

# THE CHANGING NATURE OF GM PLANTS

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## Introduction

Throughout the history of crop improvement, 'new technologies' have been regularly utilised by plant geneticists/breeders to develop new gene combinations for improving crop cultivars. Over the last 25 years rapid scientific advances in plant cell culture and molecular biology have resulted in technological developments that allow genetic engineering in virtually all crop species. This offers new opportunities for the incorporation of genes from any source of DNA into crop cultivars.

In this workshop we will describe how genetically engineered plants are produced, summarise the opportunities they present for agriculture, and outline their global adoption in recent years. We will also describe our recent results that allow the development of genetically modified (GM) plants without foreign DNA. This offers a more socially acceptable and responsible way forward for the development of GM crops. The implications of this latter approach for precision breeding of crops in the genomics era will be described.

## How crops are genetically engineered

The development of methods for the genetic engineering of crops offers new opportunities for the incorporation of genes into crop cultivars. This allows a gene of known DNA sequence to be inserted into specific vectors for transfer into the chromosomes of single plant cells. The production of transgenic crops involves a mix of tissue culture and conventional breeding techniques.

*Agrobacterium tumefaciens* mediated transformation is the most common method of transformation used for the production of transgenic plants. It involves making use of the gene transferring ability of this naturally occurring soil bacterium which causes crown gall disease on a range of plants, including grapes and roses. To cause these galls the bacterium transfers a portion of its DNA, known as the T-DNA, into the plant cell. This T-DNA is integrated into the plant chromosomes where the products of these genes cause the galls to develop and also produce a food source that can only be utilised by the bacteria. Only 25 nucleotides of DNA, repeated at each end of the T-DNA, are necessary to initiate the transfer of DNA into plant chromosomes. This discovery led to the replacement of the disease causing genes with genes for a wide range of agronomically useful traits. To produce GM plants, plant tissue is then co-cultivated with the modified *Agrobacterium* for a few days to allow for gene transfer to take place.

Other approaches that are used to transfer DNA into plant cells include:

- direct uptake of DNA into plant protoplasts (naked plant cells without their cell walls) using specific chemicals or electric shock to induce transient pores in the exposed cell membrane to facilitate DNA entry
- direct uptake of DNA into plant tissue using biolistics in which DNA is coated onto tiny metal particles and shot by explosive force under a vacuum into plant cells.

Whatever approach is used for introducing the genes into plant cells, only a very small proportion of the cells have been subjected to a gene insertion event. These rare plant cells can be conveniently identified through the use of genes that confer resistance to phytotoxic chemicals such as antibiotics or herbicides. Such genes are known as selectable markers, of which the most commonly used is the bacterial gene, neomycin phosphotransferase. This gene confers resistance to kanamycin and related antibiotics by coding for an enzyme that detoxifies these chemicals. When this gene is transferred to plant cells, the rare transformation events can be selected on culture medium containing a concentration of kanamycin that is normally toxic. In this manner the genetically engineered cells can be preferentially selected and grown in cell culture and then carefully nurtured to regenerate shoots and grown back into complete plants. These plants are multiplied in tissue culture and then grown under contained greenhouse conditions to obtain seed following conventional plant breeding practices.

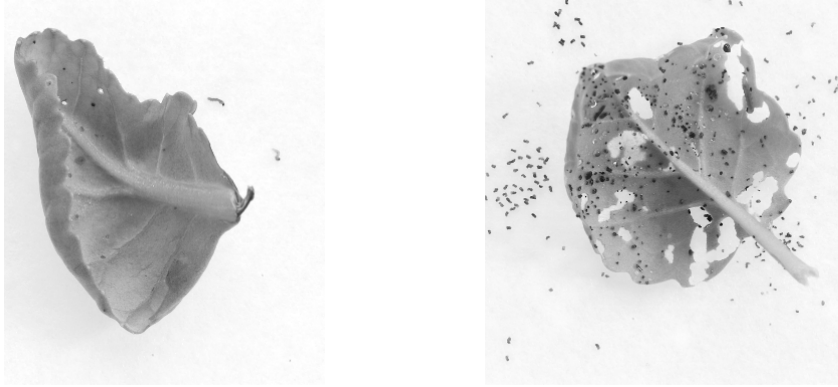
Genetic engineering offers several significant advantages to further enhance the genetic improvement of crop plants by traditional breeding. As the basic structure of DNA is similar in all organisms, genetic engineering allows traits to be obtained from any source of DNA as the techniques used in genetic engineering circumvent the usual reproductive barriers between unrelated species. Genes can be derived from: the same crop; closely related species; other unrelated plants; microbes such as fungi, viruses and bacteria; animals, including humans. In addition, artificial genes synthesised in the laboratory can be used. While the origin of some genes, especially any associated with animals, raises important ethical concerns for some people, this should not be confused with concerns associated with environmental impact and food safety.

Genetic engineering allows the immediate transfer of genes into advanced breeding lines or elite plant cultivars. The laboratory phase of this process can be achieved in four months to a year, depending on the crop. This is substantially faster than that possible for the transfer of genes from related wild species via more traditional backcross approaches which can take many generations. Furthermore, genetic engineering allows the transfer of discrete single genes with the minimal amount of DNA to effect the desired characteristic. This is in marked contrast to the transfer of genes from wild species via repeated backcrossing which results in large fragments of neighbouring DNA with undesirable genes.

## Opportunities for agriculture

Transformation systems have been developed for virtually all crop species somewhere in the world. For all the major crop plants routine procedures have been established in many laboratories. These procedures have been used to transfer genes for traits such as: tolerance to several different classes of herbicides; resistance to insects, nematodes, fungi, bacteria and viruses; altered plant processes such as flowering and altered quality traits. Transgenic crops to be granted regulatory approval overseas, for commercial release, are summarised in Appendix 1. Greater attention is now being focused on “gene stacking”, pyramiding more than one transgene into the same cultivar, e.g. herbicide resistance and insect resistance.

The introduction of these traits offers many advantages to modern agricultural practices, in particular less chemical input. For example the introduction of herbicide resistance aids weed control, enables more environmentally friendly herbicides to be used, and in some cases fewer chemical applications. The introduction of pest and disease resistance via genetic engineering has been achieved via the introduction of plant-derived genes but also by the introduction of bacterial and viral genes; sources of genes not available in traditional breeding programmes. The introduction of insect resistance into many crops, including forage kale (Figure 1), has involved transfer of the insecticidal gene from *Bacillus thuringiensis* (Bt), the active ingredient in biological insecticide products such as Dipel. Other important agronomic traits that have been improved via genetic engineering include altered oil content in seeds, drought tolerance, salt tolerance and cold tolerance. In addition, post harvest attributes such as increased shelf life have been enhanced. Researchers are also working towards developing forage crops such as ryegrass with inducible flowering. These crops would remain vegetative thus increasing yield and would require application of a trigger to induce flowering.



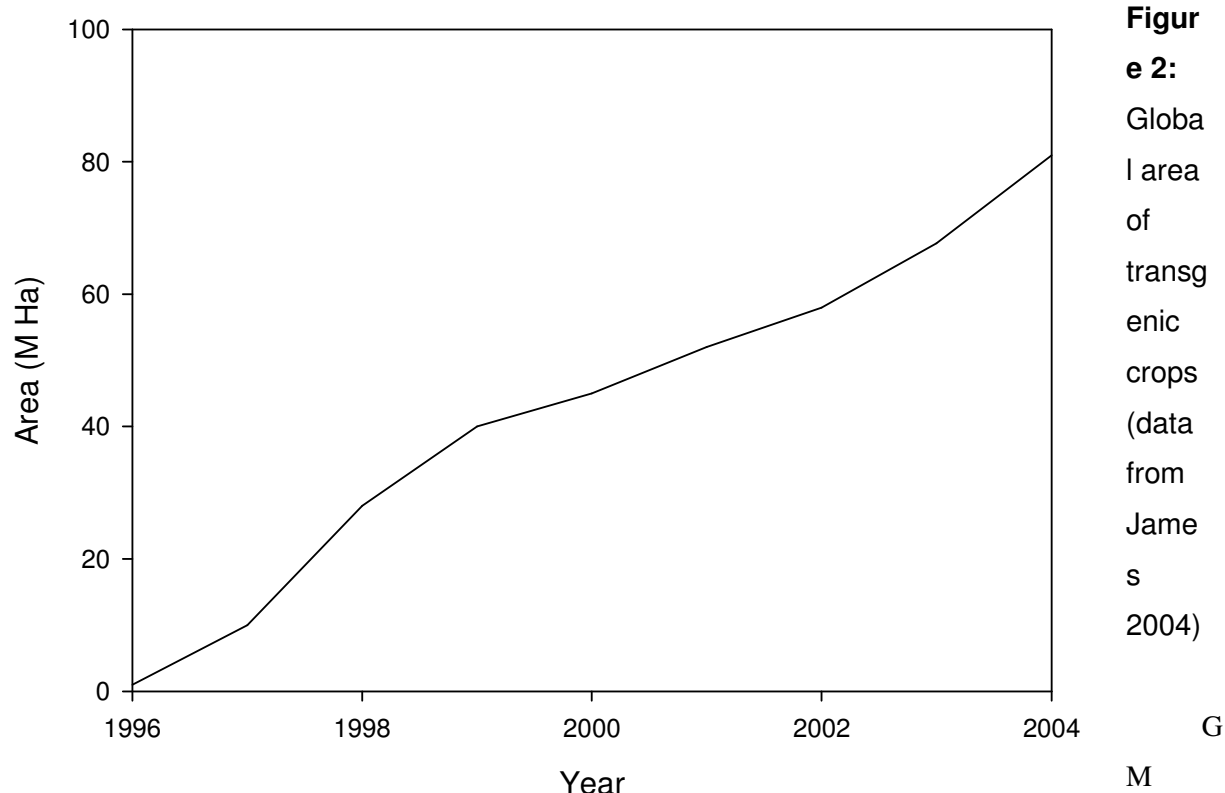
**Figure 1:** Comparison of feeding damage by diamond-back moth larvae on leaves of Bt-containing Proteor forage kale (left) and non-transgenic control (right) after 3 days (see Christey et al. 2005).

In addition to adding traits to plants, genetic engineering technology can also be used to modulate or decrease the expression of existing genes. This involves the introduction of genes in reverse orientation. The resulting gene products interact with existing plant transcripts, interfere with their expression and thereby eliminate an existing gene function within a plant. For example, in forage kale high levels of SMCOs cause kale anaemia or red water in cattle. The introduction of a gene in this pathway in reverse orientation blocked the biochemical pathway and caused dramatic decreases in the levels of SMCOs in the plants.

The tools of molecular biology also allow the way genes are controlled within a plant to be changed. Changing the control of genes involves the use of molecular switches, the DNA regions preceding each gene, known as promoters. These DNA regions define when, where, and to what magnitude the coding region of a gene turns on within a plant. By attaching a specific promoter to a specific gene, the manner in which that gene is controlled within a plant can be changed. For example, a gene for resistance to a pest or disease that attacks the roots of a plant can be signalled to only work in the roots and not the grazed leaves or harvested fruit or grain.

## Global adoption of GM crops

Since the first commercial release of a GM crop in 1994, there has been a huge adoption of the technology with 2 M ha planted in 1996 and increasing to 53 M ha in 2001, equivalent to about twice the total land area of NZ. This ranks as one of the highest adoption rates for any technology in the history of agriculture and has sustained an annual growth rate of more than 10% per year for each of the last eight years. By 2004 the global area had risen to 81 M ha - a 20% increase over 2003 (Figure 2).



**Figure 2:** Global area of transgenic crops (data from James 2004)

crops are currently grown in 17 countries with 14 countries growing more than 50,000 Ha. Five countries account for over 95% of the current area of GM crops: the United States (59% of the global total), Argentina (20%), Canada (6%), Brazil (6%) and China (5%). The main GM crops grown in 2004 were soybean (60% of global area), maize (23%), cotton (12%) and oilseed rape (5%). The major crops grown are transgenic for herbicide and/or insect resistance (Table 1).

**Table 1:** Dominant GM crops grown in 2004 (data from James 2004)

<b>Crop</b>	<b>Area grown (M ha)</b>
Herbicide tolerant soybean	48.4
Bt maize	11.2
Bt cotton	4.5
Herbicide tolerant maize	4.3
Herbicide tolerant canola	4.3
Bt/herbicide tolerant maize	3.8
Bt/herbicide tolerant cotton	3.0
Herbicide tolerant cotton	1.5

## **GM research in New Zealand**

In New Zealand, a wide range of traits has been introduced into a variety of plants using GM technology. In addition, GM crops have been field tested in New Zealand since 1988 following the granting of appropriate approvals. The majority of these GM field tests over the past 18 years have involved potatoes, but also include a wide range of other crops including: vegetable and forage brassicas, canola, asparagus, onions, petunia, peas, lisianthus, sugarbeet, kiwifruit, tamarillo, clover, and pine trees. All field trials are conducted under strict conditions to ensure no plant material including pollen is transmitted from the site. In addition, monitoring is conducted in subsequent years to ensure the removal of volunteer plants that may grow in subsequent seasons. No applications have been made for commercial release of a GM crop in New Zealand, but FSANZ (Food Standards Australia New Zealand) have approved the use of several GM derived ingredients for use in food.

## **New approaches to genetic engineering: GM crops without foreign DNA**

One of the main concerns underpinning the public antagonism toward GM crops involves the ethical issue of transferring genes across wide taxonomic boundaries. In contrast, gene transfer within species is a more acceptable concept, a viewpoint consistent in public surveys conducted throughout the world. However, the transfer of genes within crop species still requires the use of vector systems based on DNA derived mainly from bacterial systems. We therefore took up the challenge to design a vector system in which all the DNA destined for transfer to a crop originates from within the target species.

This requires the identification of DNA fragments in crop genomes with the functional equivalence of important vector components. By careful bioinformatic searches of DNA databases we have found DNA sequences within genomes of plant species that allow the design of virtual vectors with the entire DNA destined for transfer to a crop being derived from the target species. To date we have been able to design such vectors for potato, petunia, tomato, tobacco, barrel medic, apples, onions, rice, and pine trees. The vectors based on potato and petunia DNA fragments have been successfully constructed.

The complete genome sequence of all the major crop plants will be known within the next five to eight years. The real future for crop improvement lies beyond such achievements and the subsequent mining of germplasm collections of plant breeders for all the variant versions of genes with altered function. This will identify many genes for immediate transfer back into the same crop and will allow highly targeted genetic improvement of crop cultivars. Vectors based on DNA of the target crop provide a key tool for a highly efficient way to transfer genes between individual plants within species. Such gene transfer strategies provide immense opportunities to utilise the knowledge of genome sequences in crop improvement, especially the genetic enhancement of biochemical pathways to produce targeted improvements in quality traits.

The engineered plants resulting from gene transfer using vectors based on DNA of the target crop are essentially non-transgenic GM plants with no foreign DNA. This is in essence equivalent to backcrossing desirable genes into a new genome location of elite crop lines in a single step without any linkage drag. Such genetic events are similar to micro-translocations that can arise from natural or radiation-induced chromosomal rearrangements — a technology that has been widely used in plant breeding for the past 50-60 years. Theoretically, identical plants could be derived from this genetic engineering approach and traditional plant breeding methods.

## Conclusion

Crop breeding has been revolutionised by scientific advances in molecular biology over the last two decades. Some of the greatest advances include the ability to transfer DNA from any source into single plant cells from which complete plants can be regenerated via cell and tissue culture. The genetic engineering of plants offers new opportunities for the incorporation of genes into crop cultivars. We have recently developed vector systems for genetic engineering of crops that permit gene transfer without foreign DNA. We anticipate that this concept will provide a more socially acceptable and responsible way forward for the future deployment of GM crops.

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