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## **Applications of Biotechnology: Genuine Benefits or Empty Promises?**

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# Applications of Biotechnology: Genuine Benefits or Empty Promises?

E.S. DENNIS

Developing countries are faced with the formidable task of doubling their food output over the next twenty-five years and they must do this in ways that are sparing of the environment and resources. Because of intensive land use and widespread shortage of biomass, cultivated soils are being depleted of essential nutrients and organic matter. Fisheries, livestock and forestry resources are also under increasing strain.

As part of a comprehensive approach, one of the technologies that will assist in overcoming these problems will be genetic engineering of crop plants. The main objective will be to find improved varieties that give reliable high yields at the same or lower production costs through characteristics such as resistance to plant pests and diseases, and to climatic stress. The main targets will probably be rice (staple for 2.4 billion people) and cassava (staple for 500 million people).

Less than twenty years ago the first transgenic plant was produced; this was tobacco containing a gene from a bacterial parasite, *Agrobacterium*. It was only in 1995/1996 that the first transgenic crops were released; by 2002 between 30 and 50% of the major crops produced in the United States, soybean, maize and cotton, are transgenic. This

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In 2000 she, together with Dr Jim Peacock, was awarded the inaugural Prime Minister's Prize for Science for Research in Plant Molecular Biology, particularly for work on the initiation of flowering.

has been a remarkable history and we are really only at the beginning of capturing the benefits from genetic engineering of crop plants.

What is genetic engineering? Genetic engineering, or genetic manipulation, is the introduction of a gene from one organism to another, or alteration in the level of activity of a pre-existing gene by either increasing or decreasing it.

What is a gene? A gene is a piece of DNA. It has two components; firstly it has the coding region that makes a protein and, secondly, it has regulatory regions which affect the activity of a gene. We can either alter what product a gene makes or we can alter the activity of the gene.

## Changing a single gene

So far, the genetic manipulation that has been done on crops is very simple gene manipulation, introducing or altering the activity of one or few genes. To date, the best example is the insecticidal toxin gene *Bt* from *Bacillus thuringiensis* (*Bt*), where a single gene can convert a cotton plant from being devastated by a few *Helicoverpa* larvae to a protected plant in which only small bites are taken out of the leaves before the larvae die. Over 800 insect species attack growing or stored rice, and the same *Bt* gene that protects cotton can be used to protect rice against some of these insect pests.

This *Bt* gene transferred to rice gave protection against stem borers, which are chronic pests in many rice-growing regions. Protection against insect pests in stored seed, such as pea weevil, can also be achieved by introducing genes, such as an alpha amylase inhibitor, against pea weevil.

Abiotic stress, such as is caused by water deficiency or surplus, cold, heat and salinity, causes severe losses to crop production. While responses to these stresses are complex, a

remarkable result has been achieved in tomatoes by the introduction of a single gene. This single gene is an *Arabidopsis* sodium transporter and works by moving sodium into the vacuole of the cell, keeping the high sodium concentration away from other parts of the cell. The surprising result is while wild-type plants can't grow on 200 mM of salt, transgenic plants containing the added single gene grown on 100 mM salt produce almost as much fruit as the non-transgenic plants grown on 5 mM salt. The transgenic plants and non-transgenic plants perform equally well at low salt concentrations, but at high salt concentrations transgenic plants grow where wild-type plants can't.

One of the benefits of using these new molecular technologies is that it has enabled us to understand the processes that are occurring in plants and thus given us the ability to manipulate them. For example, many viruses infect plants and cause loss of yield. We have only recently discovered the mechanism by which plants resist viruses. Plants can switch off viral genes and prevent infection. This system uses an intermediate in viral replication, which is a double-stranded RNA, as a trigger against viruses. Scientists have been able to harness this novel mechanism to protect plants. Double-stranded RNA, which is produced in a hairpin form, can be used to silence any genes including viral genes. A transgenic plant containing a hairpin RNA with sequences from barley yellow dwarf virus is immune to barley yellow dwarf virus infection. In principle, this method can be used against any virus to give immunity.

This hairpin RNA can also be used to manipulate the activity of a plant's own genes, for example by switching off the activity of unwanted genes. *FLC* is a gene that delays flowering. In the presence of a hairpin RNA directed against *FLC* sequences the gene is switched off; the floral repressor is inactivated and plants will flower early. Many different traits can be manipulated using this hairpin RNA.

Next to increasing yield, improved nutritional quality is an important issue. Rice is rich in energy and is a good source of protein. In Asia, rice is responsible for 80% of calorie and 68% of protein intake. Diets that depend chiefly on milled rice lead to malnutrition with deficiencies in lysine, vitamin A, iron and zinc. Two billion people worldwide suffer from iron deficiency anaemia,

and perhaps another half-a-billion from vitamin A deficiency. We are trying to increase the level of iron in rice by adding an extra haemoglobin gene which encodes an iron-containing protein.

Rice does not contain  $\beta$ carotene (vitamin A) or its precursors. Genes for four enzymes in the pathway for vitamin A synthesis have been introduced and 'golden rice', which contains increased levels of vitamin A, produced. Nutritional testing of the GM rice needs to be done to monitor the effects on human health.

## Multiple gene changes

So far I have considered only single-gene changes. These may provide enhanced resistance to pests – insect, fungal or viral and enhancement of resistance to climatic stresses that reduce yield. Many different genes have been introduced into different species and are undergoing both field and laboratory testing.

In future, we will be looking at manipulating whole pathways; one of our targets is to engineer apomixis into rice for enhanced seed development. Apomixis is the reproduction of the female plant without any male contribution; it is clonal propagation through seed. It fixes the genotype and can maintain hybrid vigour and yield, reduce pollination problems and, perhaps, lead to rapid production of new varieties. Apomixis is present in some wild species but introducing it into crop species, such as rice, involves altering pathways of seed development. Using the model plant *Arabidopsis* we are making some progress. Normally, in the absence of fertilisation, seed will not develop; unfertilised, the seedpod stays small. We have isolated three mutants in *Arabidopsis* which allow partial seed development in the absence of fertilization. This allows endosperm development, the part of the seed that feeds the embryo, without any fertilization. We are now looking for mutants that also allow embryo development and thus complete apomixis seed development. Apomixis is one of the major goals in genetic engineering to enhance crop yield.

Another new technology that may be important is genomics where, instead of looking at single genes, all the genes in an organism are studied. Already the genome sequences of *Arabidopsis*, a model broad leaf plant and rice, a model cereal, have been published and are in the public domain. Computer predictions are of some 20–30 thousand

genes in each of these species. The task is now to establish the function of each of these genes and to see if any can be used for crop improvement. This can be achieved in several ways: Firstly, by determining the pattern of activity of each of the genes. Microarrays, where all of the genes of a genome are spotted onto a microscope slide by a robot, can be used determine which genes are active. We assume that if a gene is active in roots, its function is in roots, if it is switched on by heat it is part of a heat stress response. Using this technology we can also look for genes important in stress responses. For example, if we look at two cultivars, one with a poor stress response and one with a good stress response, we can determine which genes are switched on specifically in the good stress responder and which are switched on in the poor stress responder. These are the sorts of genes we might want to incorporate into breeding programs.

A second way of determining what a gene's function is is to mutate the gene and see if there is a mutant phenotype. The genomes of all the cereals are very similar; they have similar genes in a similar order. Rice has a small genome size so it is used as the model plant; there is much more DNA in the genome of other cereals so they are much more difficult to handle, but what we find for rice will apply to the other cereals. By inserting known DNA sequences in each of the genes of rice we will be able to determine what each of the genes does. For example, we have identified a gene that affects plant height. A plant with an intact gene without an insertion has normal height. A plant with the disrupted gene is dwarf. We can say that because disrupting this gene gives you a dwarf plant then this gene controls plant height. In this way we identify genes that are going to be important for rice and cereal improvement and in the future, we will be able to use them for crop improvement.

Identifying the function of all the genes of rice involves large amounts of infrastructure, and we already have inserts in about 1000 rice genes. But, given the number of genes as targets, it is impossible for any one laboratory to complete the task. The best approach would be a worldwide consortium to combine resources and share results. We have received a grant from the Department of Science through the Australian Academy of Technological Sciences and Engineering to convene an international workshop in November this year to set up such collaboration with

participants from all of the active rice functional genomics laboratories world wide.

Although genetic engineering has only been around a short time, we are finding that the new knowledge we gain leads to new approaches. The genes that we isolate can be important as markers for following genes in breeding. When we introduce these genes into transgenic crops it can lead to cultivars with improved pest or viral resistance or enhanced nutrition.

We must remember that genetic manipulation is just one approach in the whole spectrum of plant improvement and that gene manipulation has to be integrated into adapted cultivars containing other characteristics.

The big edge that transformation genetics has over conventional breeding is that the desired properties can be systematically sought, identified and extracted from one plant, or almost any other organism, and within a relatively short period of time, transferred to another plant. The result is the same as that achieved with conventional methods but without costly, and sometimes impossible, crossbreeding.

## **Public or private?**

As in drug development, the development of transgenic cultivars is a very expensive process and passing all the regulatory hurdles can cost in the order of ten million dollars for the release of a single cultivar. High-value crops in a developed country may recoup this expenditure, but private companies will not do a lot of development of cultivars and obtain regulatory approval of crops specifically for developing countries where they will not make a profit. Clearly, there has to be public research for public good to produce these benefits for developing countries. Some companies are contributing their intellectual property free of charge for developing countries, which is an important contribution.

What is needed to make the GM approach really work is clearly defined objectives, with important goals and research done properly. Technology needs to be developed in particular, genes and transformation technology for introducing them, intellectual property constraints clarified and management of these new technologies defined; this will involve rural officers, farmers and the public being kept informed. The technology will

need to be done in public research laboratories in developed or developing countries as a partnership with the support of private and public organisations. This will allow private companies to make available their intellectual property so there is freedom to operate. Proper field and nutritional testing must be carried out, and regulatory procedures followed. Breeding must be with cultivars that are going to be useful in a particular situation and there must be the strong management strategies to prevent, for example, the build up of insect resistance.

This needs to be done in the context of international laboratories, international trade and government. Once a cultivar is available there has to be seed production and distribution, which is one of the most important components. One way to handle all this is for the CGIAR Institutes to take the initiative to see this process through.

Only in this way will we be able to harness the potential of GM crops for food for the world.