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Influence of transgenic hybrid rice expressing a fused gene derived from *cry1Ab* and *cry1Ac* on primary insect pests and rice yield

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ABSTRACT

Although dozens of transgenic *Bacillus thuringiensis* (*Bt*) rice lines have been developed, none of them has been released to farmers. Under field conditions, we evaluated the influence of a hybrid *Bt* rice on the primary rice insect pests and rice yield in 2005 and 2006. Four treatments were evaluated, including *Bt* and non-*Bt* rice treated with insecticides when necessary, and unprotected *Bt* and non-*Bt* rice. Unprotected *Bt* rice exhibited stable and high control of the three primary lepidopteran pests, *Chilo suppressalis* Walker, *Tryporyza incertulas* Walker and *Cnaphalocrocis edinalis* Güenée. Under unprotected conditions, larval densities of these three pests in *Bt* plots decreased by 87.5–100% compared to those in non-*Bt* plots, and percentages of damaged stems and leaves remained less than 0.6% during the entire rice growing season. In early rice growth stages, populations of two important planthoppers, *Nilaparvata lugens* Stål and *Sogatella furcifera* Hovarth, were significantly affected only by protection level (protected vs unprotected). However, in late rice growth stages (filling and maturing), densities of planthoppers were significantly affected both by protection level and by rice type (*Bt* vs non-*Bt*), and densities of *N. lugens* were significantly higher in *Bt* plots than in non-*Bt* plots under unprotected conditions. Pesticide sprays were reduced by 60 and 50% in protected *Bt* vs protected non-*Bt* plots in 2005 and 2006, respectively. Yield of unprotected *Bt* rice increased by 60–65% compared to unprotected non-*Bt* rice, but decreased by 28–36% compared to protected *Bt* rice. These results show that *Bt* rice increased yield greatly, but still required pesticide sprays to avoid losses caused by non-target insect pests.

1. Introduction

Bacillus thuringiensis Berliner (*Bt*) is a spore-forming bacterium that is lethal to selected insect pests (e.g., Lepidoptera and Coleoptera). It has a long history of use since the 1930s as a microbial control agent (Shelton et al., 2002). *Bt* crops that express insecticidal protein genes derived from *Bt* have provided an efficient method for insect pest management on a global scale. Since commercially released in 1996, plantings of transgenic *Bt* crops, particularly *Bt* corn and *Bt* cotton, have increased dramatically (James, 2008). Benefits of worldwide adoption of *Bt* crops include increased crop yields, reduced pesticide use, less environmental damage and reduced labor (Garcia, 2005; Edge, et al., 2001; Huang et al., 2003; Naranjo, 2009).

Rice, *Oryza sativa* L., is a principal food crop for nearly 3 billion people (Vaesen et al., 2001). Heavy, perennial yield losses have been documented by insect pests, especially stem borers, leaffolders and planthoppers, in China (Sheng et al., 2002; Chen et al., 2003b). Many companies and institutions are developing genetically modified insect-resistant rice, and dozens of *Bt* rice genotypes with high resistance against lepidopteran pests have been developed since 1993 (summarized by Chen et al., 2006b; Cohen et al., 2008). However, *Bt* rice has not yet been released to farmers primarily because there are concerns about potential adverse ecological impacts. However, field trials with *Bt* rice began in 1998 (Chen et al., 2006b), and have not yet revealed negative impacts on non-target organisms. For example, Bernal et al. (2002) reported that brown planthopper (BPH), *Nilaparvata lugens* Stål, and its natural enemy, *Cyrtorhinus lividipennis* Reuter, were exposed to *Bt* toxins from rice lines, but exposure did not affect the fitness of either species. A recent trial also confirmed that a *cry1Ab* transgenic rice did not affect several natural enemies in paddy fields (Chen et al., 2007). Chen et al. (2006a) disproved the possibility that *Bt* rice would lead to higher populations or greater damage by rice

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planthoppers and leafhoppers. Other studies revealed that several *Bt* rice lines did not impact non-target arthropods at the population and community levels (Jiao et al., 2007, 2006; Chen et al., 2003a; Liu et al., 2002).

In the field experiments cited above, plots of *Bt* rice lines and isogenic counterparts were often managed the same way, so the only difference among treatments was crop type (*Bt* and non-*Bt*). If *Bt* rice is released for commercial planting in the future, it should not be managed the same way as conventional cultivars, particularly in terms of pest management. In China, rice is mostly grown by farmers with relatively small rice plantings. To what extent *Bt* rice can reduce pesticide use and increase yields relates directly to its adoption by farmers. In this paper, a 2-year field study was conducted to compare a hybrid *Bt* rice expressing a fused gene derived from *cry1Ab* and *cry1Ac* and its isogenic counterpart in terms of pest insect populations and damage. Under local rice pest management programs, pesticide application frequency and rice yields were also compared between the two rice types.

2. Materials and methods

2.1. Plant materials

Transgenic *Bt* Shanyou 63 and its non-transgenic counterpart were evaluated. *Bt* Shanyou 63 was produced by crossbreeding Zhenshan 97A, an elite *indica* CMS (cytoplasm male sterile) restorer line, with *Bt* Minghui 63, an elite *indica* CMS line. *Bt* Minghui 63 possessed a fusion gene derived from *cry1Ab* and *cry1Ac* driven by *actinI* promoter (Tu et al., 2000). After transformation, *Bt* Minghui 63 was cultivated for five generations in the field, and then was used to produce *Bt* Shanyou 63.

2.2. Treatments and plot design

Field experiments were conducted at an experimental farm in Wuhan Provence, China (latitude 30°34′ N, longitude 114°17′ E) in 2005 and 2006. In this area, there are three rice-cropping seasons according to sowing date, with middle and later season rice being exposed to the more serious pest insect infestations. *Bt* Shanyou 63 and the non-transgenic control were sowed as middle season rice in late April and transplanted 1 month later. Experimental plots were laid out in a completely randomized design, with four treatments, i.e., unprotected (a) and protected (b) *Bt* rice, unprotected (c) and protected non-transgenic rice (d), and four replicates. Plots with pesticide sprays were inspected every 4 or 5 days, and treated with appropriate pesticides when pest densities exceeded action thresholds according to local integrated pest management (IPM) programs (Chongqing Educational Committee, 1992). Each plot was 600 m^2, surrounded by a 1-m wide non-cropped buffer. Rice seedlings were transplanted by hand at a density of one seedling per hill. Spacing between rows was 20 cm and hills within rows were spaced 15 cm apart. All treatments were managed by normal cultural practices except for pesticide sprays. A windshield was held downwind between protected and unprotected plots when spraying.

2.3. Pest insect sampling and rice yield testing

In Wuhan of Hubei Province, rice pest insects prone to cause significant damage include two stem borers, *Chilo suppressalis* Walker and *Tryporyza incertulas* Walker, a leaffolder, *Cnaphalocrocis edinalis* Güenée, and two planthoppers, *Nilaparvata lugens* Stål and *Sogatella furcifera* Hovarth. We therefore selected these five species as the primary emphasis for field inspections. Five sampling sites, each consisting of five rice hills, were selected randomly from the central rows of each plot. Every tiller in selected hills was examined carefully for the five species and damage caused by them. A white square enamel tray (25 × 30 cm) painted with mineral oil was used to estimate *N. lugens* and *S. furcifera* densities. We placed the tray even with the rice stem base, shook the rice hill by hand three times and then counted nymphs and adults clinging on the tray. Leaves with visible scrapes or rolls were considered to be damaged by leaffolders, and were dissected to count leaffolder larvae. Meanwhile, percentage of damaged leaves was estimated. *C. suppressalis* and *T. incertulas* cause deadhearts or whiteheads when the larvae penetrate rice stems. Damaged stems were dissected to count larvae of *C. suppressalis* and *T. incertulas*. Sampled rice hills were marked with a bamboo rod to avoid being sampled a second time. Sampling was conducted during tillering, booting, blooming, grain filling and maturing. Sampling dates were selected to stagger the pesticide sprays as much as possible. When rice grain ripened fully in mid September, 10 rows (each containing 10 hills) were selected randomly in each plot to evaluate rice yield. Panicels were threshed and grain was dried in sunlight and weighed.

2.4. Data analyses

Total numbers of insects recorded in each plot on each sampling date were pooled together for statistical analysis. Means of population densities, damage rates and rice yield in different treatments were analyzed by two-way (rice type vs protection level) analysis of variance (ANOVA) using SPSS (version 12.0). Before analysis, insect count data were transformed using square root $(X + 1)$, but untransformed means are presented. When a significant difference was detected, means were compared and separated by least significant difference (LSD, $P \leq 0.05$).

3. Results

3.1. Insecticide use in protected treatments

Insecticides used in the two protected treatments, together with target species, and spraying dates are summarized in Table 1. Protected non-*Bt* rice plots were sprayed five times in 2005 and four times in 2006 targeting *C. medinalis*, *C. suppressalis*, *T. incertulas*, *N. lugens* and *S. furcifera* according to the action threshold of each pest (Jiang and Wu, 1991). Sprays in protected *Bt* plots were reduced by 50% compared to protected non-*Bt* rice in both years. Although considerable adult and egg densities of *C. medinalis*, *C. suppressalis*, and *T. incertulas* were often observed in *Bt* rice plots, the larval densities did not exceed action thresholds (Table 2).

3.2. Abundance of main target pest insects

Larval densities of three primary pests, *T. incertulas*, *C. suppressalis* and *C. medinalis*, were compared among treatments in three rice developmental stages in 2005 and 2006. In sum, *Bt* rice provided good control of the main lepidopteran pests. In most cases, control by *Bt* rice exceeded control by insecticide sprays applied three or four times during the rice growing period. The first sampling was conducted at 30 days after transplanting (DAT) in 2005 and 39 DAT in 2006, during the tillering stage when lepidopteran pests began to infest rice, before pesticides were applied. At that time, very little larval damage was observed in *Bt* rice plots, while larval densities in non-*Bt* plots approached action thresholds (Table 2).

Before the second sampling in the booting stage, protected non-*Bt* rice treatments were sprayed two times in 2005, and one time in 2006, to control lepidopteran pests. Protected *Bt* rice treatments were not sprayed because pest insect densities did not exceed