USING F1 SCREENING TO DETECT RESISTANCE ALLELES TO BT COTTON IN FIELD POPULATIONS OF THE COTTON BOLLWORM, HELICOVERPA ARMIGERA

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Abstract

To successfully apply F_1 screening using Bt plant requires an accurate standard for judging whether F_1 survivors are resistant or susceptible. Because the r allele from the wild male could confer a different level of resistance from r_1 allele of the laboratory resistant female, a low survival rate and variation in larval growth rate on Bt cotton are more commonly seen, which are associated with substantial fitness costs due to attainment of resistance to Cry1A toxins. Because the phenotypic separations were not following the Mendelian separation in F_1 generation, therefore, we analyzed larval surviving and growth rates and determined that the larval body weight of $\geq$ 0.6 mg is a critical parameter for separating potential resistant lines from susceptible lines. These potential resistant lines were further verified in F_2 re-screening by using a corrected surviving rate (>21.3%) for field-collected males to carry sr and rr genotypes.

Introduction

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is one of the economically most important insect pests on cotton in China. Transgenic cotton, expressing the Cry1Ac toxin from *Bacillus thuringiensis* (Bt) has become one of the most important tools for managing the insect in China since 1997. Since the first introduction in 1996 (John 1997, Zhang et al. 2000**)**, Bt cotton has become a dominant crop in many cotton growing areas. In North-Central China, Bt cotton, transformed with single Cry1ac toxin gene, completely replaced conventional cotton in many regions. The widespread implementation of the single toxin producing cotton has likely prompted adaptation and increased the intensity of selection for Bt resistance in target insect populations (Gould 2003, Huang 2006).

Resistance levels that increased over years (Wan et al. 2005) might be associated with increasing selection pressure due to increased Bt crop acreage over years. Other factors, such as different monitoring methods, sources of Bt toxin, background of insect control and resistance management in different regions, and genetic variability in the field populations, might also contribute to the variation of resistance levels detected.

In this study, F_1 screening was used to monitor Bt resistance in the field populations of *H. armigera* in North-central cotton growing area in China. Because of recessive or partially recessive nature of the Bt resistance (Akhurst et al. 2003, Tabashnik et al. 2005, Xu et al. 2005), it is difficult to monitor early shifts of heterozygous allele frequencies using the LC_{50} and other dose-response parameters (Roush and Miller 1986, Venette et al. 2000). F_1 and F_2 screenings (Andow et al. 1998, Gould et al. 1997) are effective and sensitive bioassay techniques in detecting rare resistance alleles at an early stage of resistance development. Considering time/cost saving and the availability of a highly resistant strain of *Helicoverpa armigera*, selection with Bt cotton leaves, in our laboratory, the F_1 screening technique was used in this study.

Because the F_1 screening has ability to detect the resistance alleles not only at the same loci as r allele in the resistant strain, but also at the other loci if they confer resistance and have dominant expression in field populations (Gould et al. 1997), separation of resistant from susceptible phenotypes was difficult. The major objective of this study was to develop a criterion for distinguishing resistance gene alleles in the field populations of *H. armigera*.

Materials and Methods

Insects. The susceptible strain of the cotton bollworm (YCS) has been reared for 145 generations on artificial diet without exposure to any insecticides including Bt toxins. This strain was used in this study as a base-line reference for comparison of larval growth rate. The resistant strain (YCR) was developed from selection of a colony for up to 102 generations with Bt cotton leaves, and was used to mate with field-collected moths to produce F_1 progenies. Male moths of field populations were collected in 2006 and 2007 from Bt cotton fields using light traps. The males were transferred to laboratory and were allowed to individually (single-pair) mate with the virgin female moths of the resistant strain. The F_1 progenies were used for F_1 screening.

Transgenic Bt cotton. Approximately 7-wk old cotton leaves were made continuously available by periodically planting variety Xinmian33^B (NuCOTN33^B, Bollgard®) in the greenhouse. Xinmian33^B leaves, used for all experiments, express a high amount of the Bt insecticidal crystal protein Cry1Ac. Bt Cry1Ac toxin expression in Bt cotton was verified using the susceptible strain to ensure that all susceptible insects were killed. A non-Bt conventional cotton was used as the control.

Growth rate of laboratory strains and hybrids on Bt cotton. A growth experiment was conducted to characterize larval growth, development, and survival on Bt cotton for the susceptible and resistant laboratory strains, and the hybrids of reciprocal crosses F_1 ($♀_{YCR}×♂_{YCS}$) and F_1' ($♀_{YCS} × ♂_{YCR}$). A total of 100 neonates from each strain were fed with Bt cotton leaves. After 5 d, the survival rate, developmental stage, and larval body weight of all survivors were recorded.

F_1 screening for resistance genotype. Field-collected males were individually mated to virgin females (r_1r_1) of the homozygous resistance strain. Their progeny were tested on Bt cotton leaves using the same method of growth rate assay. F_1 survivors were considered resistant individuals if they reached the same growth and developmental rates of the resistant strain on Bt cotton leaf for 5 days. The potential positive lines from F_1 selection were further verified with F_2 screening as described below.

F_2 rescreening for eliminating false positive families. All potential F_1 positive lines were rescreened to eliminating false positive lines. The F_1 survivors were transferred to artificial diet and reared until pupation. After moths emerged, single sib-mating pairs were set up for all potential positive F_1 families. F_2 neonates were screened on Bt cotton leaves for 5 d.

Results and Discussion

Differential growth rates of susceptible, resistant, and hybrid progenies. After 5-d feeding on Bt cotton leaves, most Bt-resistant larvae survived. All larvae from the susceptible strain (ss) were unable to survive on Bt cotton. Most heterozygous individuals died after feeding on Bt cotton. The results demonstrated that the Bt-resistant strain had significantly higher surviving rate (75±1.9%) than the Bt-susceptible strain (0%) and the other two hybrids. Similarly, the Bt-resistant strain had significantly higher mean body weight (0.707±0.01 mg) than the Bt-susceptible strain (0 mg) and other two hybrids. No heterozygous survivors reached body weight of ≥0.6 mg and developmental stage of mid-2nd instar. Whereas the body weight of resistant individuals (r_1r_1) ranged from 0.4 to 2.1 mg, and a majority of survivors (65% of total or 87% of the survivors) reached ≥0.6 mg and at least mid-2nd instar.

F_1 screening using Bt cotton leaves. After 5-day feeding on Bt cotton, seven lines completely died. Various numbers of F_1 progenies died in all other lines. Surviving rates ranged from 0.7% to 40% in 2006 and ranged from 3% to 51.8% in 2007. Scatter plot was used to show the distribution of F_1 surviving rates. The surviving rates distributed almost evenly and continuously within the range, and no clear separation could be distinguished from the total of 262 lines. Therefore, it was impossible to separate resistant lines from susceptible lines based on surviving rates.

A total of 7782 larvae survived 5-d treatment on Bt cotton. Larval body mass ranged from 0.1 mg to 2.3 mg. Results showed that most survivors had low weight with up to 38% of the larvae not growing beyond 0.1 mg. Approximately 85% survivors had a body weight of less than 0.6 mg in both 2006 and 2007.

Regression analyses indicated a correlation between surviving rate and larval body weight for each line. The R-square value reached the maximum as the body weight increased to 0.7 mg and higher (P<0.001), however, the correlation slope decreased as the weight was beyond 0.6 mg. To minimize underestimation of resistance alleles, both larval body weight of ≥0.6 mg and developmental stage of mid-2nd instar were used as a reference for differentiating positive (putative resistant) from negative (putative susceptible) lines.

Based on above criterion, F_1 progenies from 93 lines reached body weight ≥0.6 mg and developmental stage of beyond mid-2nd instar. The growth and development rates of these lines were similar to those of resistant strain, and then these lines were considered as potential positive lines with YCR-like resistance gene alleles. Other lines did not grow and develop as fast as the resistant strain, and then were considered as negative lines or susceptible lines.

F_2 verification. Due to some non-genetic factors, some heterozygous F_1 progenies (sr_1) may grow and develop as fast as homozygous (rr_1) progenies. When the adult F_1 males and females are randomly paired for sib-mating, three possible genotype pairs may occur, including (1) rr_1× rr_1, (2) sr_1× rr_1, and (3) sr_1× sr_1. Theoretically, three surviving rates (100%, 50%, and 25%, respectively) will be observed based on recessive resistance nature. Because only 75% (instead of 100%) of the resistant strain (r_1r_1) survived on Bt cotton and 88% of the resistant strain survived on conventional cotton, corrected surviving rate for resistant strain is 85.2%. The corrected surviving rate for sr_1× sr_1 genotype is 21.3%. Therefore, the F_2 progenies with surviving rate ≤21.3% indicate that the field-collected male (F_0) carried ss genotype, otherwise, the F_0 male carried sr or rr genotype if the F_2 progenies have surviving rate >21.3%. By using this criterion, approximately 23% of the putative positive F_1 lines produced F_2 progenies which had surviving rates below 21.3%. These lines were considered as false positives categorized by growth criteria of body weight ≥0.6 mg.

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