Transgenic Cotton: An Overview

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ABSTRACT

The first transgenic cotton having insect or herbicide resistance was released to the field in 1996 in the United States. Since then the rapid increase in transgenic cotton acreage within 10 years attests to the overall success of agricultural biotechnology. This review article provides an overview of genetically modified cotton and its application in agricultural production. We first critically review cotton tissue culture as the basic work of biotechnology. Then, three main transformation methods, namely, Agrobacterium-mediated, particle bombardment and pollen tube-pathway are described in this paper. The performance of transgenic cotton plants engineered for insect, disease, herbicide resistance and fibre improvement is reviewed from a perspective of the benefits and limitations. Finally, recent progress in plastid engineering research of cotton is briefly mentioned. Cotton genetic engineering shows great potential to enhance breeding programs by introducing novel traits that have eluded more traditional plant improvement methods and therefore will likely play an increasingly important role in the genetic improvement of cotton in the future.

Keywords: Gossypium, plastid engineering, tissue culture, transformation

INTRODUCTION

Cotton is worldwide one of the most important commercial crops and consequently plays a vital role economically, politically, and socially. Chiefly a fiber crop, it has been estimated to contribute US $15-20 billion to the world’s agricultural economy, with over 180 million people depending on it for their livelihood (Benedict and Altman 2001).

There are 51 diverse species in the genus Gossypium. Four are cultivated, G. hirsutum L. and G. barbadense L., which are tetraploid (2n = 4x = 52), and G. arboreum L. and G. herbaceum L., which are diploid (2n = 2x = 26). The species most widely grown around the world is G. hirsutum. More than 95% of commercial cotton is upland cotton (G.
fibre quality for the benefit of society and the environment.

The traditional breeding methods use sexual hybridization to introduce desirable agronomic traits, such as high yield, good quality and disease resistance, into new breeding lines which may be released after several years of field testing. Significant progress has been made through different breeding programs. The yield increase contributed by genetic improvement was 7-10 kg/ha/year for the USA (Wilkins 2000), 23 kg/ha/year for Australia (Constable et al. 2001), and 8-10 kg/ha/year for China (Kong et al. 2000). Conventional breeding contributed a lot to cotton improvement, but progressed slowly recently because of a shortage of germplasm and creative breeding tools.

Molecular breeding provides a new way which allows genes to be selectively and effectively reshuffled into the most desirable combinations. This may include the introduction of foreign genes into the plant genome that confer novel traits that enhance food and fiber quality directly, or through providing protection against biotic and abiotic stresses in the environment (Wilkins 2000). Since initial commercialization in 1996, global planted area of biotech crops has soared by more than fifty-fold from 1.7 million hectares in six countries to 90 million hectares in 21 countries in 2005 (James et al. 2005).

The first generation of transgenic cotton genetically engineered to provide insect or herbicide resistance was from a wild species of cotton by Price and Smith (1979); however, somatic embryos could not develop into plantlets. The first successful regeneration of whole cotton plant via somatic embryogenesis was obtained by Davidonis and Hamilton (1983). However, the method had limitation due to long incubation period of callus for induction of proembryoids and low efficiency of embryo formation. In a different study, Shoemaker et al. (1986) evaluated seventeen G. hirsutum L. cultivars for induction of somatic embryogenesis and plant regeneration. Approximately 40% of the somatic embryos underwent normal germination and the procedure was simple and rapid. To the same year, somatic embryogenesis from callus cultures of mature leaf and petiole explants from six cotton varieties has been reported by Gawel et al. (1986). Trolinder and Goodin (1987, 1988a, 1988b) found that induction of somatic embryogenesis in cotton is genotype dependent. Finer (1988) also reported plant regeneration from somatic embryogenic suspension cultures established from cotyledons of cultivar Coker 310. Some Coker varieties have been reported to have the highest regeneration potential compared to other varieties. However, plant regeneration in China cultivar of Upland cotton YZ-1 through somatic embryogenesis was first reported by Jin et al. (2006a), which showed predominant ability of somatic embryogenesis over Coker lines with a high ratio of somatic embryogenesis within two months and produced higher number of somatic embryos from one gram of embrogenic calli.

Many factors can influence the efficiency of a regeneration procedure. The main factors determining the tissue culture response in cotton include genotypes, donor plant

**Table 1 Studies on somatic embryogenesis and plant regeneration in cotton.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Explant used</th>
<th>Mode of regeneration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. hirsutum L.</td>
<td>P</td>
<td>C-SE</td>
<td>Gavel and Robacker 1990</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>H</td>
<td>C-SE</td>
<td>Zhang et al. 1991</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>H</td>
<td>C-SE</td>
<td>Voo et al. 1991</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>COT, H</td>
<td>C-PE-PT</td>
<td>Finer and Smith 1984</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>H</td>
<td>C-PE-PT</td>
<td>Friesenbach de Boer 1993</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>COT, H</td>
<td>C-SE-PT</td>
<td>Zhang et al. 2000b</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>H</td>
<td>C-SC-SE-PT</td>
<td>Sakhanokho et al. 2001</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>H</td>
<td>C-SE-PT</td>
<td>Mishra et al. 2003</td>
</tr>
<tr>
<td>G. klotzschianum A</td>
<td>H</td>
<td>C-SE</td>
<td>Sun et al. 2003</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>C-SC-SE-PT</td>
<td>Ganesan and Jayabalan 2004</td>
<td></td>
</tr>
<tr>
<td>G. davidsonii, G. raimondii, G. stocksi, G. aridum, G. klotzschianum</td>
<td>H</td>
<td>C-SC-SE-PT</td>
<td>Sun et al. 2006</td>
</tr>
</tbody>
</table>

C: callus; COT: cotyledon; H: hypocotyl; IE: immature embryo; LD: leaf disc; P: petiole; PE: pro-embryo; PT: plantlet; SA: shoot apex; SC: suspension culture; SE: somatic embryo; ST: stem

**COTTON CELL AND TISSUE CULTURE**

Somatic embryogenesis and plant regeneration

A rapid, simple and efficient plant regeneration protocol is a prerequisite for genetic manipulation in vitro. Somatic embryogenesis via a callus phase (indirect method) has been reported in several cotton species. The first report on induction of somatic embryogenesis was from a wild species of cotton by Price and Smith (1979); however, somatic embryos could not develop into plantlets. The first successful regeneration of whole cotton plant via somatic embryogenesis was obtained by Davidonis and Hamilton (1983). However, the method had limitation due to long incubation period of callus for induction of proembryoids and low efficiency of embryo formation. In a different study, Shoemaker et al. (1986) evaluated seventeen G. hirsutum L. cultivars for induction of somatic embryogenesis and plant regeneration. Approximately 40% of the somatic embryos underwent normal germination and the procedure was simple and rapid. To the same year, somatic embryogenesis from callus cultures of mature leaf and petiole explants from six cotton varieties has been reported by Gawel et al. (1986). Trolinder and Goodin (1987, 1988a, 1988b) found that induction of somatic embryogenesis in cotton is genotype dependent. Finer (1988) also reported plant regeneration from somatic embryogenic suspension cultures established from cotyledons of cultivar Coker 310. Some Coker varieties have been reported to have the highest regeneration potential compared to other varieties. However, plant regeneration in China cultivar of Upland cotton YZ-1 through somatic embryogenesis was first reported by Jin et al. (2006a), which showed predominant ability of somatic embryogenesis over Coker lines with a high ratio of somatic embryogenesis within two months and produced higher number of somatic embryos from one gram of embrogenic calli.

Many factors can influence the efficiency of a regeneration procedure. The main factors determining the tissue culture response in cotton include genotypes, donor plant...
and culture systems (Trolinder and Chen 1989; Zhang et al. 1991; Zhang et al. 1997). An in-depth study of such factors would enable the development of genotype-specific culture methods to better enhance the tissue culture response of the recalcitrant crops. Wu et al. (2004) developed a new protocol for the highly efficient somatic embryogenesis and plant regeneration of ten recalcitrant Chinese cotton cultivars. The protocol was initially developed using Gossypium hirsutum L. cv. ‘Coker 201’, then applied to recalcitrant cultivars from China. It was achieved by regulating IBA (indole-3-butyric acid)/KT (kinetin) regimes, KNO3 levels and L-asparagine (Asn)/L-glutamine (Gln) at different stages of the culture process. Studies on somatic embryogenesis in cotton so far are listed as Table 1.

### Shoot and meristem culture of cotton and its application

Though studies on somatic embryogenesis have been successfully implemented for many years, the method has been reported to generate undesirable somaclonal variations in cotton (Stelly et al. 1989; Firoozabad and de Boer 1993). An extensive seed-to-seed variability in cotton (Stelly et al. 1989) has been observed among Coker lines (Trolinder and Chen 1989). Gosal and Robacker (1990). Maintenance of callus and cell cultures for longer periods often results in plants that are morphologically abnormal and functionally sterile. Such variations pose a serious problem for maintenance of genetic uniformity in plants regenerated in vitro.

Several reports on plant regeneration via pre-existing meristems in cotton have been published (Table 2). Bajaj and Gill (1986) first obtained plant regeneration by using shoot tips from field-grown plants of G. hirsutum, and followed by Agrawal et al. (1997), Gupta et al. (1997), Zhang et al. (1996), Hemphill et al. (1998) and Hazra et al. (2000). Among the explants for meristem culture, the embryo axis explant has many advantages as that the shoot regeneration process was relatively simple and not prone to somaclonal variations and chromosomal abnormalities (Saeed et al. 1997). The process of in vitro plant propagation from pre-existing meristems mainly consists of three steps: i) induction of shoot buds and their multiplication, ii) elongation of shoot buds into shoots and iii) in vitro or ex vitro rooting of shoots to form plantlets.

The size of the explant shows a tremendous difference in the frequency of plants recovered (Gould et al. 1991). Although the number of plants regenerated from shoot apex explants is higher, rooting is still problematic and variable, suggesting that rooting is highly dependent on the genotypes (Gould et al. 1991; Hemphill et al. 1998). One potential way to surmount the rooting problem in culture is the efficient grafting of shoots to seedling rootstocks (Luo and Gould 1999; Jin et al. 2006b). Most recently, the induction of multiple shoots (3.4 to 8.3 shoots/axis) from dormant axillary buds by cytokinin (Agrawal et al. 1997; Gupta et al. 1997; Saeed et al. 1997; Hemphill et al. 1998; Morre et al. 1998) offers a promising means for increasing regeneration efficiency, especially during selection in transformation experiments.

### THE CHIEF APPROACHES TO GENETIC TRANSFORMATION OF COTTON

**Agrobacterium-mediated transformation**

Agrobacterium-mediated transformation is the most widely used method to transfer genes into cotton. Umbeck et al. (1987) for the first time described the transformation of cotton ‘Coker’ varieties, an easily regenerable genotype, by using hypocotyl sections as explants inoculated with Agrobacterium. The plasmid CMC 1204 used for transformation contained a gene for neomycin phosphotransferase (NPTII) and chloramphenicol acetyltransferase (CAT). Three transgenic plants were identified that expressed NPTII and CAT. Agrawal et al. (1990) used Agrobacterium strain LBA4404 to transform cotyledon pieces from ‘Coker 201’ in the same year, and obtained 15 transmittant plants. The whole process from infection to transfer of transgenic plants to soil took 6-8 months. Perlak et al. (1990) developed transgenic cotton of Bacillus thuringiensis cryIA (b) and cryIA (c). Under high insect pressure with Heliotis zea (cotton bollworm), these transgenic Bt plants showed effective boll protection. Bayley et al. (1992) introduced 2, 4-dichlorophenoxyacetic acid (2, 4-D) resistance into cotton cultivar ‘Coker 312’ by transferring the 2,4-D monooxygenase gene, tdfA, via Agrobacterium-mediated transformation. The transgenic plants were tolerant to three times the field level of 2,4-D used for wheat, corn, sorghum and pasture crops. Cotton is very sensitive to 2,4-D, even spraying it with a small amount can cause serious damage. 2,4-D resistance prevents cotton from 2,4-D damage when 2,4-D is applied to other adjacent crops.

In another case, herbicide-resistant transgenic cotton plants carrying mutant forms of a native acetohydroxyacid synthase (AHAS) were developed by Rajasekaran et al. (1996). Meanwhile expression of protease inhibitor gene in cotton cultivar ‘Coker 312’ was also reported (Thomas et al. 1995).

More recently, our laboratory developed a reliable and high-efficiency system of transforming embryogenic callus (EC) of an upland cotton cultivar YZ-1 via Agrobacterium tumefaciens in cotton (Jin et al. 2005a). The effects of Agrobacterium strains, acetylsyngone (AS), co-culture temperature, co-culture duration, Agrobacterium concentration and physiological status of EC on stable transformation were evaluated. An overall scheme for producing transgenic cotton was presented, through which an average transformation rate of 15% was obtained.

Although cotton has been transformed via Agrobacterium-mediated methods, Agrobacterium-mediated transformation has been associated with a few potential problems. One such problem is that Agrobacterium-mediated transformation of cotton has been limited to those specific cultivars that can be regenerated in tissue culture. To overcome this difficulty, Zapata et al. (1999) used the shoot apex as an explant for Agrobacterium-mediated transformation in cotton. Out of a total of 1010 Agrobacterium-treated shoot apices, eight plants grew on kanamycin selection at 100 mg/L and were transferred to soil. Progeny obtained by selfing were germinated in the greenhouse. Evidence for integration of the GUS gene was observed in two successive generations from the regenerants.

Cotton transformation based on the shoot apex method has the advantage of being more genotype independent than somatic embryogenesis, and potentially allows for a speed-

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**Table 2 Studies on plant regeneration in cotton via pre-existing meristems.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Explant used</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. arboreum L.</td>
<td>M, ST</td>
<td>Adventitious buds, Bajaj and Gill 1986</td>
<td></td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>multiple shoots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. barbadense L.</td>
<td>SA</td>
<td>Single shoot</td>
<td>Zhang et al. 1996</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>CN, SA</td>
<td>Multiple shoots</td>
<td>Agrawal et al. 1997</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>SA, 2C</td>
<td>Multiple shoots</td>
<td>Gupta et al. 1997</td>
</tr>
<tr>
<td>G. arborescens L.</td>
<td>SA, 1C, 2C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>PM</td>
<td>Multiple shoots</td>
<td>Hemphill et al. 1998</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>CA</td>
<td>Multiple shoots</td>
<td>Morre et al. 1998</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>SA</td>
<td>Single shoot</td>
<td>Zapata et al. 1999</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>CN, SCN, ST</td>
<td>Multiple shoots</td>
<td>Hazra et al. 2000</td>
</tr>
<tr>
<td>G. arboreum L.</td>
<td>PB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>EA</td>
<td>Single shoot</td>
<td>Hazra et al. 2002</td>
</tr>
<tr>
<td>G. hirsutum L.</td>
<td>EA</td>
<td>Multiple shoots</td>
<td>Banerjee et al. 2003</td>
</tr>
</tbody>
</table>

1C: one cotyledon; 2C: two cotyledon; CA: caulinar apex; CN: cotyledonal node; EA: embryo axis; M: meristem; PB: petiole base; PM: preexisting meristem; SCN: split cotyledonal node; SA: shoot apex; ST: shoot tip.
ier recovery of transgenic lines. However, apart from the rooting problems, the advantages are easily offset by the low frequency of stable germline transformation events (reviewed by John 1997).

The second issue of concern is that Agrobacterium continues to persist on tissue following transformation, resulting in what is called a systemic infection (Matzeke et al. 1996). However, while this is most serious in clonally propagated species, it is not a major concern in see of using embryonic meristems that the preparation of shoot tip meristems is an extremely tedious, labor-intensive task, which involves the surgical removal of leaf primordia to expose the meristem, followed by the careful excision of meristem explants from imbibed seeds. Also, the stable transformation rate is very low (0.001 to 0.01%).

The first report on particle bombardment-mediated transformation in cotton was published by Finer and McMullen (1990). They bombarded embryogenic cell sus-

pensions of ‘Coker 310’ with hygromycin genes as a selecting marker. Hygromycin resistant transgenic plants were developed via somatic embryogenesis, five months after bombardment. Three years later, McCabe and Martinell (1993) described a protocol for variety-independent transformation in cotton. They bombarded meristems (embryo axes) using the electric discharge gun for gene transfer. Integration of the GUS gene was demonstrated in R0 and R1 transformants. Progeny analysis showed the transgene in a Mendelian fashion. After that, transformation of meristems via particle bombardment were reported by Chian et al. (1995) and Keller et al. (1997). Rajasekaran et al. (2000) achieved high frequency stable transformation of cotton by particle bombardment of embryogenic cell suspension cultures. They observed an increased stable transformation frequency of 4% compared to 0.7% in an earlier report by Finer and McMullen (1990). The high efficiency of stable expression was due to the multiple bombardment of rapidly dividing cell suspension.

Reports on cotton transformation via A. tumefaciensis and particle bombardment mediated techniques were listed in Table 3.

Transformation via pollen-tube pathway

Introduction of exogenous DNA into a plant embryo through the pollen tube pathway after pollination was first reported by Zhou et al. (1983) in cotton. Hu and Wang (1999) reviewed the procedures used with this approach, the confirmation of the results, and the field performance of the transformed plants. The theory of this technique can be briefly described as follows: after pollination, the micellar cells form a pathway to allow the pollen tube passage to the embryo sac, by removing the stigma and applying a DNA solution on the severed style after pollination, the exogenous DNA could presumably reach the ovary by flowing down the pollen tube and integrating into the just fertilized but undivided zygotic cells. The transformed seeds could be obtained directly without protoplast preparation, cell culture, and plant regeneration. By introducing 3H-labeled DNA from bluish dogbane (Apocynum venetum) into cotton, Gong et al. (1988) indicated that the route of the exogenous DNA into the embryo sac was through the pollen tube and the exogenous DNA was randomly taken up by the eggs, zygotes, synergid and polar nucleus. However, those results did not provide clear evidence indicating that the DNA introduced was successfully integrated into the genome and expressed in the progeny of plants.

With the GFP gene as a reporter gene, the transgenic embryos and seeds of cotton (G. hirsutum) were obtained by the methods of pollen tube pathway with the plasmid pBIN35s-mgfp (Huang et al. 1998; Huang et al. 2001). Sothern blotting analysis proved the foreign gene had inserted into the cotton genome. Green fluorescence was detectable and screenable in cotton tissue by fluorescence microscopy and a hand-held ultraviolet lamp. These studies strongly confirmed the feasibility of pollen tube pathway method for cotton genetic transformation.

Genetic transformation through pollen tube pathway offers several advantages, namely: The technique can overcome the host-range limitations of Agrobacterium, and nearly all genotypes can be transformed. Furthermore, transformation protocols are simplified, since complex bacteria/plant relationships varying with each system are eliminated. This is why this method is very popular in China especially for cotton genetic transformation. Many useful genes have been delivered into cotton genome using pollen tube pathway, such as the Bt gene (Xie et al. 1991), Api gene (Huang et al. 2001), ipt and gus gene (Yu et al. 2000), Cpti-ibt gene (Guo et al. 1999), Chi and GNA gene (Liu et al. 2002). To date, more than half the successful genetic transformation of cotton in China were made by pollen tube pathway method. More and more results showed success of pollen-tube pathway transformation in cotton, but stable transformation, independent experiences and molecular evidence
with cotton. The most serious pests of cotton are bollworms: Heliothis zea and H. armigera Hubn. These bollworms are the caterpillars of several species of moths. The caterpillar feed in the boll damaging lint and seeds and cause a considerable reduction in yield and quality. Since the end of the 1980s, cotton production has decreased due to a decline in both yield and coverage area. The decline in yield of 15 to 30% has mainly been caused by bollworm (Helicoverpa armigera) infestation. In 1992 and 1993, outbreaks of cotton bollworm infestation in China caused direct economic losses of about $630 million. Furthermore, farmers were discouraged from growing cotton. As a result, the national growing area decreased by 10-15%, and there is a tendency for cotton production to move from relatively favourable areas towards marginal regions (Zhang et al. 2000a).

Control of insect pests in cotton cultivation depends mainly on the use of chemical insecticides that are under serious public debate for reasons of human safety and environmental pollution. Scientists have been looking for new strategies to control cotton insect pests. An attractive alternative is the production of proteins with insecticidal activity by the cotton plant itself. Numerous laboratory and field tests confirm that the most efficient and cheapest method for protecting cotton from pests is the utilization of transgenic cotton for insect resistance. The most widely favoured genes thought to be most useful for cotton are the Bt toxin genes which contains a crystalline protein toxin.

Genetically modified cotton

Transgenic cotton for insect resistance

About 100 species of insects are known to be associated with cotton. The most serious pests of cotton are bollworms: Heliothis zea Boddie and H. armigera Hubn. These bollworms are the caterpillars of several species of moths. The caterpillar feed in the boll damaging lint and seeds and cause a considerable reduction in yield and quality. Since the end of the 1980s, cotton production has decreased due to a decline in both yield and coverage area. The decline in yield of 15 to 30% has mainly been caused by bollworm (Helicoverpa armigera) infestation. In 1992 and 1993, outbreaks of cotton bollworm infestation in China caused direct economic losses of about $630 million. Furthermore, farmers were discouraged from growing cotton. As a result, the national growing area decreased by 10-15%, and there is a tendency for cotton production to move from relatively favourable areas towards marginal regions (Zhang et al. 2000a).

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**Genetic manipulation of Bt**

**Bacillus thuringiensis**, commonly known as Bt, is a bacterium that occurs naturally in the soil. It has been used for more than 50 years as a biological insecticide (Qaim and Zilberman 2003). A critical factor following transformation is the desired expression of the insecticidal gene. Bt genes are Adenine-Thymine (A-T) rich while plant genes tend to increase the GC content of their encoding genes (Perlak et al. 1991). The first results on transfer of Bt genes in tobacco and tomato were published in 1987 (Fischhoff et al. 1987). Since then Bt genes have been transferred to many crops including cotton, maize, rice and potato. Perlak et al. (1990) produced insect resistant cotton by introducing the Bt gene into the cotton genome. The Bt coding sequence was modified to increase the levels of both cry 1A(b) and cry 1A(c) insect control protein expression to 0.05-0.1% of the total soluble proteins. These truncated forms of the insect control protein genes Bt provided effective pest control. The plants with the modified cry 1A (b) gene had a 10-100-fold higher level of insect control protein compared with the wild type gene. Similar results were obtained with the cry 1A (c) gene (Perlak et al. 1991).

Chinese scientists began modification of Bt genes in 1991. Since then, tremendous progress has been made in this field. The effects of different degrees of gene modification were investigated in the cry1A genes. The results indicated that removal of the polyadenylation sites and ATTAA sequences, and changes to a total of 353 of the 615 codons, raised the levels still higher (up to 0.2-0.3% of total soluble protein) – 100-fold higher than the level for unmodified genes (Zhang et al. 2000a).

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**Table 3 An overview for approaches to cotton genetic transformation.**

<table>
<thead>
<tr>
<th>Transgenic trait</th>
<th>Introduced gene</th>
<th>Method</th>
<th>Explant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selectable markers or</td>
<td>NPTII and OCS</td>
<td>AT</td>
<td>COT</td>
<td>Firoozabady et al. 1987</td>
</tr>
<tr>
<td>reporter gene</td>
<td>NPTII and CAT</td>
<td>AT</td>
<td>H</td>
<td>Umbeck et al. 1987</td>
</tr>
<tr>
<td></td>
<td>HPT</td>
<td>PB</td>
<td>ECS</td>
<td>Finer and McMullen 1990</td>
</tr>
<tr>
<td></td>
<td>GUS</td>
<td>PB</td>
<td>ZEM</td>
<td>Chlan et al. 1995</td>
</tr>
<tr>
<td></td>
<td>NPTII</td>
<td>AT</td>
<td>ST</td>
<td>Zapata et al. 1999</td>
</tr>
<tr>
<td></td>
<td>NPTII and GUS</td>
<td>PB</td>
<td>ECS</td>
<td>Rajasekaran et al. 1996, 2000</td>
</tr>
<tr>
<td></td>
<td>GFP</td>
<td>AT</td>
<td>H</td>
<td>Sunilkumar and Rathore 2001</td>
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<tr>
<td></td>
<td>GUS</td>
<td>AT</td>
<td>H</td>
<td>Sathyavathi et al. 2002</td>
</tr>
<tr>
<td></td>
<td>GUS gene without promoter</td>
<td>AT</td>
<td>EC</td>
<td>Jin et al. 2005a, 2005b</td>
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<tr>
<td>Pathogen resistance</td>
<td>Bean chitinase gene</td>
<td>AT</td>
<td>H</td>
<td>Tohidfar et al. 2005</td>
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<tr>
<td></td>
<td>Antimicrobial peptide</td>
<td>AT</td>
<td>H</td>
<td>Rajasekaran et al. 2005</td>
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<td></td>
<td>Andochitinase</td>
<td>AT</td>
<td>H</td>
<td>Emani et al. 2003</td>
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<td></td>
<td>Antisense AV2 gene</td>
<td>AT</td>
<td>ST</td>
<td>Sanjaya et al. 2005</td>
</tr>
<tr>
<td>Insect resistance</td>
<td>Cry1Ac</td>
<td>AT</td>
<td>ST</td>
<td>Perlak et al. 1990</td>
</tr>
<tr>
<td></td>
<td>Proteinase inhibitors</td>
<td>AT</td>
<td>H</td>
<td>Thomas et al. 1995</td>
</tr>
<tr>
<td></td>
<td>Bromoxynil tolerance</td>
<td>AT</td>
<td>Cot</td>
<td>Fillati et al. 1989</td>
</tr>
<tr>
<td></td>
<td>Cry1Ac and API-B</td>
<td>AT</td>
<td>EC</td>
<td>Wu et al. 2005</td>
</tr>
<tr>
<td>Herbicide tolerance</td>
<td>t4A for 2,4-D resistance</td>
<td>AT</td>
<td>H</td>
<td>Bayley et al. 1992</td>
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acS and acB: *Acetobacterium* *saccharum* celluloses synthase; AHAS: acetylhydroxy acid synthase; API: Arrowhead protease inhibitor; AT: *Agrobacterium tumefaciens*; BAR: phosphinothrin acetyltransferase; CAT: chloramphenicol acetyltransferase; COT: cotton; EC: embryogenic callus; ECS: embryogenic cell suspension; EPSPS: 5-enolpyruvylshikimate-3-phosphatase synthase; GFP: green fluorescent protein; GUS: β-glucuronidase; H: hypocotyl; HPT: hygromycin phosphotransferase; NPTII: neomycin phosphotransferase II; OCS: octopine synthase; PB: particle bombardment; pha: polyhydroxyalkanoate synthase; SA: shoot apex; SE: somatic embryo; ST: shoot tip; ZEM: zygotic embryo meristem;
Field testing of Bt cotton

The first field trial with genetically engineered plants expressing Bt toxin was conducted in 1986 with tobacco. Since then, many transgenic crops have been tested in field in USA, Argentina, Australia and China. In 2005, Bt crops occupied 16.2 million hectares in the world (James 2005). The first field trials with Bt transgenic cotton were conducted in USA in 1988 (Jenkins et al. 1991). The cry1A proteins expressed in Bt cotton and Bt corn have been extensively tested for toxicological analysis in the laboratory and field. In China, the development of transgenic cotton that expresses Cry1A insecticidal proteins from Bt spp. *kurstaki* has resulted in new varieties or lines with improved resistance to key lepidopteran insect pests. Cotton plants expressing modified cry1A gene sequences have demonstrated excellent control of cotton bollworm (Helicoverpa zea) and pink bollworm in greenhouse and field experiments. Meanwhile, transgenic Bt cotton did not affect the natural enemies. Numerous field experiments showed that the total labour for pest control workdays could be decreased by 57% by planting Bt transgenic cotton varieties, of which the bollworm controlling labour workdays were decreased by 70% compared with planting regular cotton varieties, the total pest controlling input was reduced by 70%, of which the bollworm controlling input was reduced by 90% (Zhang et al. 2000a). Thus, Chinese breeders and farmers have more interest in the breeding and commercialization of transgenic Bt cotton.

Other insecticidal proteins

Apart from Bt genes, other genes for insect resistance such as those for proteinase inhibitors, α-amylase inhibitor, chitinases and lectins are also being used to produce transgenic insect-resistant cotton plants. The discovery of non-Bt insecticidal proteins from a host of plant and microbial sources offers a wealth of opportunity to significantly extend the range of insect pests that can be effectively controlled in transgenic crops.

Proteinase inhibitor genes

The presence of antimetabolic proteins, which interfere with the processes of digestion in insects, is a strategy for defense that plants have used extensively (Thomas et al. 1995).

Proteins can occur in tissues that are particularly vulnerable to attack, such as seeds, or can be induced by mechanical wounding in tissues attacked by chewing insect pests, such as leaves.

The first gene of plant origin to be successfully transferred to another plant species resulting in enhanced insect resistance was isolated from cowpea encoding a trypsin inhibitor (CpTI) (Hilder et al. 1987). Scientists at the Institute of Genetics of China also cloned the cowpea trypsin inhibitor (CpTI) gene. This gene was successfully engineered into cotton plants by both Agrobacterium-mediated transformation and the pollen tube pathway. The molecular data confirmed the stability of this gene and transgenic plants had increased resistance to cotton bollworm (Li et al. 1998).

The presence of serine proteinase inhibitors in plants can reduce insect attack. They are of interest because of their potent inhibitory activities against proteolytic enzymes of insects. Analysis of the effects of various proteinase inhibitors has shown that these are detrimental to the growth and development of insects, from a variety of genera including *Helicoverpa*, *Spodoptera* and *Diatribota* (Wu et al. 1997).

More recently, fourteen different cDNA fragments encoding serine proteinases were isolated by reverse transcription-PCR from cotton boll weevil (*Anthonomus grandis*) larvae. Using a combination of 50 and 30 RACE, the full-length sequence was obtained for five of the cDNAs.

Northern blotting analysis showed that for 2 genes, expression is induced upon feeding and is concentrated in the gut of larvae and adult insects. Reverse northern analysis of the 14 CDNA fragments showed that only two trypsin-like and two chymotrypsin-like were expressed at detectable levels. Under the effect of the serine proteinase inhibitors soybean Kunitz trypsin inhibitor and black-eyed pea trypsin/chymotrypsin inhibitor, expression of one of the trypsin-like sequences was upregulated while expression of the two chymotrypsin-like sequences was downregulated (Oliveira-Neto et al. 2004).

Lectin genes

Lectins are carbohydrate binding proteins and are abundant in seeds and storage tissues of some plant species. Lectins such as those purified from snowdrop or garlic are toxic to insects but not to mammals. The most effective protein tested is the lectin from snowdrop (*Galanthus nivalis agglutinin*, GNA), which gave approximately 80% mortality at a concentration of 1 g/L in the diet, when used in assays with first and third instar nymphs. GNA also had anti-metabolic effect on brown plant hopper (BPH), and green leafhopper pests of rice (Powell et al. 1995). The gna is the first transgene to exhibit insecticidal activity towards sap sucking insects in crop plant.

Recent interest has mainly concentrated on the lectin from snowdrop (GNA) in China, because it has shown activity against aphids, which are the third most important pests of cotton in China. Scientists in China have transformed the gna gene into cotton plants using the *Agrobacterium*-mediated method. Results of laboratory experiments indicated that GNA increased the resistance of cotton to aphids. Apart from the gna gene, scientists at the Institute of Genetics of CAS obtained transgenic cotton plants carrying the pea lectin (*P-Lec*) gene, which showed some resistance to cotton bollworm (Liu et al. 2002).

Second and third generation insect resistant cotton

Although constitutive expression of insecticidal transgene products has provided high levels of resistance in crop plants, tissue-specific or inducible expression might be desirable under some circumstances. Because the epidermal cells are the first to be attacked by insects, defence genes expressed under epidermal cell-specific promoters (e.g. CER6, an enzyme for cuticular wax production) might be useful. Phloem-feeding insects can be targeted using the root phloem-specific promoter AAt3 (Okumoto et al. 2004), the phloem-specific pumpkin promoter PP2. Progress is being made with chemically inducible promoters, including those induced by ethanol, tetracycline, copper, glucocorticoid steroid hormones, and steroidal and nonsteroidal ecdysone agonists (Padidam et al. 2003). Creating a ‘within plant refuge’ is a novel application of using inducible promoters whereby the transgenic plant or parts thereof can serve as a refuge plant as long as either the expression of the insecticidal gene is not induced or the induction wears off (Christou et al. 2006).

Theoretical models predict that plants expressing two dissimilar Bt toxin genes are likely to have the potential to delay resistance in target insect populations more effectively than single toxin-containing plants (Christou et al. 2006). The simultaneous introduction of three genes expressing insecticidal proteins, a *Cry2A* and *Gna*, into indica rice to control three major pests, rice leaf folder (*Cnaphalocrocis medinalis*), yellow stemborer (*Scirpophaga incertul*us) and the brown plant hopper (*Nilaparvata lugens*), has been reported (Bano-Maqbool et al. 2001). The Bt genes target the leaf folder and the stem borer, and the *Gna* gene targets the plant hopper. Triple transgenic plants were more resistant compared with their binary counterparts.

Transgenic cotton line SOK321 expressing two insecticidal proteins (*Cry1A* and *CpTI*) was commercialized in
northern China and demonstrated increased insecticidal activity on *H. armigera* relative to single gene *Bt* cotton (Liu et al. 2005).

Comparison of three different transgenic *Bt* cotton populations containing either the single *Cry1Ac* or *Cry2Ab* gene, or both genes, for fruit penetration and damage by a feral and *Cry1Ac*-selected strain of cotton bollworm revealed that transgenic cotton containing two *Bt* genes performed better (Jackson et al. 2004).

Developing some non-conventional sources of insecticidal novel proteins was another significant character of the next generation insect resistant cotton. Second generation insect-resistant transgenic plants with increased potential for durable resistance might result from the deployment of plants expressing multiple insecticidal novel proteins such as the VIP (vegetative insecticidal proteins) produced by *B. thuringiensis* during its vegetative growth. These have insecticidal activity toward a wider spectrum of insect pests, yet they have little sequence homology with the more conventional Cry proteins (Yu et al. 1997). Transgenic cotton expressing such a VIP is expected to be released commercially in the USA. *Photorhabdus* and *Xenorhabdus* bacteria are symbionts of entomopathogenic nematodes. Unlike *Bt* toxins, proteins produced by these two bacteria are not acutely toxic when ingested by the insect. Instead they cause septicemia in the insect, the insect is killed and its tissues are used as nutrients by the nematode (Chattopadhyay et al. 2004). Considerable progress has been made in the identification of several toxin genes from these two bacteria (Williamson and Kaya 2003). These genes encode large insecticidal toxin complexes with little homology to other known toxins. Arabidopsis plants expressing toxin A gene from *Photorhabdus luminescens* showed strong insecticidal activity against one lepidopteran and moderate activity against a coleopteran pest (Liu et al. 2003).

**Transgenic cotton for disease resistance**

Diseases are another important factor which causes huge yield loss. The most common diseases are bacterial blight, leaf spots, grey mildew, wilts and root rot. Fusarium wilt, caused by *Fusarium oxysporum*, is a soil-borne fungal disease. This disease causes death or stunting of the plant with yellowing and wilting of leaves. Verticillium wilt caused by *Verticillium alboatrum* is another soil borne and most common diseases in the world. The disease is aggravated by cold wet weather and irrigation. Stunting, chlorotic, mottling and shedding of the leaves, squares and bolls are the symptoms of this disease. Besides the two soil-borne diseases, nematodes also cause considerable losses in cotton yield and quality (Goodell 1993), but this disease is not serious in China.

Mycoparasitic fungi are proving to be rich sources of antifungal genes that can be utilized to genetically engineer important crops for resistance against fungal pathogens. Emani et al. (2003) transformed cotton and tobacco plants with a cDNA clone encoding a 42 kDa endochitinase from the mycoparasitic fungus, *Trichoderma virens*. Plants from 82 independently transformed callus lines of cotton were regenerated and analysed for transgene expression. Several primary transformants were identified with endochitinase activities that were significantly higher than the control values. Homozygous T2 plants of the high endochitinase-expressing cotton lines were tested for disease resistance against several fungal pathogens. *Rhizoctonia solani* and a foliar pathogen, *Alternaria alternata*. Transgenic cotton plants showed significant resistance to both pathogens.

Fertile, transgenic cotton plants expressing the synthetic antimicrobial peptide, D4E1, were produced through *Agrobacterium*-mediated transformation (Rajasekaran et al. 2005). *In vitro* assays with crude leaf protein extracts from T0 and T1 plants confirmed that D4E1 was expressed at sufficient levels to inhibit the growth of *Fusarium oxysporum* f. sp. *vasinfectum* and *Verticillium dahliae* compared to extracts from negative control plants. In *planta* assays with the fungal pathogen, *Thielaviopsis basicola*, which causes black rot root in cotton, showed typical symptoms such as black discoloration and constriction on hypocotyls, reduced branching of roots in GUS negative control T1 seedlings, while transgenic T2 seedlings showed a significant reduction in disease symptoms and increased seedling fresh weight, demonstrating tolerance to the fungal pathogen.

Recently, Tohdfar et al. (2005) introduced the *chi* gene into the cotton genome. Integration of the *chi* gene into the genome of putative transgenic plants was confirmed by Southern blot analysis. Western immunoblot analysis of leaves isolated from T0 transformants and progeny plants (T1) revealed the presence of an immunoreactive band with MW of approximately 31 kDa in transgenic cotton lines using anti-chitinase polyclonal anti-serum. Chitinase specific activity in leaf tissues of transgenic lines was several folds greater than that of untransformed cotton. Crude leaf extracts from transgenic lines showed *in vitro* inhibitory activity against *Verticillium dahliae*.

Cotton leaf curl virus (CLCuD) is one of the many important threats for cotton productivity and has emerged as a serious disease of cotton in the world. Cotton transgenics for resistance against cotton leaf curl disease using anti-sense movement protein gene (AV2) were developed in an Indian variety (F846) via Agrobacterium-mediated transformation using shoot-tips as explants (Sanjaya et al. 2005). A binary vector pPZP carrying the antisense *AV2* (350 bp) gene along with the *nptII* gene was used. Transgenic nature of the putative transgenics was confirmed by molecular analysis. Shoots were induced on selection medium and subcultured on rooting medium containing IBA and 75 mg/L kanamycin. Transgenic plants were recovered in 12–16 weeks from the time of gene transfer to establishment in pots. Preliminary analysis of the field-established plantlets was conducted by PCR. T1 plants were obtained from T0 seeds, the presence of the *AV2* and *nptII* genes in the transgenic plants was verified by PCR and integration of T-DNA with *AV2* into the plant genome of putative transgenics was further confirmed by Southern blot analysis. Several T1 lines were maintained in the greenhouse. Progeny analysis of these plants by PCR analysis showed a classical Mendelian pattern of inheritance.

**Transgenic cotton for herbicide resistance**

Glyphosate [(N-phosphonomethyl) glycine] is a nonselective, postemergent, foliar-applied systemic herbicide most commonly marketed under the trade name Roundup® and Glyphomax®. Glyphosate-tolerant cotton was widely available commercially in the U.S. for the first time in 1997. It was immediately accepted and has increased in total acres planted and in market share each year since its release. It is estimated that glyphosate-tolerant cotton planted accounted for 87.6% of the total U.S. cotton crop in 2005 (Data source from the USDA National Agricultural Statistics Service, http://wps.usda.gov/nass/pubs/).

One of the most commonly used herbicides to control broadleaf weeds is 2,4-D. 2,4-D and several related phenoxy compounds have been used extensively for more than 50 years. It is a post-emergence, translocatable herbicide and specific only to broadleaf plants. Soil organisms that degrade 2,4-D were identified more than forty years ago and the multi-enzyme pathways for 2,4-D degradation have subsequently been demonstrated in several bacterial genera. The first gene (tfk4) involved in the 2,4-D degradation pathway of soil organism *Alcaligenes eutrophus* encodes 2,4-D monooxygenase enzyme, which converts 2,4-D into less toxic 2,4-dichlorophenol and glyoxyxlate by cleavage of the aliphatic side chain. Transgenic plants resistant to 2,4-D have been developed as a tobacco model system. Cotton has also been engineered for 2,4-D resistance by *tdh4* isolated from *Alcaligenes eutrophus* plasmid pJP5 (Bayley et al. 1992; Lyon et al. 1993). Herbicide resistant transgenic
cotton harboring a single copy of the tdf4 gene is released for field trials (Bayley et al. 1992). Transformants containing the tdf4 gene were also verified to exhibit 50- to 100-fold greater tolerance to 2,4-D compared with untransformed controls by Chinese scientists (Chen et al. 1994).

Acetolactate synthase (ALS) is a central enzyme in the biosynthesis of the branched chain amino acids leucine, isoleucine and valine in plants. Herbicides targeting this enzyme are used for the control of broadleaf weeds with resistance to ALS. Gene transfer has been used to incorporate sulfonyleurea resistance into several commercially important crops including cotton (Rajasekar et al. 1996).

Resistance to bialaphos, a non-selective herbicide, was introduced into cotton through genetic engineering (Keller et al. 1997). A gene encoding phosphinothricin acetyltransferase (bar) from Streptomyces hygroscopicus was inserted into elite varieties of cotton through particle bombardment. Herbicide (Basta®) tolerance up to 15,000 ppm was demonstrated in greenhouse trials. The above studies demonstrate the potential for introducing commercially valuable biomaterials and therapeutic proteins. High-level expression of foreign DNA (Grevich and Daniell 2005).

The complete cotton chloroplast genome sequence of *Gossypium hirsutum*

Recently, Daniell’s research group first reported the complete cotton chloroplast genome map, which will provide useful information for chloroplast genetic engineering (Lee et al. 2006). The complete cotton chloroplast genome is 160,301 bp in length, with 112 unique genes and 19 duplicated genes within the IR, containing a total of 131 genes. There are four ribosomal RNAs, 30 distinct tRNA genes and 17 intron-containing genes. The gene order in cotton is identical to that of tobacco but lacks rpl22 and infA. There are 30 direct and 24 inverted repeats 30 bp or longer with a sequence identity ≥90%. Most of the direct repeats are within intergenic spacer regions, introns and a 72 bp-long direct repeat is within the psaA and psbA genes. Cotton chloroplast genome lacks rpl22 and infA and contains a number of dispersed direct and inverted repeats. RNA editing resulted in amino acid changes with significant impact on their hydrophobicity. Phylogenetic analysis provides strong support for the position of cotton in the Malvales in the eurosids II clade sister to *Arabidopsis* in the Brassicales.

Development of cotton chloroplast genetic engineering

Cotton plastid transformation has been extensively attempted using vectors containing species-specific cotton chloroplast vectors and different selectable markers. Spectinomycin had a lethal effect on cotton cultures that prevented the selection of transgenic lines. After identification of suitable selectable markers, the concept of a “double barrel” vector for plastid transformation was used for the first time. This concept employs a vector containing two selectable marker
Transgenic cotton. Zhang and Jin

Transgenic cotton is a type of genetically modified plant that has been engineered to contain specific genes that confer a particular trait, such as pest resistance or disease tolerance. The majority of commercially grown cotton in the world is transgenic, primarily due to the presence of Bt toxins, which are proteins produced by the bacterium Bacillus thuringiensis (Bt). These toxins are toxic to insects and are used in pest control in agricultural settings.

The Bt toxin could harm natural enemies indirectly

A major tactic of Integrated Pest Management (IPM) is to preserve natural enemies associated with crop pests (Bates et al. 2005). Natural enemies of pest species include generalist predators such as carabid beetles or specific parasites such as parasitoid wasps (Vojtech et al. 2005). Although insect-resistance factors expressed in crops might not have a direct effect on natural enemies of pests, indirect effects are almost inevitable.

The insect-resistant biotech cotton varieties are specific to a group of insects that includes most bollworms and budworms but excludes natural predators and parasites. The active toxin binds to receptors in the insect's midgut cells. The binding creates pores in the wall of the insect's gut, allowing ions to equalize, ultimately causing the gut to lose its digestive function (Gutierrez et al. 2006a). Once the binding has taken place after ingestion, the insect's gut is paralyzed, forcing it to stop eating. After the stomach is immobilized, the cells break open and the pH of the stomach decreases as its fluids mix with the lower-pH blood. A lower pH allows the spores to germinate and colonize the rest of the insect's cells. The bacteria spread throughout the rest of the host by the bloodstream until complete paralysis of the insect occurs (Gutierrez et al. 2006b). This process takes anywhere from an hour to a week to kill the insect. Beneficial insects might feed on insects that have taken up the toxin but have not died yet, or might digest by-products of insects such as honeydew that are contaminated with toxin. No data show that biotech toxin could kill beneficial insects, but the toxin could harm beneficial insects indirectly in the two ways described above.

The results showed that high temperature may result in the degradation of soluble protein in the leaf, with a resulting decline in the level of the toxin CrylA. It is believed that this may be the cause of the reduced efficacy of Bt cotton in growing conditions in China, where temperatures during the boll period often reach 36-40°C.

Some have predicted that Bt-insect-resistant crops would be of limited durability because mutations present at low frequency in wild pest populations would be selected and give tolerance to the toxins. When a Bt gene is inserted into a cultivar, the Bt toxin is produced throughout the cotton plant during the entire growing season. Consequently, target pests are exposed to high levels of the toxin continuously, a situation likely to elicit resistance faster than intermittent exposure to conventional insecticides.

Selection for resistance to Bt toxin is akin to the development of resistance to chemical insecticides but the process is subtle. Most models of resistance to Bt toxin assume to be recessive, autosomal and controlled by a single diallelic gene with Mendelian inheritance (Gould 1998; Tabashnik et al. 2000; Liu et al. 2001). The Bt toxin is assumed to kill susceptible heterozygous (Rr) and homozygous (rr) individuals causing rapid selection for the few homozygous resistant (RR) survivors.

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CONCERNS ABOUT THE SAFETY OF BIOTECH COTTON

Biotech cotton has multifarious advantages, and most papers and reports that have been published on this technology are favorable. The technology, however, does carry some risks, and unfortunately the negative aspects of biotechnology have not been properly covered in the scientific publications.

Unstable expressing of Bt gene and resistance to Bt toxins

Transgenic Bt cotton has been widely adopted in the world. It offers satisfactory control of major lepidopteran insects including cotton bollworm and pink bollworm. However, with millions of hectares of transgenic Bt cotton grown yearly, the possibility of insects developing resistance to Bt toxin needs to be addressed to ensure the sustainable use of Bt cotton (Gould 1998). One important principle of existing resistant management plans for Bt crops is that the Bt plants express the toxin at high and consistent levels, referred to as a “high-dose” (Gould 1998). However, He et al. (2006) found that survival of the Asian corn borer (Ostrinia furnacalis) increased as the plants aged. This phenomenon was also observed for the cotton bollworms H. armigera (Zhang et al. 2001; Wu et al. 2003) and H. zea (Greenplate 1997; Lambert 1997). Increased survival in each species may be attributed to the decline in protein expression as the growing season progresses (Greenplate 1999; Zhang et al. 2001). Chen et al. (2005) evaluated the effect of high temperature on the insecticidal properties of Bt Cotton in China. The results showed that high temperature may result in the degradation of soluble protein in the leaf, with a resulting decline in the level of the toxin CrylA. It is believed that this may be the cause of the reduced efficacy of Bt cotton in growing conditions in China, where temperatures during the boll period often reach 36-40°C.
beetle, with early instars being more sensitive than later instars and adult beetles (Meissle et al. 2005). To assess the ecological effects of Bt-cotton cultivars, the development of Spodoptera litura on transgenic Bt-cotton, the intake of Bt toxins, and the effects of Bt-cotton reared S. litura on young larvae of Propylaea japonica (a predator) were evaluated. The results suggested that the Cry1Ab/Ac fusion toxin had no direct effect on young larva of P. japonica, and a combined interaction of poor prey quality and Cry1Ac toxin may account for the negative effects observed on P. japonica development when fed NuCOTN 33B-reared S. litura (Zhang et al. 2006).

So, it can be concluded that all measures to protect crops against insect pests will reduce the numbers of available prey for predators and parasites, even if there is no direct effect (Schuler et al. 2003). This could be true particularly in cotton for the third and later generations towards crop maturity, when the amount of toxin is reduced and not all the target larvae will be killed.

Increased use of herbicide in stand of insecticides

U.S. data show that on average insecticides were applied to cotton 3 times per season to control the largest insects before the adoption of Bt cotton varieties in 1996. Five years later (2000/01), the Bt-planting area increased to 72% of the total cotton area, and insecticide use was reduced to 0.77 sprays per season against the target insects (Benbrook 2001). Bt cotton definitely reduced insecticide use. However, the introduction of herbicide-resistant biotech varieties in cotton has the potential to increase herbicide use. Herbicide tolerance, both in cotton varieties in the United States and in crops elsewhere in the world, is the most-used trait in biotechnology so far. International statistics show that of the total area of 9000 million hectares planted to biotech crops in 2005, 71% were under herbicide-resistant varieties (James 2005). Herbicide-resistant varieties make it possible for farmers to give up other control measures and rely on selected post-emergence herbicides as the backbone of weed management systems in cotton and other crops. Otherwise, long-time use of herbicide may cause environmental problems, such as polluting the soil and underground water.

Non-target pests of Bt toxin may emerge as major pests

Bt cotton is effective against a variety of budworms and bollworms, but it is not effective in controlling many secondary pests requiring insecticides use for their control (Mahaffey et al. 1995; Gianessi and Carpenter 1999; Ru et al. 2002; Gutierrez et al. 2006b).

Effectiveness of transgenic cottons with B. thuringienesis (Bt) cry1Ac gene along with non-transgenic commercial cultivars of G. hirsutum and G. arboreum for the management of cotton bollworm, Helicoverpa armigera was evaluated (Sharma and Pamapathy 2006). The results showed that Bollgard 2 and Bollgard 1 in squares and bolls was significantly lower in the transgenic hybrids than in the nontransgenic ones, especially, the larval numbers were significantly lower on the transgenic hybrids during rainy season under high infestation. However, there were no differences between the transgenic and non-transgenic hybrids in their relative susceptibility to cotton jassid, Amrasca biguttula and serpentine leaf miner, Liriomyza trifolii, white fly, Bemisia tabaci, green budhopper, Nasonia viridula, ash weevil, Myelocystis undecimpustulatus, and red cotton bug, Dysdercus koenigii.

Experience in China (Mainland) shows that populations of secondary pests such as aphids, mites, thrips, lygus bugs, whitefly, and leaf hopper, increased in Bt cotton fields after the target pests – budworms and bollworms – had been controlled (Xue 2002). Supporters and opponents of biotech varieties agree that Bt genes provide good control of target pests. But once the targets pests are controlled, minor and non-target pests may emerge as major pests. When minor pests become major ones, they may change the pest complex situation, and pests that are more difficult to control than the target pests may emerge as major pests, bringing new and difficult problems.

CONCLUSIONS

In summary, the first generation of transgenic cotton with bollworm or herbicide resistance, or both, contributed obviously to increase cotton production. Efficient transformation methods have been established meeting the needs of cotton breeding programs, but the functional genes available are still limited. In the long run, how to improve both pest and disease resistances, together with fiber quality simultaneously should be the most important objectives in future. As novel genes are cloned and transformed, more transgenic cottons with diversified input traits will be seen in the cotton field.

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