The genetic stability of transgenic Bt and Sck insect-resistant cotton

GUO JINYING¹  CHEN TAO¹  ZHANG TIANZHEN²

¹Hebei University of Engineering, Handan, CHINA; National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing,CHINA
Email: http://www.jyguo666@yahoo.com.cn

Abstract: The inheritance analysis of insect-resistance in two transgenic lines (312-5T₂ and 332-2T₂) were studied, indicating that the resistance of homologous transgenic lines to Helicoverpa armigera was controlled by one pair of dominant genes. Southern blot analysis was performed with genomic DNA extracted from the progenies of two transformed lines till T4 (312-5, 332-2), facilitating the stable inheritance of the transformed gene, and RT-PCR molecular further demonstrated that the exogenous genes had stably expressed in transformed cotton plants.

Key word: Bt  Sck  inheritance analysis  cotton

1 Introduction

Since 1996, the application and dissemination of transgenic cotton in USA, Australia and China have brought about great economic profits to the cotton production and reduced environmental pollution from synthetic insecticides[1]. Whereas, along with the commercialization on a large scale in the world of transgenic Bt cotton, the insect resistance to Bt cotton has become a serious problem in cotton genetic engineering at present. Moreover, the phenomena that ‘the efficiency of Bt transgenic cotton in the early stages was high, and then it would decrease thereafter’ is prevalence in the current commercialized transgenic insect resistant cotton, which has a direct effect on cotton yield[2-6]. At present, scientists are engaged in engineering plants with two or more resistant genes in order to delay and prevent adaptation in pest species, prolong the usage of life span of transgenic plants and broaden resistant spectrum[7-8]. The transgenic cotton harboring double genes (Bt+CpTI), SGK321, was developed and released commercially in China in 1999[9], which could greatly delay the resistance development than that of transgenic Bt cotton. In our research, the Bt and Sck genes were transformed into the cotton genome (Sumian16) by pollen-tube pathway mediated transformation, the genetic stability of two transgenic lines (312-5T₂ and 332-2T₂) demonstrated the exogenous genes had stably expressed, implying the two transgenic lines be valuable to develop new transgenic cotton varieties with high efficiency against cotton bollworm.

2 Materials and methods

2.1 Materials

312-5T₂, 332-2T₂ homologous lines were obtained in second generation progeny plants (T₃) of the cotton transformed with Bt+Sck gene, which were developed from the cotton genome (Sumian16) by pollen-tube pathway method.

The cotton conventional cultivar Sumian 16 was employed in transformation experiments. It was high yield and super fiber quality but susceptible to Helicoverpa armigera, in Yangtze-River Valley cotton growing region.
The enzymes were purchased from NEB Co. (USA), and DIG High Prime DNA Labeling and Detection Starter Kit I from Roche Co. (Switzerland).

2.2 Methods

2.2.1 Molecular identification of transgenic plants
Total genomic DNA was extracted from transformed and untransformed cotton leaves. Approximately 20 µg DNA was digested with 150 U Hind III (KpnI) and 20 µL 10× buffer, add ddH₂O to 200 µL, 37 °C over night. The CK+ was the HindIII (KpnI) fragment from plasmid pCRPBSCK35SBT. The hybridization probe was the 0.9 kpb Bt gene amplified fragment from the plasmid, PCR amplification were performed with specific primers, 5’CACAATCCCACTATC3’ (Bt forward) and 5’GGACACTGTACGGAT3’ (Bt reverse). PCR reaction was performed in 20 µL (total volume) of reaction mixture consisting of 10× reaction buffer, 20 ng plasmid DNA, 0.25 mmol/L dNTPs, 0.5 µmol/L of each primer DNA and 1 U of Taq DNA polymerase. PCR was carried out in a thermal cycle under the following conditions: 94 °C for 5 min as preheating, then 35 cycles of 94 °C denaturing for 1 min, 55 °C annealing for 1 min, 72 °C synthesis for 1 min and 10 min at 72 °C as final extension. After electrophoresis on 0.8 % agarose gels, DNA was transferred to a nylon membrane (positively charged, Roche) overnight by capillary action and later the probe labeling, prehybridization, hybridization and detection were performed according to the manufacturer’s recommendations.

Total genomic RNA was extracted from transformed, untransformed cotton leaves using the modified CTAB method (Jiang et al 2003)[10]. After reverse transcription, then diluted 1/10 as template, upstream and downstream primers used for amplification were 5’TGCGAGAGCTTCAGAGAGTG3’ and 5’ACACCTGACCTAGTGACGC3’, respectively. The reaction condition of RT-PCR was the same as the above PCR. At the same time, EF1α was amplified as an endogenous reference.

2.2.2 Bioassays
Bioassays were conducted using laboratory culture strains of H. armigera sensitive to Cry1A(c) Bt toxin as we reported before (Sun et al. 2002)[11]. Individual leaf was used for laboratory assay. The containers held at a constant temperature 27±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, larval 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: damaged area 51%~90%, but the mesophyll tissues still present in connected patches; and 4: damaged area over 90%, mesophyll tissues being not in connected patches (Tang et al. 1997)[12].

3 Results

3.1 Inheritance analysis of insect-resistance in two transgenic lines
The insect-resistant and susceptible plants were segregated out in F₂ populations produced between homologous transgenic lines (312-5T₂, 332-2T₂). The plants with 3 and 4 in leaf damage index and 3rd instar larva survival were classed as the susceptible, and those of 1 and 2 in leaf damage index and no 3rd instar larva survival as the resistant after 5-d feeding in laboratory bioassay. Following this standard, segregation of resistant and susceptible plants fits 3:1 ratio in F₂ populations crossed among the two homologous transgenic lines and Sumian16, indicating that the resistance of homologous transgenic lines to Helicoverpa armigera was controlled by one pair of dominant genes (table1). Two F₁ hybrids crossed among the 3 kinds of transgenic strains have the same resistant level to
bollworm as their parents. It means that in heterozygous status, there is no co-suppression phenomenon that will cause gene silence and resistance decrease.

3.2 Molecular identification of transgenic plants

Southern blot analysis was performed with genomic DNA extracted from the progenies of two transformed lines till T4 (312-5, 332-2). The probe was Bt gene fragment from PCR amplification. The transformed genes were shown to be stable, low copy number (in general 1–2), facilitating the stable inheritance of the transformed gene, and producing multiple desirable transgenic cotton plants (Fig.1). RT-PCR molecular detection of the progenies of transformed plants till T4 and untransformed cotton plants further demonstrated that the exogenous genes had stably expressed in transformed cotton plants (Fig.2).

Fig.1 Southern blot hybridization of transgenic cotton plants probed with Bt

1: marker; 2: Sumian16; 3: (CK+); 4-8: 312-5T0, T1, T2, T3, T4; 9-13: 332-2T0, T1, T2, T3, T4

Fig.2 RT-PCR molecular detection of transgenic cotton plants total RNA

1: Sumian16; 2-6: 312-5T0, T1, T2, T3, T4; 7-11: 332-2T0, T1, T2, T3, T4

4 Conclusion

The resistance of the two transgenic cotton plants to bollworm was controlled by one pair of dominant genes and inherited in a classical Mendelian manner in first generation progeny plants from the self-fertilized transformed parent tested by Kanamycin resistance as an indirect identification marker and bollworm bioassays, and the conclusion was further confirmed by using F2 population crossed two homologous transgenic lines (312-5T2 and 332-2T2) with original parent Sumian16, respectively, tested by the same methods. Molecular identification of transformed lines further demonstrated that the exogenous genes had stably expressed in transformed cotton plants.

References:


---

#### Table 1  Segregation of bollworm resistance in F₂ population of Bt + Sck transgenic cotton crossed with Sumian16 cotton lines

<table>
<thead>
<tr>
<th>Combinations</th>
<th>No. of resistant plants</th>
<th>No. of susceptible plants</th>
<th>Ratio</th>
<th>Chi-square</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>(312-T2×SM 16)F₁</td>
<td>28</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>(312-T2×SM 16)F₂</td>
<td>103</td>
<td>30</td>
<td>3:1</td>
<td>0.303</td>
<td>0.50-0.75</td>
</tr>
<tr>
<td>(332-T2×SM 16)F₁</td>
<td>35</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>(332-T2×SM 16)F₂</td>
<td>132</td>
<td>50</td>
<td>3:1</td>
<td>0.465</td>
<td>0.25-0.50</td>
</tr>
</tbody>
</table>

\[ \chi^2_{0.05} = 3.84 \]