

Plant biotechnology for food and environment security

Case studies from Australia

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Higher agricultural production can be achieved through the application of modern biotechnology. Gene technology has been successfully used by scientists all over the world to produce improved varieties of plants with desirable characters. These genetically modified (GM) crops have since been commercialized to a large extent in many of the developed countries. This article presents Australian case studies on the opportunities provided by biotechnology and genetic engineering for food and fibre production, particularly through the control of pests and diseases.

Introduction

For over 10,000 years, humans have been selecting and modifying crop plants to make them more suitable for cultivation and more productive. With the introduction of intensive and extensive cultivation of crops resulting from the Green Revolution that began in the 1960s, significantly higher yields have benefited both producers and consumers. This has led to increased food production as well as increased incomes for farmers. The resulting demand for higher agricultural production, achieved through using pesticides and monocultural practices, to meet the rapidly growing world population has created certain unintended consequences.

Some pests and diseases that were previously considered to be unimportant suddenly became major threats, and the use of chemicals has sometimes created damage to the environment and human health.^{1,2} The extensive clearing of land and the use of fertilisers and pesticides to boost production has also led to significant changes in the landscape. In Australia, eucalyptus woodland has given way to pastures and crops, sandplain vegetation to wheat and lupins, and riverine plain to irrigated fields. These changes have also had other consequences, including acceleration of the natural processes of soil acidification, proportionate increases in nutrient removal, and imbalances between water added in rainfall and irrigation and water use by crops and pastures. The

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imbalances in the water cycle have resulted in rising water tables and dryland salinity that is more difficult to counteract. The annual imbalance in water use in dryland farming systems may be as little as 20 mm more drainage than under native vegetation³, but the cumulative effects of this water escaping to the ground water can be serious.

Much of modern research in plant science is aimed at finding environmentally sustainable ways of controlling biotic and abiotic stresses as well as improving product quality. Various plant breeding and improvement programmes have been initiated to produce new varieties with better natural resistance against pests and diseases, such as rice tungro virus.^{4,5}

In addition, integrated pest management (using a combination of cultural practices, disease forecasting, partial natural resistance and applying safer and less chemicals) and biological control programmes have proved to be successful in many instances in the control of pests and diseases.^{6,7,8,9,10} Problems arise when there is a lack of suitable natural resistance genes or biological control agents to combat multiple strains of a pathogen, such as in many virus diseases, or when a low-value plant species such as forage crops is not amenable to the costly inputs of spray chemicals.

Cultural and management solutions are also being contemplated to reverse the environmental impact of modern agricultural production systems. These solutions include reforestation, the planting of trees in agro-forestry and the modification of existing agricultural systems to increase water use and to bring it into balance with water inputs. These are based on the notion that the soil water imbalance has resulted from replacing deep-rooted, perennial species with shallower rooted annual crops and pastures that grow through the winter and spring.³ Attempts have been made to model crop responses to fertilizer application^{11,12} with the aim of providing information to farmers on managing fertilizer application and the breeding of crops for tolerance to adverse environmental conditions. One promising modification to agricultural practice is the use of lucerne, a peren-

ennial with deep roots and a strong summer growth habit that produces a valuable herbage. Lucerne uses substantial amounts of water over the summer months and dries out the soil profile to a greater depth. Thus, when lucerne is grown in rotation with cereal and oilseed crops and managed appropriately, the water use of the system is more nearly in balance with rainfall.¹³ Lucerne and other high yielding crops and perennial pastures could help to combat the salinity problem that affects 2.5 million ha (4.5 per cent) of Australia's cultivated land. Research showed that lucerne has the capacity to dry soil to a depth 200 mm more than annual pastures and 100 mm more than well-managed crops.

Biotechnology is a recent addition to the techniques of plant improvement by genetic approaches. We define biotechnology as consisting of two distinct technologies. The first is marker technology in which genetic markers (DNA fragments) are used in marker-assisted selection (MAS) to identify and expedite the combining of existing desirable characters within a new plant line. The second is gene technology in which desirable genes are reconstructed by recombinant DNA methods and transferred into plants to provide a needed genetic trait, such as virus resistance. Plants derived using gene technology are termed genetically engineered or modified organisms (GMOs), whereas plants derived using marker assisted selection are not considered to be GMOs.

In this article we shall present case studies on the opportunities presented by gene technology for increasing food and fibre production, particularly through the control of pests and diseases of crops using added resistance genes. The use of MAS is beyond the scope of this brief article.

The first experimental plants produced using gene technology appeared in 1983. Since then a number of GM crops have been commercialized. The majority of these plants contain new characters that either provide enhanced competition with weeds or protect against insect pests or viral disease, all of which can be classified as biotic stresses. These new characters were all supplied through the introduction of single genes, most of which were derived from microbial sources.

In order to successfully use gene

technology for plant improvement, there are currently a few criteria that must be met. First, it is necessary to go through a tissue culture phase in which single cells are transformed with the gene of interest, and those cells containing the new gene are then selected on a specific growth medium and regenerated into whole plants. This phase is slow and labour-intensive and requires the use of selectable marker genes capable of permitting growth on selective agents such as antibiotics, herbicides or nutrients that can only be taken up and/or metabolized when the marker gene is expressed. New methods of gene transfer that do not involve a tissue culture step are under experimental evaluation, e.g. in barrel medic. These new procedures will require good methods to screen for the introduced character.

Tissue culture-based methods for gene transfer have been developed for many crop and pasture plants. A small selection is shown in Table 1. Only a fraction have reached the stage of commercialization. In Australia, cotton and carnation are the only plants in commercial production. Overseas, canola, soybean and maize are the dominant commercial crops. Many crops are in field trials and under regulatory evaluation prior to commercial release over the next five to ten years.

The second criterion to be met is the availability of specific genes for transfer into plants. Until recently, there were relatively few genes available. Large scale genomic sequencing projects have almost reached completion for one dicotyledonous plant, *Arabidopsis thaliana* and for one monocot, rice. This means that we will have most, if not all, of the genes present in plants. In addition, the genomes of yeast, a number of bacteria, the fruit fly and a nematode have also been completed. A knowledge of the DNA sequence of all these genes will be useful for the construction of new genes for plant improvement.

The third criterion for the successful use of gene technology in plants is a good understanding of gene function and how genes are regulated. This is currently being approached on a small scale using the techniques of biochemistry, cell biology, gene transfer, physiology and agronomy using a handful of available genes. Progress is still slow and, because the genomes of plants

contain more than 50,000 genes, there is a pressing need to speed up our progress in understanding gene function. The new developments in genome research called functional genomics will begin to provide such an understanding when this is integrated with an analysis of the proteins and the products of metabolism. An approach in functional genomics is to knock out all of the genes one at a time in one or a few model plants. Such “gene knock-out” plants have already been generated in *Arabidopsis* and are under development for rice. Analysis of the effects of each of these gene disruptions will provide an understanding of the role of the gene and could enable a rational deployment of that gene for future plant improvement.¹⁴

Finally, after obtaining the transgenic plants, several research and developmental steps are required before a GM crop is ready for commercial release. These include:

- Establishing freedom to operate for any third party-owned technology used to produce the transgenic plant;
- Molecular analysis of the transgenic plants;
- Conducting field trials of the new lines under biosafety guidelines;
- Conducting environmental and health risk assessments prior to the commercial release of the GM crop;
- Production of elite breeding lines homozygous for the added gene; and
- Establishing a breeding nursery for cultivar development.

Other biotechnology research is underway to improve plant performance through increasing tolerance to abiotic stress and to produce plants with soil remediation capacity (Table 2). In addition, there are many projects¹⁵ aimed at improving other aspects of plant performance and quality.

The following case studies illustrate the process of the development of pest and disease resistant GM crops in Australia.

Case study 1

Cotton with built-in pest protection

The use of genes that encode insecticidal proteins in transgenic crops has the potential to benefit agricultural crop

Table 1: Gene technology applications to selected plants in field trials or commercial production

Cereals	Wheat, Barley, Rice, Oats, Maize*
Oilseeds	Canola*, Rapeseed*, Soybean*, Sunflower
Grain legumes	Lupins, Peas, Chickpeas, Lentils
Pastures	Lucerne, Subterranean Clover, White Clover, Barrel Medic, Perennial Ryegrass, Fescues
Horticultural crops	Potato, Tomato*, Papaya, Pineapple, Melon, Squash*, Carnation**, Grape
Fibre plants	Cotton**, Eucalyptus, Poplar, Flax

* Already in commercial production globally

**Already in commercial production in Australia

Table 2: Gene technology potential producing plants with better tolerance for abiotic stresses

Desirable traits	Examples
Heavy metal tolerance	Metallothionein and phytochelatin genes have been shown to be involved in heavy metal (Cd, Cu and Zn) tolerance in natural plant populations, eg. in <i>Silene</i> . ^{37,38,39,40,41} Heavy metal tolerant Brassica was obtained by the transfer of a yeast metallothionein gene ⁴²
Drought tolerance	Increased water stress tolerance in GM plants under drought stress have been reported. ^{43,44,45}
Flooding tolerance	Flood tolerant <i>Arabidopsis thaliana</i> plants have been produced by expressing the gene encoding isopentenyl transferase, by reducing senescence induced by flooding. ⁴⁶
Cold stress	Suppression of proline degradation has been shown to improve tolerance to freezing. ⁴⁷ Transgenic lucerne plants which over-express various forms of superoxide dismutase ⁴⁸ , have resulted in greater winter survival than controls. The specific biochemical changes induced in the transgenics which allows this winter survival is under study. ⁴⁹
Salt tolerance	Plants transformed with the yeast regulatory genes, HAL1 (regulates cation transport) and TPS1 (regulates sugar metabolism) ⁵⁰ and over-expression of the alfalfa gene <i>Alfin1</i> (which encodes a putative regulatory protein linked with salt tolerance in this species) ⁵¹ have been shown to have improved salt tolerance.
Soil remediation	Transgenic plants with the potential to detoxify soil contaminants that limit plant growth by removing pollutants from the environment and that secrete proteins from roots aimed at improving soil-root interactions have been produced. ⁵³

production, the environment and the consumer. The benefit to the environment and the consumer will come from the reduced use of chemical sprays. Insecticidal proteins delivered in an organ-specific fashion allow only the pests of the crop to be targeted, thereby reduc-

ing the collateral damage often associated with broad spectrum chemical insecticides. The elimination of chemical sprays reduces their costs and thus provides a benefit to agriculture.

Cotton is one of Australia's main rural export earners. To protect the crop,

however, growers spend up to A\$ 125 million a year on insect control, and this figure is increasing each year as control becomes more difficult. Gene technology has been used to introduce an extra gene (Monsanto's INGARD® gene) which comes from a bacterium commonly found in soil, *Bacillus thuringiensis* (Bt). The gene was modified so that it would function in cotton plants. Home gardeners have used Bt formulations containing the whole live bacterium as a safe, natural insecticide. GM cotton containing the Bt gene produces the Bt protein in their leaves. Because it is highly specific, this protein kills the pest species but has no impact on the natural predators of heliothis such as spiders. It is also harmless to humans and other animals. Annual field trials, beginning in the 1992/93 cotton season, have shown that the protein in the plants is highly effective in preventing damage by heliothis. Just as important, cotton yields are the same as those from crops without the INGARD® gene but protected by insecticide sprays.

An important management strategy is to prevent populations of the heliothis pest from becoming resistant to the natural protein in the plants. To ensure pest populations do not develop resistance, guidelines have been developed for growers to plant crops attractive to heliothis around their INGARD® cotton fields. The heliothis moths produced in these 'refuges' will greatly outnumber any which have survived feeding on the new cotton plants. As a result, the chances of a resistant population developing will be minimal. Such management strategies will prevent resistance emerging for many years if all.

The Genetic Manipulation Advisory Committee (GMAC) has overseen all steps in the development of INGARD® cotton in Australia to ensure that the genetically enhanced plants pose minimal risk to health or the environment. Other organizations involved in ensuring reduction of risk include State environment protection agencies, Departments of Agriculture and the National Registration Authority (NRA). Commercial release of cotton with in-built protection from heliothis was approved for an initial period of five years in 1996 over a limited area, following final clearance from GMAC and NRA.

To further reduce the chance of the pest developing any resistance to the

Bt insecticide, a new variety carrying two different Bt genes will be put to trial this year. Farmers expect that this new development will further reduce the use of insecticides. For cotton growers, the prospects appear excellent for a new era of sustainable cotton production that is environmentally friendly.

Case study 2

Virus-resistant pasture legumes

Pasture legumes such as subterranean clover, white clover and lucerne are the backbone of Australia's rural industries.²¹ They provide protein for grazing animals and nitrogen for cropping, and improve soil structure and stability, all these benefits being valued at over A\$ 14 billion per annum. However, a widespread gradual decline in pasture legume yields and persistence is reducing farm productivity and profitability and adding to the process of soil degradation.

Recent surveys in Australia have shown that several economically important viruses contribute to the widespread decline.^{16,17,18,19} The viruses include alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), clover yellow vein potyvirus (CYVV), subterranean clover stunt nanovirus (SCSV) and white clover mosaic potyvirus (WCMV). These viruses directly reduce foliage production by up to 90 per cent and also reduce the quality, nitrogen-fixing capacity and persistence of the pastures.^{16,20} The viruses also affect pasture and productivity indirectly by permitting weed invasion and reducing soil quality.¹⁸ On the basis of recent economic analyses of pasture improvements²¹ it is estimated that controlling these viruses in pasture legumes could increase profitability for Australian rural industries by over A\$ 960 million (Table 3).

Control of virus diseases by eradication of insect vectors is not an environmentally or economically viable option in forage legumes. Conventional breeding programmes for viral disease resistance have not been very successful, mainly due to virus-strain limitations and lack of durability in the natural resistance genes available. In perennial forage crops the robustness and duration of the resistance is of prime importance. Any partial resistance resulting in a delay of

onset of viral symptoms by a few months will probably not provide any growth advantage to the plant in the second or third growth season.

Transformation protocols for pasture legumes^{22,23,24} and coat protein-mediated resistance strategies²⁵ were used to genetically engineer subterranean clover with resistance to BYMV²⁶, white clover with resistance to AMV and CYVV^{27,28} and lucerne with resistance to AMV. Field trials of the genetically modified AMV-resistant white clover lines have shown that the plants are immune to all three diverse strains of AMV tested.²⁸ Over 60 per cent of non-transgenic test plants were infected with virus after one growth season but the virus remained unable to replicate in all the immune transgenic plants expressing the AMV resistance gene. The growth habit and general size and shape of the transgenic plants in the trial did not differ significantly from control non-transgenic plants.

Controlling viruses in pasture legumes could increase the competitiveness and profitability of pastoral industries through improved pasture yields and persistence. Another major benefit of the virus-resistant clover varieties is the need for less nitrogen fertilizers, and less insecticidal sprays against aphids on these crops, because aphids will no longer be potential virus vectors or carriers into other crops. Dominant legume composition of pastures will reduce weed invasion and improve soil quality.

Case study 3

Weevil-free peas

Field peas are an important rotation crop in Australian agriculture. Peas are worth A\$100 million a year and they also add nitrogen to the soil and reduce root disease among crops that follow them. However, there are pests and diseases that threaten the field pea industry. Pea weevils are an important insect pest. The pea weevil (*Bruchus pisorum*) is a pest with a world-wide distribution. *B. pisorum* adults emerge from hibernation in spring and feed on pea pollen before mating and laying eggs on immature pea pods. The larvae, once hatched, burrow through the pod wall and into the seed, creating a small, dark "entry hole" approximately 0.2 mm in diameter. The larvae

develop through 4 instars inside the seed, consuming cotyledon contents and creating a cavity with a circular 'window' of testa at one end of the seed. The larva pupates behind this window. The resulting adult either remains dormant or pushes the window open and leaves the seed, creating a 5 mm 'exit hole'. The adults survive until the following spring by hibernating in available shelter including pea straw, buildings and woodlands.

Pea weevil infestation causes economic losses, partly due to the direct loss of seed contents consumed by the pest and partly due to the fact that weevil-damaged seed has lower germination rates and fetches a lower unit price. Currently, this pest is controlled using chemical insecticides.

Common bean seeds contain a protein that inhibits the activity of the starch-digesting enzyme, α -amylase. The gene that produces this potentially protective protein is called α -amylase inhibitor. This gene was isolated from beans and re-constructed for high level, seed-specific expression^{29,30} and introduced into the pea using gene technology.³¹ Field trials have shown that the genetically modified peas containing the bean gene are resistant to pea weevils.³²

The resistance gene introduced into the pea has been consumed by people for thousands of years in beans, with no ill effect. The protein, α -amylase inhibitor, whether from bean or transgenic pea seeds, has been extensively studied and has shown no health risk to humans or animals.^{33,34}

Intellectual property rights

Many techniques and gene components used in biotechnology are subject to intellectual property claims through patenting and plant variety rights. The issue of freedom to operate with affordable licences is one which requires due diligence prior to any major research undertaking that is likely to lead to a commercial outcome. Licensing requirements are in force only in countries where the patents have been filed and approved. Until recently, many of the patents were taken only in major developed and developing countries in North America, Europe and East Asia.

Table 3: Economic impact of AMV, WCMV and CYVV on Australian rural industries

Pasture spp.	Virus	Pasture loss(%)	Estimated losses p.a. to Australian industries (A\$ million)
<i>Trifolium repens</i>	AMV	12.0	104 - 109
	CYVV	5.6	52 - 60
	WCMV	6.4	56 - 77
<i>Medicago sativa</i>	AMV	9.0	78 - 133
<i>Trifolium subterraneum</i>	AMV	3.0	33 - 133
	BYMV	4.2	43 - 172
	CYVV	7.0	72 - 288
	SCSV	13.0	130 - 384

Regulatory policies and risk management

The debate on genetically modified crops has continued over the past few years and we are no closer to resolving widespread concerns about environmental and health risks as well as the perceived risks associated with multinational company control of food plants. Most scientists think that food from genetically modified plants is as safe as, if not safer than, food obtained from plants derived by breeding and selection, yet the public remains uneasy. There is an urgent need therefore for all parties - scientists, industry, media and governments - to respond effectively and responsibly to the concerns and public unease about GM technology. The potential risks associated with gene technology need to be put in a context that recognizes the fact that grain production will need to increase by 40 per cent (700 million tonnes) by 2020.

It has been suggested that an international panel of experts, representing the diversity of scientific and social opinions, be set up to offer governments and the public the best possible independent scientific assessment of the current knowledge about the uncertainties and risks of GM crops.³⁵ In the past 12 months, many international and national agencies have held conferences to assess the implications of genetically modified crops, create public debate and formulate effective regulatory policies to manage the potential risks.

Despite the potential risks, the overwhelming message from developing countries at a recent OECD conference

on GM food safety in Edinburgh was that they wanted to evaluate any technology, including biotechnology. They need to grow more food at lower cost, with less reliance on chemicals and with a reduction in potential damage to the environment.³⁵ They do not want to be dependent on aid or redistribution, but want to be in control of their own destinies.³⁶ Scientific assessments from an international panel as mentioned above and from developed countries like Australia would be most beneficial in helping developing countries in their establishment of risk management policies that are suited to their social and environmental needs. Since GM crops take at least 6-8 years to develop before they are ready for commercialization, and assessment of potential risks could take decades to complete, it would be wiser to allow limited development and commercialization of GM crops under strict, scientifically-based, safety guidelines.

In Australia, gene technology is regulated by many government bodies, including the Genetic Manipulation Advisory Committee, the Australia New Zealand Food Authority, the National Registrations Authority, Environment Australia, the Department of Health and Aged Services, the Australian Quarantine Inspection Service, and State Departments. The Federal Government's Interim Office of the Gene Technology Regulator (IOGTR), which reports to the Ministerial Council on Biotechnology, brings these organizations together to provide efficient, open and effective regulation of gene technology.

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