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PCR CONFIRMATION OF THE *cry1Ac* GENE IN TRANSGENIC BT (Bollgard®) COTTON

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Abstract

Transgenic cotton (*Gossypium hirsutum* L.) with *Bacillus thuringiensis* Berliner δ-endotoxin genes has been widely adopted for suppression of lepidopteran pests. To account for inconsistency of the endotoxin gene expression, we developed a polymerase chain reaction (PCR) for checking the purity of transgenic cotton plants. A total of eight *cry1Ac* genes were aligned for the PCR primer design. A DNA fragment was amplified from Bt cotton, sequenced, and confirmed to be a portion of the Bt gene. A total of 150 cotton plants representing five cultivars were examined for the presence of the Bt gene. Results demonstrated that all of these cotton plants harbored the Bt endotoxin gene.

Introduction

Routine methods to determine the purity of transgenic *Bacillus thuringiensis* Berliner (Bt) or Bollgard cotton (Monsanto Co., St. Louis, MO) are limited. Although enzyme-linked-immunosorbent assays (ELISA) are commercially available to detect the expressed Cry-protein(s), no published methods are available to determine if an individual plant contains the *cry1Ac* gene. Therefore, without a routine DNA assay to detect the *cry*-gene, it is hard to differentiate between plants that do not contain the *cry1Ac* gene (i.e. Bt cotton seed contaminated with conventional seed) and transgenic plants which are improperly expressing the protein product. We developed a short, robust polymerase chain reaction (PCR) protocol that selectively amplifies the *cry1Ac* gene in transgenic Bt cotton. When used in conjunction with ELISA to detect the Cry1Ac protein (Adamczyk and Sumerford, 2001), issues regarding gene purity and the possibility of gene silencing can be addressed.

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Materials and Methods

Cottons

Thirty young cotton leaves (ca. 6x6 cm from 30 individual plants) were collected from five different cotton fields. All cotton fields were planted with Delta and Pineland Co. cultivars (DP 444BRR, DP555BRR, NuCOTN 33B, and SG125 BRR).

DNA Extraction

Cotton genomic DNA was prepared using a Qiagen DNeasy plant mini kit (Cat.No. 69106, Qiagen, Valencia, CA). A fresh leave disk (9 mm in diameter, Fig. 1) from an individual plant was ground in lysis buffer. DNA precipitation and wash were conducted following manufacturer's instructions. DNA was eluted with 100 µl buffer.

Primer Design and PCR Amplification

The DNA sequence (Adang et al., 1985; GenBank accession M11068) for *cry1Ac* and seven synthetic or modified *cry1Ac* DNA sequences were downloaded from the GenBank of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). These modified Bt sequences included M60856 (Perlak et al., 1991), Y09787 (Nayak et al., 1997), AY126450 (Park et al., unpublished), AF177675 (Kemp and Sutton, unpublished), U63372 (Adang et al., 1985), AF023672 (De Rocher et al., 1998), and AF537267 (Quanhong et al., unpublished). CLUSTALW multiple alignment protocol (Thompson et al. 1994, http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalwan.html) was used to align seven modified *cry1Ac* DNA sequences with unmodified *cry1Ac* sequence. Conserved regions were revealed and used for primer design. Four primer regions were selected (Fig. 2) to synthesize two forward primers, BtF1 and BtF2, and two reverse primers, BtR1 and BtR2. These primers covered more than 62% of the N terminal coding region for the synthetic *cry1Ac* genes (Fig. 2). PCR was performed to amplify the *cry1Ac* gene fragment from cotton DNA. The DNA fragment with expected size was purified, cloned, and sequenced. The *cry1Ac* DNA sequence was confirmed by homology searching of GenBank.

***cry1Ac* Detection**

Individual cotton leaves (30) were randomly collected from each of five cotton fields planted with the different Bt cotton cultivars described previously. Two primers, BtF2 and BtR1, were used to amplify the 1061 bp *cry1Ac* gene from cotton DNA.

Results and Discussion

PCR reaction and annealing temperature were optimized, and target DNA fragments were successfully amplified. PCR amplification using combinations of forward primer BtF1 with other two reverse primers, BtR1 and BtR2, generated a major band with size at approximately 900 bp. This fragment appeared to be from non-target fragment because it was amplified from both Bt cotton and non-Bt cotton DNA preparations with the same size lower than the expected size (1165 bp for BtF1+BtR1, and 1115 bp for BtF1+BtR2).

PCR amplification using forward primer BtF2 with the reverse primers, BtR1 and BtR2, resulted in a distinct band with expected fragment size, 1061 bp for BtF2+BtR1 or 1007 bp for BtF2+BtR2. These fragments also were amplified from Bt cotton DNA, and no corresponding fragments were amplified from non-Bt cotton DNA.

The 1061-bp fragment was selected for cloning and sequencing. By using Blastx homology search of GenBank database, the 1061-bp cotton DNA fragment was perfectly matched to *cry1Ac* genes. The translated protein sequence (436 a.a. residues) was nearly identical to many Bt δ-endotoxins. By using the Blastn homology search protocol, DNA sequence identity reached almost 100% to the *cry1Ac* gene (GenBank: Y09787, Nayak et al., 1997) and an artificial *cry1Aa* insect control gene (GenBank: M60856, Perlak et al., 1991). Other highly match genes included a synthetic construct insecticidal protein Cry1Ac1 (*cry1Ac1*) gene (GenBank: AY126450, Park et al., unpublished) with 97% sequence identity, and to a synthetic construct *cry1Ac* insecticidal endotoxin gene (GenBank: AF177675, Kemp and Sutton, unpublished) with 91% sequence identity. The DNA sequence identity to wild type Bt *cry1Ac* genes was 81%. Based on sequence analysis, we concluded that the 1061-bp fragment was amplified from an artificial *cry1Ac* gene inserted into the cotton genome.

By conducting PCR amplification with forward primer BtF2 and reverse primer BtR1, we surveyed a total of 150 cotton plants from five fields. A 1061-bp DNA fragment was specifically amplified from all Bt cotton plants (100%).

In conclusion, we developed a specific DNA marker for surveying the *cry1Ac* gene within transgenic cotton genome. In future studies, we will use this artificial fragment from the *cry1Ac* gene to develop a probe to study gene expression levels and insecticidal function under different environment conditions. Furthermore, this technique will address purity issues surrounding certain insect-resistance management strategies.

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Figure 1. Cotton leaf disks were collected from cotton leaves.

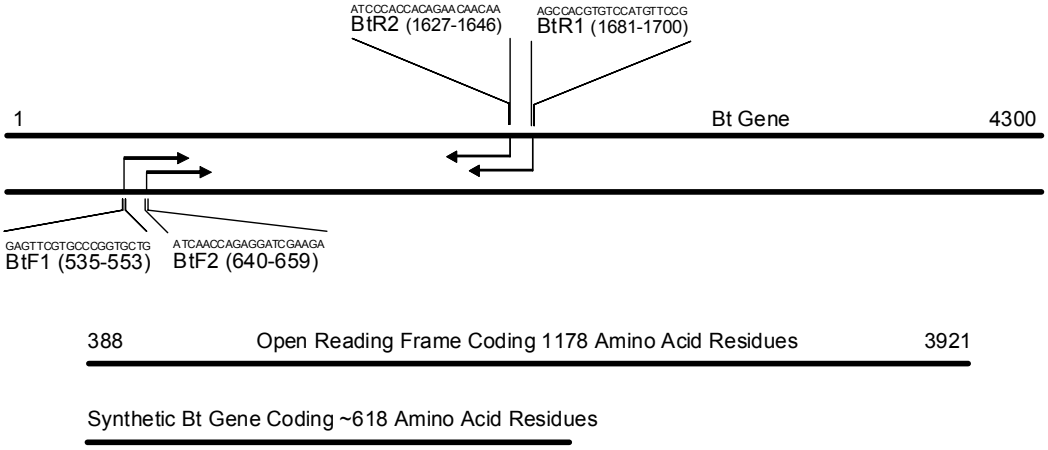

Figure 2. Bt gene and primer design. Diagram of approximate primer locality on *cry1Ac* Bt gene (Adang et al., 1985; GenBank accession M11068) and coding regions.