

## THE SCIENCE BEHIND TRANSGENIC COTTON PLANTS

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### 1. INTRODUCTION:

Traditional plant breeding techniques have had a major impact on the Australian cotton industry through the production of the widely successful CSIRO varieties Siokra and Sicala. This science will continue to provide the Industry with the most relevant varieties for Australia's unique environmental conditions, but now it will be enhanced by the new technology of genetic engineering. Breeders in the past have been very restricted in the genetic resources that they can call upon for variety improvement and have only been able to produce new re assortments of genes already present in existing cotton varieties, or at most their very close wild relatives. Potentially useful genetic resources present in other plants or even non-plants have been inaccessible because of the sexual barriers to crossing between unrelated species. This genetic resource has now become accessible because of recent advances in recombinant DNA technology, the science of studying and manipulating genetic material. It is now possible to produce transgenic organisms, organisms containing genetic material from novel sources. The technology has been used in a variety of organisms from simple bacteria, yeasts and fungi, up to more complex organisms such as plants and even animals. The techniques have recently been extended to cultivated cotton, opening up tremendous possibilities for the improvement of our existing Australian cotton varieties.

### 2. PRODUCING TRANSGENIC COTTON PLANTS

Once a potentially useful gene has been identified, how do we transfer it into a commercially useful cotton plant? The first step is the isolation of the gene from the donor organism and it is this step that is often the most difficult. Any organism, say another plant may have many hundreds of thousands of different genes and although we have ways of physically isolating large chunks of DNA (the chemical constituent of genes) that contain individual genes or pieces of genes, picking the one piece that contains a specific gene can be problematical. Simple organisms like viruses and bacteria have many fewer genes than plants or animals and are often an ideal source for useful genes for genetic engineering as will be described below. This paper is not the appropriate place to discuss the many different methods of isolating genes but in the end we end up with a piece of DNA in a test-tube that contains the coded information necessary for the production of some specific protein product, such as an insecticidal protein or an enzyme that breaks down a herbicide etc..

#### 2.1 Genes in Pieces - Customising Genes for New Roles.

Although all genes use the same basic vocabulary or genetic code to specify their gene products, different organisms regulate when and where a gene functions in slightly different ways. This regulatory information is located at the front of the gene and is called a promoter. The middle of the gene contains the information for the product of the gene and the end of the gene contains information on the boundary of the gene, so that cells can tell where one gene ends and another begins. Because plants use different regulatory information in their promoters than say animals or bacteria, a whole bacterial gene introduced into a plant will not function because the plant cannot recognise the control signals present in the bacterial gene. Genetic engineering techniques allow us to correct this by swapping parts of genes in a very precise manner. We can make a bacterial gene work in plants by chopping off the bacterial regulatory sequences and replacing them with appropriate regulatory sequences from a plant gene. This may also be important when we want to change where a gene is expressed in a plant. If we have a plant gene that is normally expressed in leaves and we would like to change its expression to be in flowers, we can remove the regulatory sequences specifying leaf expression and replace them with the regulatory sequences from a different gene that is normally expressed in flowers. When this new hybrid gene is introduced back into a

plant it will now function in those tissues specified by its new regulatory signals. Genetic engineering therefore gives us very precise control over when and where new genes will function in transgenic plants.

## 2.2 A Natural Vehicle for Introducing New Genes into Plants.

There are a number of different techniques of getting novel genes from the test-tube into the chromosomes of a transgenic plant. The method of choice depends in part upon the target species, but with cotton we have relied on a natural gene transfer agent that has evolved its own method of plant genetic engineering. The disease called Crown Gall is a plant tumour disease caused by the soil-borne bacterial pathogen, *Agrobacterium tumefaciens*. In the early 70's it was recognised that the bacterium caused the disease by transferring some of its own genetic material into the DNA of the plant cells that it infected. These parasitic genes subverted the normal biochemical machinery of the infected cells and caused them to make novel compounds that only the bacterium could utilise. This process of genetic colonisation by the bacterium was just what genetic engineers were looking for, provided that they could stop the bacterium causing the disease symptoms. After further study scientists were able to identify which genes caused the disease and because bacteria are much simpler organisms to genetically manipulate, they were able to

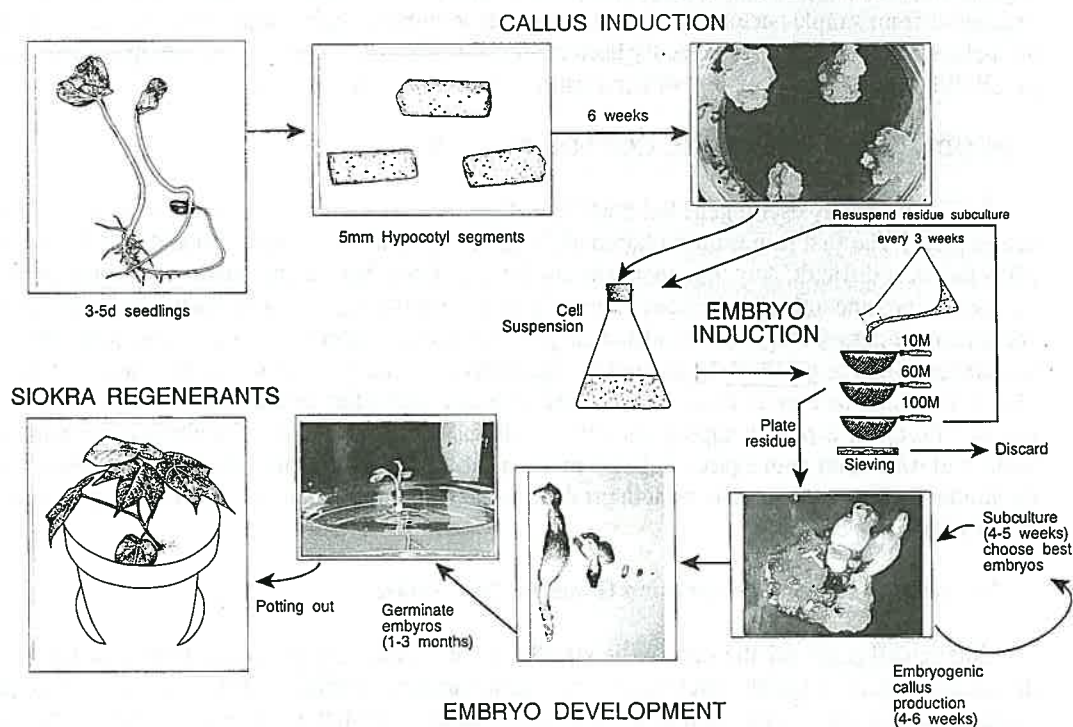


Figure 1. Schematic outline of the tissue culture regeneration cycle for Siokra cotton.

replace the disease-causing genes with the novel genes they were constructing from parts of potentially useful genes. The bacterium could then be used to piggy-back genes from the test-tube into plant cells. However not all plant cells exposed to the bacterium eventually receive the novel genes so how can we select out these plant cells from amongst a whole mass of cells that don't receive any new genes? This problem was solved in the first real application of plant genetic engineering.

Plant cells are sensitive to many of the antibiotics that are used to control bacterial infections in animals and humans. If a gene could be isolated that gave the plant cells tolerance to one of these toxic antibiotics then if physically linked to some desirable gene and inserted into the *Agrobacterium*, it would provide a useful selection system to kill off those cells that don't receive the genes during the "infection" process. Genes have been known in bacteria for many years that give the bacteria resistance to antibiotics by producing enzymes that breakdown or chemically modify the antibiotic so that it is no longer toxic. Using the techniques of gene splicing described above researchers have been able to modify a bacterial gene that encodes an enzyme that detoxifies the antibiotic kanamycin and have produced a new hybrid gene that causes the production of this enzyme in plant cells and prevents their death in the presence of potentially lethal doses of kanamycin. Combining this antibiotic selection system with plant tissue culture procedures it has been possible to use *Agrobacterium* to deliver genes into a wide variety of plants from petunias to cottons.

### 2.3 A Gene Transfer System for Cotton.

The *Agrobacterium* provides a way of getting novel genes into a single cell of a plant such as cotton, but how do we obtain a whole transgenic plant in which all of the cells of the plant contain the same new genetic information? The key to this is a good tissue culture system that allows the proliferation of a single cell and the regeneration of whole plants. With some plants, such as tobacco, tissue culture is a relatively easy and gene transfer systems were quickly developed (tobacco is still often used as a model plant system for testing out ideas and hybrid genes to see if they would be useful in more economically important crop plants). Cotton has proven to be a much more difficult species because it behaves very poorly in tissue culture, although overseas groups have had some success with a poor agronomic varieties of the Coker cultivar. At Plant Industry, however, we have been able to develop excellent, if slow, tissue culture systems for three commercially important Australian cotton cultivars, Siokra 1-3, S324 and to a lesser extent Siokra 1-4. This tissue culture system is shown in Figure 1.

Whole plants can be regenerated from small pieces of seedling cotyledons or hypocotyls by first putting them onto a medium that promotes callus production (disorganised masses of dividing cells). After about six weeks the callus is transferred to a liquid culture without the plant hormones normally necessary for the growth of the callus. In order to keep growing the callus must change its growth characteristics to become more organised so that it can produce its own hormones and converts to what we call an embryogenic state. The cells organise themselves into very tiny embryos, just like the embryos found in cotton seeds, each with a root end and a cotyledon end. These small embryos can be proliferated on a different solid tissue culture medium and then germinated like a seed to produce a small plant that can be potted in soil and eventually transferred to the glasshouse to produce a normal flowering plant. The whole process takes about 9 months. The gene transfer system is similar, if slightly slower, except that at the early stage the cotyledon or hypocotyl pieces are dipped in a solution of *Agrobacterium* engineered to contain the kanamycin antibiotic resistance gene and whatever desirable gene is being transferred to the cotton. These pieces then go onto callus induction medium containing a toxic dose of kanamycin to allow only those cotton cells receiving the engineered genes to grow. The rest of the procedure is the same as shown in Figure 1. The plants produced at the end are normal, set seed and pass the introduced genes on to their progeny in a normal manner. The only difference from non-transgenic cotton is that all of their cells contain two extra genes, the antibiotic resistance gene (that confers no deleterious properties) and the desired, hopefully beneficial, gene introduced along with it.

### 3. WHERE DO USEFUL GENES COME FROM?

As indicated earlier, useful genes for cotton improvement might come from anywhere depending on the problem being tackled. In the immediate future the main problems being investigated by us are in the area of plant protection. Protecting cotton from undesirable pests, diseases and chemicals.

### 3.1 Insect-Proofing Australian Cottons

The Australian cotton industry is currently worth over \$900 million in exports to Australia but it is also an industry under siege from insect pests that see the crop as a free meal. In order to survive, the industry has had to become Australia's biggest user of chemical pesticides, applying over \$125 million dollars worth each year. This has created enormous economic, environmental and ecological problems, not to mention image problems for the industry, and yet, Australian and International markets continue to demand the availability of natural fibres like cotton. If cultivars can be made more tolerant to insect pests using genetic engineering technologies there will be less reliance on chemicals and a more favourable environmental image for the industry as a whole, not to mention substantial savings in production costs.

Australian cottons are attacked by a number of insect pests, but the major ones are the caterpillars of the cotton budworm (*Helicoverpa* (previously *Heliothis*) *armigera*) and the native budworm (*H. punctigera*) and occasionally spider mites (*Tetranychus spp.*). The two *Helicoverpa* species are only just being kept under control by the application of toxic organochlorine and organophosphorus insecticides such as endosulfan and profenofos. The more environmentally friendly synthetic pyrethrin insecticides have been effective in the past, but there are growing fears that resistance development by the insects may soon make pyrethroids ineffective. This, together with the increasing public concern about the use of toxic chemicals, and their impact on the environment, has led to a flurry of interest in more environmentally acceptable insecticides and the development of more insect tolerant cotton varieties.

Traditional plant breeding has done much to improve the host plant resistance of Australian cottons to insect pests, and continues to provide new genotypes which require less chemical intervention than the old

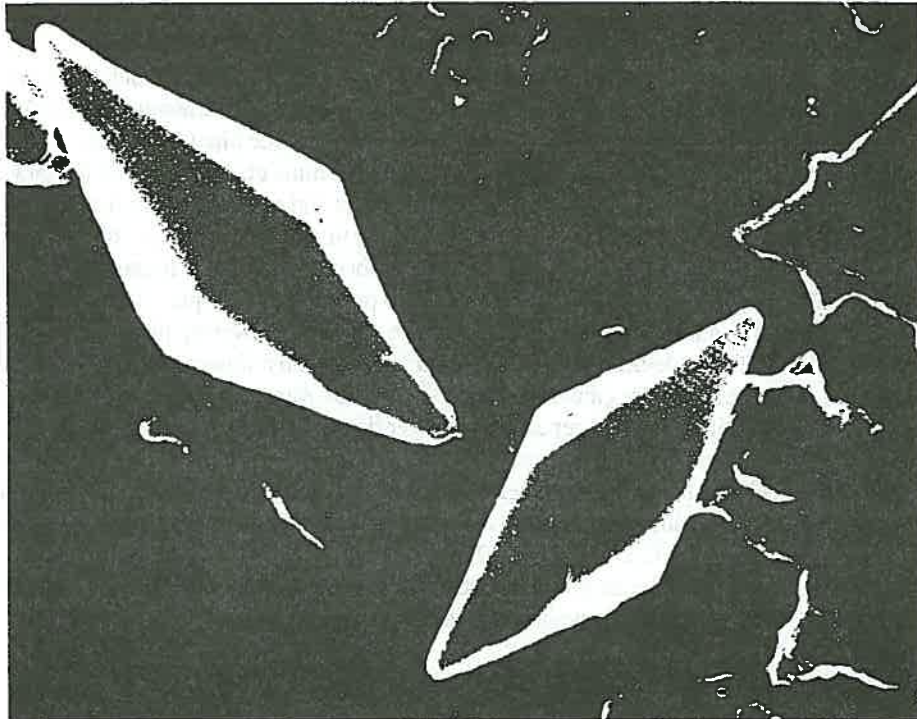


Figure 2. Insecticidal crystal proteins from *Bacillus thuringiensis* (BT).

varieties. New characters such as glabrousness (absence of hairs on the foliage), frego bract (outward bending, thin bracts around the cotton boll) and nectariless (absence of nectar forming nectary glands on the leaves and flowers) are now being assessed for their capacity to reduce the attractiveness of the cotton plant to the *Helicoverpa* species and to enhance the excellent host plant resistance provided by the okra leaf phenotype characteristic of CSIRO's best varieties. There are, however, limits to the improvements in natural resistance to insects provided by alterations in plant shape and structure. Genetic engineering should enhance our capacity to produce more tolerant plants by accessing a much wider gene pool for novel insect resistance characters. The most widely favoured genes thought to be of greatest value for cotton are the insecticidal crystal protein genes from the bacterium *Bacillus thuringiensis* (BT).

### 3.1.1. The insecticidal proteins of *Bacillus thuringiensis*.

Certain members of the gram positive bacteria belonging to the genus *Bacillus* produce proteins that are insecticidal. The most well characterised are those of *Bacillus thuringiensis* (BT), where the insecticidal proteins are found as crystalline bodies (Figure 2.) with sporulating bacteria. The insecticidal crystal proteins are characterised by their potency and specificity towards specific insect pests, many of which are agronomically important, and their relative safety to non-target insect species and vertebrates, particularly humans. They have enjoyed a long history of use in horticultural industries where the mixture of crystals and spores are sprayed just like a chemical pesticide, but they have not been used with much success on broad-acre field crops. A great deal of research is now going into improved strains and formulation technology to try to break into this lucrative market.

### 3.1.2 Mode of Action of Insecticidal Crystal Proteins.

The insecticidal crystals are composed of a large protein that is essentially inactive. When a caterpillar ingests some of the insecticidal crystals, the alkaline reducing conditions of the insects midgut cause the crystals to dissociate and release the crystal protein. At this stage the protein toxin is inactive, but specific proteases within the gastric juices of the insect chop the protein down to its protease resistant core that is

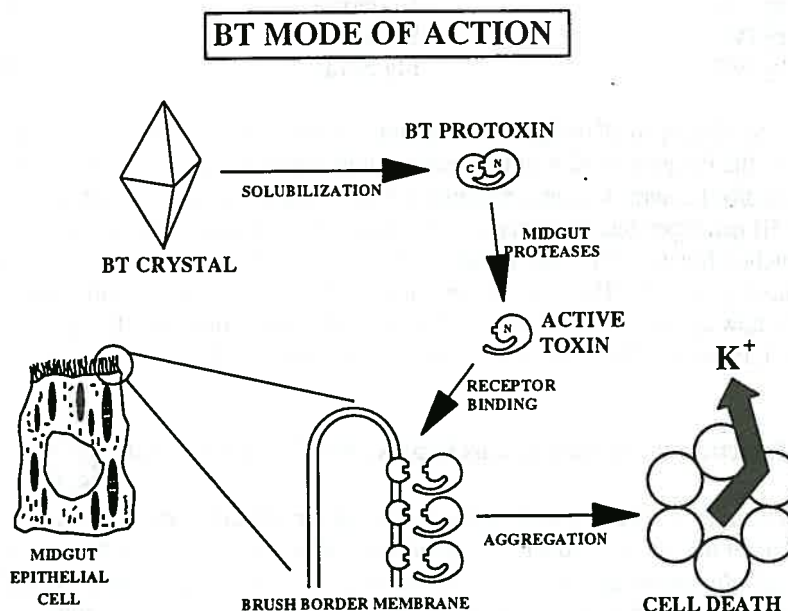


Figure 3. Schematic outline of the proposed mode of action of BT in killing insects.

now fully active. This activated insecticidal protein then binds to a specific receptor on the brush-border membranes of the cells lining the midgut and inserts itself into the cells membrane. When about eight of these aggregate together, they form a pore or channel through the membrane, and allow the cell contents to leak out (Figure 3.) causing the death of the cells essential for nutrient absorption. The insects rapidly stop feeding and eventually starve to death or die from secondary bacterial infections within about 24 hours. The processes of crystal solubilisation, proteolytic processing to an active insecticidal protein and the binding to a specific receptor, all make the BT proteins highly specific and very desirable from an environmental perspective.

### 3.1.3 Classification of Crystal Protein Genes.

Many thousands of different isolates of *B. thuringiensis* have been collected and their insecticidal protein content and activity spectrums determined. A large number of BT-insecticidal protein genes have also now been cloned and sequenced. To avoid any confusion researchers have proposed a uniform naming system for the crystal protein genes (*Cry* genes) based on their protein sequence and the types of insects for which they were toxic (Table 1.):

TABLE 1. Classification of insecticidal BT proteins.

Gene Type	Insect Host	Size (kD)
Cry IA	Caterpillars	133.2
Cry IB	Caterpillars	138.0
Cry IC	Caterpillars	134.8
Cry IIA	Caterpillars/ Fly larvae	70.9
Cry IIB	Fly larvae	70.8
Cry IIIA	Beetle larvae	73.1
Cry IVA	Fly larvae	134.4
Cry IVB	Fly larvae	127.8
Cry IVC	Fly larvae	77.8
Cry IVD	Fly larvae	72.4

The proteins encoded by the Cry I genes are all similar in protein sequence and are toxic only to caterpillars, ie. the larvae of moths and butterflies (Lepidoptera); the Cry II genes encode proteins toxic to Lepidoptera and/or Diptera (flies and mosquitoes) while the Cry IV proteins are only active against Diptera. Cry III genes produce proteins active against beetle (Coleoptera) larvae. Within these major groupings smaller divisions have been made by considering the similarities or differences between the different protein sequences. The Cry I group, for example was originally divided into Cry IA, IB and IC (although it is now up to Cry IG) where the different sub-groups may only be 50% identical at the protein sequence level. Finer sub-divisions have also been made and Cry IA now consists of Cry IA(a), IA(b) and IA(c).

### 3.1.3 Insecticidal Crystal Proteins Expressed in Transgenic Plants.

The most characterised insecticidal proteins are those encoded by the CryIA class, and these are the ones present in all the major commercial preparations of BT that are currently used to control Lepidopteran larvae such as *Helicoverpa* spp. Until quite recently the commercial preparation called DIPEL was the major BT insecticide and this was produced by *B. thuringiensis* var *kurstaki* from which the first BT gene was cloned. Once the gene was cloned researchers quickly came upon the idea to express the bacterial gene in crop plants so that the leaves of the crop would produce its own highly toxic, but very specific, biological insecticide. The first genes were equipped with promoters from the Cauliflower mosaic virus 35S gene and expressed with mixed success in transgenic tobacco or tomato plants where they gave

increased tolerance to insect pests. Pest resistance levels were not always high because of very low levels of BT-protein expressed in the transgenic tissues, levels that would probably not be sufficient for long-term protection of a field crop.

In 1990 researchers at Monsanto made a significant advance in the expression of BT genes in plants. They noticed that BT genes were excessively rich in two of the chemical components of their DNA, much more so than plant genes. Monsanto reconstructed the BT gene using a DNA synthesiser, to correct this bias but maintaining the encoded protein sequence so that the gene product was the same as that in the insecticidal crystals. This synthetic gene when expressed from a strong plant promoter in transgenic tobacco, tomato or Coker cotton plants gave exceptionally high levels of expression of BT protein in all of its tissues (over 0.2% of total soluble protein), with a corresponding increase in the effectiveness of insect control. Preliminary field tests in the U.S. on BT Coker cotton plants indicate very effective performance, even with economically important pests, like *Helicoverpa zea* (Cotton bollworm), without the use of chemical pesticides.

#### 3.1.4 BT genes in Australian Cotton Cultivars.

CSIRO has recently signed a research agreement with Monsanto to utilise some of their advanced BT-technology to improve the insect tolerance of Australian cotton varieties. This is being approached in two

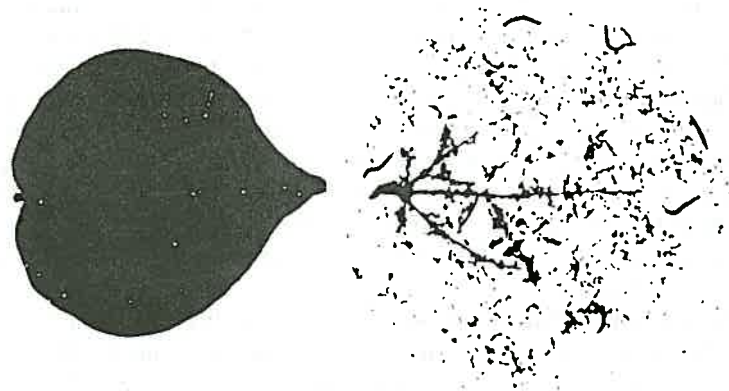


Figure 4. Cotton plants expressing BT proteins (left) showing high toxicity to *H. armigera* larvae, while non-expressing plants (right) are completely consumed.

ways, by backcrossing the BT genes from the transgenic American Coker variety produced by Monsanto, into Australian cultivars by traditional breeding procedures and by direct transformation into Australian Siokra cultivars using *Agrobacterium tumefaciens* mediated gene transfer techniques. The backcross program is only part way through (we may need to go to backcross 5 or 6, or more, before the material will be ready for commercial evaluation and release), but it would be of great value to know whether the gene being introduced will be expressed at high enough levels to control the insect pests present in Australian

cotton fields (ie. *H. armigera* and *H. punctigera*). The plants being produced are crosses with a single transgenic parent Coker plant, selected by Monsanto as the best performing plant from amongst a number of independent transgenic plants. This plant showed high levels of BT protein expression and effective killing of *H. zea*. Although we have seen impressive control of *H. armigera* in laboratory tests (Figure 4.) of some of the backcrossed material, this has been to a single isolate of the insect and we have some indications that field isolates are more tolerant to BT than our laboratory strain. It is therefore not clear whether the original plant had levels high enough to control our pests in the field. We are therefore proposing to carry a field trial on some of the early backcross material (homozygotes selected from the BC<sub>2</sub> plants) in the 1992-93 season and would like to assess the relative field tolerance of six different Australian cultivars containing the Monsanto BT gene. When we have sufficient material available we would also like to test some of the Siokra 1-3 and S324 cultivars transformed directly with a similar BT gene construct, but this will form part of a later field release as these plants are still at the first generation stage.

### 3.1.5 Countering Resistance Development to BT.

There is always the fear that resistance will eventually evolve in the *Helicoverpa* populations and make the BT transgenic cottons useless in a relatively short time. How we manage these particular plants in the field may go a long way towards delaying resistance development, but the strategies used must still be determined and will form part of the discussions at the "Managing Biotechnology" forum at the Cotton Conference. A second strategy is to develop multiple insect resistance gene that will be incorporated into new cultivars either separately or in combination with the Cry IA gene obtained from Monsanto. At CSIRO we are currently working on at least four different insecticidal genes that may be useful in transgenic cotton.

Two of the new genes are BT's but different from the Cry IA gene. Both of these genes encode insecticidal proteins that work in similar ways to the Cry IA protein but act through different receptors in the insect's midgut. In studies on populations of the Diamondback moth (*Plutella* spp.) that have evolved resistance to BT applied as a microbial spray, researchers have found that resistance was a consequence of a reduction in the number and binding strength of the BT receptors in the insect's midgut. Resistance to Cry IA does not necessarily give cross-resistance to Cry IB and Cry IC proteins and we have targeted these genes for expression in cotton. Initial studies with expressing Cry IC genes in transgenic tobacco plants (as a rapid test system) have shown some toxicity to *H. armigera*, but as yet we do not have expression levels high enough to be as effective as the synthetic Cry IA gene from Monsanto. Research is continuing to increase the expression levels of both Cry IB and Cry IC genes before they are introduced into Siokra varieties by direct transformation.

A third insecticidal protein that we have identified is a proteinase inhibitor from a tropical plant, the giant taro. This plant is highly resistant to insect attack in the tropics and produces high levels of a particular inhibitor in its edible corms. Purified inhibitor fed to *H. armigera* larvae causes a dramatic decrease in the growth of the larvae, possibly by inactivating the digestive proteases of the insect and starving it to death. We have recently cloned the gene for the giant taro inhibitor and will attempt to express it in transgenic tobacco to test its insecticidal properties. Similar inhibitors from cowpeas, potatoes and tomatoes have been shown by others to have some insect control properties against certain insects when expressed in transgenic plants and we can expect this inhibitor to have some anti-feedant effects against *H. armigera* if we can express it at high levels in transgenic cotton. Because it affects the insects in a completely different way than the insecticidal BT proteins, its activity should be unaffected by resistance development to BT.

Insect tolerant plants may be a source for other insecticidal protein genes and we are investigating specific classes of plant defence proteins from other tropical crops, such as sweet potato, for their potential use in transgenic plants. These genes are still at the early stage of characterisation and we have yet to clearly demonstrate effectiveness, but in the long-term they may be useful additions to our arsenal of defences to incorporate into commercial cotton varieties.



100 times less toxic to plants than 2,4-D. Our major goal was therefore to produce a cotton plant that would contain sufficient 2,4-D monooxygenase enzyme in its leaves to break down 2,4-D into DCP before the herbicide could damage the sensitive shoot tips.

### 3.2.2 Developing and Testing the Genetic Hardware in a Model Plant Species:

The gene for the 2,4-D monooxygenase enzyme (*tfdA* gene) is encoded on a large plasmid found in the *Alcaligenes* bacterium, and was isolated as a small fragment of DNA. As indicated above, bacterial genes do not function when introduced directly into plant cells, so the gene needed to be modified so that it would be recognised and expressed by the cellular machinery of a plant. This involved replacing the bacterial promoter with a promoter known to function in plants. We used a region from a plant virus (the Cauliflower mosaic virus) gene that has been effective in constitutively expressing a variety of genes in engineered plants, including the BT gene. The hybrid gene that we stitched together from bits and pieces of other genes then had to be introduced into *Agrobacterium tumefaciens* and used to infect tobacco as a model species. Transgenic tobacco plants could be produced in as little as six to eight weeks, compared with the year or more it takes for cotton. Several transgenic tobacco plants were produced using the *Agrobacterium* containing the hybrid *tfdA* gene and these were screened using an enzyme assay we developed to detect the presence of the 2,4-D monooxygenase enzyme. The plants expressing the highest levels of the enzyme were analysed further after being transferred to the glasshouse and the first generation of seeds harvested. When germinated on agar plates containing different levels of 2,4-D we could quickly show that the transgenic plants had an increased tolerance to 2,4-D and could germinate on media containing thirty to fifty times the level of 2,4-D that would prevent the germination of normal wild type tobacco seeds. Resistant seedlings transferred to soil in a biosafety glasshouse and then sprayed with 2,4-D at 3 weeks, also proved to be resistant to 2,4-D at thirty to fifty times the level that would damage control plants. At the highest level sprayed (1000 ppm) a small amount of damage was occurring to the transgenic plants, mostly at the shoot tips. Genetic and biochemical studies clearly demonstrate a correlation between the expression of the bacterial 2,4-D monooxygenase in the plants and their tolerance to 2,4-D.

### 3.2.3 A First Generation of 2,4-D Tolerant Cotton

Coker cotton is a poor agronomic variety but has very good tissue culture characteristics making it ideal for transformation work and it proved to be slightly easier to transform than Siokra cotton. We adopted this cultivar as a model substitute for commercial varieties, although we were aware that useful genes introduced into Coker varieties could still be moved into more commercial cultivars by traditional genetic means if this proved desirable. Using the published protocols for the transformation of Coker cotton we were able to produce about a dozen independent transgenic Coker plants containing the *tfdA* gene. Unfortunately, not all of these proved to be fertile. The first four fertile plants were analysed further, by genetic and molecular techniques, after transfer to the glasshouse and collection of progeny seeds. Two of the lines contained single insertions of the novel DNA, while the other two contained two insertions of the gene that segregated independently in the progeny. When sprayed with 2,4-D all four lines were tolerant to up to 300ppm 2,4-D, while two lines were immune to damage at 600 ppm especially when in the pure homozygous form (Figure 6.). The two other lines showed more extensive damage at 600 and 900ppm 2,4-D. The extent of damage in the best plants was similar to control plants sprayed at 10ppm so it looks as if we have around a 90-fold increase in tolerance to this herbicide. At 900ppm only slight damage occurred to the shoot tips of the best performing line and it is the homozygous progeny of this plant that we wish to field test in 1992 (if we get government approval) to get a better estimate of field tolerance levels.

Although the 2,4-D resistance engineered into Coker could be crossed into Australian varieties, the necessity for several generations of back-crossing would make this impractical. We would prefer to introduce the genes directly in well adapted Australian varieties and have concentrated our efforts over the last year into producing a large number of transgenic Siokra 1-3 containing the *tfdA* gene. The transformed Siokra material has lagged behind our Coker transgenics because the protocols used for the American

cultivar work poorly with this Australian variety and we have had to optimise the protocols specifically for the Siokra material. We have now established efficient protocols for Siokra 1-3 (as well as Siokra S324),

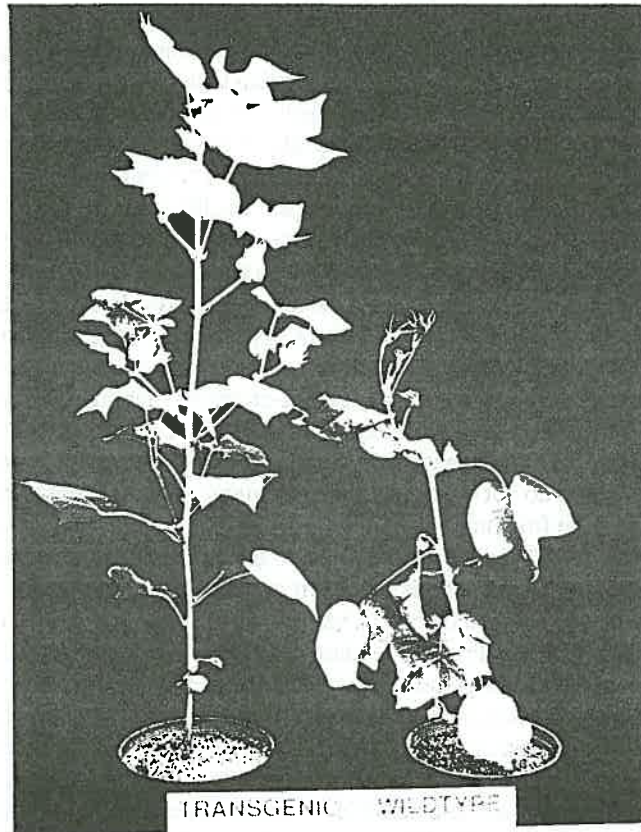


Figure 6. Transgenic Coker cotton (left) exhibiting tolerance to 300 ppm 2,4-D. Non-transgenic cotton (right) is damaged and eventually died.

and, although it still takes almost a year to produce a transgenic cotton plant, we now have several plants reaching maturity in the glasshouse which require evaluation. Over the next year we intend to produce many more transgenic Siokra 1-3 plants expressing 2,4-D tolerance and will select the best material for further study and eventual commercial evaluation in the field. The current levels of 2,4-D resistance may be adequate for drift protection, but we cannot be certain until we establish their resistance levels under field conditions using commercial formulations of 2,4-D. If the levels are insufficient in the field, it may be necessary to continue to modify the *tfd A* gene constructs to achieve higher levels of expression in cotton, and hence the results of the field trial will be necessary to guide our future research efforts on this project.

### 3.3 Future Directions.

These are just two examples of the sorts of useful genes that could be introduced into cotton using genetic engineering techniques. Other projects that have been begun at CSIRO will address the problem of Verticillium wilt disease in cotton by developing suitable artificial resistance genes and poor germination of cotton in cold soils by manipulating the oil content of cotton seeds. Many other avenues will be opened as our understanding of plant development and biochemistry increases.

#### 4. ARE TRANSGENIC COTTON PLANTS SAFE?

The techniques for producing transgenic cotton plants are now relatively routine, if a little slow, but how safe are these plants compared to plants produced by traditional plant breeding. Many people experience the "Frankenstein Factor" when considering genetically engineered organisms - could we produce uncontrollable monsters by manipulating the genetic material of the organism? This view invariably stems from a lack of information about the real nature of transgenic plants. Genetic engineering is a precise process, taking very precisely defined genes or parts of genes and introducing them into single locations in a plant's chromosomes. The behaviour of these genes is predictable from our knowledge of how genes function and the only real problem is usually how to get them to function as well as they would if nature had put them in the plant first. (Transgenes are usually expressed at much lower levels in the transgenic plants than they would in their usual genetic environment.) Traditional plant breeding, on the other hand, is a much more random process. Two plants are crossed and each of their 100,000 or so genes are completely mixed. The breeder then has to select the best possible combination for yield, quality, disease or pest resistance. Invariably the resultant plants are less well adapted than the original highly adapted cultivar because of the re-assortment of the genetic material, and it then takes a number of years to recover the desired characteristics by successive back-crosses to the original commercial cultivar. If you are fortunate enough to have a gene transfer system for an elite cultivar, such as we have for Siokra, then useful genes can be inserted directly without disturbing any of the agronomic properties of the cultivar. Those single inserted genes do not change any of the general characteristics of the plants (our transgenic cotton are indistinguishable from non-transgenic cotton) since these characteristics are specified by many hundreds of different genes. When they reach the field the behaviour of the plants in response to water, nutrient balance etc. will be identical to the plants from which they were originally derived, but they will have increased pest resistance or tolerance to a herbicide etc. In this respect we consider these plants to be as safe as any plant derived by traditional breeding. They will however create some different problems that will have to be addressed at a management level. As discussed above the BT cottons may encourage resistance development by *Helicoverpa* species and the industry will have to reach some consensus on how best to use these plants in the long term. Herbicide tolerant cottons may also create some problems unless used wisely. Growers will have to pay particular attention to the herbicides they use around transgenic cotton, particularly if they normally use herbicide mixtures. The sorts of herbicide tolerance genes we might have in cotton are very specific. The 2,4-D resistance gene gives resistance to 2,4-D and 2,4-DB but not to picloram for example. Neighbouring growers should be aware of what each other are spraying in case one is not using a herbicide tolerant crop. This may be partially overcome by incorporating resistance genes into all the current commercial varieties, but non-cotton crops will also have to be taken into account. None of these problems are insurmountable and are easily controlled by effective management strategies, something that cotton growers have already shown they are good at!

#### 5. GETTING TRANSGENIC COTTON INTO THE FIELD

A number of different transgenic cotton plants with increased tolerance to pests and chemicals have been produced and tested in laboratories and contained glasshouses, but how long will it be before growers see them in the field? Genetic engineering is a very new technology and although we do not foresee any deleterious effects from their introduction, government regulatory bodies are being understandably cautious about their release into the environment. The testing of the plants in the field is likely to take a few years starting from very small trials of only a few hundred plants and building up to more traditional sized cultivar evaluation trials. Applications have been lodged for the first trial of transgenic BT-cotton to be done this year at the Narrabri Agricultural Research Station to carry out preliminary experiments on some of the plants in our back-cross program. These initial experiments with only 200 transgenic plants will be to establish appropriate containment criteria for subsequent larger trials. A major consideration of the regulatory agencies is that all transgenic material must be contained within the test site during the initial evaluation stages. This means that we must ensure that the BT-genes in the transgenic plants do not escape to other cotton in the region. Cotton is usually an inbreeding plant but a small percentage of cross-pollination is generally carried out by bees. To ensure that bees do not transfer pollen (eg. containing BT genes) away from the site onto other cotton, the test site will be surrounded by a small field of cotton to act

as bait plants. Pollen collected by bees from any of the transgenic plants is likely to be deposited on these neighbouring non-transgenic plants which will be harvested at the end of the trial and their seeds analysed for the presence of the introduced genes. In the next year we will test 2000 transgenic plants, and in subsequent years larger numbers provided that they perform well and show good insect control. At some stage we will request a general release of the transgenic plants and then they will be treated just as any non-transgenic cultivar. During the scale-up in the field releases we will have to work out the best strategies for using the transgenic plants to delay resistance development by *Helicoverpa* species. In all it will probably take another four to five years before any seed will be available for commercial sale.

There have been some criticisms of the regulatory procedures for releasing transgenic plants into the environment and this has formed the subject of a Parliamentary Inquiry that published its report this year. The Inquiry recommended a streamlining of the release process and the institution of a single regulatory authority with strong legal powers of enforcement. The current system is a voluntary one although no-one could afford to ignore the regulations because of adverse public reaction. The release we are proposing to do this year with BT cotton will have to pass through two independent CSIRO committees, the governmental Genetic Manipulation Advisory Committee and two state Departments (Department of Agriculture and Environment Protection Authority) before any plants can be put into the field. The proposal will receive extensive assessment to ensure that the release is safe to the researchers, the cotton industry and the environment and that it will be carried out in a manner appropriate to any perceived risks.

## 6. CONCLUSIONS

Genetic engineering technologies have advanced rapidly over the last two years and transgenic cotton plants are approaching the point of contained release into the environment. The technology is producing new varieties with value added characteristics that should enhance the long-term sustainability of cotton production in Australia. Although the technology is new and should be used with caution, particularly in the early stages of release and assessment, it holds a lot of promise. The technology is not something to be frightened of, as it produces in a very precisely controlled manner cotton plants with additional features not possible by traditional breeding methods. Over the next few years genetic engineering will become a standard plant breeding tool adding to the already considerable capacity of traditional plant breeding techniques.

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