Bt Cotton is Spreading

The most efficient and cheapest way of controlling insects without insecticide use is the use of in-built genetic resistance in the plant. Unfortunately, strong genes are rarely available within the cotton genome, which could make the plant immune against one or a number of pests at the same time. In the absence of such a genetic resistance, chemical control was advocated as an interim solution to the problem until alternate methods to provide equivalent control become available. Researchers have been looking for such methods and techniques to exploit them on commercial scale. Since the Bt gene technology showed potential for genetic control to lepidopteran insects, researchers have relied on transformation technology for successful production of genetically altered plants.

Methods of Transformation

In cotton, for insertion of foreign genes into the plant, the agrobacterium-mediated transformation has been the dominant method at least during the 1980s. Unfortunately, the use of the agrobacterium-mediated transformation was limited due to genotypic specificity. Once a foreign gene has been inducted into a certain genotype, that genotype should be capable to form a plant-let from callus tissue. From the experience of working with genotypes in various countries, researchers concluded that some Coker types from the USA and some Siokra types from Australia were able to regenerate plants from the callus tissue more successfully than any other genotype. People started using Coker and Siokra types with the agrobacterium method for transforming cotton varieties. Thus, first the desired gene is transformed into a Coker or a Siokra type and then, through back crossing, the same gene is transferred into the desired commercial genotype. Fortunately, the desirable gene did not carry any undesirable linkages and 3-4 successive back crosses converted the desired commercial variety into a transformed genotype. Now, from the commercially available Bt cottons, it has been proved that at the end of the third back cross about 94% of the Bt gene effect is transferred in the recurrent parent.

Genotypic specificity of the agrobacterium-mediated transformation method tempted researchers to continue working on alternate methods for induction of foreign genes. Direct transformation of meristems with agrobacterium and transformation using particle acceleration are some of the other options, which were explored during the 80s. Direct transformation of the plant parts would eliminate the need for transferring genes via Coker or Siokra genotypes. Elimination of backcrossing means expediting the transformation process by at least three years. Among all options available for direct transformation of meristems, particle acceleration showed most promise for future success and attracted attention of private companies. According to Stewart (1991) only two laboratories in the USA were actively engaged in cotton transformation using the particle gun method in the early 90s: Hans Bohnert at the University of Arizona and John Finer at Ohio State University. Now, in every country having a program on genetic engineering of cotton, public sector research frequently uses the particle gun method for transformation of genes.

Benefits of Genetic Engineering

Utilization of the genetic material carried by chromosomes, genes and more specifically the DNA structure has existed even before researchers knew the secrets of heredity. However, since the fundamentals of inheritance of characters became known about a hundred years ago, breeding approaches applied some fundamental principles for transfer of characters from one genotype to the other. In order to apply and make use of the information, there has always been a need to understand the genetic control of a character. Genetic control of many characters is still not properly understood. Of characters, probably yield is of utmost important which is claimed to be a quantitative character and almost impossible to be transferred according to the wishes of the breeders. Utilization of information on genetic control of characters was also limited by location of characters in close vicinity with others (desirable and undesirable) on the same chromosome, an important disadvantage in conventional breeding in all crops and especially so in cotton.

Genetic engineering provides a tool for manipulation of a single gene or a group of genes coming from another genotype of the same species, a far relative or even a different living organism, including plants and animals. Since genetic engineering of cotton started, the following two areas have progressed most rapidly.

- The use of insecticides has increased to the extent that it resulted in high production costs and resurgence of secondary pests. Production of toxins from within the plant for resistance to insects, particularly bollworms, was the most promising area of research in biotechnology.
- Herbicides can be used before planting or after planting but in either case it is not sure what weeds will show up in the field and in what intensity. Herbicides cannot be applied after germination, when composition of the weed complex and level of weed infestation are known, as they will also kill cotton seedlings/plants. Thus, resistance to broad-spectrum herbicides was another area of high interest for researchers.

Insect Resistance

It is difficult to control bollworms, which enter into the bolls and are least exposed to the insecticides. Bt cotton is a solution to this problem as the toxin, protein in nature, is distributed throughout the plant. For the same reason, Bt corn has also proved to be very successful against European corn borer. NuCOTN 33^{B} and NuCOTN 35^{B} were the first Bt cotton varieties released for commercial production. They both belong to the Delta and Pine Land Company and were developed from varieties already in commercial production. More than 40 DPL Bt cotton varieties are expected to be available in 1998, but they all carry the same *B. thuringiensis* gene.

Herbicide Tolerance

Herbicide tolerance can be generated through over production of the enzyme system that is affected by the herbicide or it could also be a production of a specific enzyme within the plant that detoxifies the chemical and the cotton plant no longer remains sensitive to the herbicide. There could be other means of developing tolerance to herbicides in the cotton plant but currently only two herbicide groups are in the limelight and they are both available on a commercial scale in many varieties of cotton. Calgene in collaboration with the Stoneville Pedigree Seed Company has developed varieties resistant to bromoxynil that have been marketed under the trademark BXN cottons. Delta and Pine Land Company in collaboration with Monsanto has developed varieties resistant to glyphosate (Roundup Ready, RR).

Stoneville Pedigree Seed Company offered bromoxynil resistant varieties BXN 57 and BXN 58 for commercial cultivation during 1996/97. Both varieties together were grown on about 20,000 ha in the USA during 1996/97. BXN 58 was replaced with BXN 47 during 1997. It is estimated that BXN 47 and BXN 57 were grown on over one hundred thousand hectares during 1997/98.

Roundup Ready transgenic cotton was grown on 329,000 hectares in the USA during 1997 and it is expected that area may double during 1998. There were some problems with Roundup Ready cotton in the USA during 1997 as some fields showed excessive shedding and deformed bolls. About fifty farmers in Arkansas and Mississippi have filed claims against Monsanto and the Delta and Pine Land Company, responsible for distributing seed, for losses that they attribute to genetically modified cotton, including abnormal boll formation as well as plants that dropped bolls.

Neither Monsanto nor Delta and Pine Land Company have experienced such abnormalities during the course of experimentation. Currently, no scientific explanations for excessive shedding and malformation of bolls are available yet. The problem is being investigated by Monsanto and Delta and Pine Land Company, and early observations show that excessive shedding may be related to the timing and level of Roundup Ready application on the crop. The affected growers are only a small percentage of the total growers who planted Roundup Ready cotton, and there is still a strong faith in the technology.

According to the November 1997 issue of the Biotech Reporter, the US Environmental Protection Agency has granted a blanket exemption for Roundup Ready technology from tolerance requirements for all plants. Tolerance requirements are more important for food crops where herbicide residue has to be kept below certain limits to avoid any health risks. But, blanket exemption for the Roundup Ready technology will enhance the utilization of glyphosate over the top of other crops

Transgenic Cotton in the USA

In the USA, which is the first country to grow Bt cotton on a commercial scale in the world, Bt cotton had to have approval from the USDA, the US Environmental Protection Agency (EPA) and the US Food and Drug Administration (FDA). All regulatory formalities were completed by the end of 1995, and Bt cotton was first grown on a commercial scale in the USA during 1996/97. Practically herbicide tolerant cotton varieties trademarked as BXN also entered into commercial scale production the same year. Roundup Ready herbicide tolerant varieties were planted on a commercial scale in 1997.

Transgenic cotton having a gene from the soil bacteria *Bacillus thuringiensis* (Bt) is now well known to a majority of the cotton farmers in the USA. During 1997/98, Bt cotton resistant to lepidopteran insects was grown on about one million hectares in the USA. Last year, early season premature reports indicated inability of the Bt gene to produce sufficient toxin and provide desirable control against bollworms. But, end of the season reports confirmed effectiveness of the Bt gene and farmers concluded benefits in using transgenic cotton. Thus, area under Bt cotton increased by 60% in 1997 in the USA and unlike the previous year, there were no reports against Bt cotton with

Bollgard gene resistant to bollworms particularly the tobacco budworm, *Heliothis virescens*.

A number of Bt genes are available which can be used to confer resistance to a variety of pests. The fundamental phenomenon is based on the abilities of a particular gene to produce a specific chemical injurious for pests attacking the cotton plant. It is preferred that a desirable chemical is produced in a specific part of the plant but no such genes are available yet. It would be even more desirable that a single gene be capable to produce such a chemical(s) with a strong effect against more than one insect. From the currently available genes, none is capable to control all species of bollworms. However, the degree of effect on many species varies.

Herbicide tolerance and resistance to insects are not the only areas of research under exploration. There are many other avenues being researched mostly by the private sector. A detailed article " More Genetically Engineered Cottons" was published in the December 1996 issue of *THE ICAC RECORDER* on thermal properties of cotton. Herbicide tolerant and Bt cotton will continue to be used on a large scale but it is expected that the following new genes will become available for commercial production in the near future.

Year	**Transgenic Cotton**
1998	Herbicide tolerant BXN gene and Bt gene resistant to lepidopteran insects will become available in the same variety.
1999	Second generation of Bt cotton (with a different Bt gene) will become available.
2002	Boll weevil protected cotton will be grown.
2003	Naturally colored cotton may be grown. Blue color may be the first to become available after brown and green already available in the normal varieties.
2004	Thermal properties, improved fiber quality and leaf curl virus resistance and many new transgenics are expected.

Programs in Other Countries

Many countries have developed programs in genetic engineering. Outside the USA, most programs are in the public sector organizations. The program involves five important stages as follows:

- Identification of suitable gene or genes which are capable of producing desired action/chemical upon insertion into the cotton plant.
- Availability of desired lab facilities and technology for transformation of cotton.
- Testing of transformed genotypes.
- Government approval for commercialization.
- A system to produce and distribute seed under strict control.

Herbicides are not used commonly in many countries and consequently Bt cottons resistant to insects have a larger market. It will take many years for countries other than Australia and the USA to develop their own transgenic varieties. These countries have two choices: Wait for a few years and develop and grow their own transgenic varieties on a commercial scale, or pay the technology fee and immediately start growing the presently available varieties. Some countries, due to their specific growing conditions, cannot grow varieties from other countries and will have to develop their own programs.

Plantings in other countries are as follows:

Australia	1996/97	30,000 ha
	1997/98	60,000 ha (estimated)
China (Mainland)	1996/97	137 ha
	1997/98	4,000 ha
	1998/99	30,000 ha
Mexico	1996/97	13,000 ha
	1997/98	20,000 ha
South Africa	1996/97	Trials
	1997/98	400 ha
USA	1996/97	600,000 ha
	1997/98	980,000 ha
Zimbabwe	1996/97	Trials

DPL has now 11 programs in 16 countries. The countries where they have tried their varieties include Argentina, Bolivia, China (Mainland), Colombia, Greece, Mexico, Paraguay, South Af-

Bt Genes and their Effects on Various Insects

Insects	**Genes**							
	CrylAb	**CrylAc**	**CrylB**	**CrylC**	**CrylD**	**CrylF**	**CryIIA**	**CryIII**
H. zea	XXX	X	?	No		No	XX	
H. virescens	XX	XXX				XX	XX	
S. littoralis	No	No		XXX	X	X		
P. gossypiella	XXX	XX	?	No			X?	
A. argillacea	?	XXX						
A. gossypii								
A. grandis								
Eutinobothrus spp.								No
Conotrachelus spp.								?

rica and Zimbabwe. India, where DPL and Monsanto are working separately, may also have some experiments in the field on Bt cotton during 1998. Contacts are also being developed in Pakistan to explore the market for Bt cotton. DPL is working with Monsanto, and they are willing to share their transformed genotypes/varieties with other countries. In order to use the technology, other countries have to pay a fee for the benefit they will gain in savings on insecticides.

The fee for BXN varieties is based on the seed by weight which is calculated at about US$15/ha for all varieties with bromoxynil resistant gene. The fee for the Roundup Ready resistant varieties is about US$12/ha.

The technology fee for Bt cotton with a Bollgard gene was about US$80/ha in the USA and US$245/ha in Australia. In the USA, the technology fee for the Bt + Roundup Ready seed was US$100/ha.

The difference in the technology fee in the USA and Australia for the Bollgard gene is related to the benefit in the form of savings in production costs. It is estimated that USA growers will save at least US$80/ha worth of insecticides by planting Bt cotton resistant to lepidopteran insects. Because using conventional varieties the Australian growers have to spend more on spraying (10 sprayings per season), savings are larger in the case of planting Bt cotton and thus the technology fee is higher.

Monsanto signed contracts with all Bt cotton growers in the USA and Australia. The Australian contracts included compensation to Bt cotton growers if the cost of controlling insects exceeded conventional practices, a provision which was not included in US contracts.

How the private seed companies will be able to keep seed control in countries where landholdings do not exceed a few hectares per family is still not very clear. Growers in many countries keep their own seed. There could be a one-time fee and varieties and genes could be changed frequently. Genes have to be changed because of development of resistance to the toxin. Plant variety protection laws have to be strengthened in countries interested in growing transgenic cottons to safeguard the investment of private companies.

Additional Effects of Bt Cotton

The primary thrust of genetic engineering has been to reduce the use of pesticides, thus lowering the cost of production and minimizing environmental pollution. Colored cotton and improved fiber qualities, including changed thermal properties of the cotton fiber, are some other areas of research where extensive work has already been done and new genotypes are expected soon. Still the importance of Bt cotton resistant to pests will remain.

When Bt cotton was introduced, the only objective was to eliminate or at least minimize sprays against lepidopteran insects. Bt cotton has proved to be more effective against tobacco budworm *Heliothis virescens* than other bollworms. In the last two years, from the trials conducted in various countries, a number of other observations on the positive effects of Bt gene in cotton have been noted. These are

- Higher yield over normal varieties. In the USA, on the average many farmers noted approximately a 7% increase in yield. The basis for higher yield is better protection against insects.
- Usually one-day-old larvae are killed while feeding on cotton having toxin produced by the Bt gene. It has been observed that pollen grains are low in toxin, which sometimes allow the larvae to continue feeding on the flower buds.
- Lower use of insecticides has helped to control beet armyworm, *Spodoptera exigua*.
- The incidence of verticillium wilt is reduced.
- Alabama cotton leafworm *Alabama argillacea* has proved to be highly susceptible to the toxin.
- At lower levels of pest population, it may not be economical to grow Bt varieties but their use has reduced the pest population further.
- Insects will develop resistance to Bt toxin.
- It has been observed that micronaire is reduced by about 0.2 in Bt varieties, which may be due to higher boll set.

References

Ag Biotech Reporter, *AgBiotechnology Research/Business News*, Vol. 14, No. 11, November 1997, Freiberg Publishing Company, 2302 W. Ist Street, Cedar Falls, IA 50613, USA.

Barton, G. 1997. Bollgard™ cotton update, Monsanto Corporation, St. Luis, MO, USA. Available online: http://www.monsanto.com/MonPub/InTheNews/articles/97-03-20BollgardUpdate.html.

Belot, J. L. 1997. Advances in the biotechnology program on cotton of the CIRAD-CA. *Proceedings of the 6th Meeting of the Latin American Association for Cotton Research and Development*, Instituto Nacional de Tecnología Agropecuaria - INTA, Casilla de Correo 164, Sáenz Peña, Argentina.

Carlson, G., Marra, M. and Hubbell B. 1997. Transgenic technology for crop protection – The new "Super Seeds." *CHOICES*, The American Agricultural Economics Association, 1110 Buckeye Avenue, Ames, IA 500100-8063, USA.

Hake, K. 1997. A global review of the field performance of Bt cotton. Presentation made at the 6th Meeting of the Latin American Association for Cotton Research and Development held in Argentina, December 1-5, 1997.

Stewart, J. McD. 1991. *Biotechnology of Cotton*, ICAC Review Article on Production Research No. 3, CAB International, Wallingford, Oxon OX10 8DE, UK.