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Advances in the Genetic Engineering of Insect-Resistant Soybeans

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Abstract

This paper reviewed the recent advances in research on, and the modification of the resistance genes, in the construction of vectors, methods of genetic transformation, and the resistance in transgenic plants.

Keywords: Soybean; Insect-resistance genes; Genetic transformation; Genetic Engineering for Insect-Resistance

Soybeans usually suffer severe infestation from insect pests during growth and developmental period, and tremendous yield losses of soybean often are incurred as a result. The widespread and overdose applications of chemical insecticides not only enhanced resistance in insect pests，caused damage to beneficial insects and other animals, but also severely polluted the environment, raised production costs and interrupted the ecological balance. Conventional crop breeding and cultivation techniques are becoming increasingly unsatisfactory and unproductive due to the long time required, limited genetic resources, unclear mechanism of insect resistance and the instability of resistance caused by the emergence of new biotypes of insect pests. Newly developed plant genetic engineering techniques from the 1980s opened up a new way of soybean breeding for insect pest resistance, and attracted the attention of many plant breeders. This paper will review the recent advances in soybean breeding for insect pest resistance by way of genetic engineering.

1. Genes for insect pest resistance and their modifications

1.1 Bt gene for crystalline protein toxin

The bacterium *Bacillus thuringiensis* (Bt) is a bacillus from the soil which produces insecticidal crystalline protein. An insoluble crystalline protein – δ-endotoxin may be produced when the endospore is formed. This endotoxin may account for 30% of the weight of the endospore, and is a polypeptide protoxin which is usually composed of 600-1200 amino acids. The mechanism of the insect-resistant Bt crystalline protein is that its binding to a receptor on the epithelial brushborder membrane of the midgut of insect causes the disruption of osmotic balance in the brushborder membrane, leads to the lysis of cells and eventually kills the insect (Aronson et al., 1986). The insecticidal property of Bt toxin protein is extremely specific, and two of the variants (Bt aizawai7-29 and Bt kurstaki HD-1) are ideal

strains for killing the insects belonging to the family Lepidoptera (Rowe, 1987). In 1981, the Bt endotoxin protein gene *cryIA(b)* was cloned by Whitely. Up to now, more than 50 toxin genes have been cloned from Bt (Barton and Miller, 1993). The Bt toxin protein gene has been mapped to the plasmids within the molecular mass range of 30-150 x 10^6, and the toxic domain is located in the coding regions of the sequence corresponding to the N-terminus of the toxin protein (Vacek et al., 1987). Adang and coworkers (1985) first reported the transgenic tobacco transformed by the Bt toxin gene and both transgenic plants are resistant to the 1-instar larvae of a kind of tobacco moth. Then the Bt toxin genes were transferred to cotton, tomato, soybean and other crops by the researchers. The effects of insect-resistance were not satisfactory due to the low level of expression of the toxin protein gene of the wild type Bt (Benedict et al., 1992). The reason for that was presumed to be due to the Bt gene contains more AT bases and ATTTA sequence repeats than the normal DNA sequences in the structural genes of higher plants (Murry et al., 1991). The AT-rich regions in higher plants were regarded as introns which are not expressed (Goodal et al., 1989), and the ATTTA sequence repeats in the transcription and translation systems in higher plants would affect the stability of mRNA (Shaw et al., 1986), hence the two kinds of sequences are not used for coding in higher plants (Kunkle, 1985). Hence, researchers in Monsanto Co. strived to solve this difficult issue of low expression level of crystalline protein in two aspects: on the one hand, the promoter of Ti plasmid vector was modified by adding duplicated enhanced region and the resulted in 5-10 fold rise of the expression level of the wild type genes (Perlak et al., 1990); on the other hand, they synthesized a completely new DNA sequence by artificial methods. Altogether 390 bases were changed in the artificially synthesized new sequence compared to the wild type Bt toxin protein gene. The change involved 60% codons and the GC content was increased to 49%, the ATTTA sequence repeats were not included in this new sequence. After this completely modified Bt toxin protein gene was introduced into plants, the δ-endotoxin level was 100 folds that of the wild type, and showed strong insecticidal effects (Perlak et al., 1991)

1.2 Cowpea trypsin inhibitor

Plant proteinase inhibitors are a natural insect resistant substance (Ryan, 1989). It has some advantages overt the toxin protein from *B. thuringiensis*: wider spectrum of anti-insect pests, no side-effects to human being and it was rare for insects to develop tolerance (Gatehouse, 1988). Many kinds of genes or cDNAs encoding serine-type proteinase inhibitors have been isolated and purified from cowpea, soybean, tomato, potato, barley etc. After many years of in-depth research, it was discovered that the cowpea trypsin inhibitors (CpTi) showed the most ideal effect of anti-insect pests, because the targeting site of CpTi is active center of the enzyme. Unless the change happens to the major biochemical and physiological processes in the digestive system, it is unlikely that the insect pests would be induced to become resistant to CpTi . Therefore, the CpTi gene mediated resistance against insect pests is comparatively stable. The first cowpea trypsin inhibitor preparation was obtained by the International Institute of Tropical Agriculture (IITA) located in Nigeria from an insect pest-resistant material –TVU2027 through screening thousands of cowpea

germplasms. It was a polypeptide composed of about 80 amino acid residues, and its product can inhibit the digestive enzymes in the digestive system of insects so that the insects would starve to death due to the incapability of absorbing nutrients after feeding. Hilder and colleagues (1987) first introduced the cowpea trypsin inhibitor gene into tobacco, and obtained the transgenic tobacco which could kill a major insect pest on tobacco. Parrott and colleagues (1994) introduced the CpTi gene into soybean, but no detailed report has been published up to now.

2. Construction of vectors for insect resistance genes

Generally speaking, the genes of insect resistance separated and cloned can not be used directly, and hence some molecular rearrangements or modifications have to be performed. The gene of interest, promoter, reporter gene, terminator and enhancer have to be integrated into a suitable vector (usually the Ti plasmid from *Agrobacterium tumefaciens* is used), and the key element is promoter. Among the promoters used in soybean, the 35S promoter from Cauliflower mosaic virus (CaMV), nopaline synthase (NOS) gene promoter, light-inducible promoter and β-bean protein promoter were the most widely used ones. Several common reporter genes are chloramphenicol acetyl transferase (CAT), neomycin phosphotransferase II (*nptII*) and β-glucuronidase (GUS) genes.

3. The methods for delivering insect resistance genes

3.1 Ti plasmid from *Agrobacterium*- mediated gene transfer

Two species in the genus *Agrobacterium* — *A. tumefaciens* and *A. rhizogenes* have the capability that get a fragment (T-DNA) of their plasmids transferred into the nucleus and then T-DNA could be integrated into the genome of the host plant. The gene transfer for the genetic engineering of soybean and other crops has been carried out currently mainly mediated by the plasmids from *Agrobacterium*, and the Ti plasmid from *A. tumefaciens* was the most widely used one (Jia Shirong et al., 1992). By this approach, the explants were immersed in the solutions containing *A. tumefaciens* to get the plant tissues infected by the bacteria, then the explants were taken out, thoroughly washed, and put on the selective medium containing certain antibiotics to let grow and induce the explants to regenerate. Since the gene of interest was linked to a reporter gene (such as *nptII* etc.), any explant which developed into callus must be the one transformed — the gene of interest has been integrated into the plant genome. The advantages of *Agrobacterium*- mediated gene transfer are that it is both reliable and efficient. But only those dicotyledonous plants whose explants could regenerate can be used.

3.2 Biolistic bombardment method

Biolistic bombardment method which was also called gene-gun method, was developed by Klein and co-workers (1987) from Cornell University for the foreign genes to be delivered directly into plant cells. The major steps of this method are as follows: the gold (or tungsten) particles coated with the gene of interest in the vector；

the metal particles were bombarded into the plant tissues; regenerated plants were obtained by tissue culture. This procedure is applicable to any plant species and organs, especially suitable for the monocotyledonous plants which the *Agrobacterium* could hardly infect. But this method is not very reliable and the rate of transformation is rather low.

3.3 Pollen tube method

On the basis of extensively investigating inter-species hybridizations carried out in China and abroad, Zhou Guangyu (198, 1988) proposed a hypothesis of DNA fragments hybridization, and designed the method of direct DNA delivery into the recipient through the pollen tube. This procedure utilizes the pollen tube formed after pollination of crops and delivers the foreign DNA into the egg cell, zygote or the early embryonic cells of the plant. Lei Bojun and coworkers (1991, 1994) successfully delivered the total DNAs from sources of intra-species, inter-species, and inter-genera into recipient soybean plants, and obtained some useful genetic variations. This method is convenient, fast and may overcome some problems such as low frequency and difficulty of getting rid of bacteria in transformation by *Agrobacterium*- mediated gene transfer, and is particularly suitable for those plant species which are difficult to get regenerated or regeneration rate is extremely low.

Other methods such as electroporation and polyethylene glycol (PEG) treatment and electroporation mediated genetic transformation have been widely used for soybean, but only transformed callus and regenerated bud were obtained, and no report of regenerated plants is available (Christou, 1990; Dhir, 1991)

4. The current state of genetic engineering of soybean for insect resistance

Facciotti and coworkers (1985) first cloned genes used in the transformation of soybean by *Agrobacterium*- mediated delivery. Hinchee and coworkers (1988) first obtained transgenic soybean plants by *Agrobacterium*- mediated transformation. Three most susceptible cultivars were selected from among 100 cultivars and their cotyledons were used for co-culture with *Agrobacterium* harboring plasmids p-Ti-T37-SE and pMON9749 (containing NPT II and GUS gene respectively) or pTiT37-SE and pMON894 (containing NTP II and Glyphosate tolerance gene respectively), and transgenic plants were obtained co-transformed either with NPT II and GUS gene or NPT II and Glyphosate tolerance gene. The segregation ratio of two kinds of transgenic plants both are 3: 9 (co-transformed : non-transformed), suggesting that one copy of the foreign gene was inserted into the plant’s genome, and displayed a Mendelian inheritance.

According to the materials provided by the National Agricultural Library, breakthrough has been obtained in the research on transgenic soybean which are resistant to leaf-eating insect pests (Kalinshi, 1993). Parratt and coworkers (1994) bombarded embryo-shaped suspension cell lines of soybean cultivars of various genotypes by using micro-projectiles coated with Bt crystalline toxin protein gene and/or CpTi gene, and obtained 3 transgenic soybean cell lines transformed with Bt crystalline toxin protein gene and they have regenerated into plants. Two progenies of

the regenerated plants were used to feed the bean moth larvae, and one of them has significant inhibitory effects to the growth of the bean moth larvae, because the leaf consumption was remarkably less than those non-transformed soybean plants. The callus that carrying CpTi insect resistance gene had also been obtained, and further identification is being carried out. Experiment results showed that cotton ballworm and another insect shared the similar situation in feeding and development on different soybeans, no significant difference was found between the transgenic lines and the controls (Parrott et al., 1994). Thus, the expression of the Bt toxin protein gene in soybean plants did not reach a level that is sufficient for the δ-endotoxin to inhibit the feeding of insects or kill them. Therefore, Stewart and co-workers (1996) delivered the artificially synthesized Bt crystalline protein gene (Bt cryIAc) into the insect-susceptible cultivar Jack by means of micro-projectile bombardment, and obtained 3 transgenic lines in which the expression of the cryIAc protein reached the level as high as 46 ng/mg. The progenies of the transgenic plants showed comparatively strong inhibitory effect to several kinds of insect pests used. The percentage of leaves shed due to cotton bollworm for the transgenic plants was only 3%, that of the control lepidopteran insects- resistant line GatIR-81-296 was 20%, and that of the lepidopteran insects-susceptible cultivar Cobb was as high as 40%.

The research on foreign genes of insect resistance in soybean in China is just at the beginning, and the progress of related research has been comparatively slow. Xu Xiangling and co-workers (1997) delivered the Bt k-δ-endotoxin protein gene on the plasmid pKT54B7C5 into the Northeast soybean cultivars such as Hei-Nong 37, Hei-Nong 39 mediated by the Ti plasmid. Clustered buds and regenerated plants were obtained from hypocotyls and cotyledons by using many kinds of explants and infection methods. Preliminary results of kanamycin selection and opine detection showed that the foreign genes have been delivered into soybean. Altogether 81 regenerated plants were obtained, and 7 seeds have been harvested. Further research on these seeds is being carried out.

Taken together, some remarkable advances have been achieved for the research on the genetic engineering of soybean for insect resistance, but they were still in the infant stage. More effective insect-resistant genes need to be found and separated to open up more direct paths for killing insect pests and contributing to grain production. With the development of molecular biology, biotechnology and modern plant breeding, it can be anticipated that transgenic cultivars (lines) with stronger insect resistance will come of age in the near future. After experimental production of these cultivars in large area, the biological control of soybean insect pests will become a reality.

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