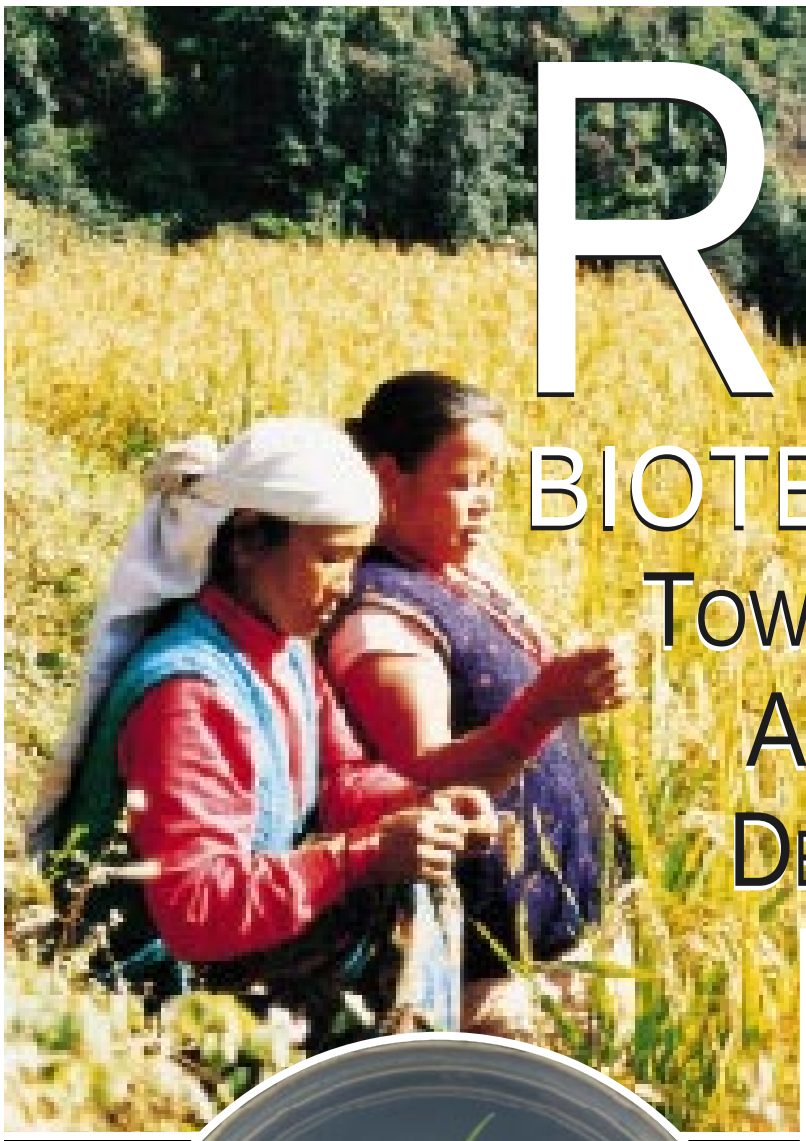
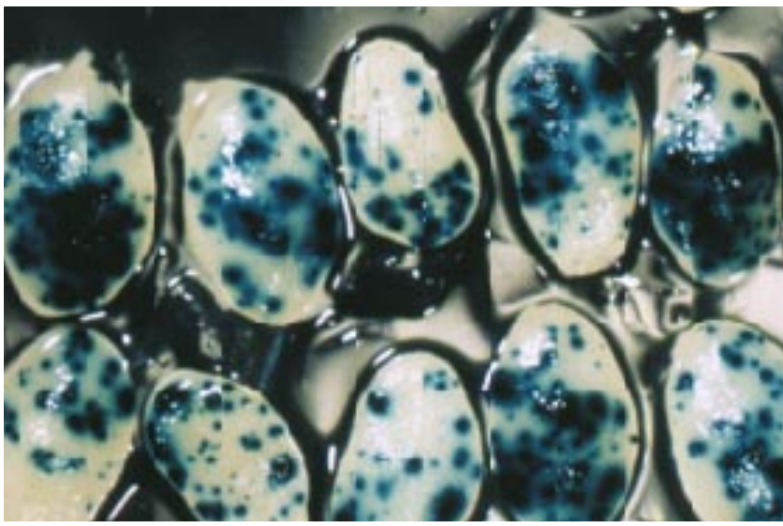
Department for International Development
Plant Sciences Research Programme

REPORT

RICE BIOTECHNOLOGY: TOWARDS SUSTAINABLE AGRICULTURE IN THE DEVELOPING WORLD





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From research to application

SUCCESSFUL laboratory studies have enabled the projects to progress to the next phase of development: assessment of biosafety and enhanced crop performance of both African and Asian rice varieties in glasshouse facilities. Such 'road-testing' has already indicated significant resistance to RYMV, and nematode pests, among the transgenic plants and is providing information to help develop yet further improvements. This extensive evaluation ensures that crops eventually released to farmers will meet their needs.

In the past year, in greenhouse tests (R6453, R6121), genetically modified African rice plants containing the cystatin gene showed a 50% reduction in nematode eggs. Other modified African rice plants showed immunity to strains of RYMV (R6355). Molecular work on understanding the mechanism of gene silencing, how transgenes are inherited from one generation to the next, and the importance to transgene activity of just where in the plant genome the transgene is inserted, has also been transferred to the greenhouse. It has been possible to 'road-test' improved designs for transgenes that confer resistance to nematodes and to rice tungro bacilliform virus, and also designs for better control sequences and marker genes. Successes with these have also made it possible to test Asian varieties for nematode resistance, and encouraged further work on improving the transgene stability and level of activity in transgenic plants.

Technology and information transfer

The success of DFID's rice-improvement programme is firmly based on the philosophy that projects are partnerships. Integrating project results into national research infrastructures in developing countries must be matched with transfer of skills to enable these countries to develop their own expertise. Short-term visits to UK labs to learn specific methods – such as the visit by an IRRI researcher to learn how to inoculate tungro virus – can be effective in removing bottlenecks to particular projects. Visits provide a two-way information flow, ensuring feedback of experience, and communication of social, economic and infrastructure constraints that may affect introduction and dissemination of techniques and crops into recipient countries. Intellectual property developed in the DFID programme, if protected, is transferred to the developing country on a royalty-free basis.

Above - Developing diagnostic testing for RYMV, without a power source, in Tanzania. (Photo: F. Kimmings, Crop Protection Programme)

Assessing rice performance in the field, in the Philippines

Biosafety

It is increasingly recognized that biotechnology can offer considerable environmental benefits in terms of reduced use of agrochemicals, and that higher crop-productivity may safeguard presently uncultivated and marginal lands. Nevertheless, due care in matching introduced genes to the agro-ecological environment for which they are intended is essential to avoid enviromental disturbance and promote stable and sustainable agriculture. Thus, strategies for ensuring the biosafety of novel crops and agricultural practice cannot be considered solely on a region by region, or country by country basis. Efforts are being made by industrialized countries to harmonize their regulatory systems to promote free trade, while at the same time protecting the environment. The same stringency of regulation and diligence in its application must be translated to countries that receive the technologies and end-results of research. Although much of the research carried out in the UK on these projects is on crops whose diseases and pests are unlikely to threaten European agriculture, the work is carried out in strict accordance with EU Directives governing the contained use (i.e. in the laboratory) and release to the environment (i.e. field trials and ultimate provision to farmers). The many complexities of moving the improved rice varieties to the ultimate beneficiary – the resource-poor farmer – are through pathways that satisfy international requirements.

Future plans

Much valuable progress has been made in developing appropriate molecular biology techniques, and gaining a better understanding of the basic biology of rice and its interactions with pests, diseases and the environment. This will enable innovative and effective research approaches to be put in place to tackle many of the complex problems faced. What's more, we are now at the stage of seeing the results of initial successful laboratory and greenhouse work being transferred to field trials, where transgenic plants will be evaluated for their stability and value under 'real' conditions. Further research is already underway, developing second and third generation 'products': these show improvements that result from more efficient methodologies, refinements in application of molecular understanding of plant function and interaction, and feedback of information from field performance of the first generation crops. Plant biotechnology can benefit the poor farmer by increasing agricultural productivity through improved crop yields, and more efficient use of inputs and resources. Much work still remains to be done, and further improvements must be made. Yet already there are tangible results of the research transferred to, and further developed in, the countries where they will make a major contribution to the achievement of stable and sustainable agriculture and economic security.

Box 2 - DFID Rice Biotechnology Achievements

- Efficient transformation of both African and Asian rice varieties
- Development of safe and efficient marker genes for research and development
- Understanding of transgene function in rice
- Strategies to develop plants with predictable and stable transgene expression
- Strategies to develop rice with enhanced grain yield
- Development of disease-resistant rice varieties
- Development of pest-resistant rice varieties
- Transfer of transgenic plants to greenhouse for stability and disease-resistance testing
- Training and technology transfer to collaborating countries

The Plant Sciences Research Programme (PSP)

THE Plant Sciences Research Programme (PSP) of the Department for International Development (DFID; formerly the Overseas Development Administration, ODA) is developing solutions that address the challenge of feeding a growing world population, within an economically and environmentally sustainable strategy. The problems faced are immense, and will not be solved quickly. Long-term solutions that enable countries in the developing world to eliminate poverty through increased self-reliance are essential. The DFID has recognized these needs and developed strategies involving new approaches to crop improvement. These strategies are driven by the needs of the recipients, and matched by appropriate scientific capability, and pathways to disseminate the technology. In accordance with the high priority given to rice to meet these aims, the PSP has invested extensively in biotechnological research for rice improvement.

THIS report summarizes the scope of the challenges being addressed, and the progress made, through DFID rice biotechnology projects.

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DFID RICE BIOTECHNOLOGY PROJECTS REVIEWED

Genetically engineered resistance to RYMV (R6355) *Y Pinto, D Baulcombe, Sainsbury Laboratory, Norwich*

Transgenic crop resistance in upland and lowland rice to nematodes (R6453)
H Atkinson, Centre for Plant Biochemistry & Biotechnology, University of Leeds

Assessing transgenic lines of rice for resistance to tungro virus disease (R6394)
R Hull, John Innes Centre, Norwich

Transgene structure and function in rice (R6343)
P Christou, John Innes Centre, Norwich

Generic system for rice transformation by particle bombardment (R6121)
P Vain, P Christou, J Snape, John Innes Centre, Norwich

Development of a simple, rapid, reproducible system for high-frequency production of transgenic rice plants (R6197)
D-F Chen, M Elliott, De Montfort University Norman Borlaug Institute for Plant Science Research, Leicester

Enhancement of grain filling in rice by genetic manipulation of phytohormone levels (R6724 [H])*
D-F Chen, M Elliott, De Montfort University Norman Borlaug Institute for Plant Science Research, Leicester

**Project funded by the Competitive Research Facility, DFID*

Developing sustainable global agriculture for the 21st century

Discussing woman farmer's experience of crop loss resulting from viral disease in Tanzania. (Photo: F. Kimmings, Crop Protection Programme)

A global problem

Many countries in the developing world are facing the challenge of rapid population growth while the agricultural support systems become increasingly fragile. Of the 4.5 billion people in the developing world, it is estimated that 700 million currently suffer from chronic undernutrition. Within the next 25 years, developing-world farmers will need to produce food for an additional 2.5 billion people (see Table 1). Although the world's food supply has tripled since the 'Green Revolution' in cereal production of the 1960s, the reliance on agrochemicals to achieve these yields imposes costs many developing countries cannot meet. This, coupled with the degradation of productive land, loss of land to urbanization, decreasing water supplies and the plateauing yield of many conventionally produced crops, poses considerable challenges. Yet the economies of these countries will continue to rely heavily on the agricultural sector. The development of high-yield, high-quality, low-input, low environmental impact agriculture is therefore essential to achieve the necessary increase in food production and employment.

Meeting the need

The potential contribution of plant biotechnology to a strategy for improving the food and economic security of the developing world cannot be disputed. Agronomic expenditure and the cost of crop losses through disease, pest and environmental damage run into tens of billions of dollars annually – costs these parts of the world can ill afford. Research supported by the Plant Sciences Research Programme (PSP) is based on socioeconomic need, and the opportunities for research to give major crop improvements.

Plant biotechnology is being applied to serious problems which currently limit crop productivity – loss of both growing and stored crops, and the inherent genetic limitations to productivity of crops improved through conventional breeding.

Key areas of research are:

- understanding and enhancing the resistance of crops to viral, fungal and bacterial diseases, insect and nematode pests; and
- genetic modification of plants to enhance the conversion of a plant's resources into nutritionally valuable protein and carbohydrate.

Much effort is also devoted to studying the effects on crop productivity of stresses inflicted by the physical environment. Such stresses, resulting from droughts, extreme temperatures, or excess minerals such as salt, are already a cause of unreliable productivity in developing-country agriculture and are likely to be exacerbated by climate change.

Facilitating self-reliance

Effective transfer of technology and materials is essential if farmers and consumers with limited purchasing power are to benefit. Applied through the international system of publicly supported agricultural research institutions – which already has a strong record of delivering improved crop varieties to developing countries – plant biotechnology can help by shifting the balance towards more efficient use of inputs, and reduced agrochemical use.

Much of the research is carried out in collaboration with agricultural research institutions in developing regions. Such links ensure that research is undertaken with emphasis on the farmers' needs and the constraints under which they operate, and provide routes to promote technology transfer within the agro-economic structures existing within the region.

Table 1 Predicted changes in population, and rice consumption*

	Population (millions)		Rice (%) total calorie supply	Rice consumption‡ (million t)	
	1995	2025		1995	2025
Asia	3443	4860	35	418.5	684.9
Africa#	719	1431	7	15.7	36.1
Cote d'Ivoire	14	34	22	1.1	2.2
Guinea	7	15	37	0.8	4.0
Liberia	2	5	40	0.3	1.0
Nigeria	111	217	10	3.4	4.6
Sierra Leone	5	10	40	0.5	1.8
World	5702	8122	21	457.5	757.5

**Source: IRRI Rice Facts 1997*

#The figures for Africa provide an average for the whole continent, where rice is not in all cases the main staple; examples of West African countries showing the dependence on rice as main source of calories follow.

‡ Rough rice equivalent

WHY RICE?

WHY is rice (*Oryza sativa* L.) such a focus of research activity? It is grown primarily in developing countries and is the main food source for three billion people – almost half the world's population. Its yield and quality are severely compromised by pests, diseases, physiological and environmental factors. It is also the world's single largest market for agrochemicals, consuming around UK£2 billion annually. Together, agrochemical costs and crop losses amount to UK£tens of billions each year. Because most methods of rice cultivation are heavily dependent on diminishing local water resources, the need to reduce agrochemicals in drinking water supplies is therefore also significant. Strategies for reducing the crop loss (almost 50%) each year are already being addressed in the laboratory, greenhouse and field through both conventional breeding and biotechnological methods.

Improvement of rice through genetic modification

The ability to isolate and transfer (i.e. to clone) useful genes into diverse species has overturned previous ideas about limits to crop improvement. It is important that, to be both useful and acceptable to the poor farmers across the target regions of Asia and Africa, the genetically modified crops developed are derived from varieties already widely grown and suited to particular agroenvironments.

Among the many rice subspecies that exist, *indica*, *japonica* and *javanica* are of greatest agronomic importance: *indica* accounts for 80% of cultivated rice. *Japonica* and *javanica* can readily be crossed to yield fertile hybrids. However, *indica* is genetically distinct, and crosses of *indica* with *japonica* or with *javanica* are less successful. The different subspecies have different advantages: for example, although *indica* is quite resistant to insects and diseases, it is less able to tolerate low temperatures and can sometimes grow too tall and collapse in response to fertilizer application. Different types of rice are needed to suit different types of cultivation: in mountain regions, rice is grown in a manner similar to wheat or maize; whereas in lowland regions, it may be grown in standing water.

Rice research is now at a crossroads: many technical barriers to biotechnological improvement of the crop have been overcome and there are numerous targets where introducing agronomically important genes into rice will provide significant benefit. Genetic modification thus offers an option complementary to conventional breeding for generating major increases in productivity and endowing the plants with inherent resistance to pests and disease. Nevertheless, there is a need for ongoing basic research to develop and optimize the technology for application to widely grown varieties of rice, and to understand the relationship between genes and the characteristics they control at the molecular level.

Gene technology

There are several methods for introducing genes into plants (see Box 1), but they vary in their usefulness. Although the first transgenic plant – tobacco – was created nearly 15 years ago, the method used did not succeed with cereals. The subsequent development of other gene-transfer methods, and technologies for regenerating whole plants from plant cells and tissues, means that it is now possible to genetically engineer most types of plants, including cereals. However, the variation found even among the agronomically important varieties of rice means that not all gene-transfer methods are equally efficient.

Optimizing techniques for gene transfer in rice is therefore a priority, and work has progressed with both *Agrobacterium* (R6197) and biolistics (R6197, R6121) approaches to develop efficient and reproducible systems for transformation of commercial varieties of rice.

A range of reporter and selectable genes (Box 1) can be used, but alternatives are required: concerns have been expressed over the use of some antibiotic-resistance genes, and selection of modified plants can require many months to distinguish between plants where gene transfer has been successful, and where it has failed. Rice varieties show different sensitivity to selective agents, and variety-independent systems are needed. Reliable, non-destructive detection methods, that could be carried out quickly on living tissue at an early stage, would also be valuable. There has therefore been much interest in using novel genes that encode proteins which fluoresce when an ultraviolet light is shone on the transgenic plant. One such protein is the 'green fluorescent protein', and work so far (R6121) shows that it can be used to detect, safely and easily, transformed rice plants just a few weeks, rather than a few months old. It also seems to be appropriate for developing rice variety-independent transformation, and may be useful for other cereals too.

Initially, problems with regenerating whole plants from plant tissues or cells were a major barrier to plant improvement. However, better protocols for regenerating *japonica* varieties from protoplasts, immature plant embryos and cells derived from mature embryos have now been developed (R6197). *Indica* varieties have proved to be more difficult, partly because of the greater variation in regeneration ability between varieties. Nevertheless, an efficient system has been developed for regenerating plants from mature embryos of commercially important *indica* varieties, including *Pusa Basmati* 1(R6197).

Getting the best results from transgenes

Efficient use of gene technology in rice requires predictable and fine control of the function of introduced genes (transgenes). Knowing the characteristic a gene is responsible for in the plant within which it is normally found, and being able to transfer that gene into a new species, is rarely sufficient to transfer the desirable characteristic to a new crop. Many factors can prevent the gene functioning in the same way, or even at all. The introduced gene may be rearranged so that it is no longer intact, or is not linked in the right order to sequences that control its activity. It may be present, and intact, but somehow 'silenced': such transgene silencing has been a focus of research for several years and only now are mechanisms by which this occurs starting to be understood. Even if transgenes are stable in the first generation of transgenic plants, they may not be stably inherited in subsequent generations. Understanding how transgenes are integrated into plants is facilitating the improved design of transgenes to avoid these problems.

Recent work (R6343) to understand how transgenes function in rice has focused on analysing the fate of model transgenes introduced into seven *indica* and *japonica* varieties. Extensive work has revealed some surprising results, including a new understanding of how transgenes behave. The main outcome is that it is now possible to design better strategies for creating transgenic rice with more stable and predictable gene expression (R6121). A further benefit is the opportunity to exploit the information derived from these experiments to facilitate the use of transgenes in other crops – including some that are the focus of other DFID inititatives.

Demonstration field plot at IRRI for testing rice variety performance

Rice productivity: overcoming physiological limits

Between 1960 and 1980, annual cereal production increased from around 800 million tons to nearly 2000 million tons. This leap in productivity was mainly the result of large-scale planting of high-yield semi-dwarf varieties, developed through conventional breeding and reliant on high levels of fertilizer input to maximize yield. Since then, further increases in yield have not been forthcoming. Because major increases in the area planted to rice are unlikely - if anything, the area available will decrease because of urbanization and industrialization – strategies to develop rice varieties with a higher yield potential are seen by the International Rice Research Institute (IRRI) as being the highest priority for research. However, rice breeding programmes worldwide indicate that we are close to the limit of yield improvement through conventional means, and attempts to increase productivity through breeding have encountered problems.

By breeding its New Plant Type (NPT) rice, IRRI aimed to increase productivity, and progress since 1989 has been rapid: NPT rice is semi-dwarf, sturdy, with all stems bearing grains and an increased number of grains per stem. However, these new varieties showed poor grain-filling. Grain yield is determined by two plant processs: photosynthesis, to produce the carbohydrate; and partitioning, which determines how much of the carbohydrate is stored in the grain.

Two main approaches are being used to address the problem:

- modifying the levels of plant hormones (auxins and cytokinins) known to play an important role in grain filling in cereals, by introducing genes for enzymes which regulate hormone biosynthesis from *Agrobacterium*. Grain development is associated with waves of hormones: a wave of cytokinin causes a rapid increase in the number of cells in the developing grain; and a wave of auxin with a massive accumulation of carbohydrate and protein.
- delaying the aging (senescence) of plant leaves, by introducing cytokinin biosynthesis genes designed to increase the level of hormone at a stage when leaves would otherwise start to die. Thus, by prolonging the time during which leaves can make carbohydrates by photosynthesis, the supply of carbohydrates to the developing grains is increased.

Growing cereals may be sprayed with cytokinins and auxins to increase yield, but this is imprecise and can have undesirable side-effects. By linking the hormone biosynthesis genes to wheat or rice promoters that control expression of the hormone, it is possible to direct the increase in hormones to just the desired parts of the plant.

Three NPT rice varieties have already been transformed using biolistics, and procedures have been optimized for *Agrobacterium*-mediated transformation of commercial *japonica* and *indica* rice varieties (R6197). Work is now under way to develop the higher yielding characteristics in widely grown varieties.

Box 1 - How to genetically modify rice

Successful genetic modification of plants depends on:

- the introduction of isolated genes into the plant's own genome
- regeneration of an intact plant from the modified tissue
- expression of the introduced genes
- stable inheritance of the introduced gene by subsequent generations.

Designer genes

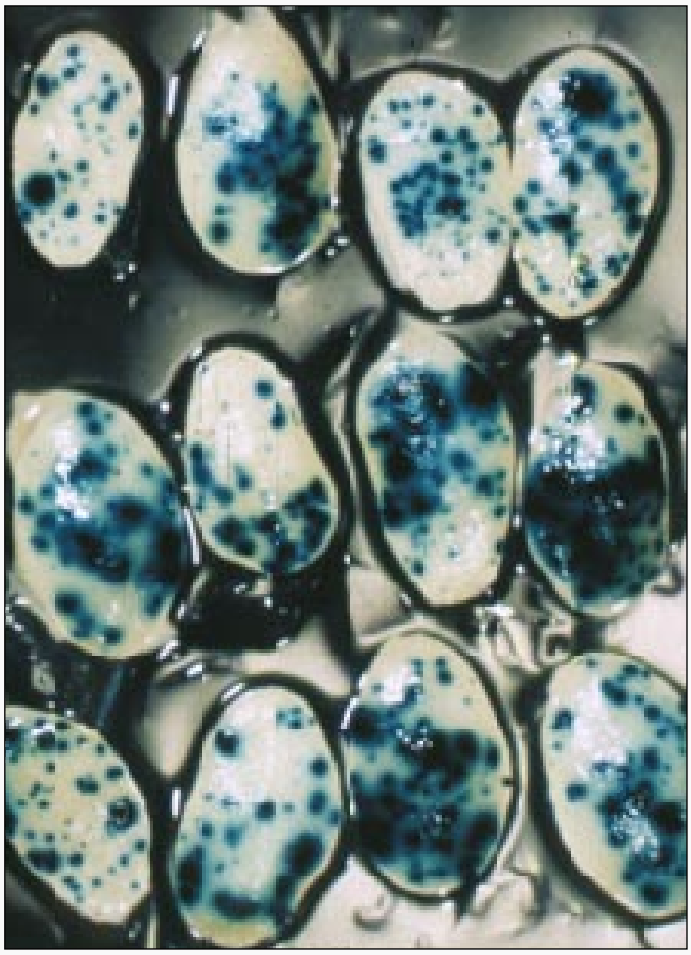
Genes isolated from the same or other species ('transgenes') have to be introduced into a plant together with sequences that control how they work. A 'promoter' is needed to activate or 'express' the gene. Some promoters cause the genes to which they are linked to be expressed all the time, whereas others allow expression only at certain stages of plant growth (e.g. flower bud formation), or in certain plant tissues, or in response to external environmental signals (daylength). Some promoters are weak, whereas others are strong, and this determines how much of the gene product is made. There are advantages in being able to control gene expression, directing the plant's resources into growing or synthesizing valuable molecules, where and when appropriate. 'Marker genes' are also linked to the foreign gene to enable detection of plant tissue into which the foreign gene is successfully introduced – these may confer resistance to a selection pressure (e.g. antibiotic resistance), or a characteristic that may be detected easily in a laboratory test. The use of marker genes enables only the tissue which has the foreign gene successfully introduced to be identified and regenerated into whole plants, thus saving considerable expense and effort. The foreign gene and associated sequences may be introduced as either naked DNA, or as part of a vehicle 'vector' which may facilitate its introduction, depending on the method used.

Getting foreign genes into plants

Gene-transfer methods must get the foreign gene past the barriers of the cell wall, cell membrane and the envelope that surrounds the nucleus, without affecting the cell's viability. One method uses the natural DNA-transfer ability of *Agrobacterium*, which has been called 'Nature's own genetic engineer'. Although *Agrobacterium* normally causes disease, its tumour inducing ability can be removed, and the resulting 'vector' used to introduce transgenes. Until recently, this approach did not work well with cereals, and other methods had to be developed. One of these uses plant cells from which the cell walls have been removed ('protoplasts') into which DNA can be introduced by application of high voltages. However, whole plants cannot be regenerated from protoplasts for all plant species. The 'gene gun' or 'biolistics' device can be used with all plant species. This uses gold or tungsten microparticles, coated with transgene DNA, which are fired into the target tissue by an explosive discharge or pressurized helium. DNA which penetrates the nucleus may be incorporated among the plant's own genes: if the introduced genes are active, and the gene-product synthesized, the plant is said to be 'transformed'. If the plant is stably transformed such that the foreign gene is inherited and active in subsequent generations, then the plant is truly 'transgenic'.

Regeneration of whole plants

The unique ability of pieces of plant tissue, or even individual cells, to regenerate into a whole plant is exploited in most techniques of gene transfer. Cells or tissue into which genes have been introduced can be regenerated by the use of media which contains appropriate plant hormones, and careful culture, into whole plants. However, there is no universally applicable method as tissues from different sources respond differently: culture and regeneration protocols must be adapted depending on both plant and cell type.



Above - Blue patches show successful expression of introduced marker genes in rice embryos

Right - Regenerating transformed rice seedlings in test tubes

Combating rice diseases and pests

RICE is vulnerable to numerous diseases and to pest predation, problems exacerbated by the pattern of intensive and continuous cultivation designed to increase productivity. Endowing plants with genetic resistance is the main biotechnological strategy for controlling nematode and insect pests and the wide range of viral, bacterial and fungal diseases. The role of insect pests as vectors for disease is often as significant as the damage they cause by direct feeding.

Developing viral resistance

RICE YELLOW MOTTLE VIRUS

Rice yellow mottle virus (RYMV) causes a serious disease in African rice: in some locations, rice production is impossible because of this disease. To address this problem, a genetic modification strategy, already successful in combatting viral diseases of other crops, has been used (R6355). Known as 'homology-dependent resistance', this strategy involves introducing fragments of virus genes into a plant. The presence of these viral genes causes the plants to be resistant to infection by the virus. In the first demonstration of homology-dependent resistance in rice, we have produced rice plants, carrying RYMV transgenes, that are resistant to RYMV. These transgenic plants are derived from rice cultivars that are grown widely in West Africa. However, before the plants can be grown as crops, we need to test the effectiveness of the RYMV resistance under field conditions, and to establish that the resistance is effective against all natural isolates of the virus. This more advanced testing of the plants will be carried out in collaboration with the West African Rice Development Agency (WARDA) and the International Institute for Tropical Agriculture (IITA).

Symptons of infection with RYMV, showing healthy uninfected rice leaf on left, and the symptoms following infection at increasing time intervals up to 25 days

TUNGRO DISEASE

Of the virus diseases that affect rice in the tropics, tungro is the most serious, leading to annual losses of £1 billion in South-East Asia. The disease is caused by a complex of two viruses, one of which affects the spread of the complex by leafhoppers, the other is responsible for the severe damage inflicted. The current method of controlling the disease relies on insecticide sprays to kill the leafhoppers: there are few examples of natural resistance in rice, and attempts to cross-breed this resistance into crops have been largely unsuccessful. Extensive molecular biological work has been carried out on tungro viruses and attempts are being made to genetically engineer virus-resistant plants by interfering with the way the viruses work (R6394). Taking this route of genetic modification, various strategies based on introducing parts of genes that code for the viral coat proteins into plants are being tested for providing disease-resistant rice varieties. This work has now progressed to the stage of evaluating the transgenic rice plants at IRRI, and the development of improved 2nd and 3rd generation constructs (R6394).

Developing nematode resistance

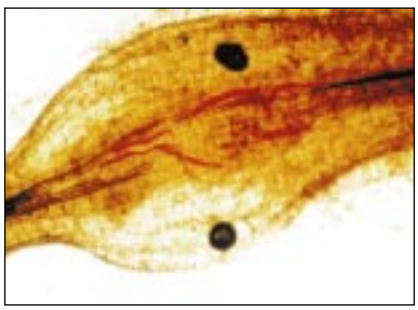
Nematodes destroy nearly UK£70 billion in crops worldwide annually, and there are few options for controlling the heavy damage inflicted. Crop protection relies mainly on some of the most toxic and environmentally hazardous pesticides in widespread use. Classical plant breeding has frequently failed to produce commercial crop varieties able to resist the pests. However, the potential of recent advances in plant biotechnology for reducing the time taken to introduce specific resistance genes into established varieties has been recognized.

Root-knot nematodes (*Meloidogyne* species) are often termed 'universal plant parasites' and are the primary pest of tropical agriculture. They damage upland rice in both Asia and Africa, causing losses of up to 70% of individual crops. Attempts to compensate for this loss by increasing the number of crops per year causes nematode numbers to increase dramatically, exacerbating the problem. These losses impact upon poor farmers for whom hazardous nematicides are inappropriate and too expensive.

One of the most promising strategies to combat nematode feeding and damage to plants is to develop transgenic plants that produce proteinase inhibitors – naturally occurring self-defence proteins produced in plants in response to damage by feeding pests. These discourage feeding by interfering with the digestive enzymes – proteinases – of the pests.

Cysteine proteinases are an essential component of the digestive system of nematodes, but are not part of digestive systems of mammals. Indeed, the inhibitors of cysteine proteinases (cystatins) are already consumed without ill effect, by billions of people, in many plant foodstuffs including rice. Although plant cystatins are not normally produced in roots, and so have no natural role against root-feeding nematodes, plants can be modified to produce them in their roots.

A cystatin gene previously cloned from rice was altered by 'protein engineering' to improve its effectiveness against nematodes (R6453). The modified gene was transferred into rice, which produced the cystatin in the roots (R6121): nematodes feeding on these plants failed to thrive and their egg production was halved in comparison with plants not producing the cystatin defence. Research is now under way to boost production of this cystatin to provide even better protection, in both old and young roots. Field trials will commence at WARDA as soon as high levels of resistance are achieved in greenhouse-trial plants, so minimising lead times to first use. Maize and sunflower cystatins are also being investigated for their usefulness in a progressive approach to high-level resistance. This new defence strategy is expected to be effective against a wide range of nematodes, a feature particularly relevant to developing world agriculture, where the farmer is often unaware of nematodes, and beyond the reach of expert advice.



Root-knot nematode worms (stained pink) beginning the feeding process that damages root growth and function.