Future Biotechnologies

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Runs on the Board for Cotton Biotechnology

Biotechnology is currently reshaping agriculture throughout the world with over 16 million hectares planted annually to genetically modified (GM) soybeans, corn and cotton, mainly in the US, but increasingly in Australia, Asia and South America. Some controversy remains towards GM food products and there is still a reluctance to embrace GM crops in Europe, but GM cotton is partially buffered from these concerns, as only its highly processed oil is used for human consumption, and it is now being widely grown in many cotton producing countries.

The first GM crop to be released in Australia was INGARD cotton in 1996 developed through partnerships between CSIRO and Monsanto and Deltapine Australia and Monsanto. INGARD contains a gene, Cry1Ac, from a natural biological control agent *Bacillus thuringiensis* that is toxic to many of the caterpillar pests prevalent in Australian cotton fields, including the two most damaging species *Helicoverpa armigera* and *H. punctigera*. Six years down the track we have seen growing acceptance of the value of INGARD to reduce the pesticide usage in cotton for the control of caterpillar pests. Continued monitoring of INGARD performance by the CRDC has shown that INGARD cotton has reduced pesticide spraying by 40-60% in comparison with nearby conventional cotton fields (CRCD, 2000), although the total industry benefit is reduced by the self imposed cap on INGARD production areas (no more than 30% of planting area). The economic benefit to growers of using INGARD appears to be increasing ($182/ha in 2000 compared with $72/ha in 1999), the real value of INGARD is still seen to be its environmental plusses and this is generally the main reason for growers choosing to grow this GM crop.

Ultimately a single gene will always remain vulnerable to the insects developing resistance and cotton varieties with two insecticidal genes are considered to be a minimum for any long term and widespread use of insecticidal GM cotton by the Australian industry. New Bollgard II varieties (INGARD cotton with an additional *Cry2Ab* gene) are very rapidly approaching commercialisation. Registration of Bollgard II for human consumption is nearing completion and applications for commercial production are currently with the NRA and OGTR awaiting assessment. Some small-scale commercial release could be expected in the 2002 or 2003 seasons. Research trials have indicated considerably better insect control for Bollgard II extending for much of the season so considerable reductions in pesticide application are to be expected. Provided that an effective resistance management strategy can be implemented that takes into account the reduced efficacy of the INGARD component of Bollgard II later in the season we can expect to see widespread adoption of Bollgard II over the next few years.

The second GM crop to be released in Australia in 2000 was the herbicide tolerant Roundup Ready cotton tolerant to glyphosate. Unlike INGARD cotton there is no imposed restriction on planting areas and it is expected that adoption will be rapid provided that varieties with suitable performance and disease tolerance are available. Roundup Ready cotton cannot be a stand-alone weed management option and must be incorporated into an integrated weed management program. Continued use of glyphosate results in a build-up in those weeds like peach vine that are more tolerant to glyphosate and these would require other control methods. The current version of Roundup Ready cotton is not completely tolerant to glyphosate and direct spraying is restricted to the four leaf stage, reducing some of the potential benefit of this technology to reduce hand chipping of weeds and the consequent exposure of farm workers to chemicals. Despite this growers have embraced RR varieties and its area grew to over 90,000 ha in 2001 and could easily double in 2002. An improved version with a wider application window has been developed by Monsanto and could be available to Australian growers by about 2007.

Both GM technologies, despite their limitations, have clearly demonstrated their benefits to Australian cotton growers in improving production capabilities, increasing profit margins (even if only a little) and considerably reducing impacts on the environment. It is likely that over half of the area planted will be GM in 2002 and move rapidly towards full adoption in the short number of years thereafter. The positive impacts on the industry and the environment will be a powerful example of the value of GM technologies and will contribute considerably to both allaying public fears of GM and promoting further investment in biotechnology research.

Consumer Attitudes to Biotechnology – Do They Really Care?

Consumer attitudes to GM crops vary considerably from country to country and from crop to crop. They tend to be difficult to judge as they are very often clouded by the media skirmishes generated by a very small number of vocal activists. This has led to the commissioning of a number of public awareness surveys to gauge the true feeling of the Australian public, not only as a guide to government regulators, research organisations and private companies in formulating and implementing regulatory oversight of biotechnology, but also in defining where to target educational programs. Significant among these were the surveys by Biotechnology Australia conducted in 1999 and 2001 (http://www.biotechnology.gov.au/MB_survey_results.ppt and http://www.biotechnology.gov.au/industry_research/reports/pYCHW_pdf.PDF).

Significant numbers of respondents in the first survey were concerned both that biotechnology would not result in improvements to society and that it involved significant risks. Fortunately, agricultural biotechnology applications, particularly those that resulted in more pest resistant plants were in general thought to be a good idea (no doubt largely due to the high media profile of INGARD cotton and the media activities of those associated with its development and deployment in Australia). Most respondents would wear clothing or eat oil /margarine derived from GM crops, but were more cautious about GM fruits or vegetables. Teachers and farmers in particular, but also the general public, considered CSIRO one of the more reliable sources of information on biotechnology. However, lack of confidence in the regulation of gene technology was prevalent and, in fact, there was surprisingly little awareness of any of the existing government regulatory agencies or their activities. Most people surveyed thought that biotechnology innovation was out of control and was being driven by large multi-national companies who were more powerful than the governments that try to regulate them. Such widespread attitudes were clearly instrumental in the demise of the voluntary regulatory system for biotechnology research under GMAC in force until 2000 (see below).

Follow-up surveys in 2000 confirmed the early results, but with small but significant increases in the awareness and acceptance of some agricultural biotechnology applications, particularly for pest control (presumably as a result of the publicity around GM cotton in Australia). The results of these surveys have been informative and highlight the improving, but essentially very low, level of understanding by the general public of many of the issues related to gene technology. There was clearly a lack of understanding of what was meant by GM foods, compounded by a general lack of knowledge of conventional agriculture in general. There was the perception that if it wasn't "organic" it was genetically modified in some negative way. Although the Office of Gene Technology Regulator (OGTR) had started operations before the last survey there was little knowledge of its existence or role, but there was a clear public expectation that any government regulator of biotechnology should be open and transparent, have financial and political independence, access to relevant expertise and enable public involvement.

We clearly have a long road to hoe if biotechnology is going to find widespread public acceptance and this can only be knowledge based, as many people surveyed still thought biotechnology was too complex and intimidating a scientific subject and that in itself engendered uncertain, cautious and often negative attitudes on a very general level to biotechnology. The onus for education should be fairly distributed across government, the cotton industry and the developers of gene technology and given the trust in CSIRO it is appropriate that we continue to be actively involved in public education.

The Changing Face of Regulation of Biotechnology in Australia – For Good or Bad?

Up until June 2000 the responsibility for the regulation of gene technology in Australia was dispersed over a number of both State and Federal Agencies. Central to the regulation was the Genetic Manipulation Advisory Committee (GMAC), a committee of scientific experts very capably led by the pragmatic Professor Nancy Millis. Under her guidance GMAC approached gene technology regulation, particularly the field release or commercialisation of GM crops, on a case-by-case basis looking at the potential risks to human health and safety and to the environment and making science based recommendations to the various Statutory Authorities such as State Departments of Agriculture, Environmental Protection Agencies and the National Registration Authority and the Food Authority. Both INGARD cotton and RR cotton were released under this system and had a relatively straightforward passage.

Growing public concern with the rate of biotechnology innovation and the perceived lack or laxity of regulation (a point that could be hotly refuted by any of us who had to tackle the regulatory system at the time) began to be heard by Federal legislators and the long heralded Office of the Gene Technology Regulator came into existence with the passing of the *Gene Technology Act 2000* (http://law.agps.gov.au/cgi-bin/download.pl?/scale/data/pasteact/3/3428). Impetus for the *Act* was provided by a number of national and international incidents involving gene technology, including the StarLink corn contamination problems in the US and failures in compliance of Australian canola field trial guidelines. The new *Act* replaced the previous partly voluntary system of regulation with a strict legal framework with impressive statutory powers of entry, seizure, monitoring and penalties to ensure compliance with the *Act* by all parties carrying out biotechnology research, development and commercialisation. These powers were also applicable to the activities of opponents of gene technology and are thought to be sufficient to deter vandalism of gene technology facilities and trials. Australia now has undeniably the most strict and open system of gene technology regulation of any country in the world, disclosing all details of activities including the exact GPS co-ordinates of any field releases of GM crops.

Such a proscriptive system has not come without cost, and it is clear that the act of legislating what is a complex and dynamic area of science bears with it a large measure of inflexibility and administrative and financial burden that will no doubt inhibit rather than promote new developments in biotechnology, particularly by smaller research groups. The legislation does not really distinguish between different scales of activity and a small field trial of just a few plants has to be treated in the same way as a full commercial release of a biotech product. Compliance monitoring and post-harvest restrictions on field trials for example will generate additional costs and in some cases may require compensation to be paid to growers for loss of income from fields removed from production during monitoring. Legislated periods for public comment on field trial applications and license conditions have easily doubled or tripled the time taken for the processing of field trials. Trials also cannot be easily moved from one location to another and this can create difficulties for researchers when applications must be submitted almost a year ahead of time before most growers have decided what and where they will plant crops. So, while we all recognise and support the need for sound regulation of gene technology, it should be tempered by commonsense and take into account the real risks associated with any particular biotech activity. At this stage it is not clear whether such commonsense can or will prevail in the light of a growing bureaucracy surrounding gene technology regulation, and while the public is concerned about gene technology it is uncertain that they will be proactive in using the facilities for public comment enacted into the *Gene Technology Act,* rather leaving it up to the same small groups of activists who often have other agendas.

New Biotech Products – What's just round the corner?

Our growing experience with the release of transgenic cotton varieties tells us that it takes about five years to produce and release new cultivars of transgenic cotton once a definitive product or event has been identified (INGARD for example was a single event). The selection of so-called "Elite Events" which are moved into a commercialisation stream are at the end of a research and development chain that might have taken up to ten years or more to first establish a proof-of-concept that the transgenic trait actually works, and then to produce a number of independent transgenic lines or events and screen through them to eliminate those that may have poor agronomic performance simply as a result of going through the genetic modification process.

In thinking about what is just around the corner for cotton then, we need look no further than at what is currently being tested in the field by various biotechnology companies or researchers. No country is as explicit in revealing details of field trials as Australia now is, but an examination of the US field trial databases, for example, would suggest that the next five to ten years will mainly bring more of the same - various modifications and improvement on technologies with which we are already familiar in cotton or other broadacre crops. Also there will be a broadening of the traits we already have into many other crops, trees and ornamentals. Several organisations are developing herbicide and insect tolerance traits for cotton and other crops but their availability to growers will ultimately depend on their access to elite cotton germplasm and their ability to compete with the products already developed and deployed by Monsanto through its various seed partners. To be competitive they would need to offer to growers the same or better performance to the Bollgard II/RR combination and, by the time they would be ready for commercial release, to the improved Bollgard II/ improved RR. It is difficult to see any of the companies doing this alone, given the enormous head start that Monsanto has in cotton biotechnology, but it might be possible if some of them get together and combine their respective technologies.

Probably a bit of a dark horse in this whole area are the Chinese, who have recently been putting an enormous effort into biotechnology and molecular biology in all of their major crops, including cotton. They have not so far been constrained by the International Patent system that restricts research in the West, and have been repeating and extending almost every cotton biotech application, including clones of many of the Monsanto insect control technologies. It is difficult to get precise information, but many transgenic lines are being evaluated in the field (their system of regulation is minimalist) and unsubstantiated reports of the development of successful products with insect, herbicide and disease tolerance, for example, are common. One interesting application has been the introduction and expression of the rabbit fur keratin gene into cotton fibres. According to press coverage 400 plants have been grown in the field at Taican in the Jiangsu province and have produced cotton that is whiter, more lustrous and longer than the corresponding conventional varieties, plans are in place for significantly larger areas next season.

Fibre quality manipulation is also an area of activity of a number of government and private cotton researchers in Western laboratories. There have been several attempts already at improving cotton fibres using transgenics, as yet without a great deal of success. This has included manipulating levels of plant hormones important for cell growth and elongation (John, 1999), putting in extra copies of genes for enzymes that cross-link the chains of cellulose in the cotton fibres or enhancing the expression of other genes normally expressed in fibres like expansins, or microfibril cross linking proteins (Mishra et al., 2001). While many of these have given measurable effects in the glasshouse, none have been translated into commercial biotech products at this stage, probably because the effects are small when assessed under field situations.

As we gain more knowledge about the genes and metabolic pathways involved in cotton fibre development we can hope to apply more targeted approaches to manipulating fibres. CSIRO and US researchers are manipulating key genes for enzymes involved in mobilizing sugars in cotton that are the necessary precursors for the production of cellulose. Simple sugars are thought to provide the turgor pressure necessary to cause the fibre cells to swell up and elongate so a number of strategies for increasing the proportion of sugars that go to the fibre cells are being explored. Haigler et al, (2001) report that over-expressing sucrose phosphate synthase, for example, results in a greater deposition of cellulose inside the developing fibre and an increase in seed cotton yield under field conditions. CSIRO researchers have concentrated on the enzyme sucrose synthase that controls the rate of sugar import into fibres (Ruan et al., 2000) and using transgenic plants have shown that this enzyme is absolutely essential for fibre development. Experiments are now underway to modify the expression of this gene in both the developing embryo and fibre to see if the balance can be shifted in the sugar flow to favour fibre elongation.

As indicated for the "rabbit hair cotton" gene technology allows for some completely novel approaches to cotton fibre improvement. However, attempts to produce naturally blue and black cottons using genes for pigment production from bacteria, while showing some promise initially, have proven to be extremely difficult. The very specialised nature of the cotton fibre and its metabolism dedicated to cellulose production has hindered success in this area and insufficient pigment has been produced to warrant further research and development (Calgene Inc, unpublished data). In a similar vein US researchers have used bacterial genes to produce small quantities of a biodegradable plastics in fibres (John, 1999) resulting in slightly enhanced thermal properties but it is unclear whether this initial research will be continued.

A new transgenic trait is being developed in Australia by CSIRO in the form of cotton with genetically modified oil (Liu et al., 2002). Cottonseed oil is essentially a by-product of lint production, but still represents the major oilseed crop in Australia. Its end uses are determined by the types of fatty acids present in the cottonseed oil, particularly its high saturated fat content and low level of the unstable linolenic acid, making it useful for frying and margarine production. Cotton oil is however partially chemically hydrogenated to improve it stability and physical properties, but this can generate nutritionally undesirable types of fatty acids so genetic modification can help by modifying the relative proportions of the different fatty acids in cottonseed oil to remove the need for hydrogenation. By switching down the activities of some of the enzymes involved in the desaturation of cottonseed oil specifically in developing seeds CSIRO researchers have produced both a high oleic acid (HO) and high stearic acid (HS) type of GM-cotton the oils from which will have specific food uses. The first field-testing of this material is likely to start in 2003 to generate sufficient material for proper food use evaluations. A similar, although less dramatically altered HO cotton, has been developed by US researchers (Chapman et al., 2001).

What will the Future Bring?

Plant biology is currently going through a new boom with rapid advances being made in the molecular characterisation of plant genomes as part of internationally co-ordinated projects. This wealth of information, and the molecular and genetic tools that go with it, have seen enormous leaps and bounds in our abilities to identify and isolate genes involved in key developmental processes in plants particularly those controlling plant growth, architecture, flowering, responses to stresses and pests and diseases, as well as primary and secondary metabolic processes. These genomic tools are so far available only for two model plants Arabidopsis and rice – the first a model for dicot plants (like cotton, canola, and soybean etc.) and the second as a model for cereals, but are beginning to be applied to other plants. It is not unreasonable to expect that most of the genes, except for those involved in specialized process unique to a particular crop (like the genes for cotton fibre development), will be common between all plants, so genes identified in say Arabidopsis, will have their direct counterparts in cotton. So using Arabidopsis we should be able to more easily work out what many of the 40,000 or more genes in Arabidopsis do and start using that information to manipulate the corresponding genes in cotton.

We are starting to develop some of the genomics tools specifically for cotton and this will allow use more encompassing whole genome approaches to systematically investigate what genes are really important at the different stages of fibre production, for example, from when they begin to form on the seed, during their rapid elongation and during the secondary cell wall filling or maturation stages that all determine fibre yield and fibre quality. So-called cotton DNA chips are being manufactured containing many thousands of cotton genes which can be used to follow changes in gene expression at different times in development, in different cotton mutants with altered fibres or even in different breeding lines with different fibre quality characteristics as well as a variety of other applications in cotton science (see paper by Wu et al., in these proceedings). These approaches should identify new target genes for manipulation in transgenic cotton as well as genes that may provide useful molecular markers to enhance the breeding of high quality, disease tolerant cotton varieties using conventional breeding.

Other Molecular Technologies – Contributing to Breeding and Biotechnology

Molecular or DNA markers are being used widely for fundamental studies in plant biology and genetics, but also increasingly in commercial breeding programs. They provide a powerful tool for crop improvement because they provide the ability to quickly track the presence of either single genes or quantitative characters through various crosses at a very early stage, independent of the expression of the trait or the growth stage of the plant. While such DNA marker techniques have become routine in many species of agricultural importance, they can be difficult to apply to cotton because of its high chemical content, and can only be transferred to cotton with some difficulty. CSIRO, however, is already pioneering the use of DNA technologies in its transgenic cotton breeding program to select homozygous transgenic lines on a large scale and to monitor the purity of transgenic breeding lines and has now established many of the techniques for high-throughput screening of individual plants using PCR techniques, techniques that would form the basis for any marker-assisted selection in either transgenic or conventional breeding.

CSIRO breeders would ideally like to have markers linked to the tolerance traits for many of the diseases of cotton, such as Verticillium and Fusarium wilts, Cotton Bunchy Top, and Alternaria. Breeding for these traits is currently difficult because of the problems of reliably screening for resistance and molecular markers would accelerate the breeding process and simplify the combining of multiple disease resistance traits into elite cultivars. However, the development of molecular markers is proving to be difficult in cotton because of the very narrow genetic bottlenecks that cultivated cotton has gone through during its development making all varieties (at least at a DNA level) appear very similar to each other. Considerably more effort therefore needs to be put into finding suitable markers linked to key agronomic and disease tolerance traits before these can be applied to breeding programs and there are international efforts underway to achieve this (eg. see http://algodon.tamu.edu/icgi/icgi.html). Some of the genomics tools being developed for cotton will certainly contribute to our abilities to find such markers.

Conclusions

Ten years ago we would have all reasonably thought that by 2000 biotechnology would have already changed our world for the better in many different ways. That promise still holds up, but we can see that the timeframes have been pushed further into the future as GM technologies have become the brunt of the political and social uncertainties characteristic of our Age. Large multinational companies have not been sensitive to consumer concerns and have pushed headlong into the deployment of what are justifiably safe and useful biotechnology products that deliver significant agricultural benefits. While as scientists we can understand their enthusiasm with this new and exciting technology the widespread lack of understanding by the general public, both lay and professional, has allowed their fears to grow out of all proportion to the risks that the technology poses (sometimes encouraged by those with different agendas than concerns about GM). The multinational's failure to understand or at least to compensate for public fears have back lashed against biotechnology and put new regulatory and public acceptance barriers in front of the release new biotechnology products that may take years to dispel. Those attitudes are slowly changing amongst the Life Science giants like Monsanto, but it may be too late now that the horse has bolted and regulation become much more restrictive.

The most significant advances in the deployment of biotechnology have clearly been in agriculture with the development and release of a variety of GM crops that improve the production side of farming, but such technologies have yet to be passed through to benefits to consumers (other than benefits to the environment). The environmental benefits of INGARD have less impact on the populous in the cities than a product that they use directly on a day-to-day basis that would be cheaper or better for their health. Vanguard crops like cotton will, however, certainly contribute towards public confidence in the safety and benefits of GM crops, but it will be essential to move some of the quality enhancement traits commercialised before GM will attain the acceptance it truly deserves.

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