Peanut Aflatoxin and Genomics Research in China: Progress and Perspectives

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ABSTRACT

Peanut is an important oil and food crop in China with a unique role in agricultural development and food security. Aflatoxin contamination in peanut, normally more serious in southern parts of the country, is a crucial factor affecting sustainable development of the peanut industry. Extensive efforts have been made at several institutions in China for aflatoxin management and related genomics research. Several local peanut germplasm lines have been identified as resistant to seed infection by *Aspergillus flavus* or aflatoxin production. Two AFLP markers have been identified that are linked with resistance to seed invasion and one was converted into a SCAR marker for more efficient breeding application. Several new peanut cultivars with improved productivity and possessing resistance to aflatoxin contamination are extensively used in production. Integrated management approaches have been recommended to farmers based on agro-ecological characteristics in different regions. More recently, molecular techniques have been extensively used in genetic diversity assessment, investigation of genetic relationships among different germplasm groups, marker development, identification of interspecific genome introgression, gene cloning and function analysis, and genetic transformation of important traits concerning productivity, quality and food safety of peanut. Special emphasis has been placed on resistance to aflatoxin, bacterial wilt, foliar diseases and fatty acid desaturase. Perspectives of peanut genetic improvement and further research priorities are also discussed.

Key Words: Peanut, aflatoxin, resistance, genomics, genetic transformation.

Peanut or groundnut (*Arachis hypogaea* L.) has been an important oil and food crop in China, and extensively grown in most provinces. Currently, China is the largest country worldwide in terms of annual peanut production, consumption, and international trade (Liao, 2003). During 2002–2006, the average annual sowing area under the crop was 4.93 million ha and the production was 14.32 million t. (FAO, 2002–2006). About 55% of the production is crushed for oil, 30% used for direct consumption or for various food processing, and 7% exported. Among the major oilseed crops in the country, peanut has obvious advantages in terms of crop yield, oil yield and crop value to the farmer as compared with soybean and rapeseed. However, peanut supply in China is still insufficient because of increased demand for both peanut oil and edible peanuts. Further increases of peanut production will be needed to meet the increased market demands.

With the growth of peanut production and human consumption, aflatoxin contamination in peanut products has attracted more concern. Aflatoxins are secondary metabolites of *Aspergillus flavus* (Link) and *A. parasiticus* (Speare) (Mehan *et al.*, 1991), and are among the most carcenogenic mycotoxins in nature. Peanut is a crop highly susceptible to *Aspergillus* infection and consequent aflatoxin contamination. Traditionally, Chinese peanuts for the export trade were mostly from the northern growing regions while those from the central and southern regions were rarely exported due to higher aflatoxin contamination (Sun, 1998; Liao, 2003). In many places, aflatoxin could be detected in nearly 50% of the samples of peanuts or peanut products (Xiao, 1996). Obviously, the warm climate in the subtropical regions encourages aflatoxin contamination (Liao, 2003). Moreover, the expanse of the peanut industry also worsens the contamination problems because limited facilities are available for proper and timely treatment of peanuts during harvesting, storing, and processing. Therefore, even in the northern growing regions aflatoxin contamination has become problematic for the industry during recent years. Correlation of aflatoxin exposure with higher incidence of human liver cancer has been reported in certain locations in southern parts of the country (Yin, 1983; Zhang and Huang, 1992). The social and economical

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importance of aflatoxin contamination in the food and and animal feed chains is widely acknowledged (Liao and Holbrook, 2006). Management of aflatoxin contamination is crucial for sustainable development of the peanut industry.

Genetic improvement for host resistance in peanut to seed colonization by the aflatoxigenic fungi species such as *A. flavus* and *A. parasiticus* and aflatoxin formation has been regarded as a valuable approach for reducing contamination in peanut even though genetic resistance is unlikely to completely resolve the problem (Liao and Holbrook, 2006; Mehan, 1986; Mehan *et al.*, 1991). Genomics is an important research area crucial for crop improvement, but at present the genomics research in peanut is not developed enough to be useful in solving the aflatoxin problem. In China, several institutions and universities are involved in research on aflatoxin and genomics. This paper reviews the recent research progress on peanut aflatoxin management and related genomics towards addressing food safety and enhancing productivity.

Aflatoxin and Host Plant Resistance.

Recent status of aflatoxin contamination. Aflatoxin contamination in peanut could be influenced by several factors (Hill *et al.*, 1983; Liao, 2003). As the natural conditions and agricultural systems in different locations in China vary dramatically, aflatoxin contamination extent in peanut could be very different across locations and seasons. Limited investigation on the toxigenicity of strains of *A. flavus* from various locations indicated that the most toxigenic strains are from Guangxi in South China and those with the least toxigenicity were from Gansu in Northwest China (Xiao, 1996). Zhuang (pers. comm.) also observed differentiation among *A. flavus* strains in toxigenicity collected from Fujian Province. In general, aflatoxin contamination in peanut has been more serious in southern parts of the country versus the northern producing regions (Liao, 2003). In particular, peanut oil was more contaminated than peanut food, especially in the southern regions. Recently, Sun (pers. comm.) investigated aflatoxin contamination in peanuts in different locations of Shandong Province and concluded that less contamination was found in the samples. A survey of aflatoxin contamination of peanut and peanut products for export trade from northern China also reported low contamination. In a recent investigation, aflatoxin B_1 was detected in 13% of 76 samples collected, and about 5% of the samples had aflatoxin B_1 concentrations higher than the European Union permission standard ($\leq$2 μg/kg) (Zhang, unpubl. data). Most contaminated samples were shelled peanuts rather than unshelled pods, indicating that the shelling processing may have at least partially caused the problem (e.g., a small scale sheller may have rehydrated the pods during processing). However, Xu *et al.* (2006) reported that aflatoxin B_1 was detected in 80% of peanut samples collected from local markets in Nanjing, ranging from 0 to 58.25 μg/kg with an average of 0.72 μg/kg. Contamination of peanuts in the small scale domestic markets has been relatively higher than those being exported (Liao, 2003). A better system is needed for monitoring the contamination in different locations with an emphasis in the south.

Screening germplasm for resistance to aflatoxin. More than 7000 accessions of cultivated peanut have been assembled in China (Liao and Holbrook, 2006). Among these peanut accessions, about 2400 have been tested for their reaction to infection by *Aspergillus* or aflatoxin production (Jiang *et al.*, 2002, 2005; Xiao *et al.*, 1999a). Modifications of screening techniques for resistance to aflatoxin formation have been used in breeding populations (Xiao *et al*., 1999b). During the past two decades, extensive screening work has been conducted at the Oil Crops Res. Instit.(OCRI) of Chinese Acad. of Agric. Sci. (CAAS) in Wuhan, the Crop Res. Instit. (CRI) of Guangdong Acad. of Agric. Sci. in Guangzhou, the Cash Crops Res. Instit.(CCRI) of Guangxi Acad. of Agric. Sci. in Nanning, the Fujian Agric. and Forestry Univ. (FAFU) in Fuzhou, and the Shandong Peanut Res. Instit. (SPRI) in Qingdao. Twenty eight peanut genotypes were identified with some resistance, including the confirmation of several previously reported in the USA and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India (Jiang *et al*., 2005; Mehan, 1986; Mixon, 1986; Wang *et al.*, 2003, 2004; Xiao *et al*., 1999a). Most genotypes resistant to seed infection and colonization belong to *A. hypogaea* subsp. *fastigiata* var. *vulgaris* and have small seeds (Jiang *et al.*, 2005). Xiao *et al*. (1999a) reported that the two lines N1211 and N1322 had the lowest aflatoxin production under artificial inoculation conditions among 1517 peanut accessions screened. They also found that the resistance in these two genotypes was more stable across seasons than other entries tested. Lei *et al*. (2004) and Liao *et al.* (2003) reported that Taishan Zhengzhu and Zhonghua 6 (with resistance to bacterial wilt) were relatively resistant to aflatoxin formation under artificial inoculation conditions. The hybrid progenies of entries with bacterial wilt resistance were relatively more resistant to aflatoxin formation than progenies from parents susceptible to bacterial wilt (Wang *et al.*, unpubl. data). Wang *et al.* (2003)

found that several peanut lines showed resistance to aflatoxin formation *in vitro* after having been stored under conventional conditions for 4 yr such that the seed were no longer viable . They indicated that the durability of the resistance in these lines might be related to storage proteins. Jiang *et al.* (2006b) investigated persistence of seed coat resistance during storage and impact of intact testa on aflatoxin contamination among peanut genotypes with diverse reactions to infection of *A. flavus*. The results indicated that the resistance to *A. flavus* could persist for at least 7 mo in resistant germplasm lines under conventional storage conditions in Wuhan. No significant difference between the seed samples stored for 7 mo and freshly harvested ones were observed in terms of *in vitro* invasion ratio and infection index. However, the resistance could decline or be lost after 9 mo storage. The quick decline of the resistance was due to the change of environmental temperature. Peanut genotypes with high oil percentage were generally more susceptible to *A. flavus* invasion. Two genotypes with resistance and good seed quality, G845 and G8, were identified (Jiang *et al*., 2006b). Several resistant germplasm lines have also been identified in Guangdong (Li, 2006). Mechanisms of resistance to aflatoxin contamination also have been investigated (Liang *et al.*, 2002; 2005).

Genetic markers for resistance to aflatoxin. Even though peanut genotypes have been reported as resistant to seed infection by *Aspergillus*, the progress in breeding for resistance has been slow due to various reasons, including the lack of a cost-effective method to identify resistance in segregating progenies. Hence, there is a need to develop a rapid and reliable indirect screening method for selection. Molecular markers could be an important selection tool for crop breeding. Lei *et al.* (2005a) reported two DNA markers closely linked with resistance to *A. flavus* infection using the Amplified Fragment Length Polymorphism (AFLP) technique based on bulked segregate populations derived from F_2 progenies of Zhonghua 5 $\times$ J11 (J11 is resistant to seed infection). The two specific fragments linked with seed infection resistance were E45/M53-440 (about 440 bps) and E44/M53-520 (520 bps). The linkage distance of the former marker to the resistant gene was 6.6 cM, while that of the latter was 8.8 cM. Twenty peanut genotypes with resistance to infection of *A. flavus* and seven susceptible genotypes were used to verify the reliability of the resistance markers, and high correlations between the molecular markers and the resistance screening were observed. To provide a more effective molecular assisted selection tool for breeding, the E45/M53-440 band was isolated from the PAGE gel and sequenced. According to the sequencing results, four primer pairs were designed for SCAR (Sequence Characterized Amplified Region) analysis, from which the primers D1 (GGATGGCTAGATTATTGCCGTAT) and D2 (AATTCATGTCCCTAGTGGCTGAT) showed correlations to the screening results and AFLP analysis. The amplified 412bp products were sequenced and corresponded to the sequence of marker E45/M53-440. Hence, it was confirmed that the AFLP marker E45/M53-448 was successfully converted to a SCAR marker, named as AFs-412. For confirming potential application of the AFLP and SCAR markers in the other resistant genotypes, 20 peanut accessions with resistance to seed infection by *A. flavus* and seven susceptible ones were selected for AFLP and SCAR analysis. The specific band was found in 16 of 20 resistant genotypes whereas it was not found in any of the susceptible ones (Lei *et al.*, 2006). The marker E45/M53-440 and AFs-412 could be used for a marker assisted selection breeding program.

Breeding for resistance. Genetic enhancement for resistance to infection of mycotoxin-producing *Aspergillus* and to aflatoxin formation in peanut seeds has been recognized as a valuable approach for reducing contamination, even though the available resistance may not completely resolve the problem. Progress has been made on selecting resistant germplasm and cultivars in China. Several elite peanut germplasm lines with resistance to seed invasion and aflatoxin formation have been identified and used in breeding.

At OCRI, a new cultivar, Zhonghua 6, with resistance to aflatoxin formation and bacterial wilt has been developed (Liao, 2003). In seed inoculation tests under laboratory conditions across years, Zhonghua 6 had a reduced aflatoxin content which was only 10% or less of the susceptible control. To date, it has the highest level of resistance to aflatoxin formation among the improved peanut cultivars. Very low natural contamination of aflatoxin in Zhonghua 6 in farmers' fields has also been observed. This cultivar has been widely cultivated in eastern Hubei Province in central China where bacterial wilt is a major problem. Several breeding lines involving parents resistant to seed invasion or aflatoxin formation have been developed for further selection in both OCRI and other institutions.

Yueyou 9 is a new peanut cultivar with resistance to seed invasion of *A. flavus*. It was developed in the CRI of Guangdong Acad. of Agric. Sci. (Li, 2006). Under laboratory conditions, the seed infection ratio of Yueyou 9 was less than

19%, which is regarded as highly resistant, while the susceptible control was 100%. It has been released in Guangdong Province. Field trials are underway for investigating the role of resistance in reducing natural contamination in farmers' fields.

Kanghuang No 1 is an improved peanut cultivar with aflatoxin resistance released in Fujian Province in 2003. It is also moderately resistant to bacterial wilt. It was developed from the cross combination Yueyou 169 × ICGV94449A (Tang, pers. comm.).

Because nearly 60% of the peanuts are used for oil extraction (Liao, 2003), integration of aflatoxin resistance with high oil content in high yielding genetic backgrounds is of high priority. Unfortunately, no high oil germplasm line has been identified as resistant to aflatoxin and no combination of aflatoxin resistance with high oil content has been selected among numerous hybrid progenies in several breeding programs. This illustrates the need for more diverse germplasm with aflatoxin resistance for further varietal improvement of peanut.

Management of aflatoxin. Integrated management approaches are necessary for any production and processing system for minimizing aflatoxin contamination in peanut. In northern parts of China, preharvest contamination is generally less serious and more efforts are made to prevent contamination during harvest and storage (Liao, 2003). Rehydration during shelling is believed a key factor contributing to aflatoxin contamination even in seed lots being exported. In the central and southern parts of the country, the situation is variable and more complex. In upland fields, drought stress during the late growing stage in combination with high temperature may occur and lead to preharvest contamination. Various foliar diseases and soil-borne diseases could also worsen the contamination. Integrated management approaches including using peanut cultivars with resistance to aflatoxin contamination, drought tolerance, resistance or tolerance to other diseases and pests, proper irrigation, control of diseases and pests, timely harvest, quick drying after harvest, and control of conditions of storage and processing have been generally recommended to farmers and extension systems (Liao, 2003).

More recently, more farmers are using mulch cultivation technology in both paddy rice and upland fields for improving peanut yield in central and southern parts of China. As mulch could increase soil temperature and may cause heat stress during the maturing period, it may increase aflatoxin contamination. On the other hand, the drought stress could be alleviated by mulch and thus the preharvest contamination could be reduced. Therefore, the impact of mulch on aflatoxin contamination may be different at various locations. A recent investigation indicated that peanut seeds harvested from mulched cultivation supported less aflatoxin production in *in vitro* inoculation tests (Wang, unpubl. data), indicating that mulch cultivation could reduce the risk of contamination of peanuts if end-season drought stress exists. Based on this result, the mulch approach is highly recommended to farmers.

Peanut Genomics and Related Research.

Genetic diversity assessment of peanut germplasm by molecular markers. There have been several reports on peanut genetic diversity at the molecular level (Han *et al.*, 2004; He and Prakash, 1997; Hopinks 1999; Lei *et al.*, 2005b; Wang *et al.*, 2005b; Jiang *et al.*, 2006a). Chen *et al.* (2003) reported genetic diversity of 32 peanut cultivars from different areas in China by AFLP fingerprinting analysis. The results showed that the similarity of all the plant samples was 35%, and the samples could further be divided into three groups by 45% similarity, indicating considerable diversity exists among these peanut genotypes.

Tang *et al.* (2007) assessed the diversity among different botanical types of the cultivated peanut through Single Sequence Repeat (SSR) profiling. They concluded that SSR markers are a useful tool for peanut because each botanical type of *A. hypogaea* could be further divided into subclusters based on genetic distance. Tang *et al.* (2007) also compared the effectiveness of SSR and AFLP markers and found that the clusters were mostly consistent with the current classification system based on morphological differentiation of *A.hypogaea*. Based on the genetic similarity revealed by SSR and AFLP markers, the diploid species, *A. duranensis* Krapov. and W.C. Gregory, was elucidated as one ancestor of *A. hypogaea*. Variety *hirsuta* was considered as the most primitive botanical type and var. *vulgaris* the most advanced type. Among the wild related species in genus *Arachis*, section *Procumbentes* was closer to section *Arachis* than other sections, and followed by *Heteranthae, Rhizomatosae*, *Extranervosae*, and *Caulorrhizae* (Tang *et al.*, 2007). Jiang *et al.* (2006a) assessed genetic diversity of peanut genotypes with resistance to bacterial wilt with AFLP and SSR markers. They found the clusters generated through SSR profiling were more consistent with the morphological classification than through AFLP profiling. Zhang *et al.* (2006) also reported the effectiveness of SSR markers in identifying relationships among improved peanut cultivars.

Molecular marker development. Gao *et al.* (2003) reported methods for developing SSR markers in peanut. Wang *et al.* (2005b) tried to identify molecular polymorphism in peanut using the RGA (resistance gene analog) approach but the technology still needs refinement. Using hybridization enrichment, library construction along with the published SSR markers, Huang *et al.* (2006) found 44 polymorphic SSR markers effective to distinguish 12 peanut lines. Hong *et al.* (2007) reported an SSR marker (PM93/630-600) for deep purple testa color in peanut. Jiang *et al.* (2007) identified two SSR markers for resistance to bacterial wilt in peanut based on the results through resistance evaluation of F_6 and F_7 recombinant inbred lines (RILs) and DNA markers. They developed a map including eight linkage groups covering a distance of 603.9 cM containing 29 markers (28 SSR markers and one phenotypic marker). Xia *et al.* (2007) identified three AFLP markers linked with resistance to late leaf spot (LLS) in peanut. They found that the high level of resistance to LLS in an interspecific hybrid derivative, ICGV 86699 (*A.batizocoi* Krapov. and W.C. Gregory and *A.duranensis* Krapov. and W.C. Gregory were in the pedigree), was controlled by a single recessive gene based on resistance identification and analysis of segregation in the F_2 population of Zhonghua 5 $\times$ ICGV86699. The three AFLP markers, E35/M51, E37/M48 and E41/M47, were also linked with each other on the same linkage group. The three markers could be detected in many interspecific hybrid derivatives with LLS resistance regardless of the cultivated parents, but were absent in the cultivated genotypes of valencia type with LLS resistance, indicating the genetic background of resistance in the wild related species was different from that of the cultivated species. Two AFLP markers (M3L3-460 and M8L8-645) for rust resistance in ICGV86699 have also been identified by Hou *et al.* (2007). It is generally believed that SSR markers are reliable (Furguson *et al.*, 2004; Hopkins *et al.*, 1999), and thus are commonly used for map based gene cloning in model plants such as *Arabidopsis* and rice. Due to the lack of genomic sequence information for peanut, there are too few SSR markers to construct a fine genetic map. With the large scale sequencing of peanut cDNA and DNA libraries, more data will be available and additional SSRs (or other cost effective markers) should become available for assisting peanut research.

Gene introgression into A. hypogaea from wild species. Molecular approaches have been used to detect genomic introgression from wild species of *Arachis* into the cultivated peanut. Wu *et al.* (2003) reported molecular identification of genomic ingression of *A. cardenasii* Krapov. and W.C. Gregory in 8126 (an interspecific hybrid derivative) using the Random Amplified Polymorphic DNA (RAPD) approach. They found that the RAPD primers including OPE2(GGTGCGGGA), OPF8(GGGATATCGG) and OPF20 (GGTCTAGAGG) could be used as markers of *A. cardenasii* intrgressions.

He *et al.* (2005) reported molecular evidence of gene introgression from wild *Arachis* species into the cultivated species. Among progenies derived from a cross with *A. correntina* (Burkart) Krapov. and W.C. Gregory as the pollen parent, several morphological traits and resistance to foliar diseases were similar with the diploid parent. Nineteen hybrid derivatives and their parents were tested for DNA variation by using SSR markers. Among 40 SSRs, three produced bands in *A. correntina* and the hybrids, but not in the cultivated parent, indicating genomic introgression had occurred. Yin *et al.* (2003) identified 27 polymorphic RAPD markers on the progeny of interspecific hybrids.

Gene cloning. The techniques for gene discovery have progressed well for many plants, and many research groups are conducting research for gene cloning and function analysis. Yin *et al.* (2007) reported a full-length sequence coding of a Δ^{12}-fatty acid desaturase (FAD2) gene from peanut which was cloned into the plant expression vector, pRSETB, to generate recombinant plasmid pRSET/HO-A. This fatty acid desaturase expressed in high level in *E. coli* BL21(DE3) with induction of isopropyl-D- thiogalactopyranoside (IPTG).

Xie *et al.* (2007) also reported cloning of Δ^{12}-fatty acid desaturase gene and construction of vectors. Genomic DNA was extracted from peanut leaves and two fragments of FAD2 were isolated by PCR application. Antisense FAD2-1 and FAD2-2 were cloned under the soybean oleosin gene promotor. The constructs were then transferred into *Agrobacterium tumefaciens* LBA4404 by the frozen-fusion method and have been used for peanut transformation. Based on the sequence of Δ^{12}-fatty acid desaturase gene in GenBank (AF248739), the promoter region and partial exon of the gene in peanut (by using Yuhua 4 as template) were cloned, and inverted repeats of the Δ^{12}-fatty acid desaturase gene fragment were further cloned into the plant binary vector pCAMBIA1301, and are ready for use in peanut transformation (Xie *et al.*, 2007).

A full-length cDNA of an arachin gene was cloned from the immature peanut seeds cDNA library by plaque *in situ* hybridization. The cDNA contained an ORF (open reading frame) of 1886 nuclotides with a deduced protein of 536 amino

acids. Results from northern blot analysis showed that the expression of the gene occurred at an early stage of seed development and increased dramatically over time (Quan *et al.*, 2006).

A full-length resveratrol synthase gene of 1537 nucleotides was amplified from peanut by the Polymerase Chain Reaction (PCR) approach and cloned into vectors (pMD18-T and pBluescript II KS+). Analysis of the nucleotide sequence indicated that the sequence contained two exons and one intron and the reading frame encoded a protein of 389 amino acid whose molecular weight was 42,800. The amino acid sequence GVLFGFGPGLT was found at position 368-378 which was the family signature sequence for stilbene synthase (Wang *et al.*, 2005a). In other research, cDNA of resveratrol synthase was cloned by over-hang extension PCR protocol using total DNA as the template. The PCR products were cloned into pBS-T vector and its sequence was determined. Then the cDNA of resveratrol synthase gene was subcloned into plant expression vector pBI121 and named pBI121L which could be used for plant transformation.

Two heteromorphic CaM genes in peanut were cloned by PCR reaction(Meng *et al.*, 2004). The first strand of cDNAs was obtained by reverse transcription of the RNA from leaflets of peanut using primers synthesized according to the CaM gene sequence of barley. It was shown that the two different genes were all composed of 447 nucleotides encoding 148 amino acids. In addition, the two CaM genes, PCaM-1 and PCaM-2 shared 84% identity at nucleic acid sequence level and 97% identity on amino acid level with only five substitutions.

Using RACE (Rapid Amplification of cDNA End), Huang (2007, unpubl. data) isolated 15 copies of the resveratrol synthase gene from peanut line H2007. One of the genes has been expressed in *E. coli.*

Although much progress has been made in gene cloning in peanut in China, most of genes cloned were derived from homologous sequences in GenBank via bioinformatic analysis, and this tendency will continue in the near future. Other avenues such as construction of T-DNA insertion mutation library, EST sequencing, differential display and proteomics analysis may also help to discover functional genes in peanut. Functional gene isolation and promoter identification and isolation also is very important. In most cases, control of temporal and spatial expression of exogenous gene is crucial. For example, constitutive expression of a cold tolerance gene DREB1A led to the dwarf trait in *Arabidopsis*, while using a cold responsive promoter led to the enhanced cold tolerance and normal growth. In peanut, several promoters with the expression only in seeds have been cloned and their functional analysis is underway (Huang, pers. comm.).

Expressed sequence tag (EST) is a new strategy for isolating functional genes and has been extensively used in model animal and plant gene discovery. The ESTs are rapidly being deposited in GenBank. One EST project has been carried out in Bio-Tech Center of Shandong Acad. of Agric. Sci. located in Jinan, in which about 4000 ESTs from developing seeds of peanut have been sequenced. Another project at OCRI aiming to find disease resistance genes is also under way with a goal to sequence 60000 EST sequences from root, leaf and developing seeds (Huang, pers. comm.).

A peanut seed (including several developmental stages) cDNA library was constructed at the Bio-Tech Center of Shandong Acad. of Agric. Sci. (Wang, pers. comm.). To date, 10,000 ESTs were sequenced representing about 600 contigs and 2100 singlets, among which, 4406 ESTs have been submitted to the dbEST database (access number EE123340-EE127745). Two hundred and eighty cDNAs sequences were submitted to GenBank. From the EST sequences of 23 seed storage protein genes, there were six allergen genes identified. This information should be useful in research to down regulate the allergen expression and accumulation by antisense RNA or by RNAi techniques to produce a peanut with low allergen levels. Twelve genes involved in lipid metabolism and storage were also obtained from these ESTs.

Genetic transformation. Genetic transformation is of vital importance in molecular breeding, gene functional analysis, and gene discovery (Xu *et al.*, 2007a). The first peanut transformation in China was reported in 1996 (Fang *et al.*, 1996). Several transgenic peanut plants with *gus* gene were obtained by *A. tumefaciens* mediation. During the past decade, more research on peanut transformation has been conducted by various institutions. The mediation approaches in peanut transformation including particle bombardment, *A. tumefaciens*, pollen tube pathway, and ovary injection have been used (Xu *et al.*, 2007a). Several target genes have been attempted in transforming peanut including *peanut stripe virus* (PStV) coat protein gene (Chen *et al.*, 2004), chitinase gene and β-1,3-glucanase gene (Shan *et al.*, 2003), Cowpea trypsin inhibitor (CpTI) gene (Xu *et al.*, 2003) , Δ^{12}-fatty acid desaturase gene (Xie *et al.*, 2007), γ-tocopherol methyltransferase gene (Liu *et al.*, 2005), hepatitis B surface antigen (*HBsAg*)(Chen *et al.*, 2002), and resveratrol synthase (RS) gene (Xu *et al.*, 2007b). Different tissues of peanut including leaflet,

somatic embryo, embryo axis, cotyledon, and hypocotyl have been successfully used as explants in generating transgenic lines. However, further research efforts are needed for improving the transformation efficiency of peanut and more reliable plant regeneration protocols.

Conclusions and Perspectives

Peanut production in China is expected to further increase in the near future because peanut is competitive to other oilseed plants in many aspects. As the domestic vegetable oil production and supply in China is expected to remain in short supply during the next decade, the ratio of peanut crushed for oil should remain as high as around 60%. Since relatively high ratio of contaminated unrefined peanut oil samples from southern and central parts of China have been found (largely due to preharvest contamination and poor drying of the raw peanuts before processing), it is essential to strengthen management of contamination in peanut oil by using integrated approaches. More efforts are needed to screen and develop high oil germplasm with desirable resistance to aflatoxin and other biotic and abiotic stresses. Genetic manipulation of fatty acid components is important to enhance the nutrition quality and may also contribute to improve the reaction of peanut to aflatoxin contamination. Genomic approaches should be very promising in many aspects for genetic enhancement. EST sequencing, gene cloning, functional analysis and genetic engineering will concentrate on peanut quality and resistance enhancement.

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