Field Crops Research 118 (2010) 215–220

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Yield benefit and underlying cost of insect-resistance transgenic rice: Implication in breeding and deploying transgenic crops

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ARTICLE INFO

Article history:
Received 11 March 2010
Received in revised form 17 May 2010
Accepted 18 May 2010

Keywords:
Oryza sativa
Insect resistance
Transgene
Transgenic breeding
Genetically modified crop

ABSTRACT

The rapid development of transgenic biotechnology has greatly promoted the commercialization of genetically modified (GM) crops including the insect-resistant crops worldwide. Apart from the enormous yield benefits brought by the GM crops, the cryptic fitness cost associated with transgenes has also been detected under experimental conditions although it is considered to be rare. To estimate the yield benefit and cost of insect-resistant GM rice, we studied field performances of three insect-resistant GM rice lines, involving their non-GM parental variety as comparison. Great benefits as estimated by the yield-related traits were observed in the GM rice lines when high insect pressure was recorded, but a cryptic yield loss was detected when the level of insect pressure was extremely low. Given the fact that cryptic yield loss presented in the GM rice lines under the low insect pressure, a strategic field deployment should be required when insect-resistant GM rice are commercialized to circumvent the unnecessary yield losses. This is probably true for other insect-resistant GM crops. Effective biotechnology and breeding measures are also needed to particularly minimize the potential underlying cost of an insect-resistance transgene before commercial production of the GM crops.

1. Introduction

The rapid development and application of transgenic biotechnology have greatly promoted the field production and commercialization of genetically modified (GM) crops over the world (Nap et al., 2003; Brookes and Barfoot, 2009; James, 2009). The application of transgenic biotechnology and GM products is very important in terms of its potential for poverty alleviation and solving the problems of malnutrition at large. By the end of 2009, the estimated global area of GM crops has exceeded 130 million hectares and generated about US$52 billion economic gains worldwide (James, 2009). Insect-resistant GM crops are among the most successful transgenic products, accounting for 15% of total global cultivation area of GM crops. For example, the cultivation of insect-resistant GM cotton has led to reduced applications of pesticides that can harm human health and agricultural ecosystems (Huang et al., 2003; Brookes and Barfoot, 2009). Additionally, a considerable decrease in regional outbreaks of cotton ball worms in cotton and many other crops was found to be associated with the extensive cultivation of GM *Bt* cottons (Wu et al., 2008).

The huge yield benefits brought by the cultivation of insect-resistant GM crops appear obvious (Brookes and Barfoot, 2009; James, 2009). However, transgenes may also bring "fitness cost" to GM crops, probably due to (1) the added burden by the constitutive over-expression of the alien transgene (Gurr and Rushton, 2005); and (2) disruption to the function of native gene by random insertion of transgenes into the host genome (Marrelli et al., 2006). The above-mentioned two types of fitness cost brought by a transgene have been detected in a number of studies under experimental conditions. For example, a reduction of fruit number was detected in two cold tolerant transgenic (*CBF*) *Arabidopsis thaliana* lines, which was considered as the fitness cost by the constitutive expression of the transgenes (Jackson et al., 2004). A significant reduction in 1000-kernel weight was also detected in a transgenic wheat line, which was likely connected with the over-expression of a leaf-rust resistance transgene (*Lr10*; Romeis et al., 2007). In addition, transgenic (*chitinase IV*) silver-birch lines that had increased resistance to fungal diseases also exhibited a lower growth rate than their non-transgenic controls, possibly caused by the position effect of the inserted transgene (Pasonen et al., 2008). All these studies demonstrated the underlying cost of a transgene that may cause yield reduction of a GM crop at a different extent if the cost is significant.

The field performance of insect-resistant GM crops, particularly in rice under conditions with low or no insect occurrence,

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doi:10.1016/j.fcr.2010.05.008

in the event that an underlying fitness cost associated with these transgenes might become visible, is less understood. In fact, the occurrences of insect pests fluctuate significantly among years in ago-ecosystems (Utida, 1958; Zhu et al., 2003, 2008). Therefore, exploring the potential underlying cost caused by a transgene is important and practical for the effective application of an insect-resistant GM crop. Rigorous tests of inherent fitness cost of novel transgenes in commercialized crops in field are rare in peer-reviewed literature (Chen et al., 2006; Kim et al., 2008), although some researchers consider such costs to be absent or negligible (Qaim et al., 2006). Some previous studies did not include rigid controls on insect pressure (Andersen et al., 2007; Gomez-Barbero et al., 2008), so that any cost brought by a transgene may not be easily detected, which is probably the reason for the expectation of negligible underlying cost of transgenes. Whether or not there exists an underlying fitness cost of insect-resistance transgenes, which may lead to a yield loss under certain environmental conditions, needs to be determined by well-designed studies under field experiments involving different GM crops.

Rice is an important cereal crop providing a staple food for nearly one half of the global population (FAO, 2004). In China, many insect-resistant GM rice lines have been developed and some are in the pipelines waiting for commercialization (Lu and Snow, 2005; Wang and Johnston, 2007). Recently, two GM *Bt* rice lines were granted biosafety certificates by the Ministry of Agriculture in China. Therefore, before extensive commercial production, it is important to estimate GM insect-resistant rice for the underlying cost from any transgene that may result in yield losses under low or no target insect occurrences. On the basis of our previous results that have shown yield benefit and underlying cost of three GM rice lines containing insect-resistant transgenes, under environmental conditions with different target insect occurrences (Chen et al., 2006), we have conducted additional 2-year field experiments at a larger scale involving the three GM rice lines under the similar experimental designs of insect pressure. The objective of this study was to determine the fitness benefit brought by the transgenes under normal insect pressure and any underlying cost of the transgenes under low insect pressure in field conditions. This knowledge is very important to facilitate the design of strategic field deployment of insect-resistant GM rice varieties based on information of insect occurrences, and to avoid the unnecessary yield losses caused by the underlying cost when insect pressure is low.

2. Materials and methods

2.1. Plant materials

In this study, we investigated three GM rice lines: 86AB1 with a *Bt cryIAc* (*Bacillus thuringiensis*) gene, 86AS2 with a *CpTI* (cowpea trypsin inhibitor) gene, and Kefeng-6 with both transgenes in a double insertion, *Bt*/*CpTI*. A selectable marker gene (*hy*, for hygromycin resistance) was tightly linked to the target transgenes. The *Bt*, *CpTI*, and *hy* genes were under the promoter of ubiquitin (derived from maize), *ActID* (derived from rice), and *CaMV35s*, respectively. The three insect-resistant GM lines were produced from a rice variety Minghui-86 *via* the agrobacterium-mediated transformation technology. The transformation-generated T0 individuals with the target transgenes were maintained for further improvement. The three insect-resistant GM lines were bred through consecutive selfing and selection for stable resistance to target insects and the desired agronomic traits beyond the T7 generation. Consequently, the three insect-resistant GM lines had ideal field performances and were genetically stable compared to other lines. Our previous study using the sandwich enzyme-linked immunosorbent assay (ELISA) showed that the expression level of the *Bt* transgene in the GM rice lines, as well as in its hybrids with wild rice (*Oryza rufipogon*), was consistently high ranging from 0.015 to 0.22% of total soluble protein in leaf samples (Xia et al., 2009). The expression level of the *CpTI* transgene in the GM rice lines were also very high, ranging from 0.05 to 0.53% of total soluble protein in leaf samples (F. Wang, unpublished data). These insect-resistant GM lines were produced to effectively deter lepidopteran pests such as rice stem borers (*Scirpophaga incertulas*, *Chilo suppressalis*, and *Sesamia inferens*), and rice leaf-folder (*Cnaphalocrocis medinalis*). The parental variety Minghui-86 was used as the non-GM control.

2.2. Field experimental design

Field experiments were conducted in the designated Biosafety Assessment Centers in the Wufeng Village and the Fujian Academy of Agricultural Sciences in Fuzhou, Fujian Province of China in 2004 and 2006, respectively. To test the potential benefit and underlying cost brought by the transgene, two environmental conditions were created: (1) normal insect pressure in rice fields at the Wufeng site; and (2) low insect pressure in a rice plot on the rooftop of a building in the Fujian Academy of Agricultural Sciences more than 10 km away from any cultivated rice fields (rooftop site). To create environments with different competition strength, two cultivation modes were employed: (1) pure cultivation of either GM or non-GM rice plants; and (2) mixed cultivation of equal number of GM and non-GM rice plants. In each field experiment of different years, we included four treatments of pure cultivation (one non-GM control and three GM lines), and three treatments of mixed cultivation respectively with GM lines and their non-GM control, under each environmental condition of insect pressure. Consequently, a total of 14 treatments for the 2 environmental conditions (normal insect versus low insect pressure) were included in the field experiment.

The experimental layout in the field followed the complete randomized block design. Each treatment included five replicates in 2004 and three replicates in 2006. Each replicate included 169 rice individuals planted as 13 × 13 plants with 20 cm between rows and hills. From the field plots with normal insect pressure, a total of 60 plants from all pure cultivation treatments, and 30 GM plants and 30 non-GM plants each from all mixed cultivation treatments were randomly sampled for characterization in 2004, while in 2006, 63 plants from all pure cultivation treatments, 42 GM and 42 non-GM plants each from all mixed cultivation treatments were sampled for characterization. From the field plots with low insect pressure, a total of 60 plants from all pure cultivation treatments, and 30 GM and 30 non-GM plants each from all mixed cultivation treatments were randomly sampled for characterization in 2004, while in 2006, 48 plants from all pure cultivation treatments, 24 GM and 24 non-GM plants each from all mixed cultivation treatments were sampled for characterization. In the 2004 experiment, rice seeds were sowed on June 30th, seedlings were transplanted to the field on July 27th, and rice plants were harvested on October 27th. In the 2006 experiment, rice seeds were sowed on June 25th, seedlings were transplanted to the field on July 20th, and rice plants were harvested on October 31st.

Cultivation management in the experiment followed the routine procedure of local farmers. About 2.4–3.0 kg nitrogen (urea) per 100 m^2 was applied at different rice growth stages (40% before transplanting, 40% at tillering stage, and 20% before flowering). In addition, phosphorus (P_2O_5) and potassium (K_2O) were also applied at various rice growth stages, and the approximate proportion of different fertilizers was: nitrogen 40%, phosphorus 30%, and 30% potassium. Weeds occurring in the field plots were hand-removed.

2.3. Data collection and analysis

The same rice plants from different plots were sampled and measured at different times to estimate the insect pressure in different environments and field performance for the yield-related traits, respectively. Insect pressure in different environments was estimated only based on the percentage of damaged leaves (by leafrollers) and blasted tillers (by stem borers) separately in non-GM rice plants in pure cultivation, 60 days after transplanting of rice seedlings. The percentage of damaged leaves and blasted tillers of the three GM rice lines was also measured as controls. The insect index (%) was applied to reflect the general insect pressure in different environments and calculated as the average percentage of both damaged leaves and blasted tillers. The use of this index to reflect insect pressure could avoid the misestimate of insect occurrence by counting the actual number of insects that could move between the fields. Four yield-related traits were characterized from mature plants to estimate the field performance of the GM and non-GM rice. These traits were: number of panicles per plant, panicle fertility determined as the proportion of filled grains out of the total number of grains per plant, number of filled grains per plant, and grain yield as measured by the weight of filled grains per plant. In addition, the percent increase or decrease of the GM lines relative to the non-GM control for the four yield-related traits was also calculated based on the results of 2004 and 2006 field experiments, and extracted data from the 2003 experiment (Chen et al., 2006). Significant differences between GM lines and the non-GM controls were determined by the independent *t*-test (two-tailed) for the pure cultivation, and the paired *t*-test (two-tailed) for the mixed cultivation, in which the Levene test was included for the equality of variances. All statistical analyses were conducted using the software SPSS Ver. 15.0 for windows (SPSS Inc., Chicago, IL, USA, 2006).

3. Results

3.1. Insect pressure in the experiments

In general, dramatic differences in insect pressure were observed between the non-GM controls under the two environmental conditions: normal insect pressure versus low insect pressure, as revealed by the insect index (%) in the two experimental years (Fig. 1). Under the normal insect pressure, the insect index was considerably high (13.9–23.3%) in the non-GM control in pure cultivation. In contrast, under the low insect pressure, the insect index was extremely low (ca. 0%) in the non-GM control. This indicated that the two environmental conditions with normal and low insect pressure were successfully created in our 2-year experiments. The differences in insect pressure provided an ideal system for the comparison of yield-related traits between GM rice lines and their non-GM control.

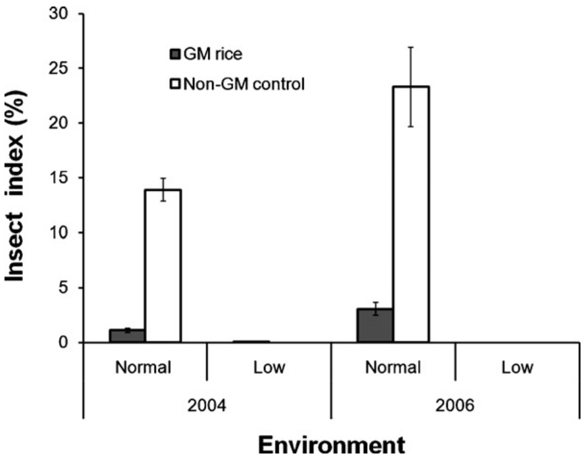


Fig. 1. Insect pressure under different environmental conditions at the experimental sites (normal versus low insect) in 2004 and 2006, as estimated by the "insect index" that was calculated as the averaged percentage of damaged leaves and tillers of the three insect-resistant GM rice lines or non-GM controls in pure cultivation plots. The white columns represent non-GM controls and the gray columns represent GM rice lines. Bars indicate the standard errors.

Noticeably, the insect pressure as indicated by the index in the non-GM control was much higher in 2006 (23.3%) than in 2004 (13.9%), suggesting the fluctuation of insect occurrences between years. In addition, there was a very low percentage of observed insect damage in the GM rice lines, particularly in the year of 2006, which is possibly caused by the non-target insects occurring in the experimental fields. The significantly lower insect damage in the GM rice lines than in the non-GM control under normal insect pressure demonstrated the effectiveness of transgenes to deter the target insects.

3.2. Performance of GM rice under normal or low insect pressure

The yield benefit or underlying cost of the insect-resistant GM rice lines was estimated by their key yield-related traits under

Table 1
Values of yield-related traits of GM rice lines and their non-GM control in pure-planting under different insect pressure.

Year	Insect pressure	Rice line	Grain yield/plant (g)	No. of seeds/plant	Panicle fertility (%)	No. of panicles/plant
2004	Normal	*Bt*	23.8 ± 3.3	701.5 ± 94.0	78.3 ± 2.0	7.9 ± 1.0
		CpTI	23.8 ± 1.7	689.1 ± 48.1	81.9 ± 2.1	7.5 ± 0.3
		Bt/CpTI	23.1 ± 2.1	729.3 ± 64.8	78.4 ± 1.8	7.7 ± 0.3
		Minghui-86	25.3 ± 1.4	732.5 ± 49.5	79.8 ± 1.4	7.8 ± 0.4
	Low	*Bt*	**7.0 ± 1.2**	**265.1 ± 39.6**	**74.6 ± 3.9***	**3.5 ± 0.2**
		CpTI	**10.2 ± 0.8**	**392.6 ± 27.9**	77.8 ± 2.8	4.3 ± 0.3
		Bt/CpTI	9.5 ± 1.2	**386.0 ± 48.4**	77.7 ± 3.6	**4.8 ± 0.8**
		Minghui-86	8.7 ± 1.2	312.5 ± 41.9	84.9 ± 2.1	4.0 ± 0.3
2006	Normal	*Bt*	**25.4 ± 2.0**	**935.5 ± 71.3**	86.6 ± 1.6	8.4 ± 0.5
		CpTI	**28.6 ± 2.7***	**1110.3 ± 127**	92.9 ± 1.1*	8.2 ± 0.7
		Bt/CpTI	**28.5 ± 1.8***	**1143.5 ± 68.8***	87.4 ± 0.7	9.1 ± 0.4
		Minghui-86	21.0 ± 2.2	788.6 ± 89.1	86.8 ± 1.0	8.3 ± 0.8
	Low	*Bt*	19.5 ± 3.2	724.3 ± 116.5	90.1 ± 2.6	6.0 ± 0.9
		CpTI	19.4 ± 0.7	717.9 ± 29.3	94.6 ± 0.8	5.9 ± 0.3
		Bt/CpTI	17.8 ± 2.2	733.0 ± 84.7	86.9 ± 1.0*	5.9 ± 0.5
		Minghui-86	19.0 ± 2.0	701.4 ± 71.9	95.4 ± 0.8	5.6 ± 0.3

Values 10% higher or less than those of the non-GM control are indicated by bold letters.

* Significant differences ($P < 0.05$) between GM rice line [86AB1 (*Bt*), 86AS2 (*CpTI*), or Kengfeng-6 (*Bt/CpTI*)] and their non-GM control (Minghui-86) based on independent *t*-test.

Table 2
Values of yield-related traits of GM rice lines and their non-GM control in mixed-planting under different insect pressure.

Year	Insect pressure	Rice line	Grain yield/plant (g)	No. of seeds/plant	Panicle fertility (%)	No. of panicles/plant
2004	Normal	*Bt*	25.6 ± 2.6	723.3 ± 70.1	77.1 ± 1.7	**7.7** ± **0.3**
		Minghui-86	25.6 ± 1.8	736.9 ± 46.4	77.4 ± 4.7	9.2 ± 0.7
		CpTI	21.0 ± 3.7	595.8 ± 101.0	79.8 ± 1.9	**7.8** ± **1.2**
		Minghui-86	21.4 ± 3.3	617.3 ± 95.3	76.0 ± 1.6	6.9 ± 1.2
		Bt/CpTI	21.3 ± 1.6	645.2 ± 45.6	73.5 ± 3.5	**7.2** ± **0.3**
		Minghui-86	22.0 ± 3.2	628.6 ± 90.6	71.2 ± 3.3	8.1 ± 0.9
	Low	*Bt*	**5.0** ± **0.9**	**184.6** ± **32.8**	77.4 ± 3.3	**2.4** ± **0.3**
		Minghui-86	10.3 ± 2.0	386.7 ± 71.6	77.8 ± 4.0	5.1 ± 1.0
		CpTI	**6.3** ± **2.0**	**269.9** ± **91.5**	77.1 ± 7.7	**3.4** ± **0.6**
		Minghui-86	10.2 ± 1.4	373.9 ± 52.7	78.5 ± 2.9	5.3 ± 0.6
		Bt/CpTI	**4.0** ± **1.1**[*]	**157.8** ± **41.9**	**71.9** ± **3.9**[*]	**2.3** ± **0.3**[*]
		Minghui-86	9.2 ± 1.1	339.2 ± 41.6	82.1 ± 3.2	4.5 ± 0.5
2006	Normal	*Bt*	**19.2** ± **2.5**	**691.9** ± **89.9**	81.8 ± 1.4	7.0 ± 0.7
		Minghui-86	16.8 ± 1.8	620.4 ± 70.3	82.9 ± 2.9	6.6 ± 0.7
		CpTI	18.6 ± 1.9	710.1 ± 67.8	86.8 ± 3.7	7.2 ± 0.2
		Minghui-86	20.4 ± 3.0	770.7 ± 98.8	86.2 ± 2.6	7.7 ± 0.8
		Bt/CpTI	**22.1** ± **2.0**	**889.2** ± **78.8**	84.2 ± 2.1	7.7 ± 0.4
		Minghui-86	18.2 ± 0.4	668.8 ± 31.4	85.3 ± 1.3	7.2 ± 0.3
	Low	*Bt*	16.6 ± 3.0	663.4 ± 125.7	91.6 ± 0.5	5.8 ± 0.8
		Minghui-86	18.2 ± 2.7	688.5 ± 129.3	91.2 ± 1.9	5.6 ± 0.5
		CpTI	**16.2** ± **2.2**	**609.6** ± **79.4**	93.1 ± 2.5	**5.3** ± **0.6**
		Minghui-86	23.6 ± 3.8	862.9 ± 137.3	95.0 ± 0.7	6.3 ± 0.8
		Bt/CpTI	**13.0** ± **3.3**[*]	**543.2** ± **136.9**[*]	**83.9** ± **1.5**[*]	**4.8** ± **0.8**
		Minghui-86	25.3 ± 2.0	936.3 ± 84.4	93.7 ± 1.9	7.3 ± 0.6

Values 10% higher or less than those of the non-GM control are indicated by bold letters.

[*] Significant differences ($P<0.05$) between a GM rice line [86AB1 (*Bt*), 86AS2 (*CpTI*), or Kengfeng-6 (*Bt/CpTI*)] and their non-GM control (Minghui-86) based on the paired *t*-test.

normal or low insect pressure. Under the environmental conditions with normal insect pressure, nearly all the yield-related traits showed much higher values in the GM lines [86AB1 (*Bt*), 86AS2 (*CpTI*), and Kefeng-6 (*Bt/CpTI*)] than in their non-GM counterpart (Minghui-86) in the 2-year field experiments, particularly in the mixed cultivation mode (Tables 1 and 2), although many values of the traits did not show significant differences between the GM lines and their non-GM control. In contrast, many yield-related traits showed lower values in the GM lines than in the non-GM counterpart under the environmental conditions with low insect pressure. Such decreased field performances of the key traits were particularly outstanding in the mixed cultivation mode (Table 2). It is necessary to point out that no obvious enhanced performances for the yield-related traits was detected in the GM lines under the environmental conditions with normal insect pressure in the 2004 experiment (Tables 1 and 2) when relatively low insect pressure was recorded in that specific year. On the other hand under the environmental conditions with low insect pressure, the insect-resistant GM rice lines showed generally lower values for the yield-related traits than their non-GM controls, particularly in the mixed planting mode with competition (Tables 1 and 2), indicating an obvious underlying cost in the GM rice lines.

When values of the yield-related traits of GM rice lines were transformed into the percent increase or decrease relative to the non-GM control, the increase/decrease in field performances between GM rice lines and their non-GM control under the different field experimental conditions was clearly demonstrated (Table 3). In general, the three GM rice lines showed enhanced yield perfor-

Table 3
Grain-yield-related traits of GM rice plants as indicated by the percent increase or decrease (shaded areas) of GM lines relative to non-GM controls. Only values with changes >10% (which may be meaningful to agriculture) are shown. Underlined values indicate a significant increase or decrease at $P<0.05$ based on the independent *t*-test (pure cultivation) or paired *t*-test (competition mode). The values with gray background highlight the negative values (cost). The statistical process was conducted on the software SPSS Ver. 15.0 for windows (SPSS Inc., Chicago, IL, USA, 2006). "Mixed" refers to competition mode between GM and non-GM plants. Relevant data in 2003 experiment was extracted from Chen et al. (2006) to compare differences in grain-yield-related traits between 3 years.

Traits	Normal insect						Low insect					
	86AB$_1$ (*Bt*)		86AS$_2$ (*CpTI*)		Kefeng-6 (*Bt/CpTI*)		86AB$_1$ (*Bt*)		86AS$_2$ (*CpTI*)		Kefeng-6 (*Bt/CpTI*)	
	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed	Pure	Mixed
2003												
Grain yield of a plant (g)	38.2	68.0	29.1		34.5	−17.9	19.6	−10.0		−15.2	13.7	−33.3
Number of grains per plant	35.7	65.1	21.0		60.5		14.6			−14.9	18.6	−30.0
Panicle fertility	34.4		12.7	−11.1	15.6		11.0		−11.1	−10.2		−13.0
Number of panicles per plant	21.7	55.8		27.3	38.2							−18.5
2004												
Grain yield of a plant (g)							−19.4	−51.5	18.0	−38.4		−56.3
Number of grains per plant							−15.1	−52.3	25.6	−27.8	23.5	−53.5
Panicle fertility							−12.2					−12.4
Number of panicles per plant		−17.0		12.5		−10.3	−11.5	−52.5		−35.9	20.2	−48.1
2006												
Grain yield of a plant (g)	21.2	14.3	36.2		36.2	21.2				−31.3		−48.6
Number of grains per plant	18.6	11.5	40.8		45.0	33.0				−29.4		−42.0
Panicle fertility			6.9								−8.9	−10.6
Number of panicles per plant										−16.0		−33.3

mances as reflected by more positive values under normal insect pressure, but reduced yield performances as indicated by more negative values under low insect pressure (Table 3). Noticeably, 86AB1 and 86AS2 that contained one insect-resistant transgene (*Bt or CpTI*) showed significantly lower value only for the panicle fertility trait in mixed cultivation (Table 3). However, the *Bt*/*CpTI* GM Kefeng-6 line that contained the double-inserted transgenes showed significantly lower values for nearly all the traits in mixed cultivation (Table 3), indicating more considerable underlying cost of the double-transgenes in this GM line.

4. Discussion

Our data from the 3-year experiments (including results in Chen et al., 2006) clearly demonstrated a great yield benefit of the three insect-resistant GM rice lines in terms of the measured yield-related traits under the environmental conditions with normal insect pressure, most likely due to the high-dose toxin of the two insect-resistant transgenes that have effectively deterred the target insects. The high-level expression of insect-resistant transgenes detected in our previous GM rice study, with >0.01% of Bt protein (Xia et al., 2009) and >0.3% of CpTI protein of total soluble protein, as well as that in other reports (Xu et al., 1996; Nayak et al., 1997; Alam et al., 1998), strongly supports this conclusion. The yield benefit was particularly evident with up to 68% increase in grain production per plant when the GM rice was exposed to the environmental conditions under normal insect pressure and competition between GM and non-GM plants in this study. These results indicate that insect-resistant GM rice can play a very important role in maintaining its high yield potential that might be threatened under severe attacks by target insects in rice ecosystems, as has been pointed out by many other researchers (e.g., Huang et al., 2005, 2008; Wang et al., 2010).

However, our 2-year experiments plus that in 2003 (Chen et al., 2006) also demonstrated a reduced field performance of the three insect-resistant GM rice lines under extremely low insect pressure regarding the yield-related traits, especially under the mixed cultivation condition with competition that can enlarge the differences between GM rice lines and their non-GM control. The reduced yield performance was most likely caused by the underlying cost of the insect-resistance transgenes, particularly in the *Bt*/*CpTI* double-transgene GM lines under competition, because the three GM lines were selected from hundreds of transgenic lines with good agronomic traits. In this case, the yield advantage of insect-resistant GM rice may not be realized when the pressure of target insects in the fields is insufficient, as indicated by our yield results of the experiment in 2004. It is however necessary to point out that there was a certain degree of decrease in yield-related traits in both GM and non-GM rice lines at the site under low insect pressure, compared to that under normal insect pressure. This was most likely caused by the slightly poor soil condition on the rooftop plots, in comparison with that in the field plots (normal insect site). However, the observed decrease in yield traits at the low insect site will not affect our general findings concerning the yield benefit and cryptic cost because our results were obtained through comparison of the yield-related traits of GM and non-GM plants within the same environment (site).

The yield cost was also observed in other insect-resistant GM rice lines (Kim et al., 2008), although it was referred to as ‘yield drag’ by the authors. The reduced or only equal yield performance of GM crops, compared with their non-GM counterpart under insufficient target insect pressure, was also observed from the assessment of field performances of other insect-resistant GM crops. For example, yield reduction of a *Bt* cotton variety was reported from field experiments conducted in India (Qayum and Sakkhari, 2005), where the low level of cotton bollworm infestation was considered as one of the possible reasons for the yield reduction (Khadi, 2006). In addition, the yield of *Bt* maize was found only being equivalent to the non-transgenic maize when target insects in field were controlled by the spray of chemical insecticides to reach moderate insect pressure (Andersen et al., 2007; Gomez-Barbero et al., 2008). All these together with other reported results mentioned earlier (Jackson et al., 2004; Romeis et al., 2007; Pasonen et al., 2008) indicate that the phenomenon of underlying fitness cost from transgenes is not rare. Possibly, the underlying cost could also exhibit in GM crops with transgenes tolerant to abiotic stresses under environmental conditions without such stresses, e.g., GM rice bred for withstanding drought stress may not perform well under ideal irrigated conditions.

The evident underlying costs of transgenes estimated from our field experiments in GM rice and other GM crops raise a common question: could a cryptic yield loss of insect-resistant GM crops happen under insufficient target insect stresses? In other words, insect-resistant GM crops may not provide a direct yield increase under low insect occurrence (Andersen et al., 2007; Gomez-Barbero et al., 2008). If this finding holds true, biotechnology developers are encouraged to further improve transgenic biotechnology for minimizing or reducing the potential underlying cost of a transgene(s), and biotechnology users are also encouraged to apply strategies to circumvent such a possible yield loss of insect-resistant GM crops under the changing agricultural environmental conditions with substantial fluctuation of target insect pressure.

To optimize the yield benefit of GM crops, breeders could select in the early generations the most beneficial transgenic events or lines that show no underlying cost for further breeding of commercial GM crops. Prior to commercialization, rigorous field assessments should be followed to test the potential benefit and underlying cost of transgenes to the GM crops under different environments, with or without insect pressure. More advanced transgenic technologies, such as site-specific insertion, inducible promoters, and regulated expression of transgenes with tissue specific promoters, can also be applied to develop transgenic crops, which can further reduce the underlying cost of transgenes caused by their constitutive expression (Gurr and Rushton, 2005). Promisingly, all these biotechnologies are available to meet the objectives when produce the next generation of GM crops.

In addition, a strategic field deployment, meaning alternating cultivation of GM crops and non-GM crops depending on the local insect pest prediction, can also be applied to circumvent the possible yield loss, even though there exists a slight cost in the GM crops, given that in reality the occurrence of insect pests in rice ecosystems can largely be variable among different years in a given region (see Appendix 1). Once the selective deployment strategy was applied, growers could circumvent the possible yield loss from planting the insect-resistant GM varieties, which might save money if less expensive non-GM varieties perform as well as GM varieties in some seasons when the target insect pressure is insufficient. Furthermore, alternating cultivation between GM and non-GM varieties could help to delay the evolution of resistance in target insects (Cerda and Wright, 2004). According to this selective deployment strategy, knowledge and prediction of spatial and temporal dynamics of target insects are needed prior to cultivation of insect-resistant GM rice because the decision of growing GM crops depends essentially on the information of dynamics of target insects. This can make the modern agriculture with a strong component of adopting biotechnology more profitable under a designed manner.

5. Conclusions

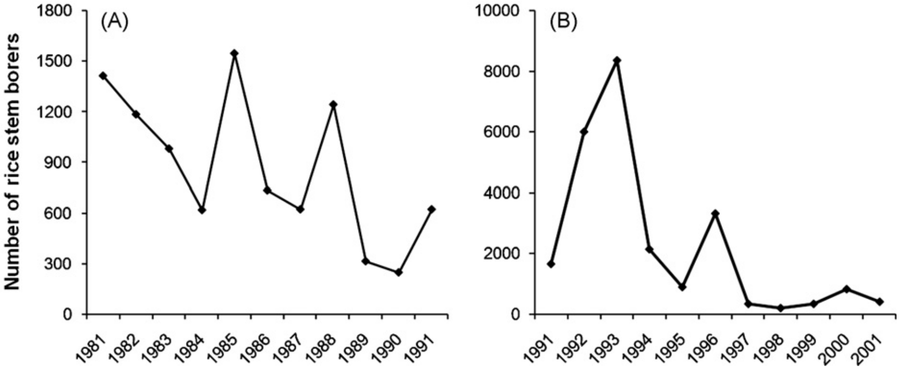
Selective use of insect-resistant GM crops may help circumvent unnecessary yield losses caused by the underlying costs of constitutive transgene expression or associated genetic (pleiotropic) effects in some GM varieties, particularly in the extensive agricultural ecosystems, where the high-input and high-output farming style is pursued. Biotechnology developers and breeders should also strive to minimize such underlying costs and select the GM crops most appropriate for particular agro-ecosystems for commercial production. Further field studies of pre-commercial GM varieties are needed to determine how extensive these yield costs may be at different scales of production, and how often the low frequencies of target pests could result in economically important yield losses in insect-resistant GM crops. This will facilitate the safe and effective application of GM crops in a sustainable way.

Acknowledgments

This work was funded by the Natural Science Foundation of China (30730066), Ministry of Science and Technology (2007CB109202), and the National Program of Development of Transgenic New Species of China (2008ZX08011-006). We thank A. Snow of Ohio State University, D. Soleri of University of California, Santa Barbara, and D. Vaughan of Japanese National Institute of Agrobiological Sciences for their critical review of the manuscript.

Appendix A.

Illustrations indicating the dramatic fluctuation of rice stem-borers between years in the important rice cultivation regions in China [Jiangxi (A) and Hunan (B) Provinces], based on 10-year field survey data by Zhu et al. (2003, 2008).



References

Alam, M.F., Datta, K., Abrigo, E., Vasquez, A., Senadhira, D., Datta, S.K., 1998. Production of transgenic deepwater indica rice plants expressing a synthetic *Bacillus thuringiensis cryIA(b)* gene with enhanced resistance to yellow stem borer. Plant Sci. 135, 25–30.

Andersen, M.N., Sausse, C., Lacroix, B., Caul, S., Messean, A., 2007. Agricultural studies of transgenic maize and the field experimental infrastructure of ECOGEN. Pedobiologia 51, 175–184.

Brookes, G., Barfoot, P., 2009. Global impact of biotech crops: income and production effects, 1996–2007. AgBioForum 12, 184–208, http://www.pgeconomics.co.uk/pdf/2009socioeconimpactsagbioforumpaper.pdf.

Cerda, H., Wright, D.J., 2004. Modeling the spatial and temporal location of refugia to manage resistance in *Bt* transgenic crops. Agric. Ecosyst. Environ. 102, 163–174.

Chen, L.Y., Snow, A.A., Wang, F., Lu, B.R., 2006. Effects of insect-resistance transgenes on fecundity in rice (*Oryza sativa*, Poaceae): a test for underlying costs. Am. J. Bot. 93, 94–101.

FAO (Food and Agriculture Organization of the United Nations), 2004. The State of Food and Agriculture 2003–2004. Agricultural Biotechnology: Meeting the Needs of the Poor? (http://www.fao.org/docrep/006/Y5160E/Y5160E00.HTM).

Gomez-Barbero, M., Berbel, J., Rodriguez-Cerezo, E., 2008. *Bt* corn in Spain - the performance of the EU's first transgenic crop. Nat. Biotechnol. 26, 384–386.

Gurr, S., Rushton, P., 2005. Engineering plants with increased disease resistance: how are we going to express it? Trends Biotechnol. 23, 283–290.

Huang, J.K., Hu, R.F., Pray, C., Qiao, F.B., Rozelle, S., 2003. Biotechnology as an alternative to chemical pesticides: a case study of *Bt* cotton in China. Agric. Econ. 29, 55–67.

Huang, J.K., Hu, R.F., Rozelle, S., Pray, C., 2005. Insect-resistant GM rice in farmers' fields: assessing productivity and health effects in China. Science 308, 688–690.

Huang, J.K., Hu, R.F., Rozelle, S., Pray, C., 2008. Genetically modified rice, yields, and pesticides: assessing farm-level productivity effects in China. Economic Development and Cultural Change 56, 241–263.

James, C., 2009. Global Status of Commercialized Biotech/GM Crops: 2009. ISAAA Brief No. 41. ISAAA: Ithaca, NY, USA.

Jackson, M.W., Stinchcombe, J.R., Korves, T.M., Schmitt, J., 2004. Costs and benefits of cold tolerance in transgenic *Arabidopsis thaliana*. Mol. Ecol. 13, 3609–3615.

Khadi, M., 2006. Impact of Bt-cotton on agriculture in India. In: Proceedings of the 9th International Symposium on the Biosafety of Genetically Modified Organisms: Biosafety Research and Environmental Risk Assessment, Jeju Island, Korea, pp. 96–100.

Kim, S.J., Kim, C.J., Li, W.N., Kim, T.Y., Li, Y.S., Zaidi, M.A., Altosaar, I., 2008. Inheritance and Weld performance of transgenic Korean *Bt* rice lines resistant to rice yellow stem borer. Euphytica 164, 829–839.

Lu, B.R., Snow, A.A., 2005. Gene flow from genetically modified rice and its environmental consequences. BioScience 55, 669–678.

Marrelli, M.T., Moreira, C.K., Kelly, D., Alphey, L., Jacobs-Lorena, M., 2006. Mosquito transgenesis: what is the fitness cost? Trends Parasitol. 22, 197–202.

Nap, J.P., Metz, P.L.J., Escaler, M., Conner, A.J., 2003. The release of genetically modified crops into the environment - Part I. Overview of current status and regulations. Plant J. 33, 1–18.

Nayak, P., Basu, D., Das, S., Basu, A., Ghosh, D., Ramakrishnan, N.A., Ghosh, M., Sen, S.K., 1997. Transgenic elite indica rice plants expressing Cry1Ac delta-endotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirpophaga incertulas*). Proc. Natl. Acad. Sci. U.S.A. 94, 2111–2116.

Pasonen, H.L., Vihervuori, L., Seppanen, S.K., Lyytikainen-Saarenmaa, P., Ylioja, T., von Weissenberg, K., Pappinen, A., 2008. Field performance of chitinase transgenic silver birch (*Betula pendula* Roth): growth and adaptive traits. Trees' Struct. Funct. 22, 413–421.

Qaim, M., Subramanian, A., Naik, G., Zilberman, D., 2006. Adoption of *Bt* cotton and impact variability: insights from India. Rev. Agric. Econ. 28, 48–58.

Qayum, A., Sakkhari, K., 2005. *Bt*-cotton in Andhra Pradesh. A three year assessment. Deccan Development Society and A.P. Coalition in Defense of Diversity, Booksline Press, Hyderabad, India. http://www.grain.org/research_files/BT_Cotton_-_A_three_year_report.pdf.

Romeis, J., Waldburger, M., Streckeisen, P., Hogervorst, P.A.M., Keller, B., Winzeler, M., Bigler, F., 2007. Performance of transgenic spring wheat plants and effects on non-target organisms under glasshouse and semi-field conditions. J. Appl. Entomol. 131, 593–602.

Utida, S., 1958. On fluctuations in population density of the rice stem borer *Chilo suppressalis*. Ecology 39, 587–599.

Wang, Y.Q., Johnston, S., 2007. The status of transgenic rice R&D in China. Nat. Biotechnol. 25, 717–718.

Wang, Y.M., Zhang, G.A., Du, J.P., Liu, B., Wang, M.C., 2010. Influence of transgenic hybrid rice expressing a fused gene derived from *cry1Ab* and *cry1Ac* on primary insect pests and rice yield. Crop Prot. 29, 128–133.

Wu, K.M., Lu, Y.H., Feng, H.Q., Jiang, Y.M., Zhao, J.Z., 2008. Suppression of cotton bollworm in multiple crops in China in areas with *Bt* toxin-containing cotton. Science 321, 1676–1678.

Xia, H., Lu, B.-R., Su, J., Chen, R., Rong, J., Song, Z.P., Wang, F., 2009. Normal expression of insect-resistant transgene in progeny of common wild rice crossed with genetically modified rice: its implication in ecological biosafety assessment. Theor. Appl. Genet. 119, 635–644.

Xu, D.P., Xue, Q.Z., McElroy, D., Mawal, Y., Hilder, V.A., Wu, R., 1996. Constitutive expression of a cowpea trypsin inhibitor gene, CpTi, in transgenic rice plants confers resistance to two major rice insect pests. Mol. Breeding 2, 167–173.

Zhu, F.Z., Zhu, J., Guo, P.Z., Zhou, G.B., Yang, H.Y., 2008. Analysis and research on the historical forecast data of rice stem borer in North Hunan. J. Anhui Agric. Sci. 36, 5487–5491 (in Chinese, with English abstract).

Zhu, J.S., Yuan, W.L., Wang, M.H., Kong, X.M., Zhang, Y.J., 2003. The historical occurrence of *Chilo suppressalis* in Jiangxi province and the strategy of management. Jiangxi Plant Prot. 26, 73–76 (in Chinese).