High pest resistance transgenic cotton transformed with \textit{Bt} and \textit{Sck} gene

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Abstract: In this experiment, 301-5T\textsubscript{2}, 305-5T\textsubscript{2}, 312-5T\textsubscript{2}, 314-4T\textsubscript{2} and 332-2T\textsubscript{2} homologous lines were obtained in second generation progeny plants (T\textsubscript{2}) of the cotton transformed with \textit{Bt+Sck} gene, which were developed from the cotton genome (Sumian16) by pollen-tube pathway method. Pest resistant bioassay and the detection of \textit{Bt} toxoprotein concentration showed that the resistance to bollworm of all the transgenic plants was obviously higher than Sumian16 and SGK321 cotton, implying that the transgenic cotton transformed with \textit{Bt+Sck} gene were valuable to produce multiple desirable transgenic cotton plants.

Key word: \textit{Bt}  \textit{Sck}  pest resistance  cotton

1 Introduction

China is one of the main countries for cotton production in the world, and cotton is one of the most important industrial crops. During the recent several years, due to the changes of climatic and ecological condition, the resistance of \textit{Helicoverpa armigera} to chemical pesticides increased 100 times than that in 1980 in China. In spite of the increase in pesticide dosage used and spraying times, the bollworm still outbroke violently into calamity and caused high losses. So, application of chemical pesticides is not only of low effect and costly, but also causes environmental pollution, and disrupts ecological equilibrium. Therefore, the bollworm damage has become one important factor affecting the cotton production in China.

Since 1987, both full-length and truncated \textit{CryIA} genes from \textit{Bacillus thuringiensis} (\textit{Bt}) have been introduced into many crops such as tobacco and tomato by \textit{Agrobacterium tumefaciens}-mediated transformation (Vaeck et al 1987, Fischoff et al 1987)[1-2]. In collaboration with Cotton Research Institute, Shanxi Academy of Agricultural Sciences (SAAS), and Industrial Crop Institute, Jiangsu Academy of Agricultural Sciences (JAAS), they transferred GFM \textit{Bt} gene to such commercial varieties as Simian 3, and Ginnian 7 etc, via \textit{Agrobacterium tumefaciens} mediated transformation (Jiao et al. 1997)[3] and pollen tube pathway methods (Ni et al. 1996, 1998)[5]. Many varieties and hybrids have been developed, and being commercially grown in China (Zhang & Tang 2000)[4]. The Bollgard\textsuperscript{TM} cotton developed in American DPL Inc. has also been commercially grown in China. In 1999, an estimated 24\% of corn and 5\% of cotton grown worldwide contained \textit{Bt} gene (Gould 2000)[5]. There is a greater chance for bollworm to develop the resistance to the \textit{Bt} cotton than to any of the foliar pesticides because it is in the field all season long and every insect is exposed to it for its entire life cycle. To delay or avoid the development of resistance, a number of general strategies have been proposed. Having two
biochemical unrelated toxins in all Bt-producing plants, each at a high dose, is expected to significantly decrease the rate of resistance development (McGaughey et al. 1998, De Maagd, Bosch, Stiekema 1999)[6-7]. The computer simulation demonstrated that the utility of two different genes to the same plant could prolong 12 to 20 years longer for resistant development than that of using a single gene (Zhang et al. 2000)[4]. The transgenic tobacco harboring double genes (Bt+CpTI) were developed and laboratory selection indicated that such transgenic tobacco could greatly delay the resistance development than that of transgenic Bt tobacco (Zhao et al. 1995; Zhao et al. 1998)[8-9]. Guo (1998) [10] at the Biotechnology Research Institute of CAAS constructed the plant expression vector harboring both synthesized Bt insecticidal protein gene and modified CpTI gene and developed transgenic cotton plants by pollen tube pathway method in 1995 (Guo et al. 1999)[11]. It was reported that the transgenic Bt+CpTI cotton plants showed excellent resistance to bollworm (Li et al. 1999)[12], and transgenic cotton variety expressing these genes, SGK321, was released commercially in China in 1999.

SGK321, both the insect resistant genes were driven by Cauliflower Mosaic Virus ‘CaMV’35S promoter. Vaucheret et al (1993)[13] reported that this phenomenon still happened even if there was homology sequence of 90bp in size of promoter region. Elmayan and Vaucheret (1996)[14]also found that UidA gene driven by double CaMV 35S promoters was silenced in transgenic tobacco .The Bt gene driven by double enhanced CaMV 35S promoters was silenced in the later stage of plants growth and development (Xia et al 2005)[15]. Thereafter, it is urgent to avoid the same promoter in polygenic transformation in order to reduce the gene silencing level. In this experiment, the plant expression vector pCRPBSCK35SBT harboring both synthesized Bt insecticidal protein gene and modified CpTI gene(Sck) driven by CaMV35S promoter and CLCuV promoter ( PRPB ) respectively, was transformed into the elite cotton (Gossypium hirsutum L. cv. Sumian 16) by pollen-tube pathway method. Then the resistance to boll worm (H. armagera) was analyzed and the Bt toxin protein concentration was also detected. The purpose of this study was to show whether or not the resistant efficiency of transgenic cotton plants with Bt+Sck were elevated subsequently.

2 Materials and methods

2.1 Materials

301-5T_2, 305-5T_2, 312-5T_2, 314-4T_2 and 332-2T_2 homologous lines were obtained in second generation progeny plants (T_2) of the cotton transformed with Bt+Sck gene, which were developed from the cotton genome (Sumian16) by pollen-tube pathway method. ‘SGK321’ was a transgenic Bt+CpTI double gene line.

The neonate of bollworm was purchased from Prof. Chune Shu (Institute of Plant Protection of Jiangsu Academy of Agricultural Sciences).

A PathoScreen kit for Cry1Ab/Cry1Ac (Agdia Co., USA) was used to detect recombinant protein expression according to the manufacturer’s instructions.

2.2 Methods

2.2.1 Laboratory bioassay

Bioassays were conducted using laboratory culture strains of H. armigera sensitive to Cry1A (c) Bt toxin as we reported before (Sun et al. 2002)[16]. Individual leaf was used for laboratory assay. The containers held at a constant temperature 27°C±1°C with relative humidity 60%~85%, and light for 14 h. Five neonate larvae per leaf were generally added to each container. After 3 days in culture, the number of surviving larvae, larvae age, and the leaf damage index were recorded. The leaf damaged degree was divided into four grades: 1: leaf damaged area below 10%, with only small needle-like damaged parts could be observed; 2: damaged area about 11%~50%, damaged parts distributed in small patches; 3:
2.2.2 Measurement of Bt toxin content
A PathoScreen kit for Cry1Ab/Cry1Ac (Agdia Co., USA) was used to detect recombinant protein expression according to the manufacturer’s instructions. This kit represents a double-antibody sandwich quantitative ELISA for Cry1Ac/Cry1Ab in sample extracts. Extracts were made from weighed leaf samples, taken from the youngest, fully expanded leaves from the progenies of transformed plants, untransformed cotton and SGK321 cotton. The intensity of colour development was measured spectrophotometrically at 450 nm. The levels of Cry1Ac protein in the leaf extracts were determined by extrapolation to a standard curve based on sample and standard absorbance values. The OD value of the untransformed control was subtracted before determining the concentration of Cry1Ac protein.

3 Results
3.1 The analysis of the content of Bt toxin protein of transgenic cotton T_2 plants
In order to search for the situation of Bt gene translation, the Bt toxin protein concentration of the functional leave, bud, flower, and boll was measured according to the manufacturer’s recommendations. The Bt toxin gene expression in functional leave was higher than that of bud, flower, and boll, this data showed a similar result of cotton resistance to boll-worm in the field. The data also indicated the Bt gene expression in different organs of transgenic cotton plants with Bt+Sck was obviously higher than that of SGK321, and showed a significant difference (P<0.01). The expression pattern in different transgenic cotton plants with Bt+Sck was different and they displayed different significant level (Table 1).

3.2 Insect resistant bioassay
At peak flowering and boll developing stages, the transgenic homologous lines with Bt+Sck bioassay showed a high insecticidal activity against cotton bollworm (table 2). The larvae death rate was higher than 90% during the peak flowering and boll developing stages, which indicated the transgenic homologous lines were highly toxic to bollworm larvae. The datum of larvae death rate, 3rd instar larvae rate and damaged degree of leaf showed a significant difference (P<0.01) among the transgenic homologous lines and untransformed Sumian 16. The datum also indicated that the efficiency of leaf of transgenic cotton plant with Bt+Sck was higher than that of SGK321, there was a significant difference (P<0.01), too, indicating that the transgenic cotton transformed with Bt+Sck gene were valuable to produce multiple desirable transgenic cotton plants.

4 Conclusion
In this study, the plant expression vector PCRPBSCK35SBT harboring both Bt gene and Sck gene driven by CaMV35S promoter and PRPB promoter respectively, was introduced into Sumian 16 by pollen-tube pathway method, which would broaden resistant spectrum, and be valuable to develop new transgenic cotton varieties with high efficiency against cotton bollworm.

References:


Table 1  The analysis of the concentration of Bt toxin protein (ng/g)

<table>
<thead>
<tr>
<th>Transgenic plants</th>
<th>Function leaf</th>
<th>Bud</th>
<th>Flower</th>
<th>Boll</th>
</tr>
</thead>
<tbody>
<tr>
<td>301-5T2</td>
<td>3335.2±107.4 AB</td>
<td>1786.0±89.8 A</td>
<td>1266.6±108.0 CDE</td>
<td>2719.2±102.7 ABC</td>
</tr>
<tr>
<td>305-5T2</td>
<td>3049.0±131.0 AB</td>
<td>1644.6±75.7 AB</td>
<td>1890.5±88.1 BC</td>
<td>3146.6±12.5 A</td>
</tr>
<tr>
<td>312-5T2</td>
<td>3467.7±17.8 A</td>
<td>1628.2±8.2 AB</td>
<td>934.2±165.7 BC</td>
<td>3124.0±9.4 A</td>
</tr>
<tr>
<td>314-4T2</td>
<td>3320.6±96.5 AB</td>
<td>1385.1±67.4 C</td>
<td>1784.0±165.5 BC</td>
<td>2297.5±436.0 ABC</td>
</tr>
<tr>
<td>332-2T2</td>
<td>3435.2±74.6 A</td>
<td>1702.6±44.0 AB</td>
<td>1542.2±260.8 BCD</td>
<td>2506.0±278.9 ABC</td>
</tr>
<tr>
<td>SGSK321</td>
<td>1811.0±97.2 C</td>
<td>458.3±7.4 E</td>
<td>221.3±2.1 F</td>
<td>732.9±163.0 D</td>
</tr>
<tr>
<td>Sumian16</td>
<td>0 E</td>
<td>0 F</td>
<td>0 G</td>
<td>0 E</td>
</tr>
</tbody>
</table>

Notes: Zero represents the concentration of Bt toxin protein less than 50ng/g; A, B, and so on represent significantly different (p < 0.01)
Table 2  Evaluation on pest resistant cotton plants with $Bt+SCK$

<table>
<thead>
<tr>
<th>Transgenic plants</th>
<th>Larvae death rate (%)</th>
<th>3rd instar larvae rate (%)</th>
<th>Damaged degree of leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>301-5T2</td>
<td>100A</td>
<td>0</td>
<td>0 C</td>
</tr>
<tr>
<td>305-5T2</td>
<td>93.3±10.3 A</td>
<td>0</td>
<td>0.5±0.4 BC</td>
</tr>
<tr>
<td>312-5T2</td>
<td>98.6±1.6 A</td>
<td>0</td>
<td>0.7±0.6 BC</td>
</tr>
<tr>
<td>314-4T2</td>
<td>96.7±4.5 A</td>
<td>0</td>
<td>0.5±0.4 BC</td>
</tr>
<tr>
<td>332-2T2</td>
<td>98.9±1.0 A</td>
<td>0</td>
<td>0.5±0.5 BC</td>
</tr>
<tr>
<td>SGK321</td>
<td>74.0±9.7 B</td>
<td>0</td>
<td>1.4±0.5 B</td>
</tr>
<tr>
<td>Sumian16</td>
<td>15.5±2.2 C</td>
<td>86.3±5.5</td>
<td>3.7±0.6 A</td>
</tr>
</tbody>
</table>