enzyme A as a co-factor. Two similar genes encode PAT, *bar* (isolated from *Streptomyces hygroscopius*) and *pat* (from *S. viridochromogenes*), which confers resistance to BASTA. In the field, BASTA is used as a weed control. It has been field evaluated and found to be environmentally friendly as it is metabolizable in nature (De Greef *et al.*, 1989).

Glyphosate (N-phosphinomethylglycine) inhibits the enzyme 5-enolpyruvyl shikimate 3-phosphate synthase (EPSPS), which is involved in the shikimate pathway. Glyphosate is the active compound in the well-known herbicide Roundup™, produced by Monsanto. Resistance to glyphosate is not based on inactivation of the herbicide, rather, the gene inhibits EPSP synthase (Wilmink and Dons, 1993). This is a single dominant gene stably inherited and shown to work in many crops, and it has been evaluated in the field. The possibility of unintended harm to crops and human food seems remote. Currently, among the transgenic crops, there have been some noteworthy developments in traits: herbicide tolerance contributed the most (77% or 40.6 million hectares), with 7.8 million ha planted to *Bt* crops, and the stacked genes of insect resistance and herbicide tolerance in both maize and cotton contributed 8% (2.6 million hectares) as reported by James (2001).

Weeds cause severe problems in rice and reduce yield considerably. Transplanting is the traditional method of rice cultivation in Asia and it can partially control weeds. Direct seeding, however, is becoming more inevitable because of increased mechanization, which reduces labour costs. Direct seeding now covers about 29 million hectare in Asia (21% of the total rice area in the region). However, the chemical costs of weed control in direct-seeded rice tend to be higher than those of transplanted rice, and these chemicals threaten human health and the environment (Pandey and Velasco, 1999). Direct seeding will expand in Asia because of water shortages, high labour costs, and efforts to increase productivity.

Genomics-based strategies for gene discovery, coupled with the high throughput transformation process, have accelerated the identification of candidate genes. The present gene technology has revolutionized the concept of biology and accelerated the progress of plant breeding. However, the controversy around this technology probably results from the way in which this technology and its application to crop improvement has developed faster than the public's perception. Plant breeding for crop improvement involves phenotypic, physiological or biochemical strategies, including feedback inhibition and genetic changes (unknown mutants, gene silencing, sterility, pleiotropic effect, etc.). However, once the breeders have succeeded in selecting a fertile line with

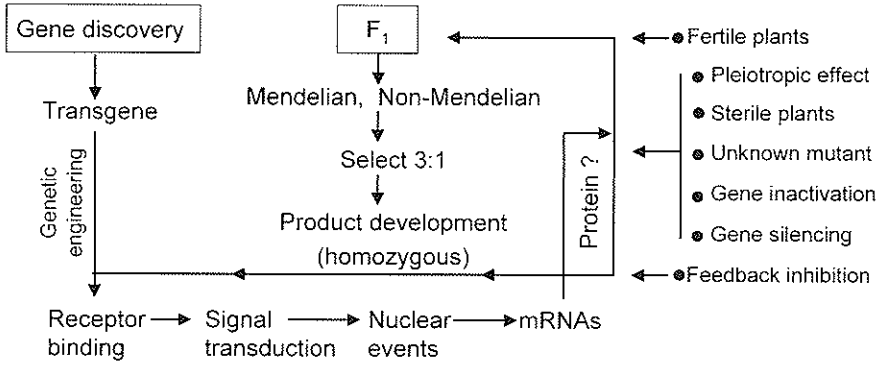

Figure 13.5. Bioengineered product development.

predictable segregation data, the improvement continues into subsequent generations. Such an approach, indicating the use of genetic engineering to complement the conventional breeding procedures for crop improvement, is summarized in *Figure 13.5*. The transgenic approach may give farmers an excellent opportunity to modernize agricultural technology with reduced use of agrochemicals for an environmentally friendly system.

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