GENETIC ENGINEERING OF COTTON: MYTH OR REALITY?

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Genetic engineering involves the agronomic improvement of crop plants through the introduction of new genetic information (genes) originating from other organisms. Unlike traditional plant breeding, this is not restricted to genetic material from plants that can be sexually crossed with the crop plant, be they other varieties or wild relatives, but extends to all living organisms, from the simplest virus to the most complex animal. The universal code for all genetic information means that a plant can decode these foreign genes to produce a new product in its cells. Although the functional segment of the foreign genes might be recognised, the gene signals that control the switching on and off of genes tend to be unique to different types of organisms. To make a bacterial gene, say, function in a plant it is necessary to remove its own bacterial gene controls and replace them with appropriate gene controls isolated from a plant gene. Even genes from other plants may require some modification of the gene controls if the donor and recipient plants are very distantly related. This construction of new gene combinations in a test tube represents the first stage in genetic engineering; the second stage is to get these essentially synthetic genes into the crop plant. This latter process, called genetic transformation, is often difficult for crop plants, especially cereal crops, but has now been demonstrated for a wide variety of crop and pasture species such as rice, maize, lucerne, white clover, tomatoes, potatoes, brassicas, soybean and cotton.

WHAT HAS GENETIC ENGINEERING ACHIEVED SO FAR?

Genetic engineering is not well suited to tackling complex multiple gene characters such as yield, but it has had success in three main areas

that relate to improving crop productivity by manipulating single gene characters. These are herbicide resistance, virus resistance and insect resistance.

The increasing costs of mechanical weed control make herbicide resistant crops particulary attractive, especially if this involves cheap broad spectrum herbicides. This may also be important to protect a crop against residual herbicides used in a previous crop rotation. Two types of herbicide resistance genes have been constructed. Firstly, many herbicides have very specific targets in the plant and they often inactivate key metabolic enzymes thus depriving the plant of metabolites essential for its growth. If a gene for a similar enzyme that is insensitive to the herbicide can be introduced, the plant should be able to grow in the presence of otherwise lethal doses of the herbicide. The resistant genes can be isolated from bacteria, yeast, or even tissue cultured plant cells selected for resistance to the herbicide. Quite acceptable levels of resistance have been generated in tobacco and tomato to the herbicides glyphosate, chlorsulfuron and atrazine using this technique. The second approach is to engineer into the plants an enzyme system that will degrade the herbicide before it has had a chance to act. Herbicide detoxifying genes have generally been isolated from soil bacteria that naturally degrade herbicides in the environment. This system has been used to confer high levels of Basta resistance to tomatoes, potatoes and tobacco.

Virus diseases can cause considerable losses in yield in many crops, even when the level of infection is so mild that no obvious disease symptoms are present. Now that we have a greater understanding of the detailed molecular biology of plant viruses and how they function, we can use their own genetic material against them to give plants resistance to viral infection. For example, making plants produce the outer protein coat

of viruses appears to give them a remarkable capactiy to resist infection, presumably by not allowing the virus to uncoat its genetic material in the plant cell. Such a system has been used in tobacco, tomato and lucerne to give resistance to tobacco mosaic virus, tomato mosaic virus and alfalfa mosaic virus, respectively. Small molecules (satellite RNAs) that act as parasites on viruses have also been used with some success against tobacco ring spot virus and cucumber mosaic virus.

The third area where genetic engineering has been successful is in the construction of novel genes conferring insect resistance to plants. *Bacillus thuringensis* spores have been successfully used as an insecticide for a number of years because of the presence within the spores of a toxic protein (BT-toxin). When plants are engineered to produce this protein in their own tissues, specific groups of insects that eat them will die or atleast be deterred from feeding. A less dramatic feeding deterrant can be achieved by causing the plants to produce proteinase inhibitor proteins in their leaves (these are proteins often produced in the seeds of mahy plants). The proteinase inhibitors stop the insects from properly digesting their food and so deter them from further feeding.

All of these new genes for herbicide, virus and insect resistance were developed in model plant species such as tobacco, the plant genetic engineers equivalent of the laboratory rat. Nearly all of them have now been tested in tomato and/or potato, more commercial crops, and are now under field test in the U.S.. There is some way yet to go before they can be considered as real breeding stock for cultivar production, even in these relatively simply engineered species. Not the least problem being the acceptance for human consumption of these modified crops by Public Health authorities. Non-edible fibre crops such as cotton may have a distinct advantage in this respect and already a number of U.S. companies

are actively putting their herbicide and BT-toxin genes into cotton.

WHERE ARE WE UP TO IN THE GENETIC ENGINEERING OF COTTON?

Even before we consider what genes we might like to put into cotton, we need a gene transfer system. Most gene transfer techniques for plants include a protocol to regenerate whole plants from small tissue samples. In cotton, this process is very slow and apparently restricted to a few specific genotypes. Some of the more genetically diverse, but less commercial, cottons such as the American Coker cottons, have been successfully regenerated and genetically transformed with BT-toxin genes (as yet we know little of their effectiveness for insect control). Obviously, it is important to be able to regenerate and transform high yielding commercial cultivars and we have concentrated our efforts in this area using lines derived from Siokra and Sicala. Yvonne Cousins has regenerated plants from some of these Australian cottons and is in the process of adapting her protocols for use with gene transfers. The regeneration process in cotton is quite slow, but we already have promising results with the transfer of antibiotic marker genes into cotton cells (not yet whole plants).

If a reliable system for the genetic transformation of Australian cottons were available tomorrow, what would we want to put into them? The obvious first choice would be the BT-toxin genes, but there are a number of factors that mitigate against this. Firstly, the genes available are from strains that are not particularly active against the insect pests of cotton and further screening will probably be necessary to find the best BT strains for cotton (this is currently happening at the Division of Entomology). Second, as with disease resistances, it would be unwise to release a BT-producing cotton without adopting an integrated approach with several

levels of resistance combined in one plant. The breakdown in any one resistance would not then make the plants completely suseptible. Insects can develop resistance to BT-toxin, as has been shown in treated stored grains, where the selection pressures for resistance are high, and this would most likely happen very quickly if every cotton plant in a field were producing the protein toxin. We have therefore opted for a two pronged approach, on the one hand, waiting for the best BT strains for cotton to be identified, and trying to use proteinase inhibitors to provide a second level of resistance.

In the short term a herbicide resistance for cotton would seem to provide the greatest immediate opportunities for genetic engineering to contribute to cotton production. Unfortunately many of the herbicide resistance genes already described are proprietry products of American companies, and although negotiations for their use in Australian crops are on-going, as yet, we do not have ready access to glyphosate, chlorsulfuron or atrazine resistance genes. The Division does, however, have a Cotton Industry funded project (covered in a poster by Dr. Bruce Lyon) to develop a new herbicide resistance gene to the relatively cheap, broad-spectrum, broadleaf herbicide 2,4-D. We hope to have this gene ready for testing in cotton in the not too distant future.

Genetic engineering obviously has a lot of potential to do things that would be impossible by traditional plant breeding, yet it must only be considered as another arm of plant breeding technology. Genetically engineered plants still have to be incorporated into a breeding program, field tested, trialled and registered as cultivars in the same way as a traditionally generated variety. The strength of this new technology is that it now provides us with the opportunity to specifically manipulate single genes without disturbing all those other genes that make any variety as

good as it is. In the future, given the appropriate level of research backup, I'm sure it will be used successfully to control, from within the plant, a variety of pests and diseases; allow the use of cheaper and more effective herbicides; and even to start manipulating the quality and character of the two major products from the cotton plant, the fibre and the cottonseed oil. Genetic engineering of cotton is certainly no myth and will undoubtedly be a reality within the next few years.