Plant Omics Journal POJ

POJ 4(6):329-338 (2011) ISSN:1836-3644

Invited Review Article

Advance in research and utilization of cotton biotechnology in China

Shui-jin Zhu*, Ling Li, Jin-hong Chen, Qiu-ling He, Xian-xian Fang, Chun-yan Ye, Shu-feng Yan, Zhuang-rong Huang, and Lei Mei

College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China

***Corresponding author: shjzhu@zju.edu.cn**

Abstract

Cotton (*Gossypium spp.*) is an economically important crop that is grown throughout the world. It is the main material in textile and the main economic resource for more than 100 millions of cotton farmers in China. Due to its main role in our economy, cotton biotechnology has been significantly improved in the last decades in China, which led to a significantly progress both in cotton research and production. Cotton biotechnology started from 1970's in China has been improved greatly in various aspects such as tissue culture, protoplast culture and somatic hybridization, and recombination technology or gene engineering. In this paper, the cotton biotechnology status in China has been taken into account by keeping in view the work done by Chinese scientist, especially in cotton somatic culture, cotton protoplast culture and somatic hybridization, and transgenic cotton with insect resistance, diseases resistance and fiber quality improvement in China.

Keywords: cotton; tissue and somatic culture; somatic hybridization; gene engineering; insect resistance; Verticillium wilt; fiber quality.

Abbreviations: BR: Brassinolide; EPSPS: 5-Enolpyruvylshikimate-3-phosphate synthase; IAA: Indoleacetic acid; KT: Kinetin; NAA: Naphthalene acetic acid; QTL: Quantitative trait loci; RT-PCR: Reverse Transcription Polymerase Chain Reaction; RACE: Rapid-amplification of cDNA ends; SSR: Simple sequence repeats; 2, 4-D: 2, 4-Dicholrophenoxyacetic acid; 6-BA: 6-Benzyl adenine.

Introduction

China is the largest cotton producing and consuming country in the world, and the gross cotton production and consumption is about one fourth of the world total. So, the cotton production in China relates directly to the textile industry, the people's living standard, the economic prosperous, the foreign trade, and farmer's income. It has a great effect on the world cotton trade as well. The cotton production in China has a history of more than 2000 years. At present, the cotton growing acreage is about 5 million hectares, about 15% of the world's total cotton acreage and about 25% of world lint production. It constitutes an important source of income for 100 million farmers and provides employment for 19 million workers in the textile and related industries. Since 1980's, the cotton production in China has been developed greatly, mainly due to the progress of cotton science and technology, especially the modern biotechnology, such as somatic culture, protoplast culture and somatic hybridization, shoot tip culture and haploid culture, and gene engineering.

1. Cotton tissue culture

Plant tissue culture is a method of culturing isolated plant organs, tissues, cells, and protoplasts in an artificial medium to regenerate into a new plant, which includes somatic cell culture, protoplast culture and somatic hybridization, shoot tip culture and anther or pollen culture (Qin and Liu, 2006). Cotton tissue culture is the basis of cotton genetic engineering, and plant regeneration is the first and the most important step in genetic improvement. An efficient regeneration system is a prerequisite for genetic transformation of plants (Firoozabady and DeBoer, 1993). Cotton tissue and somatic culture has developed rapidly since Beasley (1971) who was first one to induce the callus from upland cotton. Up to now, the systems for cotton somatic culture (Chen et al, 1987), anther culture (Zhang, et al, 1998a; Zhang, et al, 1998b) and protoplast culture (Zhang et al, 1991) have already been established in China, which made a good foundation for the cotton genetic engineering.

1.1 Cotton stem-tip culture

Scientists have now focused on such techniques which can improve the plant regeneration in somatic culture in order to establish a more perfect genetic transformation system. Tip meristem of cotton is a group of meristematic cells that can quickly form a complete plantlet with less variation. Compared with normal somatic culture using plant parts or callus as foreign gene acceptor in gene engineering, the tip meristem can avoid the limitations of genotype in plant regeneration, low regeneration rate and somatic mutation during the culture and subculture. Some perfect genetic transformation system using stem tip culture combined with other transformation techniques such as microparticle bombardment and *Agrobacterium*-mediated have been made in China (Wu et al, 1994; Yu et al, 2003; Bao and Zhang, 2009, Zhou et al, 2009).

1.2 Cotton ovule and embryo culture

There are many excellent traits in the wild cotton species and sexual crossing is the common method to transform these traits from wild cotton species to cultivated cotton. However, due to the distance of genetic relationship between the wild cotton species and upland cotton, the interspecific hybrid seeds are difficult to obtain, mainly because of the abnormal development of the hybrid endosperm. Furthermore, embryo culture in vitro is an important experiment method to study the fertilization, single fiber development, and embryo growth potential in vitro. In China, Qian et al (1988) obtained a number of hybrids between cultivated species, *G. hirsutum* and *G. arboreum*, and many diploid wild species via culture of interspecific hybrid embryos on the white medium. Up to now, many interspecific hybrid plants including *G. hirsutum* × *G. arboreum*, *G. hirsutum* ×*G. thurberi*, *G. hirsutum* × *G.klotzschianum*, *G. hirsutum* × *G. bicki*, *G. arboreum* × *G. harknessii*, *G. herbaceum* × *G.armourianum*, and *G. barbadense* × *G. australe* have been obtained, and many new germplasms with good abilities of disease resistance, insect resistance, drought and salt tolerance etc. have been derived from these interspecific hybrids, and some of them have been widely applied in breeding program (Hu et al, 1991).

1.3 Cotton somatic culture

One of the main bases of modern biotechnology is the callus induction and plant regeneration of cotton through somatic embryogenesis. Cotton is one of the most difficult regeneration crops, mainly due to the lower frequency of embryogenesis, the longer period of regeneration, the higher frequency of abnormal somatic embryos. Liu (1983) was the first one who induced the callus from wild cotton species, *G. davidsonii*, and got the regenerated plantlets afterward. Wu et al (1988), Tan and Qian (1988), and Lu and Xia (1991) induced the somatic embryos or regenerated plantlets from the callus of different explants of different cotton species included *G. arboreum*, *G. hirsutum*, *G. raimondii*, *G. gossypioides*, and *G. davidsonii* etc. They did a number of experiments in order to find out suitable culture conditions for embryogenesis and plant regeneration. Resultantly, protocols for cotton somatic embryogenesis and plant regeneration system were initially established and improved. At the same time, the regenerated plants were obtained from domestic cultivars that were considered to be difficult in plant regeneration such as Jinmain 11, Jimain 312, Lumain 6, Simian No 2, Simian 3, CCRI 12, and CCRI 13 (Feng et al, 1997; Zhang et al., 1999, 2001, 2009). Up to now, the regenerated plants or somatic embryos from eight cotton species included three cultivated species, *G. hirsutum*, *G. barbadense* and *G. arboreum*, and many cultivars or/and germplasms of upland cotton species have been obtained in China (Cui et al, 2001). In addition, suitable explants including hypocotyls, cotyledon, petiole, leaf, radicle, and stem have been used in plant regeneration, but hypocotyl has been found to be the best explant in cotton somatic culture. The problem of genotypes in cotton plant regeneration is still a limitation in cotton somatic culture. Jia and Zhao (2004) found that cotton cultivars with good embryogenesis has serious regional limitation, he indicated that cultivars from the Yellow River region were much more easily in plant regeneration than that from the Yangtze River region. Only those with efficient and stable plant regeneration system can be used in gene transformation directly, and be more useful in cotton breeding program. However, many factors in cotton somatic culture including genotype are still the key problems in cotton tissue culture, which may restrict the application of the *Agrobacterium*-mediated method and other transformation method in cotton genetic improvement.

1.4 Protoplast culture and somatic hybridization

Somatic hybridization via protoplast fusion makes it possible to combine the good traits from different species, which is very difficult in sexual hybridization. She et al (1989) was the first one to obtain successfully the regenerated plantlets from protoplast culture of upland cotton. Li et al (1995) and Meng et al (1996) obtained the embryos from protoplast culture of more than 20 cultivars of *G. barbadenese* such as Xinhai 3, Xinhai 6, Xinhai 7, 282, K253, Junhai 1, and Giza 70 etc, through the establishment of embryogenesis somatic suspensions. Wang (1998) isolated the protoplast from the embryonic callus of upland cotton, Lumain 6, and the regeneration plants were obtained after several subculture of protoplasm culture. Lv et al (1999) regenerated plants from protoplasts of upland Cotton var. Coke 201. They indicated that IAA and 2, 4-D had a positive effect on callus induction of protoplast culture, 2, 4-D was stronger than IAA, but KT showed a negative effect on protoplast culture. All those plant growth regulators had negative effect on embryogenic callus suspension culture. Due to the success of cotton protoplast culture, somatic hybridization was carried out successfully in China. Sun et al (2004) obtained 18 symmetric somatic hybrid plants between *G. hirsutum* var. Coker 201 and *G. klotzschianum* by the method of electrofusion, and 16 plants were true somatic hybrids by analysis of molecular biology techniques. In the succeeding year, they isolated protoplasts from different explants, which were cultured on liquid KM8P medium. And finally the hybrid plants between upland cotton (Coker 201) and *G. klotzschianum* were regenerated by the protoplast fusions and somatic embryogenesis (Sun et al, 2005a; Sun et al 2005b). Fu et al (2009) tried to make the non-symmetric somatic hybridization in cotton protoplast fusion using the technique of inactivation both donor and recipient parent protoplasts and fusion by electricity, and the hybrid plants were regenerated from upland cotton (YZ-1) and *G. davidsonii*. It was a novel progress in somatic hybridization following the symmetric fusion using the asymmetric fusion technique based on UV radiation.

1.5 Cotton anther culture and haploid breeding

Anther culture is an important method to obtain haploid plants, which can be used to get stable and genetically homozygous diploid plants through the chromosome doubling. Resultantly, the period of breeding program can be shortened greatly with high efficiency in selection using haploid breeding method. Also, the population with doubled haploid plant lines is a valuable population in theoretical research of plant hereditary and QTL mapping, as well as the studies of differentiation of cotton pollens or microspores and the mechanism for formation of somatic embryo. There are only a few publications on cotton anther culture. Zhang et al (1998a, 1998b) reported the process and morphological characteristics of embryogenesis and organ differentiation in the anther culture of *G. klotzschianum*, and the differentiated embryoids, adventitious buds, and the haploid plants from the anther calli of *G. klotzschianum* were obtained (Zhang et al., 1995, 1996). However, there are no any reports on the other culture of upland cotton up to now. The key problem of upland cotton anther culture is the division of the pollens or microspores inside the anther. Many factors such as genotypes, developing time of anther, medium and its composition, and culture method etc. may affect the anther culture. It was concluded that the technologies of cotton anther culture and inducing of cotton haploid line are valuable in the

research of cotton biotechnology, especially for the study of differentiation of cotton pollens or microspores and the mechanism for formation of somatic embryo.

1.6 Factors affecting cotton tissue and cell cultures

1.6.1 Culture condition and explant types

Among the physical factors, Zhang et al (1993) found that the suitable temperature for somatic culture was 28-30℃, when the temperature was below 25℃, the callus grew slowly and was difficult to induce somatic embryos. He further noticed that the temperature above 30℃ could easily accelerate aging of callus. Also other factors could play important role in somatic culture, such as the culture condition for germ-free seedlings, explant types, and explant age etc.

1.6.2 Hormones

Hormone is an important factor that affects the induction and growth of callus as well as the embryogenesis (Zhang et al, 2000). For the auxins, 2, 4-D is the most effective in order to induce both non-embryogenic and embryogenic callus. However, 2, 4-D may inhibit severely the embryogenic callus differentiating. In a medium with 2, 4-D, it is very difficult to form embryoid from callus, even if in a low concentration. Thus the medium for embryoid growth should be supplied with IAA instead of 2, 4-D (Wang et al 1992). IAA is very efficient in inducing embryoids from embryogenic callus, while NAA is poor in embryoid inducing (Cao et al, 1998). BR can extend the explant survival time, and it is good in cotton somatic culture when BR combined with other plant growth regulators. For the cytokinin, KT is better than the 6-BA, and normally the combination of auxin and cytokinin is suitable for callus induction and embryogenic formation during the cotton somatic culture (Zhang et al, 1993, 2000).

1.6.3 Culture subculture time

Culture time is also very important in the callus induction and somatic embryogenesis. Zhang et al (1993) reported that the optimal time for first subculture was 40 days. When the explant cultured on the medium for 20 to 30 days, the callus was too small to die. While somatic embryos subculture for a long period on solid medium, the viability may become poor and it was difficult to regenerate anymore. The culture method of "solid-liquid alternative culture" is good one in subculture, which can maintain the callus viability for relative long period (Zhang et al, 1993). Wang et al (1994) found that embryogenic callus of upland cotton would change from light-green or gray loose particles into yellowish compacted lump form, and embryogenesis was increased with the increasing of the culture period, but decreased when the subculture was done after a longer period. After two and half years of culture or subculture, three different calli were isolated. The first callus could produce a large number of embryoids, the second one could produce a small amount of embryoids, and the third one lost the ability of embryogenesis. Based on the experiment, they established a high frequency somatic embryogenesis system for several upland cotton cultivars such as Lumain 6 and Lu 1024.

1.6.4 Genotype

Genotype is another major factor that affects callus induction and embryogenesis. Zhang et al (1994) indicated that there was a large difference in the ability of embryogenesis in 50 upland cotton cultivars. Among the introduced foreign cultivars or germplasms, Coker pedigree germplasms can easily regenerate, and that of Acala and Stoneville cotton are more difficult ones in plant regeneration. The cultivars from Deltapine Land Co. are hard to regenerate. Among the domestic culivars, those from Yellow River region are easy to regenerate, especially that from Shandong, while those from Yangtze River region are more difficult to obtain embryogenic callus than others.

2. Cotton genetic engineering

Since the first commercial cultivation of genetically modified (GM) crops, the acreage of GM soybeans, cotton, and corn and rape etc. has increased dramatically (Zhang et al., 2000). The total acreage of the GM crops in the world was 1.7 million hectares in 1996, it increased to 134 million hectares in 2009, exceeding the total area of cultivated land in China. In 2009, there were about 14 million farmers planting GM crops in 25 countries, about 90% of them are under developed countries. Currently, there are four types of GM crops allowed for commercial production in China. Among them, GM cotton is the only one, which has been used in large-scale commercial production. Since the GM was first produced on large scale in 1997, acreage of GM cotton has been increasing dramatically. Up to now, there was more than 100 GM cotton cultivars have been approved at national and provincial levels. These GM cotton cultivars have a number of traits, which fulfill the needs of different cotton production region and different plant system. Hybrid cotton, short season and early maturity cotton, long staple cotton, glandless cotton, and colorful cotton are being under cultivation on large scale in Yellow River region, Yangzi River region, and other cotton production region. At the beginning, the major GM cotton cultivars were those introduced from Monsanto of USA (such as Nucott 33B and Nucott 99B etc.). In 2004, the total acreage of domestic transgenic cotton cultivars was surpassed to those from Monsanto of USA, 52.4% for domestic GM cotton and 47.6% for introduced GM cotton. That ratio was changed to 64.8%/35.2% in 2005, 70%/30% in 2006, and 80%/20% in 2007. Due to the national special fund for transgenic crop breeding programs, 28 approved new insect-resistant transgenic cotton cultivars were developed in 2008 and 2009, and the domestic transgenic cotton cultivars shared 93% of total GM cotton market in 2010. For the domestic transgenic cotton, SGK 321 was the first insect resistant cotton cultivar with double insect resistant genes, *Bt*+*CpTi*, and CCRI 41 is the second generation of the *Bt* cotton cultivars to extend in very large scale due to its adaptability to different environmental condition in China, and many new cultivars were derived from it, such as CCRI 63, a main production commercial cotton cultivar in Yangzi River region, and CCRI 50, a short season and high yield cotton cultivar suitable for double cropping system cotton cultivar with wheat in Yellow River region. Up to now, the total acreage of transgenic insect resistant cotton in China is around 21 million hectares, and the total new output value was more than 44 billion RMB annually and more than 250 million RMB was obtained due to reduction of chemical pesticides about 10,000~15,000 tons annually, only about 7.5% of the total chemical insecticide used in China, while it was used to be more than 25%. Statistics show that genetically modified cotton has reduced pesticide usage to around 80%, and the population of natural enemies of cotton bollworm in the cotton field has increased from 20% to 40%. Moreover, recent studies have shown that widely extending of transgenic cotton with *Bt* also can protect other crop without *Bt* from bollworm damage.

2.1 Herbicide resistant cotton

2.1.2 Gene cloning for herbicide tolerance

The *bxn* gene encoding bromoxynil-specific nitrilase was cloned from genomic DNA of Klebsiella ozaenae by PCR. The cloned gene expressed a high yield of this specific enzyme with high activity, which could degrade bromoxynil into non-noxious substances (Zhang et al 2006). The *als* gene was cloned from rice. Data for the bioassay of Sulfometuron-methyl showed that transgenic tobacco displayed notable resistance to herbicide compared to the control, with highest content of 50 mg/L(Song et al, 2007). Using the PCR method, fragments that encode the ALS were cloned from *Monochoria korsakowii,* it was found that the nucleotide sequence of R M. korsakowii biotype differed from that of the S biotype by three nucleotide substitutions. The amino acid substitution of Pro197 (CCT) by His (CAT) had been reported in many other resistant weeds. It is clear that the substitution of Pro197 may be responsible for the resistance to bensulfuron-methyl in the R M. korsakowii biotype. The functions of the other two mutations in the R M. biotype need to be further investigated (Lu et al, 2009). The glyphosate N-acetyltransferase gene was acquired from soil total DNA by PCR and inserted into *Escherichia coli* expression vector pGEX-6p-1 (Lei et al, 2007). The staggered PCR extension process was performed using *Escherichia coli* and *Salmonella typhimurium aroA* genes as templates to obtain one variant that exhibits significantly enhanced tolerance to glyphosate (He et al, 2002). The glyphosate-resistant EPSPS gene was cloned from *Pseudomonas* spp (Zhao, 2008), *Pseudomonas fluorescens* (Zhu, 2002), *Pseudomonas stutzeri* (Sha, 2008), *Halomonas Variabilis* (Liu, 2004), *Vibrio anguillarum* (Ye et al, 2008)and *Sclerotinia sclerotiorum* (Yu, 2006)). Other researchers also cloned glyphosate-resistance *EPSPS* gene from *Allium macrostemon Bunge*, *Orychophragmus violaceus*, *Abutilon theophrasti Medic*, *Brachypodium Distachyon*, *Nicotiana tabacum*, *Gossypium hirsutum* L., *Dunaliella salina*, *Oryza.sativa* L., *Brassia campestris* L., *Phaseolus vulgaris* L. and *Brassia* L.(Table 2).

2.1.2 Herbicide tolerant cotton in China

2.1.2.1 2, 4-D tolerant cotton

2, 4-D is an auxin using in plant tissue and cell culture. Cotton is so sensitive to 2, 4-D that only 1 ppm of 2, 4-D can cause distortion and withering of seedlings, leaves and buds of cotton, and resulting in no harvest. Laurent et al (2000) isolated *tfdA* gene from a bacteria of soil. The *tfdA* gene codes for a dioxygenase catalyzing the degradation of 2, 4-D to 2, 4-dichlorophenol (2, 4- DCP). The first metabolite was the glucoside conjugate of 2, 4-DCP (2, 4-DCP–β-O-glucoside). The major terminal metabolites were two more complex glucosides, 2, 4-DCP– (6-O-malonyl) glucoside and 2, 4-DCP–(6-O-sulfate) glucoside. Bayley et al (1992) successfully transformed tfda gene into cotton. The transgenic cotton plants can tolerate the 2, 4-D by 3 times of field usage level. The tfda gene was also successfully introduced into the Jinmian 7 by Chen et al (1994) and several stable tolerant transgenic cotton plants were obtained. Zhang et al (2000) and Wang et al (2001) showed that the tfda gene is a single dominant gene and is not affected by cytoplasm, which is consistent with Mendelian segregation.

2.1.2.2 Bromoxynil tolerance cotton

Bromoxynil is a nitrile herbicide used in broad-leaf weed control. The mode of action of this herbicide is to occupy the Q_B site of D_1 protein for photosystem II, thus preventing the plastoquinone from reacting with the Q_B site to facilitate electron transport in photosystem. The Bromoxynil was first reported in 1963 and utilized since 1994 in China (Lu et al, 2008). The United States started to grow bromoxynil-resistant cotton since 1995 (Su, 2009). In China, there are no any reports about the transgenic cotton with bromoxynil resistant gene up to now.

2.1.2.3 Glufosinate tolerant cotton

Glufosinate is a non-selective herbicide that inhibits glutamine synthesis. It blocks the biochemical reaction that combines glutamate and ammonia into glutamine, resulting in a high accumulation of ammonia in plant tissues, which lead to the damage of cell membrane, severely reduce photosynthesis, which led to plant death. Two glufosinate resistant genes (*bar* and *pat*) from different species of *Streptomyces* were cloned, and both of which encode phosphinothricin acetyltransferase process. Keller et al (1997) successfully transferred the *bar* gene into upland cotton. AgrEvo derived glufosinate resistant cotton cultivars and commercialized them in the United States in 2000. In China, these genes have been used as the selection markers in cotton engineering as those herbicides are not popular in China. Guo et al (1999) used an expression vector containing the *GNA* and *Bar* genes to transform the upland cotton cultivar "699" and 24 Glufosinate-resistant cotton plants were obtained. From them they developed a transgenic cotton germplasm with aphids resistance. Jiang et al (2007) transferred the salinity tolerant *BADH* gene combined herbicide resistant *bar* gene to the upland cotton by the method of pollen tube pathway. With the help of herbicide resistance *bar* gene in selection, they obtained several transgenic cotton germplasm with salinity tolerance, through bioassay and molecular analysis.

2.1.2.4 Sulfonylurea tolerant cotton

Sulfonylurea and imidazolinone herbicides can inhibit the activity of acetolactate synthase (ALS), leading to the toxic accumulation of a-ketoglutarate which resulting to decreasing of protein synthesis and plant death. Using recombinant DNA technology, DuPont transformed chlorsulfuron-resistant gene (*als*) into Coker 312 and developed cotton germplasm, 19-51a, with sulfonylurea herbicide resistance. Lian et al (2008) introduced both drought and sulfonylurea herbicide resistant gene into upland cotton Lumian-19 and seven transgenic lines with herbicide resistance and good agronomic characters were obtained.

2.1.2.5 Glyphosate tolerant cotton

Glyphosate is an organic non-selective post-emergence herbicide that can kill all green plants. According to NASS statistics in 2005, more than 90% of soybean, 30% of maize fields, and 75% of cotton were produced with the help of glyphosate herbicide control in the United States. Monsanto isolated the 5-enolpyruvylshikimate-3-phosphate synthase gene (EPSPS) from the *Agrobacterium* var. CP4, transferred it to Coker 312 through *Agrobacterium*-mediated technique, and several upland cotton cultivars resistance to glyphosate were obtained. The glyphosate resistant cotton was firstly commercialized in 1997, and the acreage was increased dramatically afterward. In China, Xie et al (2004) obtained 65 regenerated plants using the hypocotyl of CRI 35 as explant. Zhao et al (2005) introduced the *aroAM12*, a glyphosate

resistant gene coded by themselves, combined with an insect-resistant gene, *Bts1m*, into Shiyuan-321 by *Agrobacterium*-mediated method and attained 52 regenerated plants. Among them, 38 plants had both *aroAM12* and *Bts1m* genes through molecular analysis. Liu et al (2007) obtained 3 glyphosate-resistance transgenic plants, using the pollen-tube pathway transformation technique. So far, the transgenic cotton with glyphosate resistance is still on the research and trial stages. In 1995, the Shanghai Institute of Plant Physiology and Ecology obtained a glyphosate tolerant germplasm by radiation breeding methods. But the capacity of glyphosate tolerance was only 90% of the field concentration. There has not been any further report on this subject since then. While treating 31 upland cotton cultivars by different concentrations of glyphosate, Zeng et al (2001) screened 10 candidates and found that there was much more possibility to get natural herbicide resistant materials at the seedling stage. Zhu et al (2003) got a non-transgenic glyphosate resistant cotton mutant (R1098), through callus screening and Co^{60} irradiation method. Tong et al (2009) conducted a cloning of EPSPS gene and tissue-specific expression analysis. Wu et al. (2006) used R1098 as male and selected a Glyphosate-resistant cotton hybrid cotton (ZD-14).

2.2 Insect resistant cotton

The length of a growth period and insect infestations can cause serious losses to cotton production. Transgenic insect-resistant cotton has been proven to be a better alternative to insecticides since it can enhance the cotton resistance and reduce cost of cotton production. Currently, the main insect-resistant genes used in cotton are those derived from *Bacillus thuringiensis* (*Bt*) insecticidal gene, protease inhibitors (*PI*) gene from other plants oeanimals, *lectin* gene (*lectin*) and *cholesterol oxidase* (*cho*) are some of examples. Most of these genes have been successfully transferred into cotton and are effectively involved in insect control (Li et al, 2009).

2.2.1 Insect resistance with Bt

Bt gene has an excellent resistance to tobacco bud worm, cotton bollworm and pink bollworm. Fan et al (1990) isolated the *Bt* gene from *Bacillus thuringiensis*. Xie et al (1991) firstly reported the transgenic cotton with *Bt* toxin gene by means of Pollen Tube Pass-way method in China. Guo et al (1999) obtained the strong insect resistant *Bt* (*Cry IA*) gene and constructed an efficient plant expression vector after a series of modifications for the *Bt* gene. Since 1993, the Chinese Academy of Agricultural Sciences and other Research Institutes transformed that modified *Bt* gene into Chinese cotton cultivars by *Agrobacterium*-mediated method and Pollen Tube Pass-way method and 12 cotton cultivars including CCRI 19, CCRI 36, Simian 3, and Simian 6 etc. had been derived (Gao et al, 1995; Zhang et al, 2003). Wu et al (2008) monitored the bollworm population dynamics for ten consecutive years in Chinese *Bt* insect-resistant cotton growing areas Tanaka, and found that *Bt* Insect-resistant cotton is a lethal trap for the bollworm. It can also reduce bollworm damage to other crops and minimize the use of chemical pesticides. This provided a theoretical basis for the limited / localized and sustainable control of cotton bollworm. It also served as an example of the usefulness of genetically modified crops and helped to explain scientifically their benefits to the public. Up to 2009, more than 100 new transgenic cotton cultivars with *Bt* gene have been examined and approved in China.

2.2.2 Insect resistant cotton with PI

Protease inhibitors (*PI*) can interact the insect's protein digestive enzymes in the digestive tract and produce the Enzyme-Inhibitor complex (EI), which can block or weaken the hydrolysis of the digestive enzyme. Once the insect eats the protease inhibitors, its normal protein digestion is affected. The two types of genes used are *CpTI* gene from Cowpea bean and proteinase inhibitor gene (*API*) from arrowhead (*S. latifolia*). Wu et al (2003) transformed the *API* insect-resistant gene into high-quality cotton germplasm, and an insect resistance, disease resistance, high yield, and good fiber quality was developed as W-SO367. In addition, Chinese scientists have also isolated and cloned soybean trypsin inhibitor (*SKTI*), rice cysteine protease inhibitors, amylase inhibitor gene, potato proteinase inhibitor gene (*PI-II*) etc, and the transformation of those genes in cotton are carrying out in different research institutes.

2.2.3 Insect resistant cotton with double or triple genes

Bivalent and trivalent insect-resistant genes together with RNAi technology make the insect resistance of cotton cultivars more stable and durable. In 2001, the insect-resistant BT and CpTI gene was transferred into cotton and the new transgenic cotton cultivars are already in large production (Jia et al, 2001). Wu et al (2003, 2008) combined the Cry1Ac insecticidal protein gene with the chimeric gene (*Bt29K*) and the arrowhead proteinase inhibitor B (*AP1-B*) genes, constructed a doubled insect resistant expression vector. By the *Agrobacterium*-mediated transformation method, a new insect resistant cotton cultivar Ji321 has been derived commercially. Liu et al (2004) obtained cotton germplasm with high resistance to bollworm and cotton aphid through a better inhibitory effect of the double genes, *Bt+GNA*. They verified that the two insect-resistant genes (*Bt+GNA*) in transgenic cotton can inherit and express stable. Wu et al (2006) transformed the triple insecticidal genes (*Bt+CpTI+GNA*) to upland cotton by *Agrobacterium*-medium method, the regenerated transgenic cotton plants with three genes expressed were obtained and the triple genes were inherited to the next generations.

2.2.4 Insect resistant cotton with other genes

Cholesterol oxidase (*Cho*) gene is a new class of insecticidal substance, which can catalyze the cholesterol of insect intestinal epithelial cell membrane and extract (Tomoyukil) - steroid ketones and hydrogen peroxide, resulting in destruction of insect intestinal cells, leading to the death of the insect. Presently, at least three cholesterol oxidase genes had been cloned, Vipl, Vip2 and Vip3A (Mao et al, 2007). Lectin is a class of non-immune globulin which can specially recognize and reversibly bind to complex carbohydrates. When the insect eates plants containing lectin, it will bond the cell membrane around the glycoproteins of the insect's gut membrane. This affects the absorption of nutrients, and results in disease or even death of the insect. At present, pea lectin (P-lec), Pinellia ternata agglutinin (PTA) and snowdrop lectin (GNA) had been isolated in China for the improvement of plant resistance to insects by genetic engineering (Zhnag et al, 2003; An, 1997; Sun et al, 2005), but the transgenic cotton with those insect resistant genes have not been reported in China up to now.

2.3 Verticillium wilt resistant cotton in China

There are mainly two kinds of diseases in cotton production in China, Fusarium Wilt and Verticillium Wilt. Cotton breeding for Fusarium wilt resistance is very successful, and many high resistant cotton cultivars have been released and the disease has been controlled well with the extending of the resistant cotton cultivars. However, the breeding progress for resistance to Verticillium Wilt is not as good as that for resistance to Fusarium Wilt caused by the Verticillium dahliae Kleb, mainly due to the luck of the resistant cotton germplasm. Gene engineering is the usable way to produce the upland cotton germplasm with Verticillium Wilt resistance, which can be used in cotton breeding program. The active function gene is the key problem in gene engineering to produce Verticillium Wilt resistant cotton. Zhou et al. (2002) obtained a resistant gene, Rs-AF1, which can restrain the mycelium growth and spore germination of the Verticillium dahliae Kleb in the experiment from radish by the method of PCR. Dou (2002) transformed the *NDR1* and *PR1* from *Arabidopsis thaliana* to upland cotton, and the resistance to Verticillium Wilt in transgenic cotton germplasm was much better than the background one. In addition, Zuo et al. (2002) cloned a gene related to polygalacturonase (PGIPs) from *G.barbadense*, which can combine the inner polygalacturonase of the fungi and led to decreasing of this enzyme activity greatly. However, the resistance to Verticillium Wilt was increase insignificantly in the transgenic cotton materials.

Many research works on gene transformation for Verticillium Wilt resistant cotton were carried out in China, using variant genes related to chitinase, glucanase, plant defensin, thionin, and glucose oxidase etc., but few high resistant transgenic upland cotton germplasms which can be used in breeding program have been released (Wang, 2000; Zhang et al., 2001; Le et al, 2002; Zhe et al, 2004; Cai et al, 2000; Cui et al, 2001; Qu et al, 2006).

2.4 Improvement of cotton fiber quality in China

Cotton fiber, a single elongated cell, is the main product of cotton production. It is difficult to improve the cotton fiber quality by the traditional method. So many research works have been carried out on cotton fiber genomics and gene engineering in order to improve the cotton fiber quality genetically in China recent years, and some progresses have been made already.

2.4.1 Improvement of cotton fiber with SPS gene

Sucrose phosphate synthase（SPS） is one of the key enzymes in the sucrose biosynthesis pathway, which catalyzes Fructose-6-phosphate into sucrose. Haigler et al (2007) transformed the SPS gene from Spinacia oleracea L.into upland cotton, the SPS activity in leaves and fiber cells of the transgenic cotton were higher than that of check. At the condition of 15~19℃ at night, the seed cotton weight, fiber maturity, and fiber strength of the transgenic cotton line were better than that of non-transgenic cotton check. In China, Li et al (2010) cloned a cDNA of BvSPS1 from Beta vulgaris L. by the method of RT-PCR. No other reports about the utilization of this gene in cotton have been found up to now.

2.4.2 Improvement of cotton fiber with SPS gene

The enzyme of cellulose biosynthesis, cellulose synthase, affects the cellulose synthesis and fiber strength of cotton significantly. The cell of *Acetobacter xylinum* can produce the fiber-like cellulose, and the related genes of cellulose synthase operon such as *acsA*, *acsB* and *acsC* have been cloned. Li et al (2004) transformed the *acsA* and *acsB* genes into color cotton germplasm (G007, upland cotton) by means of vacuum infiltration, and the staple length and fiber strength of the transgenic cotton lines were enhanced by 15%, compared with the non transgenic color cotton check.

2.4.3 Improvement of cotton fiber with the genes form animals

Wang et al (2002) transformed the keratin gene from rabbit hair cloned by into upland cotton SGK321, the transgenic cotton with *Bt+CpTI*, the handle and sense quality of the transgenic cotton fiber were improved, but the staple length and strength were not enhance significantly. Zhnag et al (2004) transformed the rabbit keratin gene with a fiber specific E6 promoter to upland cotton cultivar Sumian 16, and three transgenic plants were obtained with molecular testing. According to the evaluation of fiber quality, the fiber quality of the rabbit keratin- transgenic cotton were, to some extent，improved, especially the average fiber strength was increased by 6.3 cN/tex to that of Sumian 16. Huang et al (2004) obtained the transgenic several transgenic sea island cotton (*G.barbadense* L.) plants with spider silk protein gene *ADF3* based on microprojectile bombardment. The fiber strength of the transgenic cotton was increased, but the different was not significant statistically.

2.4.4 Improvement of cotton fiber with the genes related to hormones

It is greatly affect by the IAA and other plant growth regulators on the cotton fiber from the cell differentiation to fiber maturity. John (1999) transformed the *iaaM* and *iaaH* which related to the synthesis of IAA to cotton plant, the IAA content in the transgenic cotton plant were increased significantly, but the staple length, fiber strength and micronair were not improved significantly. While the same genes with fiber specific promoter were done by Southwest University, and the results showed that the ratio of fiber initiation cells in the transgenic cottonseed epidermis was increase by 10%, which lead to a significant increase of lint percentage of transgenic cotton (Xiao, 2010, personal communication).

2.4.5 Cloning of genes related to cotton fiber quality.

Due the important of fiber quality, many scientists in China endeavor to clone the gene for improvement of fiber quality，and about 40 genes related to fiber quality were cloned in recent years included *β-canaliculus protein*，*E6*，*GhbZIP*，*GhIAA16*, *ACC1* and etc (Chen et al, 1999). Some of them have been transformed in to upland cotton, either by over-expression or repressive, the results showed that most of the genes have some function to improve the fiber quality if the background receptor was poor (Li, 2010, personal communication).

Conclusion

China is a great country with a population of 1.4 billion and has a broad domestic market. Cotton and textile production should first guarantee the domestic consumption. China is characteristic of a dense population and scarcity of arable land, food is a big problem to Chinese people. Consequently, enough area of land must be allocated to produce grains, and the acreage planting cotton cannot be expanded anymore, while the demand for cotton product in China will be increasing

sequentially. Breeding for high yield, better quality, and disease and insect resistant cotton cultivars is the key step to solve this problem. Biotechnology especially the modern biotechnology such as gene engineering etc. plays an important role in cotton breeding. So the modern biotechnology in China will develop even faster than what we have already with the effects of the cotton scientists and increasing of research funds supported by national and local government, as well as the various corporations.

Acknowledgements

This work was financially supported by National Basic Research Program (973 Program. 2010CB126006), National Natural Science Foundation of China (306713), National key project for transgenic crop breeding (2011ZX08005-005)

Reference

An DC, Huang ZH (1997) The rise of biotechnology in China. *Biotechnology Bulletin* 1: 10-16.

Bao M, Zhang BJ (2009) The Establishment of shoot tip culture system of upland cotton Simian 3. *Anhui Agricultural Science Bulletin* 15: 44-45.

Bayley C, Trolinder N, Ray C, Morgan M, Quisenberry JE, Ow DW (1992) Engineering 2,4-D resistance into cotton. *Theoretical and Applied Genetics* 83: 645-649.

Beasley CA (1971) In vitro culture of fertilized cotton ovules. *Bioscience* 21: 906-907.

Cai YF, Ye PS, Jiang HZ, He HH, Tan YJ, Pei Y, Guo YL, Li MY, Hou L (2000) The preliminary study of anti-fungi genes introduced to cotton to develop transgenic cotton with high resistance to Verticillium wilt. *Southwest China Journal of Agricultural Sciences* 13: 45-49.

Cao JL, Zhang XL, Jin SX，Yang XY, Zhu HG, Fu LL (2008) An efficient culture system for synchronization control of somatic embryogenesis in Cotton (*Gossypium hirsutum* L). *Acta Agronomica Sinica* 34(2): 224-231.

Chen ZX, Danny JL, Fan YL, Li SJ, Zhao JX (1994) 2,4-D Resistant transgenic cotton plants produced by *agrobacterium*-mediated gene transfer. *Scientia Agricultura Sinica* 27(2): 31-37.

Chen ZX, Li SJ, Trolinder NL (1987) Cell suspension culture and plant regeneration from embryo of certain characteristics on cotton. *China Agricultural Science* 20(5): 6-11.

Cheng HG, Zhang R, Luo SP, Dai PH, Guo SD (2007) Expression of *EPSPS* gene from *Abutilon theophrasti* Medic in the *Pichia pastoris*. *Journal of Xinjiang Agricultural University* 30(4): 52-56.

Cui BM, Zhu JB, Xiao L, Wang AY, Zhu XX, Yue XX, Yue JH, Chen ZH (2002) Cotton (*Gossypium hirsutum*) transformed withβ-1,3-glucanase gene and chitinase gene. *Biotechnology* 12(4): 1-2.

Cui R, Xing GS, Zhou GK (2001) Biochemical basis of somatic embryogenesis. *Life Sciences* 12: 6-8.

Dou DL (2002) Genetic engineering cotton for resistance to Verticllium wilt and cloning of genes related to cotton defense response. A doctoral dissertation submitted to the Committee of Institute of Botany, the Chinese Academy of Sciences.

Feng R, Zhang BH, Zhang WS, Wang QL (1998) Genotype analysis in cotton tissue culture and plant regeneration. In: Larkin PJ (ed.) Agricultural Biotechnology: Laboratory, Field and Market. Proceedings of the 4th Asia-Pacific Conference on Agricultural Biotechnology, Darwin 13-16 July 1998. Canberra, UTC Publishing, p 161-163.

Firoozabady E, DeBoer DL (1993) Plant regeneration via somatic embryogenesis in many cultivars of cotton (*Gossypium hirsutum* L.). *In Vitro Cellular and Develmental Biology-plant* 29(4): 166-173.

Fu LL, Yang XY, Zhang XL, Wang ZW, Feng CH, Liu CX, Jiang PY, Zhang JL (2009) Regeneration and identification of interspecific asymmetric somatic hybrids obtained by donor-recipient fusion in cotton. *Chinese Science Bulletin* 54(15): 2219-2227.

Guo SD, Cui HZ, Xia LQ, Wu DL, Ni WC, Zhang ZL, Zhang BL, Xu YJ (1999) Study on bivalent insect-resistant GM cotton. *Scientia Agricultura Sinica* 32 (3): 1-7.

Guo SD, Zhang XM, Cui HZ, Xu QF, Ni WC, Zhang ZL, Zhang BL, Xu YJ (1999) Research on Aphid resistance and herbicide resistance of transgenic cotton. *Journal of Yunnan University* 21(3): 127-128.

Guo SD (1995) Engineering of insect-resistant plant with *bacillus thuringiensis* crystal protein genes. *Scientia Agricultura Sinica* 28 (5): 8-13.

Haigler CH, Singh B, Zhang DS, Hwang S, Wu CF, Cai WX, Hozain M, Kang W, Kiedaisch B, Strauss RE, Hequet EF, Wyatt BG, Jividen GM, Holaday AS (2007) Transgenic cotton over-producing spinach sucrose phosphate synthase showed enhanced leaf sucrose synthesis and improved fiber quality under controlled environmental conditions. *Plant Molecular Biology* 63: 815-832.

He JX, Liang ZL,Sun CW, Jiang RQ, Zhong WN (1991) Interspecific incompatibility in crosses between the cultivated and the wild species in *Gossypium*. *Acta Genetica Sinica* 18(2): l40-148.

He M, Zeng HY, Xu PL, Ye CM (2002) Cloning and Mutagenesis of the *aroA* Gene. *Acta Scientiarum Naturalium Universitatis Sunyatseni* 41(2): 76-79.

Huang QS, Liu X, Wang YQ, Wang DM, Yang KR, Li JP, Ge F (2004) Transformation of spideIt silk protein gene into the apical meristems of gosspium barbadens via particle bambardment. *Xinjiang Agricultural Science* 41(4): 248-250.

Jia SR, Guo SD, An DC (2001) Transgenic cotton. Beijing: Science Press.

Jia XH, Zhao GD (2004) Application and progress of utilizing high and new technique in cotton heredity breeding. *Jiangxi Cotton* 26(2): 3-7.

Jiang YR (2007) Studies on the method of genetic transformation and utilization of salt-tolerant and herbicide-resistant genes in cotton. PhD dissertation, Zhejiang University.

John ME (1999) Genetic engineering strategies for cotton fiber modifcantion. In: Basra AS, ed. Cotton Fibers: Developmental Biology, Auality Improvement, and Textile Processing. New York: Food Products Press, p 271-292.

Keller G, Spatola L, Mccabe D,Martinell B, Swain W, John ME (1997) Transgenic cotton resistant to herbicide bialaphos. *Transgenic Research* 6(6): 385-392.

Laurent F, Debrauwer L, Rathahao E, Scalla R (2000) 2,4-Dichlorophenoxyacetic acid metabolism in transgenic tolerant cotton (*Gossypium hirsutum*). *Agriculture Food Chemistry* 48(11): 5307-5311.

Lei KJ, Lu WW, Hong YZ, Liu ZD (2007) Cloning of the *Glyphosate N- acetyltransferase* Gene from Soil Total DNA and Its Characterization of Enzyme Activity. *Journal of Agricultural Biotechnology* 15(1): 153-156.

Li J, Liang A H, Wu J H (2009) Insect-resistant gene and its application in transgenic cotton. *Anhui Agricultural Science Bulletin* 15(7): 40-41.

Li RJ, Meng QY, Li SJ (1995) Plant regeneration from protoplasts derived from cell suspensions on long-staple cotton in Xinjiang. *Chinese Journal of Cell Biology* 1-27.

Li X, Wang XD, Zhao X, Dutt Y (2004) Improvement of cotton fiber quality by transforming the *acsA* and *acsB* genes into *Cossypium hirsutum* L. by means of vacuum infiltration. *Plant Cell Reports* 22: 691-697.

Lian LJ, Lu SL, Li RZ, Zhang KW (2008) Observation on the agronomic traits of *BetA/Als* transgenic cotton. *Acta Gossypii Sinica* 20(6): 447-451.

Liu GY (1983) The occurrence of cotton embryoid. *China Cotton* 10: 24-25.

Liu XJ, Liu YH, Wang ZX, Wang XJ, Zhang YQ (2007) Generation of Glyphosate-tolerant Transgenic Tobacco and Cotton by Transformation with a 5-enolpyruvyl-shikimate-3-phosphate Synthase (EPSPS) Gene. *Journal of Agricultural Biotechnology* 15(6): 958-963.

Liu XJ (2002) Cloning and analyzing of EPSP synthase from *Orychophragmus violaceus*. PhD dissertation, Sichuan University.

Liu Z, Guo W Z, Zhu X F, Zhu Z, Zhang TZ (2004) Stable inheritance and expression of *Bt* and *GNA* resistance genes in transgenic cotton line. *Acta Agronomica Sinica* 30(1): 6-10.

Liu Z (2004) Cloning a novel 5-enolpyruvylshikimate-3-phosphate synthase gene conferring increased glyphosate tolerance from *Halomonas variabilis* and its expression in *Escherichia coli* and its glyphosate-tolerant mechanism. PhD dissertation, Sichuan University.

Lu ZQ, Liang H, Hu GX (2008) Research on bromoxynil toxicology. *Chinese Journal of Public Health* 24(8): 1014-1015.

Lu ZX, Xia ZA (1991) Research on tissue culture and protoplast culture in G. *davidsonii*. *Acta Plant Sinica* 33(2): 98-103.

Lu ZZ, Zhang CX, Fu JF, Li MH, Li GJ (2009) Molecular basis of resistance to bensulfuron- methyl in *Monochoria korsakowii*. *Scientia Agricultura Sinica* 42(10): 3516-3521.

Lv FB, Zhang XL, Liu JL (1999) Protoplast culture and plant regeneration in upland cotton. *Acta Agriculturac Boreali Sinica* 14(1): 73-78.

Mao YB, Cai WJ, Wang JW, Hong CJ, Tao XY, Wang LJ, Huang YP, Chen XY (2007) Silencing a cotton bollworm p450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nature Biotechnology* 25(11): 1307-1313.

Meng QY, Sun Y , Li RJ (1996) Culture and regeneration from protoplasts derived from cell suspensions on long-staple cotton in Xinjiang. *Science of Xinjiang agriculture* 6: 249-251.

Pan G (2005) Two types of herbicide resistant rice gene isolation and cloning of *cyp81a6*. PhD dissertation, Zhejiang University.

Qin YH, Liu JY (2006) Cotton tissue culture and plant regeneration. *Molecular Plant Breeding* 4(4): 583-592.

Qin YH, Qiao ZX, Liu JY (2007) Application of genetic transformation in cotton breeding. *Cotton Science* 19(6): 482-488.

Qu ZL, Zhong NQ, Wang HY, Chen AP, Jian GL, Xia GX (2006) Ectopic expresion of the cotton non-symbiotic hemoglobin gene *GhHbd1* triggers defense responses and increases disease tolerance in Arabidopsis. *Plant and Cell Physiology* 47(8): 1058-1068.

Sha JY (2008) Cloning and expression of the *EPSPS* in *Pseudomonas stutzeri* A1501. PhD dissertation, Sichuan Normal University.

She JM, Wu JY, Wang HB, Zhou HY, Chen ZX, Li SJ, Yue JX (1989) Somatic embryogenesis and plant regeneration with protoplast culture of cotton. *Jiangsu Journal of Agricultural Sciences* 5: 54-60.

Song GS, Feng DJ, Wei XL, Tang JB, Zhu Z (2007) Isolation and functional analysis of rice acetolactate -synthase (*ALS*). *Journal of Agricultural Science and Technology* 9(3): 66-72.

Su SQ (2009) New development of non-glyphosate herbicide-resistant crops. *Agrochemicals* 48 (12): 859-863.

Sun SJ, Li SG, Zhu SW, Sun JS, Lu Y (2005) Plant Genetic Transformation methods and their application in cotton breeding for cultivars improvement. *Molecular Plant Breeding* 3(2): 233-239.

Sun YQ, Zhang XL, Huang C, Nie YC, Guo XP (2005) Factors influencing in vitro regeneration from prototlasts of wild cotton (*G. klotzschianum* A) and RAPD analysis of regenerated plantlets. *Plant Growth Regulation* 46: 79-86.

Sun YQ, Zhang XL, Huang C, Nie YC, Guo XP (2005) Plant regeneration via somatic embryogenesis from protoplasts of six explants in Coker201 (*Gossypium hirsutum* L.). *Plant Cell, Tissue and Organ Culture* 82: 309-315.

Sun YQ, Zhang XL, Nie YC, Guo XP, Jin SX, Liang SG (2004) Production and characterization of somatic hybrids between upland cotton (*Gossypium hirsutum*) and wild cotton (*Gossypium klotzsehianum* Anderss) via electrofusion. *Theoretical and Applied Genetics* 109: 472-479.

Tan XL, Qian YQ (1988) Effects of different explant sources and culture conditions on quasi plant regeneration on cotton. *Acta genetica Sinica* 5: 81-87.

Tang H (2008) Ultrastructure changes of tobacco leaf after glyphosate treatment and *EPSPS* Cloning. PhD dissertation, Guangxi University.

Tong XH, Wu YX, Zhu SJ (2009) Cloning of 5-enolpyruvylshikimate-3-phosphate synthase genes *EPSPS* from the upland cotton and its expression in different cotton tissues. *Acta Gossypii Sinica* 21(4): 259-264.

Tong XH, Wu YX, Zhu SJ (2009) Cloning of 5-enolpyruvylshikimate-3-phosphate synthase genes *EPSPS* from the upland cotton and its expression in different cotton tissues. *Cotton Science* 4: 259-264.

Wang J, Feng HW, Zhao GZ, Chen XY (2002) The study of breeding high quality cotton cultivation by transferring animal keratin. *China Cotton* 29(10): 8-9.

Wang JZ, Li KQ, Zhang DL, Hao LF, Guo DZ, Liu QH, Xiao YP, Zhang SF (1994) Variation and high frequnency somatic embryogensis of embryogenic callus Gossypium hirsutum. *Acta Plant Sinica* 36(5): 331-338.

Wang PZ, Zhang SF, Hu ZH (1998) Isolation, culture and plant regeneration of protoplasts from an embryogenic callus tissue of *Gossypium hirsutum*. *Acta Botanica Sinica* 40(3): 234-240.

Wang QL, Zhang BH, Guo TL, Xu XR (2001) Inheritance of exogenous genes in transgenic cotton. *Life Science Research* 5(4): 345-350.

Wang W, Zhang X L, Liu J L (1992) Effect of Brassinolide on somatic embryogenesis of *Gossypium hirsutum*. *Plant Physiology Communications* 28(1): 15-18.

Wang ZX (2000) The study of breeding transgenic disease resistant cotton and insect resistant potato lines. Postdoctoral Research Report in Institute of Botany, the Chinese Academy of Sciences.

Wu JH, Luo XL, Wang ZA, Tian YC, Liang AH, Sun Y (2008) Transgenic cotton expressing synthesized scorpion insect toxin *AaHIT* gene confers enhanced resistance to cotton bollworm (*Heliothis armigera*) larvae. *Biotechnology Letters* 30(3): 547-554.

Wu JH, Tian YC, Luo XL, Guo HN, Shi YJ, Chen XY, Jia YT, Xiao JL Zhang XL (2003) Selection of homozygous cotton lines transformed with two types of insect-resistant genes. *Scientia Agricultura Sinica* 36(6): 651-656.

Wu JY, Chen S (1988) Long-term effects of hormone and directional selection on cotton somatic embryogenesis ability to maintain. *Jiangsu Journal of Agricultural Science* 4: 9-15.

Wu JY, Zhu WM, She JM, Cai XN (1994) Culture of apical meristems of *Gossypium hirsutum* and its application in gene introduction. *Acta Gossypii Sinica* 6(2): 89-92.

Wu W, Zhang Y, Zhu SJ, Tong XH (2006) Studies on the judging technique and controlling results for the seed purity of upland hybrid cotton. *Acta Gossypii Sinica* 18(1): 27-31.

Wu X, Li YE, Wang JJ, ShangGuan XX (2006) Genetic characters of transgenic upland cotton cultivars carrying triple genes (*Bt+CpTI+GNA*). *Chinese Journal of Eco-Agriculture* 14(1): 21-23.

Xiang WS, Li C, Tao B, Wang XJ, Li JP, Su SQ, Zhang WJ (2001) Studies on molecular mechanism of glyphosate tolerance in bean. *Chinese Journal of Pesticide Science* 3(1): 23-29.

Xie DX, Fan YL, Ni WC, Huang JQ (1991) *Bacillus thuringinesis* insecticidal crystal protein gene into cotton to obtain transgenic plants. *Scientia Sinica* (4): 367-373.

Xie LX, Li YF, Xu PL (2004) Glyphosate-resistant cotton (*Gossypium hirsutum* L.) transformed with *aroAM12* gene via *Agrobacterium tumefaciens*. *Journal of Plant Physiology and Molecular Biology* 30(2): 173-178.

Xu JW, Feng DJ, Li XG, Chang TJ, Zhu Z (2002) Cloning of genomic DNA of rice 5-enolpyruvylshikimate-3-phosphate synthase genes and chromosomal localization of the gene. *Science in China (series C)* 45(3): 251-259.

Ye XH, Mao YX, Mo ZL, Hao B, Zou YX, Li J (2008) Screening *aroA* gene from *Vibrio anguillarum* fosmid library. *Chinese High Technology Letters* 18(9): 985-990.

Yi G (2007) Cloning, functional characterization and spectroscopy analysis of the *Dunaliella salina* 5-enolpyruvylshikimate-3-phosphate synthase gene. PhD dissertation, Sichuan University.

Yi L (2005) Cloning and analyzing of EPSP synthase from *Brachypodium distachyon*. PhD dissertation, Sichuan Agriculture University.

You DH, Qian Y, Wang JM, Guo JY, Yang Y, Li XF (2004) The cloning and analysis of the cDNA of *EPSP*s gene from *Brassica campestris*. *Journal of Sichuan University (Natural Science Edition)* 41(3): 661-664.

You DH (2004) The cloning, analysis of the full-length cDNA of *EPSP*s from *Brassica campestris* and its expression in *Escherichia coli*. PhD dissertation, Sichuan University.

Yu HY (2006) Characteristics of Mycoherbicide *Sclerotinia sclerotiorum* and cloning, expression of the corresponding Gene. PhD dissertation, Harbin Institute of Technology.

Yu Y, Liu CL, Ma ZY, Li FG (2003) The culture of cotton apical meristem and its application in particle bombardment transformation, *Acta Gossypii Sinica* 15(5): 274-278.

Yue JH, Zhu JB, Cui BM, Zhu XX, Wang AY, Chen ZH, Liu GY, Jian GL, Wu G (2002) Breeding fusarium wilt and verticillium wilt of cotton-resistant strains with transgene technique. *Jounal of Shihezi University (Natural Science)* 6(3): 173-178.

Zeng Q, Xiao CH, Zhang XL, Li JB, He SJ (2001) Preliminary report of breeding for the herbicide-resistant cotton. *Crop Research* 15(4):25-28.

Zhang BH, Feng R, Li F L, Li FG (1995) Anther culture and plant regeneration in *G. klotschianum* (D3-k). *Chinese Science Bulletin* 40(17): 1612-1614.

Zhang BH, Feng R, Li XH, Li FL (1996) Anther culture and plant regeneration of cotton (*Gossypium klotzschianum* Anderss). *Chinese Science Bulletin* 41:145-148

Zhang BH, Guo TL, Wang QL (2000) Inheritance and segregation of transgenic cotton. *Life Science Research* 4(2): 136-142.

Zhang BH, Li FL, Li XL, Li FG, Liu F, Yao CB, Liu CL (1999) Effect of genotype on cotton anther response in *in vitro* culture. *Acta Gossypii Sinica* 11(2): 92-99.

Zhang BH, Li XL, Li FG (1995) Cotton situation and prospects of tissue culture. *Plant Physiology Communications* 31(1): 56-61.

Zhang BH, Liu F, Liu ZH, Wang HM, Yao CB (2000) Hormone regulation on cotton somatic embryogenesis. *Acta Gossypii Sinica* 12(1): 17-21.

Zhang BH, Liu F, Yao CB, Gong WK, Liu ZH, Wang HM, Liu YL (2001) Obtained callus resistant to kanamycin from cotton transformation by *Agrobacterium tumefaciens* with go gene. *Cotton Science* 13(2): 78-81.

Zhang BH, Feng R, Liu F, Wang QL (2001) High frequency somatic embryogenesis and plant regeneration of an elite Chinese cotton variety. *Botanical Bulletin of Academia Sinica* 42:9-16

Zhang BH, Feng R, Liu F, Yao CB (1999) Direct induction of cotton somatic embryogenesis. *Chinese Science Bulletin* 44:766-767

Zhang BH, Guo TL, Wang QL (2000) Inheritance and segregation of exogenous genes in transgenic cotton. *Journal of Genetics* 79:71-75

Zhang BH, Liu F, Wang QL (2000) Germination of somatic embryos and plant recovery in cotton (*Gossypium hirsutum* L.). *Phyton-International Journal of Experimental Botany* 68:39-46

Zhang BH, Liu F, Yao CB (2000) Plant regeneration via somatic embryogenesis in cotton. *Plant Cell Tissue and Organ Culture* 60:89-94

Zhang BH, Liu F, Yao CB, Wang KB (2000) Recent progress in cotton biotechnology and genetic engineering in China. *Current Science* 79:37-44

Zhang BH, Wang QL, Liu F, Wang KB, Frazier TP (2009) Highly efficient plant regeneration through somatic embryogenesis in 20 elite commercial cotton (*Gossypium hirsutum* L.) cultivars. *Plant Omics* 2:259-268

Zhang BH, Liu F, Yao CB, Li FG, Liu CL, Li FG (1998a) Effects of sugar sources on cotton anther culture. I: Efects of sugar sources on induction of cotton anther callus. *Acta Agriculturae Universitatis Jiangxiensis* 20(3): 311-316.

Zhang BH, Liu F, Yao CB, Li FG, Liu CL, Li FG (1998b) Effects of sugar sources on cotton anther culture. II. Effects of sugar sources on anther callus growth and embryogenesis. *Acta Agriculturae Universitatis Jiangxiensis* 20(3): 317-321.

Zhang J M, Sun J Z, Liu J L, Zhang XL (1994) Studies on Plant regeneration from somatic cells and transferring technique of plants in upland cotton. *Acta Agronomica Sinica* 20(2): 210-216.

Zhang JM, Sun JZ, Liu JL (1991) Somatic embryogenesis and plant regeneration in upland cotton. *Acta genetica Sinica* 18 (5): 461-467.

Zhang JW, Xiong CR, Li JW, Wang WT, Meng YX, Chen ZH (2006) Cloning of bromoxynil- specific nitrilase gene (*bxn*) from *Klebsiella ozaenae* and expression in prokaryotic cells. *Acta Prataculturae Sinica* 15(6): 87-92.

Zhang L (2006) Fine Mapping and Cloning of the Rice Bentazon Resistance Gene *Bel*. PhD dissertation, Huazhong Agriculture University.

Zhang XL, Zhang J M, Yao MJ, Sun JZ, Liu JL (1993) Somatic cell culture and basic research of cotton. *Journal of Huazhong Agricultural University* 12(5): 421-426.

Zhang Y, Lu XJ, Hu CZ, Han WB, Ting DS (2003) The research, application and prospect of transgenic technology in China. *Anhui Agricultural Science Bulletin* 9(6): 124-126.

Zhang Z, Ye GY, Hu C (2003) Research progress in insecticidal activity of agglutinin from *Galanthus nivalis*. *Chinese Journal of Biological Control* 19(4): 197-201.

Zhang ZL, Liu ZL, Zhou BL, Chen S, Zhang XG (2004) Transgenic cotton (*Gossypium hirsutum* L.) with strength-enhanced fiber expressing rabbit keratin gene. *Cotton Science* 16(2): 72- 76.

Zhao FY, Xie LX, Tian YC, Xu PL (2005) Glyphosate-resistant and bollworm-resistant transgenic cotton plants with the *aroAM12* and *Bts1m* genes. *Acta Agronomic Sinica* 31(1):108-113.

Zhao T (2008) Identification of a Gene Encoding EPSPS with resistance of glyphosate and development of transgenic glyphosate-resistant rice plants. PhD dissertation, Zhejiang University.

Zhen XH, Li YZ (2004) Ultrastructural changes and location of beta-1,3-glucanase in resistant and susceptible cotton callus cells in response to treatment with toxin of *Verticillium dahliae* and salicylic acid. *Journal of Plant Physiology* 161(12): 1367-1377.

Zhou XF, Jin SX, Li X, Li BC, Lian WR, Ning XZ, Liu L (2009) Multiple-shoot induction and genetic transformation of island cotton (*Gossypium barbadense* L.) in Xinjiang. *Cotton Science* 21(4): 324-329.

Zhou XH, Huang LH, Jiang X, Li YQ, Zhang XW (2009) Cloning and prokaryotic expression of EPSP synthase gene cDNA of *Allium macrostemon Bunge*. *Scientia Agricultura Sinica* 42(7): 2297-2304.

Zhou XJ, Lu S, Xu YH, Wang JW, Chen XY (2002) A cotton cDNA (*GaPR-10*) encoding a pathogenesis- related 10 protein with in vitro ribonuclease activity. *Plant Science* 162(4): 629-636.

Zhu SJ, Wang JE, Yu ZH, Ji DF (2003) A cotton mutant resist to glyphosate and its utilization in cotton heterosis. *Acta Gossypii Sinica* 15(4): 227-230.

Zhu Y (2002) Identification of glyphosate-tolerant psedomonas fluorescens strain G2 from extremely polluted environment and cloning of its *EPSP* synthase Gene. PhD dissertation, Chinese Academy of Agricultural Sciences.

Zuo KJ, Wu F, Tang K-X，Sun X-F (2002) Root full-length cDNA library construction of seaisland cotton variety 7124 induced by *Verticillium dahliae* Kleb. *Cotton Science* 14(5): 291-294.