GENETIC ENGINEERING OF PLANTS FOR WEED, DISEASE AND PEST CONTROL: SCIENCE VERSUS POLITICS?

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SUMMARY

Considerable progress has been made toward the development of genetically engineered crop plants for weed, disease and pest control. The release of such plants to the agricultural industry has raised a number of environmental and food safety issues which can be largely dealt with by appropriate management of the technology. Interim measures have been developed to permit field testing of engineered organisms until legislation governing regulation of the technology is passed. An issue yet to be properly addressed by regulatory authorities involves food safety of produce harvested from genetically engineered plants. A survey of New Zealand public found support for the development of this technology in New Zealand.

INTRODUCTION

Over the past decade there has been substantial advancement in the science of plant molecular and cellular biology, resulting in the ability to genetically engineer many crop species. This has culminated in the transfer of genes which confer an agricultural advantage into various crop plants from diverse sources such as animals, viruses and bacteria. The scientific progress has been so rapid that it has been difficult to develop and implement regulations governing the research at the same rate as the technological advances.

In this article we review progress towards the development and release of crop plants genetically engineered to facilitate control of weed, disease and pest control. We discuss the precision of the technology and ways of managing it to help prevent pests and diseases overcoming engineered resistance mechanisms, to prevent the spread of herbicide resistance to weeds, and to avoid increasing the weediness of crop plants themselves. The scientific advances are also considered in relation to current regulations and safety issues associated with laboratory experimentation, environmental release and food safety of genetically engineered crops, as well as the response of the public to an opinion poll on these issues.

WEED, PEST AND DISEASE CONTROL VIA GENETIC ENGINEERING

Status of crop plant transformation

Several independent lines of research must be used to successfully genetically engineer crop plants including the identification and cloning of target genes to be transferred, the correct alignment of their coding regions between appropriate regulatory elements, the transformation of these genes into plant cells, and the selection and regeneration of complete plants from individually transformed cells. A discussion of these technologies can be found elsewhere (Conner and Meredith 1989; Gasser and Fraley 1989) and they have resulted in the regeneration of transgenic plants in a wide range of crops (Table 1). In many instances, however, considerable research is still required to allow the efficient recovery of transgenic plants in agriculturally useful genotypes.

Strategies for weed, pest and disease resistance

1. *Herbicide-resistant crops.* There are two approaches to engineering herbicide resistance in crop plants which provide sufficient resistance in the crop to allow weed control. Inserting genes which code for enzymes that metabolise the herbicide to non-toxic compounds has been a very successful approach (e.g. resistance to bromoxynil, Proc. 43rd N.Z. Weed and Pest Control Conf. 1990: 200-208

TABLE 1: Examples of crops in which transgenic plants have been regenerated. From Gasser and Fraley (1989) with several additions.

Vegetables	Arable crops	Fruit and trees
potato	sunflower	pepino
tomato	sugarbeet	tamarillo
eggplant	oilseed rape	apple
lettuce	soybean	walnut
celery	moth bean	poplar
carrot	cotton	raspberry
horseradish	flax/linseed	blackberry
cauliflower	rice	
broccoli	maize	Pasture crops
cabbage	rye	alfalfa/lucerne
bean		white clover
pea	Ornamentals	<i>Lotus</i>
cucumber	petunia	<i>Stylosanthes</i>
asparagus	<i>Nicotiana</i>	orchard grass/ cocksfoot
	<i>Kalanchoe</i>	
	morning glory	

phosphiothricin, 2,4-D). An alternative approach involves altering herbicide target sites by transferring mutant genes that code for enzymes that are not inactivated by herbicide because the herbicide can no longer bind (e.g. resistance to glyphosate, chlorsulfuron, atrazine).

2. *Insect-resistant crops.* There have been some spectacular examples of improving insect resistance in crop plants by genetic engineering. The transfer to plants and expression of the genes encoding the insecticidal BT protein from *Bacillus thuringiensis* has provided excellent control against larvae of Lepidoptera and Coleoptera. The manipulation of proteinase inhibitor genes in plants has enhanced their resistance to a wide range of insect pests. Their expression is thought to interfere with the digestive enzymes in the guts of grazing insects.

3. *Virus-resistant crops.* The transfer and expression of DNA sequences corresponding to specific genetic components of plant viruses has repeatedly demonstrated the induction of virus resistance in plants. The most successful approach has involved the transfer of viral coat protein genes, but success has also been achieved using DNA sequences corresponding to satellite RNA and viral antisense RNA. Genetically engineered plants have been produced with improved resistance to at least nine viruses.

4. *Bacterial- and fungal-resistant crops.* Internationally there are many research programmes attempting to clone genes conferring resistance to bacterial and fungal diseases of plants. These range from targeting biosynthetic genes for "antibiotic-like" compounds from other microbes, to the disease resistance genes that have been utilised by plant breeders for many years.

Successful examples

To date most of the successful examples of genetic engineering crop plants for resistance to herbicide, insect and disease have involved plants of the solanaceous family (Table 2). This is because these species provide convenient "model systems" due to the ease with which they can be transformed. However, there is considerable effort throughout the world to extend these results to other crops, especially those listed in Table 1.

PRECISION OF GENETIC ENGINEERING TECHNOLOGY

Control of gene expression

Genetic engineering not only allows the transfer of genes from any source of DNA into crop plants, but also allows the control of their expression (Gasser and Fraley 1989). This control of gene expression is largely determined by promoter regions

upstream of the coding sequences. Molecular biology technology allows the construction of chimeric genes in which promoters and coding regions can be mixed and matched as required to achieve the desired expression of a gene. Consequently it is possible to manipulate genes so that they express in a constitutive manner (all the time throughout a plant) or regulated manner (in specific tissues at specific stages of development and/or in response to specific environmental stimuli). It is also possible to control the magnitude of gene expression (low versus high expression).

Development of isogenic lines

One of the principal advantages of genetic engineering is that single genes can be added to existing elite genotypes with no or only minimal disturbances to their genetic background. This offers the potential for the rapid development of isogenic lines in which a series of genes can be independently added to the same genotype (Conner and Meredith 1989).

Implications for weed, pest and disease control

Genetic engineering is clearly a powerful technology for developing novel germplasm for crop improvement. The ability to control the manner of gene expression and to develop isogenic lines has important implications for managing genetic engineering technology for weed, pest and disease control, as well as food safety of the harvested produce.

TABLE 2: Examples of crop plants genetically engineered for herbicide, insect and virus resistance to have reached field testing stage of development. Extracted from OECD (1990).

Resistance phenotype	Crop plants
Herbicide resistance	
phosphinothricin	tobacco, tomato, potato, oilseed rape, sugarbeet, alfalfa, poplar
glyphosate	tobacco, tomato, oilseed rape, flax, sugarbeet, alfalfa, soybean, cotton
sulphonylurea	tobacco, tomato, potato, flax
bromoxynil	tobacco, tomato, cotton
atrazine	tobacco
Insect resistance	
insecticidal BT proteins	tobacco, tomato, potato, cotton
proteinase inhibitors	tobacco
Virus resistance	
alfalfa mosaic virus	tobacco, alfalfa
tobacco mosaic virus	tomato
tomato mosaic virus	tomato
potato virus X	potato
potato virus Y	potato
potato leaf roll virus	potato
rhizomania virus	sugarbeet
cucumber mosaic virus	cucumber

MANAGEMENT OF THE TECHNOLOGY

Pest and disease resistance

The most significant application of plant genetic engineering in the near future will be the development of pest and disease resistant crops. Substantial progress has already been made in a few crops (Table 2). Extension of these results to the other crops listed in Table 1 promises to have a major impact in world agriculture. However, this technology is based on single-gene resistance. Reliance on single genes for pest and disease resistance in conventional plant breeding over the past 30-40 years has often resulted in the evolution of populations capable of overcoming the resistance mechanism. Likewise, plant pests and pathogens have evolved chemical resistance following the

repeated use of certain pesticides and it would be naive not to consider this possibility for genetically engineered crops.

Preparations of *Bacillus thuringiensis* have been used on crops in the field as a safe biological insecticide for over 20 years without any resistant insects being reported. In contrast, the intensive use of *B. thuringiensis* preparations in stored grain resulted in the rapid appearance of resistant insects (McGaughey 1985). The widespread planting of crops expressing BT insecticidal proteins will place a similarly intensive selection pressure for resistant pests to evolve rapidly. From the outset it will be important to manage the technology to minimise these risks.

1. *Multiple resistance.* The probability of pest and disease populations overcoming the resistance of genetically engineered crops would be substantially reduced if several different resistance genes were simultaneously or sequentially transformed into the same plant. Combinations of insecticidal BT proteins and proteinase inhibitor genes from different sources may provide a useful "cocktail" for durable resistance against insect pests. Likewise for virus resistance, coat protein, satellite RNA and antisense approaches could be used simultaneously in varying combinations. Furthermore, these genes may originate from a range of different viral strains or even closely related viruses. It is also feasible to construct "hybrid" genes with components from different viral sources.

2. *Multilines.* The concept of multilines was developed in the 1950s as an approach to combat rust pathogens in cereals (Poehlman 1979). It involves a composite of isogenic lines with each possessing a different gene for resistance to specific races of the pathogen. The mixture of genotypes provides a buffering effect against rapid disease development and extends the "life" of resistant genes. However, multilines never realised their full potential due to the difficulty in developing isogenic lines via backcrossing in conventional plant breeding programmes. Since isogenic lines can be conveniently developed using genetic engineering technology, and several resistance genes against specific pests and diseases are potentially available, the development of multilines by genetic engineering may become an attractive management option. This clearly provides a useful approach if populations of pests and diseases readily overcome the resistance of genetically engineered plants. Since multilines can be reconstructed each year, it would be possible to change the composition of isogenic lines as changes occur in pest and disease populations. Alternatively in annual crops, isogenic lines genetically engineered with different resistance mechanisms could be alternated each year.

3. *Controlling gene expression.* The ability to precisely manipulate gene expression via genetic engineering also has implications on the management of the technology. High level expression of insecticidal BT proteins may impose intensive selection pressure for the evolution of resistant pest populations. It may be more appropriate to use low level expression which may fail to kill the pest, but has sufficient inhibitory effect to slow growth rates and greatly reduce reproductive potential, thereby maintaining pest populations at low levels. The control of gene expression is also important to minimise the risks of harming beneficial organisms. For example pollinating insects are important in many crops, so it may be necessary to ensure that genes conferring insect control are not expressed in pollen or nectaries of flowers. Likewise, expression of antimicrobial proteins in plant roots may interfere with *Rhizobium* or mycorrhizal fungi.

Herbicide-resistant crops

The development of herbicide-resistant crops via genetic engineering is often perceived as having environmental risks. Since a general release of genetically engineered crops to the agricultural industry requires the approval of regulatory agencies (see below), these risks should be carefully considered before a genetic engineering approach is initiated. Several important issues relating to the responsible use and management of the technology are discussed below.

1. *Increased herbicide use.* The development of a crop with resistance to a specific herbicide for the purpose of controlling weeds may presumably result in an increased use of that herbicide but possibly with a consequential decrease of other herbicides. It is

therefore important to carefully choose the herbicide to which resistance is sought, and consider potential cross resistance to other chemicals. Regulatory agencies are unlikely to approve release of engineered plants perceived as increasing the use of hazardous chemicals in agriculture. However, genetic engineering technology may help promote a switch from environmentally unsound herbicides to more "environmentally friendly" herbicides that are used in very low concentrations, have low mammalian toxicity and/or rapid degradation in soil. An example of this involves our programme on the development of glyphosate-resistant asparagus, which will overcome the current dependence on more hazardous chemicals such as paraquat and diquat for non-selective weed control in asparagus.

2. *Herbicide-resistant weeds.* The natural transfer of herbicide resistance genes from engineered crop plants to closely related weed species could make the chemical control of such weeds more difficult. If such gene transfer occurred, the commercial viability of the herbicide-resistant crop would be in jeopardy. It is therefore in the best interests of genetic engineers to consider whether any weeds in the local flora naturally hybridise with the crop to be manipulated. For example, it would be irresponsible to transfer herbicide resistance into cultivated blackberries given the noxious weed status of naturalised weedy blackberries in New Zealand. Clearly regulatory agencies would not approve such a release.

3. *Increased crop weediness.* Some crop plants (e.g. potato and clover) become weeds in their own right as volunteers in subsequent crops. Whether the development of herbicide resistance in such crops will compromise the chemical control of such volunteers needs careful consideration. In fact, genetic engineering for herbicide resistance offers a novel management approach for the control of volunteers in some crops such as potatoes.

Volunteer potatoes arise from tubers left in the ground after harvest of the previous season. They do not remain dormant and always sprout the next season and are a major problem in subsequent potato crops. Not only do they compete with the current crop, but carry-through fungal, viral and nematode inocula that can affect the health of following potato crops. Since isogenic lines can be conveniently developed using genetic engineering technology, the same potato cultivar can be independently engineered with resistance to two different herbicides. When two such potato lines are alternated each growing season and the appropriate herbicide is applied, herbicide-resistant volunteers carried through the previous season can be readily eliminated. Furthermore, alternating the use of two herbicides greatly reduces the chance of herbicide-resistant weeds becoming established, as well as the "escape" of herbicide-resistant crop plants.

Other management strategies to eliminate herbicide-resistant volunteers or weeds that may establish following release of genetically engineered crops can be designed. One approach could involve the simultaneous transfer of a gene conferring sensitivity to a second herbicide alongside the gene for herbicide resistance. Due to the extremely tight linkage between these genes, increased sensitivity to one herbicide would be co-inherited with increased resistance to another, thereby providing a convenient opportunity to eliminate chemically herbicide-resistant plants should the need arise.

CURRENT REGULATIONS AND SAFETY ISSUES

Laboratory experimentation

An Advisory Committee on Novel Genetic Techniques (ACNGT) was established by cabinet in July 1978 to oversee guidelines for laboratory containment of recombinant DNA research in New Zealand. Any public institute involved in this area of research must appoint a Supervisory Committee and a Biological Safety Officer who have the responsibility for enforcement of the recommendations made by ACNGT. The current New Zealand guidelines were last revised in 1982 and are fully set out in ACNGT (1982). They establish five levels of containment (C0-CIV) and provide guidance notes for categorisation of specific experiments into an appropriate containment level. ACNGT have delegated local Supervisory Committees power to certify laboratories and approve experiments involving C0 and CI level containment. Virtually all experiments involving genetic engineering of plants are performed under C0 containment. Unfortunately the

guidelines are written primarily for microbial systems and in many ways are inappropriate for plant and animal research. They are in urgent need of revision.

Field testing

In 1985 scientists involved in genetic engineering of plants realised that rapid progress was being made toward the transfer of agriculturally useful genes into crops. Political pressure was directed at appropriate authorities in order to establish guidelines for the field testing and release of "genetically modified organisms" (GMOs). The Minister of Science and Technology established a Field Release Working Party (FRWP) in February 1986, with the brief to develop recommendations on how the field testing and release of GMOs should be regulated in New Zealand. Their report was published a year later (FRWP 1987) and recommended that no field testing should be allowed in New Zealand until legislation or interim measures are in place. An Interim Assessment Group (IAG) administered by the Ministry for the Environment was established by the Minister for the Environment in October 1988. The interim system is based on the recommendations of FRWP (1987). The legislation governing GMOs is a component of the Government's Resource Management Bill which had its first reading in Parliament in late 1989. This Bill establishes a Hazards Control Commission which will assume responsibility for all phases of genetic engineering research (laboratory experimentation, field testing and general release) (Taylor *et al* 1990).

In the last two years the IAG have considered 14 applications for field testing genetically engineered plants (Gibbs and Roberts 1989; Taylor *et al* 1990). Ten of these proposals were approved, of which nine involved vegetables (potatoes, broccoli, asparagus) from our own programme. Strict conditions were imposed concerning the isolation distances of the field plots from other crops of the same species, as well as environmental monitoring of the experimental site for pollen dispersal from the transgenic plants and appearance of volunteer plants in subsequent growing seasons.

Food safety

When genetically engineered plants are used as a food source, especially for humans, the potential for adverse effects resulting from the consumption of such produce raises another regulatory issue. Regulatory agencies to date have been largely preoccupied with environmental issues relating to field testing and given little attention to food safety. Recently a consortium of major biotechnology and food companies established the International Food Biotechnology Council to provide scientific leadership on these issues. They commissioned respected academic and industry based scientists to draft a report which provides a scientific basis to guide regulatory agencies when assessing food safety of genetically engineered crop plants (IFBC 1990). An important component of the report is a set of decision trees to streamline the regulatory process for safety evaluation. "Health risks" are perceived to arise from three main mechanisms.

1. *Products of the inserted genes.* The transferred genes themselves pose no risk since their DNA is intrinsically identical to that of the recipient crop plant. It is the proteins produced by these genes which are important to consider. In many instances it will be possible to manipulate the promoter of a transferred gene so that it is not expressed in the grain, fruit or tuber harvested. The expression of plant viral genes in transgenic plants is unlikely to be a concern, since food sources are often virus infected and already contain these genetic components and their expression products. Transferred viral coat protein genes typically express at levels of 0.01% to 0.5% of the total protein in transgenic plants, which is well below levels often found in virus infected plants. In the cases of plants engineered with insect resistance, proteinase inhibitors are commonly ingested as existing components of the human diet (especially from grain legumes) and insecticidal BT proteins are not toxic to other animals, including humans. For transgenic herbicide-resistant plants, existing chemical residue regulations for herbicide registration will presumably apply. When the nature of engineered resistance involves a detoxification mechanism it will be important to analyse for the expected metabolites of the herbicide.

2. *Pleiotropic and secondary effects of the inserted genes.* Pleiotropic effects of inserted genes will be usually non-existent or irrelevant. The biochemical basis for gene

action of the inserted genes is usually well understood and potential pleiotropic effects will be largely predictable. An example of such an effect is the substantial reduction in arginine levels in transgenic asparagus plants expressing a nopaline synthase gene (Conner *et al* unpublished results).

More difficult to predict and detect will be secondary metabolic effects of the inserted genes. The enzymes encoded by many genes transferred to plants will comprise part of biochemical pathways. For example, engineering of herbicide-resistant plants can involve the expression of genes for glyphosate insensitive 5-enolpyruvyl-shikimate-3-phosphate synthase or chlorsulfuron insensitive acetolactate synthase. These coding regions are usually under the control of regulatory signals that confer a high level of constitutive expression. Such expression could result in flow through effects of metabolites in downstream biochemical pathways. In the case of glyphosate-resistant plants this may mean increased activity of the shikimic acid pathway and the pathway to aromatic amino acids. For chlorsulfuron-resistant plants there may be increased metabolite flow through the biosynthetic pathway to branched-chain amino acids. In some instances increased amino acid biosynthesis may divert a greater flow of metabolites to secondary pathways. For example, a glyphosate-resistant tobacco may have a higher nicotine content. There are numerous examples of selecting amino acid overproducing mutants in plant cell culture (using amino acid analogues as selection agents) which have resulted in the downstream accumulation of secondary compounds (e.g. indole alkaloids, anthocyanins, biotin, cinnamoyl putrescines, flavanoids) (Conner 1986). Such secondary effects will be dependent upon the key regulatory points and rate limiting steps in any particular pathway. These control points in biochemical pathways are not well understood in most plant species and different responses could even be expected in different genotypes of the same crop.

3. *Insertional mutagenesis*. Transferred genes are inserted into the recipient plant's genome in a random manner. This has been documented as a potential method to induce mutations in the existing genome of the recipient plant (Feldman *et al* 1989). As a consequence random insertion events could activate or inactivate genes in the recipient plant. Such effects will be rare with inactivation events expected to predominate. Activation may result from enhancement effects as regulatory sequences of the transferred genes affect flanking genes of the recipient plant which are normally not expressed due to malfunctioning regulatory regions. Of potential concern for food safety is the activation of genes encoding secondary compounds (often toxic) which have been "bred out" of crop plants during their domestication. For this reason the analysis of food for changes in levels of significant nutritional components and natural toxicants in the genetically engineered crop is recommended (IFBC 1990).

Biotechnology and public opinion

Genetically engineered crops are being developed at a time when a lack of understanding regarding the importance of agricultural research exists (Gasser and Fraley 1989). Current issues, including production surpluses, farm economics, agricultural subsidies, environmental and health concerns about farming systems, and general distrust for new technologies overshadow the progress made in the development of economical, healthy and high quality food products.

It is widely accepted that public attitudes should have some input into the regulatory process. Where there is little understanding of the benefits and risks of the technology, public opinion may be easily led by public interest pressure groups. Thus many organisations working on biotechnological developments are nervous about the impact public attitudes may have on their research and regulations governing its control (Tait 1988).

Relatively little public opinion poll data has been published. A 1986 American Office of Technology Assessment poll (OTA 1987) found that while the public is concerned about genetic engineering in the abstract, most people approved nearly every specific application explored. The report concludes that the public is sufficiently concerned about the potential risks to say that strict regulation is necessary, yet a majority agreed that unjustified fears of these new technologies have seriously impeded developments and that the risks have been exaggerated.

In 1988 a UK survey of public perceptions (RSGB 1988) investigated awareness, understanding and level of interest in biotechnology. Only 38% of the British sample had heard of biotechnology compared with 66% who understood the term "genetic engineering" in the OTA survey. This survey indicated a range of public acceptance of applications ranging from 77% support for drugs produced from altered micro-organisms used to treat chronic diseases; 68% approval for altering plant cells to produce disease resistant crops to a low of 24% approval for changing the genetic make-up of animal cells to breed more productive animals.

In New Zealand a comprehensive survey of public attitudes to biotechnology has recently been completed (Couchman and Fink-Jenson 1990). Public awareness of genetic engineering was high, 74% of those questioned claimed to have a knowledge of the technology and 20% felt they could explain it to a friend. The comparative figures for other technologies is listed in Table 3.

TABLE 3: Public awareness of technology in New Zealand, 1990 (after Couchman and Fink-Jensen 1990).

Technology	Heard of; know a little about (%)	Heard of; could explain (%)	Familiarity total (%)
Genetic engineering	54	20	74
Agricultural pesticides	61	30	91
Biological pest control	65	21	86
Biotechnology	54	9	57

When asked about concerns on impacts of genetic engineering, 55% of those people who had heard about it are either slightly worried, somewhat worried, very worried or extremely worried. Comparative figures for the other technologies were: biological pest control, 49%; agricultural pesticides, 67% and biotechnology, 30%. The fact that the level of concern about genetic engineering and biological pest control were similarly high, was a surprising result indicating that technology in both areas requires greater explanation and a better record of success before they will gain wider public acceptance.

A final interesting note was that 57% of all respondents agreed with the statement that genetically modified plants may be preferable to agricultural pesticides.

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