

# Recent progress in cotton biotechnology and genetic engineering in China

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*During the past two decades, China has made great progress in cotton biotechnology and genetic engineering, obtaining first regenerative plants from cotton anther and protoplast culture, and also obtaining regenerative plants from many domestic elite cotton varieties. After transgenic cotton carrying the insect-resistant (*Bacillus thuringiensis*, Bt) gene was commercialized in 1996, at least ten Bt-cotton varieties were planted in China. In 1998, over 100,000 hectares of Bt-cotton were planted. Two kinds of bivalent insect-resistant transgenic cotton have been obtained. These new bivalent insect-resistant transgenic cotton carried two insecticidal genes, Bt gene and CpTI gene, or pea lectin (P-Lec) gene and soybean Kunitz trypsin inhibitor (SKTI) gene respectively, and will be commercialized in 2000. Herbicide-resistant varieties for 2,4-D and Bromoxynil are under development and are expected to reach the market by 2001 or 2002. Disease-resistant transgenic cotton is being developed and tested in laboratories and fields, and is also expected to reach the market by 2000 or 2001. Fibre improvement, stress resistance, and male sterility and fertility for hybrid cotton are the next targets for cotton biotechnology. Several genes for fibre improvement and hybrid cotton are being tested in various laboratories. New genes for insect, herbicide and disease resistance are being sought.*

COTTON is an important economic and fibre crop, grown in 70 countries in the world. Over 180 million people are associated with the fibre industry that produces 20 to 30 billion dollars worth of raw cotton<sup>1</sup>. Although great progress has been made in the field of improvement of cotton with conventional breeding methodology, it is time-consuming and commercialization of new cotton varieties often takes 6 to 10 years. Compatibility limitations narrow the gene pool available for this process. A number of these shortcomings may be overcome by plant biotechnology. For example, control can be exerted over selection of the gene(s) and its expression. The gene pool can be expanded to all living organisms (plants, animals, bacteria and fungi). As technology is refined, custom-made synthetic genes will become another source for desired traits<sup>2,3</sup>. Thus, cotton biotechnology can be significantly applied for the improvement of cotton.

China is the largest cotton producer in the world, with about 50 million farming households growing cotton. The crop is of primary importance to the Chinese textile industry, which is the largest in the world. This industry employs nine million workers, and its contribution to

China's export volume comprises about 25% of the total<sup>3</sup>. Thus, cotton biotechnology has received more attention, and has made great progress in the past two decades.

## Somatic embryogenesis and plant regeneration

Somatic embryogenesis and plant regeneration are fundamental to the genetic improvement of cotton using biotechnology and genetic transformation. A well-defined, reproducible, and highly efficient somatic embryogenesis and plant regeneration scheme is a prerequisite for genetic transformation because plant cell transformation is a relatively rare event.

In China, cotton tissue culture began in the 1970s, and became important in the fields of cotton anther culture, protoplasm culture, etc<sup>3</sup>. Currently, this work is being conducted in about 20 laboratories, including universities and institutes.

## Somatic cell culture

The first regenerative plant was obtained in 1986 from cotton somatic cell culture via somatic embryogenesis<sup>4</sup>. Three years later, regenerative plants were obtained from cotton tissue culture by Chinese scientists<sup>5</sup>. During the next ten years, there was a huge growth in research in this field. Some important aspects and factors, such as explant, genotype, medium, hormones and their effects

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on cotton somatic embryogenesis and plant regeneration have been studied<sup>6-25</sup>. Based on this, an efficient protocol for cotton tissue culture and plant regeneration has been established<sup>3,17</sup>. Whole plants could be regenerated within 60–80 days using this protocol<sup>17</sup>.

In order to determine the potentiality of somatic embryogenesis and plant regeneration, over 30 Chinese commercial cotton cultivars have been investigated. Regenerative plants have been obtained via somatic embryogenesis from about 20 cotton cultivars. Out of these, as far as the somatic embryogenesis and plant regeneration characteristics are concerned, at least three cultivars, including CCRI 19, Jihe 321 and Simian 3 have been found as good as Coker varieties. Most of cultivars, such as CCRI 12, Jinmian 7, Jinmian 11, Henan 79, Lumian 6 and CCRI 13 could produce somatic embryogenesis and regenerative plants also, but they were not as easy as Coker cotton or CCRI 19 (ref. 15). Out of the cultivars, which have been induced to produce regenerative plants via somatic embryogenesis, CCRI 12, CCRI 19 and Simian 3 are the major ones in China.

Somatic cell culture has been used to select somaclonal variants. It is an excellent system to select the variations against adverse conditions, such as salt stress, low temperature stress, or toxins produced by *Fusarium* or *Verticillium* wilt. Callus derived from Coker 201 and other varieties was exposed to stepwise levels of toxins of *Fusarium* or *Verticillium* wilt or salt concentrations. After 20–30 days of culture, the surviving calli were transferred into the next higher concentration. After 3–5 times of selection, somaclonal variations with resistance to *Verticillium* wilt or NaCl were obtained<sup>26-29</sup>.

Various physiological or genetic variations exist in the regenerative plants and their progenies, but the physiological variations always convert into normal one after transplanting into the soil. In cotton somaclonal variations, the variations of leaf and fertility were always physiological variations, while most of the variations of plant shape, maturation, yield characters and fibre quality characters were genetic variations. Most of the variations were disadvantageous to breeding, but some of them could be applied in cotton breeding and production. Through continuous self-cross and screening, some fine new lines with high lint, high fibre quality, long fibre, etc. have been obtained<sup>30,31</sup>.

On the basis of cotton somatic embryogenesis and plant regeneration, Chinese scientists have succeeded in encapsulating cotton somatic embryos and have obtained cotton artificial seeds. The germination capacity of the cotton artificial seeds under sterile conditions reached 40.0% (ref. 32).

#### Protoplast culture

Protoplast culture is required for obtaining somatic hybrid cells. The first paper on cotton protoplast culture

was published in 1974 by Beasley *et al.*, but they only obtained callus. Although EI-Shihy and Evans reported that they obtained regenerative plantlets from protoplast culture of island cotton (*Gossypium barbadense* L.)<sup>33</sup>, the details were not reported. In 1989, regenerative plants were obtained and reported in detail from protoplast culture of *G. hirsutum* L. by Chinese scientists<sup>34</sup>. Since then, the methods for cotton protoplast culture have improved, and the procedure for cotton protoplast culture has been established<sup>35-37</sup>.

#### Anther culture

Outside China, haploid callus was obtained in 1987 (ref. 38), but up to now there have been no reports about the embryogenesis and plant regeneration from cotton anther culture. In China, research on cotton anther culture started in the 1970s. Haploid plantlets were obtained from a wild species, *G. klotzschianum* Anderss in 1995 (ref. 39), and regenerative plants were obtained from the callus of *G. hirsutum* L. cv. Coker 201, Siokral 1–3 and Lumian 6 (ref. 40–42) via cotton anther culture.

#### Shoot and meristem culture

Compared with somatic cell culture, shoot and meristem culture is an easier method to obtain regenerative plants. Scientists in China have developed shoot and meristem culture for genetic engineering and conservation of cotton germplasm materials<sup>43-45</sup>. Transgenic cotton plants have been obtained by transforming shoot meristems by the method of particle bombardment<sup>44</sup>.

#### Ovule culture

In China, cotton ovule culture is used to study the development of cotton fibre and obtain cotton fibre *in vitro*<sup>46-49</sup>, and to obtain interspecies hybridization<sup>50</sup>.

Since Beasley and coworkers developed an ovule culture system to study the development of cotton fibres<sup>51</sup>, studies using their system have greatly improved the understanding of the physiology of fibre development. Since Shen obtained fibres from cultivated ovule *in vitro*<sup>52</sup> in 1978, great progress has been made in this field. Scientists in China have obtained a cotton fibre, which is as long as the fibre *in vivo*, but the strength was not as good as the fibre *in vivo* (Chunian Xu, pers. commun.).

Another application of cotton ovule culture is to obtain hybridization through the culture of the young ovule to maturity of the enclosed embryo. The culture system was subsequently applied successfully to the wide hybridization of wild *Gossypium* species with cultivated cotton<sup>46-49</sup>. This technique overcame the physiological barriers to most crosses, and as a result, the diversity and availability

of germplasm for cotton improvement was greatly increased. Several Chinese institutes used ovule culture to introgress the fine characteristics of wild species into upland cotton, and obtained hybridization, which had at least one of the following characteristics: pest resistance, disease resistance, resistance to adverse conditions, the glandless seed/glanded plant trait<sup>3,46</sup>. Some of these were successfully bred.

### Genetic engineering in cotton

The two areas that have advanced most rapidly are (a) insect resistance based on the toxins produced by the bacterium *Bt* and (b) resistance to herbicides. Other strategies are now coming to light.

#### *Transgenic cotton for insect resistance*

Insect pests are a major problem in cotton production in China. 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* Hubner) 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<sup>53,54</sup>.

Chinese cotton are attacked by a number of insect pests, the major ones are the cotton bollworm, cotton pink bollworm (*Pectinophora gossypiella* Saunders), and cotton aphid (*Aphis gossypii*) which cause extensive damage if left uncontrolled. *H. armigera*, in particular, is becoming a serious threat as it is resistant to all of the currently available chemical pesticides, such as synthetic pyrethroids and organophosphates. Almost \$ 50 million is spent each year on chemical pesticides to control cotton insect pests, a significant proportion of it being toxic organosulphur and organophosphorus insecticides such as endosulfan and pyrethroid<sup>53,54</sup>. The more environmentally friendly synthetic pyrethrin insecticides have been effective in the past, but there are growing fears that development of resistance by the insects may soon make pyrethroids ineffective. This, together with the increasing public concern about the use of toxic chemicals and their impact on the environment, has led to a flurry of interest in more environmentally acceptable insecticides and the development of more insect-tolerant cotton varieties.

Traditional breeding has done much to improve the host plant resistance of Chinese cotton to insect pests, and continues to provide new varieties which require less chemical intervention than old varieties. New characters such as glabrousness (absence of hairs on the foliage),

frego bract (outward bending, thin bracts around the cotton boll), nectariless (absence of nectar-forming nectary glands on the leaves), and high gland content are now being assessed for their capacity to reduce the attractiveness of the cotton plant to insects pests<sup>53</sup>. There are, however, limits to the improvement in natural resistance to insect provided by alterations in plant shape and structure. Up to now, none of the cotton varieties developed so far has shown more than a moderate level of resistance. Therefore, 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. Genetic engineering should enhance the capacity to produce more tolerant plants by accessing a much wider gene pool for novel insect resistance characters not present in any of the *Gossypium* species or their close relatives. 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.

#### Isolation of insect-resistant genes to obtain 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. *Bt* toxins are insecticidal proteins found as parasporal crystalline inclusions in sporulated *Bt* strains. They are characterized by their potency and specificity towards specific insect pests, many of which are agronomically important, and their relative safety to non-target insect species and vertebrates, particularly humans<sup>55</sup>. They have been used to control cotton pests for more than 30 years.

A large number of *Bt* genes have been identified, cloned and characterized, and a few have been expressed in transgenic tobacco or tomato plants where they have increased tolerance to insect pests. China started developing transgenic *Bt* cotton in the late 1980s, and the first transformed cotton plants were reported in 1991 (ref. 56). Fan and coworkers obtained transgenic *Bt* cotton carrying *cryIA(b)* and *cryIA(c)* genes from *Bt* spp. *kurstaki* strains HD-1 and HD-73, respectively<sup>56</sup>. Although these transgenic cotton plants containing truncated *Bt* genes showed some measure of protection against insect predation in laboratory test<sup>56</sup>, it soon became clear that the expression level of unmodified *cryIA* genes was too low to confer sufficient protection against cotton pests under field conditions. This is because *Bt cry* genes are typical bacterial genes in that they have a high A/T-content compared with plant genes (typically values are 60–70% for *Bt* genes and 40–50% for plant genes). As a consequence, *cry* gene codon usage is inefficient in cotton plants, and the A/T-

rich regions may contain transcription termination (polyadenylation) sites (AATAAA and variations thereof), cryptic mRNA splice sites, and mRNA instability motifs (ATTTA)<sup>57</sup>. Chinese scientists began modification of these *Bt* genes in 1991, supported by high technology R&D programmes in China. Since then, tremendous progress has made in this field. The effects of different degrees of gene modification were investigated in the *cryIA* genes. The results indicated that removal of the polyadenylation sites and ATTTA 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 (Table 1). These effects were also observed in transgenic tobacco, tomato and cotton by American scientists<sup>58</sup>.

Modified *Bt* genes for a number of different *Bt* toxins have been transformed into cotton plants by *Agrobacterium tumefaciens*-mediated, pollen-mediated or by the method of pathway of the pollen tube<sup>59,60</sup>. Field performance of transgenic cotton containing *Bt* genes has shown significant caterpillar resistance<sup>61–66</sup>. Incorporation of *Bt* genes into major commercial cultivars is expected to reduce insecticide applications for lepidopteran pests by more than 60–70% (refs 53, 65, 67). The resistance was steadily inherited. The character was controlled by a pair of dominant genes, and was not affected by cytoplasm (Zhang *et al.*, unpublished).

Apart from *Bt* genes, other genes for insect resistance such as those for proteinase inhibitors,  $\alpha$ -amylase inhibitor, and lectins are also being used to produce transgenic insect-resistant cotton plants in China. Among these, lectins and plant proteinase inhibitors are of particular interest because they are a part of the plant's natural defence system against insect predation. Insects use many hydrolytic enzymes such as chymotrypsin or trypsin present in their gut for digestion of food protein. Earlier studies on the effects of the inhibitors and lectins induced by the diets of insects showed their effectiveness against these pests. The scientists at the Institute of Genetics of the Chinese Academy of Sciences (CAS), and Biotechnology Research Centre (BRC) of the Chinese Academy of Agricultural Sciences (CAAS) cloned cowpea trypsin inhibitor (*CpTI*) gene<sup>68</sup>. This gene was successfully engineered into cotton plants by *A. tumefaciens*-mediated transformation

and the pollen tube pathway method. The molecular data confirmed the stability of this gene and transgenic plants had increased resistance to cotton bollworm<sup>69</sup>. Wang *et al.*<sup>70</sup> transformed cotton hypocotyl segments via the *Agrobacterium*-mediated method with soybean Kunitz trypsin inhibitor (*SKTI*) gene, and obtained transgenic cotton plants; molecular data indicated that this gene inserted stability into the cotton chromosome. The results of bioassay demonstrated that the transgenic plants showed significant resistance to the larvae of cotton bollworm<sup>70–72</sup>.

Lectins are carbohydrate-binding proteins, some of which are toxic to insects. Various lectins have shown some toxic activity against species of the insect orders Homoptera, Coleoptera, Lepidoptera and Diptera. Recent interest has mainly concentrated on the lectin from snowdrop (*GNA*), because it has shown activity against aphids, which are the second most important pests of cotton in China. Scientists at the Cotton Research Institute (CRI) of the CAAS, cooperating with scientists at Fudan University, have transformed the *GNA* gene into cotton plants using the *Agrobacterium*-mediated method. Meanwhile, scientists at the BRC of the CAAS also obtained transgenic plants carrying *GNA* gene. 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<sup>73</sup>.

Although *Bt* cotton appears to be extremely effective in controlling *Lepidopteran* larvae like cotton bollworm and armyworm, there is considerable concern among the scientific and environmental groups that a single *Bt* gene is not sufficient for the long-term protection of cotton. When used like a chemical insecticide, *Bt* toxins rarely invoke resistance mechanisms in insect pests because of their short field life, which is mainly a consequence of rapid degradation by ultraviolet light. When expressed in a transgenic cotton plant, however, the insects will be constantly exposed to the toxin and this would favour the selection of resistant individuals. In order to cope with this potentially serious problem, scientists have been trying to transform two different insect-resistance genes into the same cotton plant<sup>74</sup>. Wang *et al.*<sup>75</sup> infected cotton hypocotyl segments using *A. tumefaciens* with pBinLK carrying two insecticidal genes, *P-Lec* gene and *SKTI*

**Table 1.** Comparison of wild type *Bt* gene, *Bt* genes modified by Monsanto and Chinese scientists<sup>58</sup>

	Wild type <i>Bt</i> gene	<i>Bt</i> gene modified by Monsanto scientists			<i>Bt</i> gene modified by Chinese scientists		
		Number	Change	% Change	Number	Change	% Change
Number of base pairs	1845	1845	390	21.1	1845	392	21.5
Number of codons	615	615	356	57.9	615	353	58.1
G + C	37	49			49.7		
PPSS	18	1			0		
ATTTA sequences	13	0			0		

gene, and successfully transferred the genes into 4 upland cotton cultivars, 'Xinluzao-1', 'Xinluzhong-2', 'Jihe-321' and 'Liao-9' via *Agrobacterium*-mediated transformation. Transgenic cotton plants harbouring two insecticidal genes were confirmed by NPT-II ELISA, PCR and PCR-Southern. The results of bioassay demonstrated that the transgenic plants showed significant resistance to larvae of cotton bollworm. Meanwhile, Guo *et al.*<sup>76</sup> successfully transformed the plant expression vector pGBI121S4ABC, harbouring both the synthesized *Bt* insecticidal protein gene and the modified *CpTI* gene, into the elite cotton cultivars Shiyuan 321, CCRI 19, 3517 and 541 by the pollen tube pathway method. Resistance of the leaves of these transgenic plants to cotton bollworm was identified. The results showed that some cotton plants were highly toxic to the insect with larvae death rates up to 96%. The results of PCR, PCR-Southern, and RT-PCR analyses confirmed the integration and expression of the two genes in these insect-resistant cotton plants.

### Breeding and commercialization of transgenic cotton for insect resistance

The development of transgenic cotton that expresses CryIA 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 *cryIA* gene sequences have demonstrated excellent control of pests such as bollworm, tobacco budworm 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% (refs 53, 65, 67). In addition, the introduction of commercial cotton varieties producing CryIA insecticidal proteins is expected to reduce environmental pollution from synthetic insecticides, increase worker safety, and improve grower profitability. Thus, Chinese breeders and farmers have more interest in the breeding and commercialization of transgenic *Bt* cotton.

Once the *Bt* gene was inserted into the elite Chinese-developed cotton varieties, scientists embarked on a series of tests to demonstrate the usability of the genetically modified cotton. Researchers conducted initial experiments in the laboratory and then in restricted-access greenhouses. Lastly, they ran small- and large-scale field trials.

Two methods were selected for breeding *Bt* cotton in China. First, *Bt* genes were directly inserted into elite Chinese cotton varieties by pollen pathway method or

*Agrobacterium*-mediated transformation method. This method was selected by about 8 institutes, and has bred some elite varieties or bred lines, such as Jingmian 26, GK-1 and GK-12. The second method is cross breeding. Once transgenic *Bt* cotton plants were obtained, scientists undertook a cross and back-cross programme to introduce the *Bt* toxin genes into genotypes of the current major Chinese varieties developed by Cotton Research Institute of CAAS. This method has been selected by most of institutes and universities, and more than 10 varieties (such as CCRI 30, CCRI 31 and CCRI 32) or lines have been bred and are being commercialized.

While breeding regular transgenic *Bt* cotton, scientists in China are at the same time developing transgenic hybrid *Bt* cotton. Compared with regular *Bt* cotton, transgenic hybrid *Bt* cotton has many fine characteristics, such as high yields, short duration for obtaining a variety, etc. Thus, breeding of transgenic hybrid *Bt* cotton was paid more attention in China, and more than 3 transgenic

hybrid *Bt* cotton varieties have been bred and commercialized. About 50% of transgenic *Bt* cotton in China is transgenic hybrid *Bt* cotton.

Hebei Province of China has signed a research agreement with Monsanto Company to utilize their transgenic *Bt* cotton, and established a joint enterprise named 'JiDai'. In 1995, Monsanto's *Bt* cotton first entered into China. In 1997, JiDai received the Chinese national government's permission to commercialize its Bollgard cotton in Hebei. In 1998, Monsanto's Bollgard cotton was cultivated on approximately 33,000 hectares.

Field experiments showed that the performance of transgenic *Bt* cotton varieties was the same as other cotton varieties except for high resistance to bollworm and budworm, and an average of 70% less chemical insecticide used than conventional cotton varieties<sup>53</sup>. Transgenic hybrid *Bt* cotton varieties can increase yield 10–20% over regular *Bt* cotton or conventional cotton varieties.

Up to now, nine new varieties (Table 2) and at least 20 breed lines (Table 3) with the *Bt* gene have been bred by Chinese scientists, and ten *Bt* cotton varieties CCRI 29, CCRI 30, CCRI 31, CCRI 32, CCRI 38, Jiza 66, Jimian 26, GK-1, GK-12 and NewCotton 33B were allowed to be planted in China. One of these, NewCotton 33B directly came from Delta and Pine Land Co, USA. The transgenic line, GK-321, carrying both insecticide genes *Bt* and *CpTI* in one cotton plant, that has the fine characters of yield and fibre quality, was planted on 400 hectares in 1999. GK-321 was bred by the Biotechnology Center of CAAS and Jiangsu Academy of Agricultural Sciences, and will be commercialized in 2000.

Once the commercial release had been approved, a restricted area of 200 hectares was planted in 1994. In 1997, the fourth year of commercial release, over 20,000 hectares of *Bt* cotton were planted, and in 1998 about 100,000 hectares were planted in China. In 1999, about 350,000 hectares or 8% of the total cotton area was

growing transgenic *Bt* cotton. Of these, 20,330, 10,330, 6700, 4700 and 4700 hectares of *Bt* cotton were planted in Hebei, Shandong, Henan, Anhui and Shanxi Provinces, respectively, which are the major areas for transgenic cotton. Each year, the demand for *Bt* cotton cottonseed greatly outstrips supply.

#### *Transgenic cotton for herbicide resistance*

Another great progress of cotton genetic engineering in China is the breeding of transgenic cotton for resistance to herbicide. In China, three institutes or universities are developing herbicide-resistant cotton and others are likely to enter this area in the near future. 2,4-dichlorophenoxyacetic acid (2,4-D) is a popular herbicide that is useful in controlling weeds. Currently, 2,4-D is little used in the Chinese cotton industry because cotton is notoriously sensitive to 2,4-D and was easily damaged by drift of 2,4-D. An increase in the tolerance of cotton to 2,4-D would minimize spray drift damage, and may even allow the application of 2,4-D directly onto cotton for the control of serious broad-leaf weeds. Scientists in China have adopted a programme to engineer a herbicide detoxification pathway in cotton using a degradative gene isolated from a bacterium. Scientists at the CRI of Shanxi Academy of Agricultural Sciences, and coworkers at CSIRO in Australia and the BRC of the CAAS have succeeded in imparting 2,4-D tolerance to cotton using a gene (*tfdA*) encoding 2,4-D monooxygenase, which was obtained from the bacterium *Alcaligenes eutrophus*, and have obtained transgenic cotton resistance to 2,4-D. The progeny were subjected to a drenching spray with 800 ppm solution of 2,4-D and gave no indication of injury. Transformants containing *tfdA* gene exhibited 50- to 100-fold greater tolerance to 2,4-D compared with untransformed controls<sup>77,78</sup>. This indicated that these new constructs should ensure that the resistance gene will be highly effective in protecting cotton from damage by 2,4-D, whether it is encountered accidentally as spray drift or

from deliberate application by the farmer to control weeds. From the progenies of these transgenic cotton plants, scientists in the CRI of the CAAS and Shanxi have bred some fine lines with high-yielding and good agronomic performance.

The genes for resistant herbicide bialapho and bromoxynil have been isolated, and are being transformed into cotton by the *Agrobacterium*-mediated method.

#### *Transgenic cotton for disease resistance*

Diseases are another important factor which cause huge yield loss. Two of the major diseases of cotton in China are *Fusarium* and *Verticillium* wilt. Some resistant genes have been isolated, and are being transformed into cotton plants.

Plants ward off pathogen infections by eliciting an array of defence mechanisms, including reinforcement of the wall, synthesis of phytoalexins and oxidation of phenolic compounds, activation of defence-related genes and localization of cell death or the hypersensitive response. Associated with these reactions is a rapid and transient production of active oxygen species (AOS), such as the superoxide anion radical ( $O_2^-$ ), hydroxyl radical ( $OH^\cdot$ ), and hydrogen peroxide ( $H_2O_2$ ). The accumulation of  $H_2O_2$  and related AOS has been determined to be one of the earliest events that occurs at the host-pathogen recognition; it has been postulated to play an important role in plant defence. In addition to its oxidative potential in killing or inhibiting the growth of pathogens,  $H_2O_2$  has been shown to be involved in a number of plant defence response processes. Glucose oxidase (GO) catalyses the oxidation of  $\beta$ -D-glucose by molecular oxygen, yielding gluconic acid and  $H_2O_2$ . A number of bacteria and fungi produce GO, which has a putative antibiotic function, but GO has not been found in plants<sup>79</sup>. Murray *et al.*<sup>80</sup> isolated the GO gene from *Talaromyces flavus* and *in vitro* experiments showed GO can inhibit the growth of *Fusarium* and *Verticillium* wilt. We have isolated GO genes from *Talaromyces flavus*, and have transformed these GO

**Table 2.** *Bt* cotton varieties in China<sup>1</sup>

Variety	Breeder*	Year	Maturity (day)	Planted area (hectare)		Note
				1997	1998	
CCRI 29	CRI, CAAS	1998	130–135	4375	16667	Hybrid cotton
CCRI 30	CRI, CAAS	1998	118	–	3750	
CCRI 31	CRI, CAAS	1998	117	–	–	
CCRI 32	CRI, CAAS	1998	135	–	–	
CCRI 38	CRI, CAAS	1999	Full season		800	Hybrid cotton
Jiza 66	CRI, Hebei	1998	Full season		3000	Hybrid cotton
Jimian 26	CRI, Shanxi	1998	Full season		5100	
GK-1	BRC, CAAS	1998	Full season		2666	
GK-12	BRC, CAAS	1999	Full season		1666	
NewCot 33B	Delta and Pine Land Co	1997	122	4437	33000	
Total				8812	66649	

\*CRI = Cotton Research Institute; CAAS = Chinese Academy of Agricultural Sciences; BRC = Biotechnology Research Center.

**Table 3.** Some elite transgenic *Bt* cotton lines tested in field in China

Breed lines	Breeder	Note
GK-2	BRC, CAAS	
GK-14	BRC, CAAS	
GK-19	BRC, CAAS	
GK-21	BRC, CAAS	
GK-321	BRC, CAAS	<i>Bt</i> and <i>CpTI</i> genes
Nankang 3	Nanjiang Agricultural University	Hybrid cotton
6H1	Shandong Cotton Research Center	Hybrid cotton
Zhong 1861	CRI, CAAS	

genes into cotton plants. The offspring of transgenic cotton plants have shown some resistance to *Fusarium* and *Verticillium* wilt. Meanwhile, scientists in the BRC of the CAAS have also obtained transgenic cotton plants carrying the *GO* gene. These disease resistance transgenic cotton plants are being tested in the greenhouse and in fields, and will be commercialized by 2001.

#### Transgenic cotton for modifying cotton fibres

Based on accumulated knowledge of the biochemistry of fibre formation and development, an investigation of the genes critical to fibre differentiation and development has begun in several Chinese institutes and universities. Apart from these, some genes relevant to fibre strength and length have been sought and cloned. Rabbit hair keratin gene has been cloned and transformed successfully into cotton plants by scientists at the Shanghai Institute of Plant Physiology of the CAS. The length of the transgenic cotton fibre is 60% higher than normal cotton fibres, and their elasticity and thermal preservation are better than normal cotton fibre, just like rabbit hair.

#### Hybrid cotton

A novel concept for inducing male sterility and fertility for hybrid seed production has been developed by Plant Genetic Systems (Gent, Belgium). At the biotechnical level, the A line is transformed with a vector that carries genes which confer herbicide resistance and induce pollen sterility. A constitutive promoter is used to control expression of the resistance gene, but for sterility, an anther-specific promoter is used to control a gene that codes for ribonuclease. The ribonuclease protein degrades the RNA that is normally produced by the plant's genes for pollen development. Because proteins essential for pollen development cannot be produced as a result of the RNA destruction, the plant is male sterile. The second parent, or R line, is transformed with the same constitutively expressed herbicide resistance gene and a gene under anther-specific control that produces the antisense copy of the ribonuclease gene. In the A

and R cross, either DNA-RNA or RNA-RNA sense-antisense hybridization occurs so that the ribonuclease is not produced, and the pollen develops normally<sup>81</sup>.

Scientists in China have isolated sterile gene *Barnase* and fertilize gene *Barster* from genomic DNA of *Bacillus amyloliquefaciens*, and have obtained transgenic tobacco and oilseed rape plants; the transformation of cotton is under progress.

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