APPLICATIONS OF GENETIC ENGINEERING IN HORTICULTURE: A PRACTICAL PERSPECTIVE

ROGER DUTCHER

Calgene Pacific Pty Ltd
16 Gipp Street
Collingwood, Victoria 3066

The commercial opportunities available from the application of biotechnology to agriculture are large. Groups working with this technology are looking far beyond simply increasing yields. Those yield increases are certainly possible, and in many cases undoubtedly will occur, but the real benefits will come from improved quality resulting in better prices, reduced costs of producing a crop, and a greater reliability of harvest resulting from greater tolerances to a wide range of pest and stress phenomenon.

RECOMBINANT DNA TECHNOLOGY FOR PLANT IMPROVEMENT

The use of recombinant DNA technology, or genetic engineering, is an important step in widening the base from which genetic traits can be mobilized for use in plant improvement. The tools of recombinant DNA and the universality of the genetic code make it possible to express in plants genes from essentially any organism, including unrelated plants as well as animals, bacteria, and viruses. Proper expression of foreign genes in plants requires the use of appropriate regulatory sequences which are fused to the DNA fragment encoding the structural gene.

TOOLS FOR PLANT GENETIC ENGINEERING

Genetic engineering of plants today is largely based on the use of a tool called a vector, which is constructed from part of a common bacterium. This vector is usually referred to as the Ti plasmid (called Ti for tumor-inducing). This tool, or vector, has been isolated, and subsequently modified in the laboratory, from the common soil bacterium, *Agrobacterium tumefaciens*. This bacterium is the cause of the disease called crown gall in plants. When the normal bacterium infects a plant, it transfers a portion of its genetic code, or DNA, to the plant chromosomes. Expression of the genes carried on this foreign bit of DNA cause the crown gall disease. In the laboratory, the Ti plasmid has been "disarmed" by deleting the bacterial genes that when expressed in the plant lead to tumor formation, while keeping the basic ability to transfer foreign genes. Any foreign gene or DNA properly linked in the laboratory to the Ti plasmid vector is transferred when the vector is allowed to infect plant material in culture.

As a plant pathogen, *A. tumefaciens* has a wide but not global

host range. Crown gall disease occurs primarily in dicotyledonous plants. The monocotyledonous plants, which include our major cereal grain species such as maize, wheat, barley, and rice, are not susceptible. This does not necessarily mean that transfer of the T-DNA does not occur in these crops, however. Even in dicots, certain combinations of Ti plasmid strains and plant cultivars do not give rise to crown galls, but the transfer of T-DNA occurs. Several laboratories are testing whether Ti-based vectors will be useful for monocot transformation.

There is interest in other vector methods for transferring foreign DNA to plants, including the use of viruses, transposable elements, microinjection, and direct uptake of DNA from the culture medium (or via liposomes) by plant cell protoplasts. These methods, which work efficiently in animal systems, are however not yet as well developed for use in plants.

Genetic engineering in plants requires a manipulation in the laboratory to first transfer the gene(s) of interest into cells or tissues in culture, followed by manipulation of those cultures to obtain again whole, fertile plants. This is now possible for an increasing number of species, including many important in agriculture. This second process, called regeneration, is achieved by manipulation in the medium on which cultures are grown of the levels of growth substances, or phytohormones, which govern expression of functions required for morphogenesis. Regenerated plants can be derived from single totipotent cells in culture in several plant species. With a much greater number of species it is possible to maintain in culture clumps of cells or cells in suspension that maintain the ability either to proliferate structures that mimic embryogenesis or that are organogenic (adventitious production of organized shoots and roots). Thus, plant cells can be manipulated, as by exposure to Ti-based vectors containing foreign genes, and subsequently whole plants containing the desired gene can be obtained. For many applications it will not be necessary to go to single cells for routine transformation because organized plant tissues can be exposed to the vector in culture and regenerated transformed plants obtained directly from these treated tissues.

HERBICIDE TOLERANCE: A CASE IN POINT

To illustrate this technology, let me take the case of work done at Calgene, Inc. in California. Prior to the "invention" of recombinant DNA gene transfer methods for plants, the utility of herbicides was based on screening plants and organic compounds to look for combinations that were workable. Different species of plants, both crops and weeds, are naturally differentially sensitive to phytotoxic chemicals. This differential sensitivity, or selectivity, is the basis of herbicide use. Thus, naturally-occurring herbicide

tolerance has been sought and easily found. What we now can do that is different is to engineer plants rather than chemicals—with the result that, at least in certain cases we can doctor crop plants to go with the chemicals of choice—indeed the chemicals that are *environmentally* of choice—rather than be limited to the compromises dictated by the limitations of naturally-occurring genetic variability and organic synthesis.

The objective of the Calgene group's research is to obtain plants having tolerance to a very broad-spectrum and environmentally-safe herbicide, N-phosphonomethyl glycine, or glyphosate. The herbicide acts by specific inhibition of an enzyme, 5-enolpyruvyl-shikimate 3-phosphate (EPSP) synthase, in the shikimate pathway for biosynthesis of aromatic amino acids. This is a pathway present in plants and bacteria, but not in animals.

The strategy taken was to seek a mutated version of the bacterial gene encoding EPSP synthase that was less inhibited by glyphosate. This gene was obtained by mutagenesis of *Salmonella typhimurium,* cloning from a gene library, and subcloning into plasmids in *Escherichia scherichia coli.* Through a series of manipulations the gene was obtained on a small piece of DNA, completely sequenced, fused to regulatory sequences designed to give expression in transformed plant cells, inserted into a Ti-based vector, and transferred to tobacco cells in culture. Regenerated tobacco plants expressing the mutant bacterial gene exhibited tolerance to spraying with the herbicide. The tolerance levels achieved so far are insufficient for commercial use. But this result demonstrates conclusively that a plant metabolic pathway can be complimented by a bacterial gene product, and is the first instance in which an agriculturally useful trait has been expressed *via* genetic engineering in a crop plant.

ENGINEERING OTHER TRAITS

The choice of herbicide tolerance as an early objective in the development of this technology was not an accident. Few desirable plant traits are encoded by single genes. In addition to herbicide tolerance, single-gene determinants are known for resistance to certain plant diseases, tolerance to other environmental stresses, and certain other characteristics such as flower colour, dwarfism, and various simple morphological traits. The traits of major commercial interest, on the other hand, are often quantitative in nature; that is, they are determined by the coordinated expression of many genes. Yield, photosynthetic efficiency, the ability to make important secondary products (steroids, terpenoids, alkaloids, etc.), and the biosynthesis of important primary plant products (fatty acids, seed proteins, etc.) are examples.

It would, however, be incorrect to conclude that genetic engineering technology will not be useful in obtaining new quanti-

tive phenotypes. For example, once the control mechanisms in a complex pathway are understood, it may well be possible to modify the pathway usefully by modulating the expression of a key enzyme in that pathway. Use of chimeric genes that are differently (e.g. developmentally) regulated, or of genes encoding enzymes that are regulated by substrate or product in a different way, are two possible strategies. Use of technology to turn genes down or off in pathways that lead to undesirable products (for example, cyanogenic glycosides in cassava) is another.

I have mentioned flower colour as a character controlled by a single gene. Calgene Pacific was established in 1986 to work specifically on ornamental and forestry crops as targets for the application of its genetic engineering technology. Flower colour was chosen as its first project.

To establish a business base in the ornamentals area, Calgene Pacific created a Horticultural Group in July 1987, with the purchase of major holdings in three different domestic nursery operations. These are Plant Growers Australia in Melbourne, a supplier of containerized nursery stocks with particular emphasis on Australian native ornamentals; Bloomfields Nurseries (Australia) in Sydney, a large greenhouse operation with heavy emphasis on supply to cut-flower growers; and Biotech Plants in Somersby, one of the largest tissue culture labs in Australia and the producers of the "Bush Gems" range of hybrid kangaroo paws. The Calgene Pacific Horticultural Group is producing a wide range of products for the Australian domestic market, and has moved into the export market, where most of its future growth will occur.

With this base business in place, and with particular emphasis on the cut flower market, Calgene Pacific is well placed to commercialize its first genetically engineered products. These will be a range of elite cut-flower lines in which the colour has been specially modified. The first target is the colour blue which does not exist in carnation, chrysanthemum, or rose. This work is well advanced in our laboratories and is in collaboration with scientists at the Knoxfield Horticultural Research Station in Victoria.

The cut flower market is an attractive target for Calgene Pacific. The wholesale value of cut-flowers worldwide is in excess of US$12 billion per year. The industry demands approximately US$1 billion per year in propagating materials. Novelty is one of the most important factors involved in the marketing of cut-flowers. Thus new colours are expected to be well received by growers and by the consumer.

The future holds a long list of promising opportunities for the use of genetic engineering in the horticultural industry. In addition to colour modification, Calgene Pacific is working on a program to greatly enhance the post-harvest life of cut-flowers. These two projects alone offer extensions into other horticultural crops.

Colour is important in fruits and vegetables as well as in flowers. Enhanced post-harvest life is important in almost all horticultural crops. Horticultural crop growers will also benefit from herbicide tolerance, disease and insect resistance, drought and frost tolerance, and other novel traits being introduced to vastly improve the quality of their crops, to reduce their costs of production, and to help them to reliably produce their crops in the face of the every day uncertainties of weather, pests and disease. The tools exist to develop these products. Those groups willing and able to commit the funds and energy to applying these tools have a most attractive and potentially rewarding opportunity.

CHEMICAL CONTROL OF PLANT GROWTH

M. W. BARRETT

Mike Barrett and Associates
14 Kedron Avenue
Beecroft N.S.W. 2119

This paper reviews current commercial developments in plant growth control of nursery plants in Australia, particularly in New South Wales.

MATERIALS AVAILABLE

Growth regulator chemicals available in Australia were reviewed recently (9). Regarding synthetic plant hormones of the gibberellins only GA_3 is used to a very limited extent for plant growth control. This also applies to the cytokinin N6 benzyl adenine (BAP). Otherwise, growth regulator chemicals such as paclobutrazol (Bonzi®) (now registered), chlormequat (CCC), and daminozide (SADH) would be preferred. Dikegulac-sodium (Atrinal®), is not available commercially in Australia.

It should be noted that, with the exception of paclobutrazol, which has been developed specifically for use on ornamentals, registered uses and formulations of both chlormequat and daminozide are limited. In New South Wales use permits are available for out-of-label usage.

Paclobutrazol is formulated as a 4g per litre suspension concentrate registered as Bonzi®. It is taken up passively through leaves, stem tissue, or roots. That which enters through stems and roots is transported in the xylem to growing points. Active compound reaching sub-apical meristems inhibits gibberellin production.