Development of Transgenic Insect-protected Cotton Plants

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ABSTRACT

An artificially synthesized Bacillus thuringiensis insecticidal protein gene, co-constructed with the GUS reporter gene (Bt/GUS), was transferred into fertilized ovaries of the elite cotton (Gossypium hirsutum L.) cultivars Simian 3 and Zhongmiansuo 12 by the pollen tube pathway. Transgenic cotton plants were recovered from the seeds in the treated bolls. Histochemical analysis for GUS activity indicated that the gene was expressed in the transgenic R_1 plants of the two recipient cultivars. The presence of the Bt gene in the GUS-positive R_1 plants was confirmed by PCR and the same results were obtained in the R_2 plant populations. This indicated stable integration of the Bt gene into the recipients and its inheritance from R_1 to R_2 generations. Resistance to the cotton bollworm (Helicoverpa armigera Hübner) was identified in these transgenic plants. In R_1, five plants highly toxic to the insect were found: S545, S591, S636, and S1001 from "Simian 3+Bt/GUS", and Zh1109 from "Zhongmiansuo 12+Bt/GUS", with larva mortality up to 91.6%, 93.8%, 92.3%, 85.7% and 75.0%, respectively. Insect-resistant R_5 strains were derived from the R_1 transgenic insect-resistant plants via selfing and breeding, showing the maintenance of the transgene and insect-resistance and the practical potential in cotton production.

Introduction

The breeding and application of insect-resistant cotton (*Gossypium hirsutum* L.) cultivars is an important aspect in the integrated control of bollworm (*Helicoverpa armigera* Hübner). In addition to reducing the cost of cotton production and increasing yield, it avoids pollution of the environment, making it one of the most active research areas of agriculture in the world. However, as natural resources of resistance to bollworm combined with other desired traits have not been found in the *Gossypium* genus, conventional breeding for bollworm-resistant cotton based on morphological traits and metabolites of small molecules has not yet been successful. Recent developments in biotechnology enable the transfer of genes between various species. The gene for insecticidal crystal proteins in *Bacillus thuringiensis* (Bt) has been transferred to cotton plants to produce transgenic plants. However, the insect resistance of those transgenic plants was not enough to control bollworm. Based the principle of optimized codons of higher plants, Perlak *et al.* (1990) modified the Bt insecticidal protein gene, transferred it into cotton, and obtained transgenic plants with enhanced toxicity to bollworm. In our study (Ni *et al.*,1996, 1998), the artificially synthesized whole-sequence gene for the Bt insecticidal proteins was introduced into elite cotton cultivars to develop transgenic bollworm-resistant cultivars.

Materials and Methods

Source of insecticidal Bt gene(s). The insecticidal Bt gene used for cotton transformation was whole-sequence synthesized based on optimized codons for higher plants, co-constructed on a plant expression vector with the GUS gene as the reporter gene.

Cotton transformation. Cotton cultivars Simian 3 and Zhongmiansuo 12 most widely grown in China, were selected as the recipients of the Bt protein gene. The method for introducing the Bt gene was the pollen-tube pathway (Zhou *et al.*, 1983).

Analysis of GUS gene expression was by the method of Jefferson *et al.* (1987).

PCR analysis was by the method of Saiki (1995).

Identification of resistance to the bollworm followed the method of Su *et al.* (1997).

Results and Analysis

Cotton transformation. The pollen tube pathway introduced the synthetic Bt gene into the fertilized ovaries of cotton cultivars Simian 3 and Zhongmiansuo 12. In Simian 3+Bt/GUS, 1000 fertilized ovaries were treated and 4200 seeds were produced, whereas Zhongmiansuo 12+Bt/GUS yielded 3852 seeds from 900 fertilized ovaries.

GUS expression analysis in transgenic plants. Following germination, root and leaf sections were assayed for GUS activity. Out of 3545 seedlings of Simian 3+Bt/GUS, 53 plants were GUS positive and 21 plants survived. From 1360 seedlings of Zhongmiansuo 12+Bt/GUS, 17 were GUS positive, and 13 plants survived. In total, the GUS positive rate was 1.4%.

Evaluation of resistance to bollworm. The surviving GUS positive plants were tested for resistance to the bollworm, with Simian 3 and Zhongmiansuo 12 as the control. Five R_1 plants were verified to be highly toxic to the insect, four from Simian 3+Bt/GUS with bollworm mortality levels at 91.6%, 93.8%, 92.3%, 85.7% and one from Zhongmiansuo 12+Bt/GUS with 75.0% bollworm mortality. These were designated S545, S591, S636, S1001 and Zh1109, respectively. In the five plants, leaves were damaged slightly but bollworms damaged the boll surface of the transformed cotton plants only slightly. Bollworms bored into the centre of the control plant bolls, causing severe damage.

Resistance to the bollworm was observed in the R_2 progeny derived by selfing the five R_1 plants. There were remarkable differences among the R_2 populations. All plants in the four R_2 populations from Simian 3+Bt/GUS were resistant with high mortality rates of bollworm. However, in the R_2 population from Zhongmiansuo 12+Bt/GUS, great differences existed among individuals with 8.9% of the plants being nontoxic to the bollworm and only 10% highly toxic. These results showed that resistance to bollworm in the transgenic Bt cotton plants was inheritable but it performed differently among different transgenic events, possibly due to differences in site of transgene integration and genetic background.

The R_3, R_4, and R_5 generations were established in a breeding program focusing on resistance and agronomic traits. Resistance screening was carried out in successive generations. The results show that the transgenic, insect-resistant cotton could retain and stably inherit its transgenic resistance (Table 1).

Molecular screening of transgenic Bt cotton. On the basis of resistance analysis, some resistant plants were sampled for the presence of the Bt transgene with PCR. The amplified band of the Bt transgene appeared in the samples of R_1 plants S545 and S591 and in the samples of R_2 plants from S545, S591 and Zh1109. The PCR products were subjected to Southern blot analysis with the labelled Bt transgene as probe. All the amplified bands could hybridize with the probe, proving proved that the PCR bands were not amplified from contaminating DNA but from the Bt transgene, thus establishing that the transgene was heritable through selfing.

Field test of transgenic insect-resistant cotton. A field test was carried out for one R_5 transgenic insect-resistant cotton strain with its transgene recipient Simian 3 as control. During the damaging period of the second bollworm generation in the growing season, the control with no insecticide had 96 larvae per 100 plants, bud damage up to 81.6%, and terminal damage up to 48.1%. compared to 22.3, 20.2% and 18.8%, respectively, on the transgenic strains. During the damaging period of the third bollworm generation with one spray on the transgenic strain and four sprays on the control, the control had 15 larvae per 100 plants and 44.4% bud abscission while the transgenic strain only had 3 larvae and 6.4% bud abscission. These results indicate that the transgenic insect-resistant cotton could decrease bollworm damage and the amount of insecticide required, controlling the pest in practice.

Discussion

Inheritance and stability of a transgene integrated into a recipient plant is of importance to the transgenic plant itself and for breeding new plant strains with the target phenotype. It has been reported that marker genes could be inherited when transferred into cotton plants. Results showed the presence of the transgene in R_1, R_2, and R_5 generations, indicating that the Bt transgene could pass to progenies by sexual propagation and that the resistance to bollworm was the result of Bt transgene expression in the transgenic cotton plants.

The results also showed that only about 15% of the GUS-positive transgenic plants were highly toxic to the insects. This could be due to the construction of the Bt transgene and GUS gene

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in the vector with their own promoter and could be expressed individually with no correlation between them. This suggested that it would not be necessary to have a reporter gene in the vector construction and that breeding for the target phenotype by selection of reporter gene expression is not reliable.

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Table 1. Evaluation for insect-resistance in different trangenic cotton generations.

Sources		Plants tested	Corrected larva death rate (%, 3d)			
			40	<40~20	<20	0
R3	S545	134	119	11	4	0
	S591	183	149	28	6	0
R4	S545	128	97	23	8	0
	S591	135	99	32	3	0
R5	S545	67	61	6	0	0
	S591	60	55	2	3	0