THE UTILISATION OF TRANSGENIC PLANTS WITH RESISTANCE TO PESTS AND DISEASES IN DEVELOPING COUNTRY AGRICULTURAL SYSTEMS

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ABSTRACT

Transgenic crops are now on the market in the US and a number of Western European countries. Already, commercialised crops (and those anticipated to reach commercialisation in the next year or so) contain first generation traits, in most cases specified by one or a small number of genes. It is clear that a logical strategy has been developed in most industrialised nations in an attempt to reconcile technological achievements, market forces, intellectual property rights, concerns over the regulation of transgenic plants and their release into the environment, and the public perception of these issues. Despite obstacles and misconceptions, the products of genetic engineering will continue to make the transition from the laboratory to the market place, probably with greater ease in the US than in Europe. However, the driving forces behind agricultural biotechnology in developing countries are radically different to those experienced in the West. Issues of economic growth and development, national and international competition, and food security, have a much greater impact on policy-making decisions in developing nations compared to the industrialised world. In this paper, some of the differences between industrialised and developing countries are addressed in the context of agricultural biotechnology within the framework of both sustainable and intense cultivation systems in the tropics. Specific applications using rice as a model exemplify some of the most important issues in developing country agriculture and the potential role of biotechnology in these countries.

INTRODUCTION

Daily *et al.*, in a recent policy forum on the global food supply identified two criteria by which one can judge humanity's success in feeding itself: (i) the proportion of people whose access to basic nutritional requirements is secure, and (ii) the extent to which global food production is sustainable (Dailey *et al.*, 1998). Global demand for food is projected to double over the next 40 years. In Africa, Central and South America and Asia, plant-derived food energy requirements are expected to increase by a factor of 2-5, with a more than sevenfold increase expected in some countries. Increases in food production will have to come from increased yields from land already in production and also from improved efficiency in the use of existing water supplies. Plant genetic engineering provides a very attractive option for increasing crop productivity within these constraints.

Applied genetic engineering traits in plants fall into three broad categories: agronomic improvement, food modification and industrial exploitation. Agronomic traits such as weed control, insect pest resistance and resistance to diseases caused by bacteria, fungi or viruses, are

usually controlled by single genes. More complex traits, controlled by multiple genes, include tolerance of biotic stresses (e.g. heat, cold, drought, salt) and abiotic stresses (e.g. heavy metals). Hybrid technology will also benefit from wider applications of genetic engineering, particularly the concept of molecular apomixis which has the potential to maximize yields. Food modification traits include enhanced nutritional quality of food crops, delayed ripening of fruits, increased solids for processing vegetables, changes of colour, flavour and texture, as well as modification of oil and starch composition. Safety issues can also be addressed by eliminating toxic or anti-nutritional factors from food products. Finally, industrial uses include metabolic engineering to produce high-value chemicals, the creation of modified and specialty oils, the industrial production of recombinant or engineered proteins including enzymes, and the production of recombinant macromolecules such as antibodies and vaccines for human and animal healthcare. An additional application of plant genetic engineering is bioremediation to repair environmental damage caused by the activities of man. For instance, transgenic plants expressing metallothionenes (proteins that bind toxic heavy metals) might be used to clean up contaminated sites.

It is important to realize that although transformation technology now exists for an impressive range of species, it is still labour-intensive, time consuming and not very efficient. It is apparent that gene transfer technology is not accessible to all, and that a technology gap exists between industrialised and developing countries, and between industrial and academic institutions in the West. This gap must be bridged before the achievements of contemporary plant molecular biology seen in model laboratory species can be extended to important crops. Training and technology transfer is emerging as an important issue, and links between corporate research organisations, academic institutions and international organisations need to be strengthened to maximize the efficient use of limited resources, and to avoid duplication of effort.

TRANSGENIC RICE - ITS ROLE IN DEVELOPING COUNTRY AGRICULTURE

The last decade has witnessed significant progress in rice genetic engineering projects due, at least in part, to the establishment and implementation of the Rice Biotechnology Programme by the Rockefeller Foundation. This programme assured funding for many laboratories worldwide, allowing the development of different aspects of rice biotechnology, including genetic engineering. Despite early successes, however, rice genetic engineering has remained a challenge. Traditional and widely used gene transfer methods, such as electroporation and PEG-mediated transformation of protoplasts, were limited by constraints imposed by the culture systems. As a result, only a few japonicas and an even smaller number of indica varieties could be routinely engineered. This problem was solved by the development of particle bombardment, which allowed the production of transgenic rice plants from many important cultivars, including indicas and elite japonicas. Unfortunately, particle bombardment technology has not yet become widely available, but the dissemination of this technology is essential for the advancement of rice improvement programmes, especially in developing countries which depend on rice for feeding their populations. A number of laboratories are committed to bringing the necessary technology and training to rice growing countries. These include CAMBIA (Center for the Application of Molecular Biology in International Agriculture), ILTAB (International Laboratory for Tropical Agricultural Biotechnology) and the JIC (John Innes Centre). The Rockefeller Foundation is supporting training and technology transfer programmes in these and

other organisations, to allow scientists from rice-growing countries to acquire the means and expertise to carry out similar research at their home institutions. It is therefore likely that, as a result of these efforts, rice genetic engineering will become routine in many laboratories in the developing nations.

Hybrid rice is likely to have the same effect on the development of the seed industry in developing countries that hybrid corn had in North America and hybrid sugar beet in Europe. The International Rice Research Institute has identified hybrids which give a yield increase of 15% or more over the best available varieties. In China, increases of greater than 30% due to heterosis have been reported. If these achievements can be sustained, the private sector may be attracted into hybrid rice seed production. Hybrid seed is priced normally at 10 to 30 times the value of the crop's commodity price. With a five ton per hectare yield base and 25% heterosis, the farmer's benefit can be four times his investment in seed, making it an attractive option for both farmers and seed companies. Current hybridisation technologies are very tedious and inefficient. However, with molecular techniques for creating hybrids now being tested, it is reasonable to expect that the very encouraging results obtained with other crops such as tobacco, brassica, and maize will also be applicable to rice.

In applying recombinant DNA methods to rice improvement, it is important to consider the potential risks that transgenic rice plants may pose to the environment, the farmer, or the consumer. In the case of cultivated rice, one of the major issues that needs to be addressed is possible pollen transfer to its close relative red rice, a weed also of the genus and species *Oryza sativa*. Red rice grows commonly in the southern United States and in many other rice-producing areas of the world. It gets its name from the red colour of the pericarp, caused by anthocyanin pigmentation. This an undesirable agronomic trait which reduces the value of the rice crop. Where herbicide resistance is a target for rice improvement, there is a possibility that pollen transfer from cultivated to red rice would produce herbicide resistant weeds. It should be possible, however, to devise an optimum window for herbicide application by gaining a thorough understanding of pollen dispersion and out-crossing rates between cultivated and red rice. Transgenic rice plants with easily scorable characteristics will provide some of the answers needed to make a critical assessment of the risks and benefits of introducing herbicide-resistance traits into rice.

Plant genetic engineering is now approaching a crossroads. The limitations imposed by inefficient gene transfer have been removed from a number of important cereals. Technical problems still remain, but they are not insurmountable. The attention of the scientific community is gradually shifting to more complex questions, such as the identification and cloning of genes responsible for polygenic traits, and studies of gene expression and regulation, particularly in the field over a number of generations. One area which should not be neglected encompasses the public perception of recombinant DNA technology and the environmental risk assessment of products thus derived. The first transgenic plants were recovered in 1983. It is indeed remarkable than in just over one decade the tools of recombinant DNA technology and molecular cell biology are now at the disposal of plant breeders. Important issues such as defining appropriate and relevant targets for rice genetic engineering can now be addressed and this will result in increased agricultural productivity, enabling developing countries in particular to sustain increasing populations. In addition, alternative uses for surplus crops resulting from recombinant DNA technology have the potential to provide new resources for industry and the

consumer, thus expanding the economic basis in industrialised countries.

Nematode-resistant transgenic rice

In the tropics, nematodes reduce average crop production by 11-25%. *Meloidogyne* spp. (root-knot nematodes) account for the majority of the US$ 100 billion annual crop yield loss attributed to nematode damage. This genus is the major player among the collection of root-parasitic and foliar nematodes causing severe crop losses in the various rice ecosystems, and the majority of *Oryza sativa* cvs. are susceptible. *M. graminicola* infests upland and lowland rice in Asia either before flooding or in intermittent cultivation systems. *M. incognita* is primarily restricted to upland rice, where it can reduce plant height and yield by 60% in West Africa. *Meloidogyne* spp. have a broad host range and, therefore, also affect other crops used in rotation with rice. Their control is, therefore, vital for the development of sustainable agricultural systems. Since chemical control is unsuitable on economic and environmental grounds, biotechnology provides an important opening for improved rice production. The introduction of pest-specific anti-nutritional factors into crop plants offers a promising approach for the control of a wide range of pests. Protease inhibitors have been used to engineer both insect resistance (e.g. Hilder *et al.*, 1987) and nematode resistance (Hepher & Atkinson, 1992). Cowpea trypsin inhibitor, expressed in transgenic potato, was shown to affect the fecundity and sex ratio of *Meloidogyne* spp. and *Globodera* spp. (Hepher & Atkinson, 1992). Cysteine proteinase inhibitors (cystatins) may offer a safe defence strategy against specific pests because they are the only class of proteinases not expressed in the digestive system of mammals. However, plant cystatin expression is often developmentally regulated. For example, rice cystatin (Oryzacystatin-I, OC-I) is expressed in rice seeds but is deficient in leaves, stems and roots. Rice (OC-I) or maize (CC-I) cystatin genes have been introduced and constitutively expressed in model rice varieties (e.g Hosoyama *et al.*, 1994) potato and tobacco. A variant of Oryzacystatin-I (OC-I 86) produced by site directed mutagenesis, was shown to confer nematode resistance when expressed into tomato hairy roots and *Arabidopsis* plants. Vain *et al.*, (1998) used a genotype-independent transformation system to engineer rice for resistance against nematodes. They expressed a variant of Oryzacystatin-I (OC-I 86) in African elite rice cultivars. This strategy seeks to provide a basis for concomitant control for *M. incognita* and other root parasites of rice.

Transgenic rice resistant to sap-sucking insects

Among the many insect pests of cultivated rice, the most damaging in terms of crop losses is the brown planthopper (BPH) *Nilaparvata lugens.* BPH not only causes direct damage to rice by draining phloem sap and blocking phloem vessels, resulting in so-called *hopperburn* but it also causes damage indirectly, by acting as a vector for various stunt viruses. BPH has proved to be difficult to control with insecticides, as it has rapidly acquired resistance to common field sprays. Furthermore, the indiscriminate and excessive use of pesticides has caused drastic reductions in the populations of natural BPH predators and resulted in a number of human health problems (IRRI, 1992). Alternative BPH control strategies are thus highly desirable. Conventional plant breeding programmes to produce BPH-resistant lines have been underway for approximately 30 years and a number of resistant lines have been made available. However, the effectiveness of these lines has been limited by the evolution of BPH biotypes which are able

to overcome the resistance genes.

The possibility of introducing new BPH-resistance genes into rice by transformation offers a means of generating new resistant varieties. Sap-sucking insects generally have very low levels of proteolysis in their guts, as they utilize free amino acids in the phloem as a nitrogen source. Consequently, strategies based on inhibition of insect gut proteolysis protease inhibitors produced in transgenic plants (Hilder *et al.*, 1987) are unlikely to be successful. However, bioassays carried out in artificial diet systems have shown that plant lectins can be effective in perturbing development and decreasing the survival and fecundity of BPH, aphids and other homopterans. Not all lectins show insecticidal effects, and those that are effective show different levels of toxicity towards different insect species.

Of a series of lectins tested against BPH, the one found to be the most effective was GNA, from the snowdrop *Galanthus nivalis*. The mechanism of lectin toxicity in insects is not clear, but seems to involve binding to the gut surface. An antifeedant effect against BPH has also been suggested. There is no evidence for GNA toxicity towards higher animals so this protein would be suitable for incorporation into a transgenic crop. Transgenic potato plants constitutively expressing *gna* under control of the cauliflower mosaic virus 35S promoter (CaMV 35S) have been shown to inhibit the growth and fecundity of aphids feeding on them (Gatehouse *et al.*, 1996). These results, taken in conjunction with the data from artificial diet studies, suggest that the expression of GNA in transgenic rice could protect the plant against BPH. Since BPH is a phloem-feeder, specific expression of GNA in the phloem would deliver the protein efficiently to the insect while minimising undesirable accumulation in other tissues. To this end, the rice sucrose synthase 1 promoter (RSs1) has been used. This promoter has been shown to direct phloem-specific expression of the *gusA* and *gna* transgenes in transgenic tobacco plants. Large numbers (over 200) of transgenic plants expressing GNA were generated using particle bombardment (Sudhakar *et al.*, in press). Transgenic plants which expressed GNA at high levels (up to 2% of total leaf protein) were identified. GNA at 0.1% has been shown to be antimetabolic in artificial diets to members of three distinct families of homopteran pests including the BPH. These plants were shown to affect survival and fecundity of BPH. Subsequent testing in greenhouse and field trials will determine if any of this germplasm can be deployed successfully in large scale field trials in the tropics.

Transgenic rice carrying *Bacillus thuringiensis* (Bt) insect-resistance genes

The production of plants expressing specific Bt crystal protein genes was one of the early targets of plant genetic engineering. The value of this technology to the seed/biotechnology industry, the farmer, the environment and the consumer is evident. Crops expressing Bt genes result in significant financial, time and labour savings compared to conventional chemical-based crop protection strategies (Peferoen, 1997). An important consideration in any strategy involving insecticidal transgenes is the evolution of insect resistance to the transgenic plants. This is of particular importance when single genes are used, because their insecticidal products often interact with a single target on susceptible insect cells. A number of reports describe the evolution of resistance to Bt transgenes. For example, the evolution of resistance to *cry1Aa*, *cry1Ab*, *cry1Ac* and *cry1F* transgenes in open field populations by the diamond back moth (*Plutella xylostella*) has been described (Tabashnik, 1994). A number of laboratory-based selection experiments have also demonstrated evolution of resistance to Bt in a number of insect

species (Tabashnik, 1994). Therefore, it is important to adopt experimental strategies which will delay or perhaps prevent evolution of resistant insect populations. This may be achieved by transgene pyramiding, the use of multiple resistance genes with different modes of action against the same insects, in combination with integrated pest management.

Among the most destructive insect pests of rice are the lepidopteran stem borers (*Tryporyza incertulas* and *T. innotata*) and the rice leaf folder (*Cnaphalocrocis medinalis*) which cause annual losses in the order of 10 million tons. Complete crop failure is rare, but occasional outbreaks can destroy between 60 and 95% of the crop. Traditional breeding for leaf folder and stem borer resistance in rice has not been successful. Over the last 30 years, IRRI has screened more than 30,000 rice accessions for resistance to different stem borers, but no rice variety with a sufficient level of resistance has been developed. A number of recent reports describe rice transformation with Bt genes including *cry1Ab* and *cry1Ac* (Ghareyazie *et al.*, 1997). The regenerated transgenic plants expressed the Bt transgenes at different levels (0.01-1% total cellular protein) and demonstrated effective control of insect pests (yellow stem borer and rice leaf folder). In our laboratory we have generated transgenic indica rice (Basmati 370) expressing high levels of the novel Bt protein Cry2A and these plants show complete resistance to the yellow stem borer and the rice leaf folder. The Cry2A toxin has unique binding sites in the midgut of target insects, distinct from those of the related toxins Cry1A and Cry1C. In addition, Cry2A has a unique mode of action, forming voltage-dependent channels instead of the highly cation-selective channels formed by Cry1A and Cry1C in the lipid bilayers of insect midgut cell membranes. Due to its unique properties, Cry2A can be used in combination with other Bt toxins (e.g. Cry1Aa, Cry1Ac and Cry1C) for pyramiding resistance in transgenic plants. Insect bioassays confirmed the efficacy of insect control by transgenic rice plants expressing Cry2A (Bano-Maqbool *et al.*, in press). Primary transformants and R1 progeny were tested for their pesticidal activity. We observed that plants expressing Cry2A at moderate to high levels caused 100% insect mortality, with very little damage to the leaves tested.

CONCLUSIONS

Tremendous advances in gene transfer technology have taken place during the past few years. We are now in a position to embark on meaningful improvement programmes for a number of species, using the tools of molecular and cell biology. In some crop species, such as soybean, rice, maize, and cotton, gene transfer methods appear to be genotype- and variety-independent. A number of important crops, including most of those cultivated in developing countries for human consumption, have received very little attention. It is reasonable to expect that given enough resources, practical gene transfer methods for all the important plant species will be developed. This is of extreme importance, particularly in developing countries where populations derive most of their nutritional calories from grain legumes and cereals.

As discussed above, plant gene transfer technology is approaching a new era, where the obstacles and limitations are falling and more and more species are becoming amenable to genetic manipulation. The perspective of the scientific community is shifting from the simple strategy of engineering single gene traits to complex projects involving multiple genes. At the same time, the general public is becoming more aware of the issues which drive biotechnology and it is critical that the role of the public should be nurtured, not ignored. It is now essential

to implement broad strategies to disseminate technology and training to the developing world. A clear description of this technology and an emphasis on its potential to improve under-exploited crops, crucial for the survival of people in developing countries, may help to alleviate public concern. Technology transfer is now receiving more attention through generous funding by international organisations, such as the Rockefeller Foundation. Technology transfer is not straightforward. Some would argue that increasing the food supply in developing countries through the application of biotechnology might delay any political moves to control third world populations. The issue is indeed controversial, but serious discussions need to take place in order to ascertain the best possible way of transferring the benefits of this technology to developing countries.

Plant genetic engineering represents a unique opportunity to increase crop productivity and thus contribute towards global food security. The rice crop is an excellent example to demonstrate the benefits of a transgenic approach to pest and disease resistance which should in principle result in significant reductions in health and environmental hazards associated with pesticide misuse. Amongst the ecologist community, there is a strong belief that increased production from transgenic crops, threatens the very existence of genetic diversity as a result of monoculture. Developing agricultural systems are usually comprised of small subsistence growers who have their own favourite cultivars. Therefore, it is essential that uptake mechanisms are developed which would allow these farmers to benefit directly from the advantages of transgenic crops. Therefore, efficient technology to engineer local cultivars is vital. The Asian Biotechnology Network, national rice improvement programmes in Asia, The Department of International Development (DFID, UK) and other organizations are addressing this very issue through investment in training and technology at the local level.

Another issue whose importance is growing, is the intellectual property protection of concepts, methods, procedures and products resulting from plant genetic engineering. Patents are designed to increase innovation and competitiveness, and also to reward inventors for risk taking in developing novel and useful technologies and products. In the context of plant genetic engineering, patents will most likely act as catalysts to develop new procedures to introduce genes into plants, circumventing existing technology controlled by specific organisations. Important practical issues can now be addressed, and increased agricultural productivity should be the direct beneficiary of advances in this field. Alternative uses for surplus crops resulting from recombinant DNA technology have the potential to provide new resources for industry and the consumer, thus expanding the economic basis in both industrialised and developing countries.

REFERENCES

Bano-Maqbool S; Husnain T; Riazuddin S; Masson L; Christou P. Effective control of Yellow Stem Borer and Rice Leaf Folder in transgenic rice indica varieties Basmati 370 and M 7 using the novel d-endotoxin cry2A *Bacillus thuringiensis* gene. *Molecular Breeding (in press).*

Daily G *et al.* (1998). Food production, population growth and the environment. *Science*, **281**, 1291-1292.

Gatehouse AMR; Down RE; Powell KS; Sauvion N; RahbY; Newell CA; Merryweather A;

Gatehouse JA (1996). Effects of GNA-expressing transgenic potato plants on peach-potato aphid, *Myzus persicae*. *Ent. Exp. Appl.*, **79**, 295-307.

Ghareyazie B; Alinia F; Menguito CA; Rubia LG; De Palma JM; Liwanag EA; Cohen MB; Khush GS; Bennett J (1997). Enhanced resistance to two stem borers in an aromatic rice containing a synthetic CryIA(b) gene. *Molecular Breeding*, **3**, 401-414.

Hepher A; Atkinson HJ (1992). US patent PCTWO 92/15690.

Hilder VA; Gatehouse AMR; Sheerman SE; Barker RF; Boulter D (1987). A novel mechanism of insect resistance engineered into tobacco. *Nature*, **330**, 160-163.

Hosoyama H, Irie K, Abe K, Arai S (1995). Introduction of a chimeric gene encoding an oryzacystatin-β-glucuronidase fusion protein into rice protoplasts and regeneration of transformed plants. *Plant Cell Reports*, **15**, 174-177.

IRRI (1992). *IRRI 1990-1991: A continuing adventure in rice research*, International Rice Research Institute, Manila, Philippines.

Peferoen M (1997). Progress and prospects for field use of Bt genes in crops. *Trends in Biotechnology*, **15**, 173-177.

Sudhakar D; Duc L; Bong B; Tinjuangjun P; Bano S; Valdez M; Jeffereson R; Christou P. An efficient rice transformation system utilizing mature seed-derived explants and a portable, inexpensive particle bombardment device. *Transgenic Research* (in press).

Tabashnik BE (1994). Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology*, **39**, 47-79.

Vain P; Worland B; Clarke MC; Richard G; Beavis M; Liu H; Kohli A; Leech M; Snape J; Christou P; Atkinson H (1998). Expression of an engineered cysteine proteinase inhibitor (Oryzacystatin-IΔ86) for nematode resistance in transgenic rice plants. *Theoretical and Applied Genetics*, **96**, 266-271.