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Assessment of yield advantages of *Bt*-MH63 with *cry1C** or *cry2A** genes over MH63 (*Oryza sativa* L.) under different pest control modes

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ABSTRACT

Transgenic *Bacillus thuringiensis* (*Bt*) rice had been successfully cultivated to maintain yield potential of rice under pest invasion. Yield and resistance performance of the transgenic *Bt* rice are important to be meticulously evaluated under different pest pressures. Field experiments were conducted to investigate field performances of *Bt*-MH63 with *cry1C** or *cry2A** genes under four pest control modes: chemical control for all the pests (MPC), no chemical control for the target pests (MNTP), chemical control for the target pests (MTP) and no chemical control for all the pests (MNPC). The results showed that the maximum yield advantages of MH63 (*cry1C**) and MH63 (*cry2A**) over MH63 were 8.4 and 25.4% ($P < 0.05$) under MNTP, respectively. The grain yield of MH63 (*cry1C**) was lower than that of MH63 under MPC and MTP. Moreover, the grain yield of MH63 (*cry2A**) was lower than that of MH63 under MTP only. The correlation analysis revealed that the yield advantages of *Bt*-MH63 over MH63 were positively correlated with the damage to MH63 (expressed as percentage of white leaves) caused by leaffolders (*Cnaphalocrocis medinalis Guenee*). Although MH63 (*cry1C**) and MH63 (*cry2A**) showed great differences in *Bt* protein contents in their leaves, they had high effective resistances to leaffolders. It can be concluded that *Bt*-MH63 had obvious yield advantages over MH63 when no pesticides were applied against the target pests. However, yield reductions in *Bt*-MH63 were existed when pesticides were applied against the target pests.

1. Introduction

Leaffolders could cause a yield loss of rice by 40.6% as reported by Pandi et al. (2009), and the use of chemical pesticides is still a basic way to prevent leaffolders in China. However, extensive application of chemical pesticides can lead to a series of environmental and social problems. Recently, transgenic rice with *Bt* genes, which had high resistance to leaffolders were rapidly developed (Tu et al., 2000; Chen et al., 2005; Tang et al., 2006). For example, *cry1C** and *cry2A** were successfully transferred into MH63 (Minghui63), a widely used *indica* cytoplasm male sterile (CMS) restorer line in China (Chen et al., 2005; Tang et al., 2006).

Yield advantages of genetically modified crops are affected by environmental factors and agricultural practices (Zheng et al., 2013). The yield advantages of transgenic *Bt* crops were obvious compared with their counterparts under severe infestation of target pests (Brookes and Barfoot, 2009; James, 2009; Wang et al., 2012a; Jason and Frederick, 2013). For example, Wang et al. (2012a) found that *Bt*-SY63 [*Bt*-Shanyou63, produced by crossbreeding *Bt*-MH63 with Zhenshan97A (an elite CMS line)] had higher yield by about 20% than their counterparts under the non-control condition of target pests. However, no yield advantages of *Bt* transgenic cultivars were found when pesticides were applied against the target pests (Lauer and Wedberg, 1999; Ma and Subedi, 2005). Furthermore, yield losses were observed in some *Bt* rice when pests were strictly controlled (Chen et al., 2006; Xia et al., 2010). The yield losses of *Bt* transgenic crops were possibly resulted from the added burden by the constitutive over-expression of the alien transgenes (Gurr and Rushton, 2005), and from the disruption of native gene functions by the random insertion of transgenes into the host genome (Marrelli et al., 2006). From these previous studies, it can be indicated that *Bt* rice showed varying yield advantages over their non-*Bt* counterparts under different pest pressures. So, a rigid control of pests is essential to evaluate the field performance of *Bt* rice.

Potential impact of transgenic *Bt* rice on non-target pests is an increasing concern. Planting *Bt* rice might affect non-target pests

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by changing the tritrophic transmission and potentially releasing competition pressure in agricultural ecosystems (Cheng and Zhu, 2006; Chen et al., 2011). There were different views recorded about the influence of *Bt* rice on non-target pests (Liu et al., 2002; Schoenly et al., 2003; Chen et al., 2009; Tian et al., 2010). Planthoppers (*Nilaparvata lugens*, *Sogatella furcifera* and *Laodelphax striatellus*) were identified as key groups of the non-target pests, which could cause great damage to rice (Heinrichs, 1979; Sogawa et al., 2003). The change of densities and species dominance of planthoppers may influence the yield performance of *Bt* rice.

The objectives of this study were (1) to evaluate yield advantages of *Bt*-MH63 with *cry1C** or *cry2A** genes over MH63 under different pest control modes and (2) to find out the main reasons of the yield advantage changes of *Bt* rice.

2. Materials and methods

2.1. Plant materials

Three strains, MH63 (*cry1C**), MH63 (*cry2A**) and their non-transgenic counterpart MH63 (Minghui63) were used in this current study and provided by National Key Laboratory of Crop Genetic Improvement, Wuhan, China. The *cry1C** and *cry2A** genes for improving the resistance to lepidopteran pests were synthesized on the basis of wild-type *cry1Ca5* and *cry2Aa* genes of *Bt*, respectively (Chen et al., 2005; Tang et al., 2006).

2.2. Experimental site and treatments

Field experiments were conducted from May to October in 2011 and 2012 at Junchuang town (31°69′ N 115°33′ E), Suizhou city, Hubei Province, China. The main soil properties of the experimental site were as follows: pH, 5.33; organic C, 16.21 g kg^{-1}; total N, 1.85 g kg^{-1}; total P, 0.63 g kg^{-1}; and available K, 83 mg kg^{-1}. Treatments were arranged in a split-plot design with pest control modes as the main plots and strains as the subplots with four replicates. The pest control modes were chemical control for all the pests (MPC), no chemical control for the target pests (MNTP), chemical control for the target pests (MTP) and no chemical control for all the pests (MNPC). In MPC and MTP, chlorantraniliprole (20% suspending agent) produced by DuPont Company (Wilmington, USA) was sprayed at a dose of 150 ml ha^{-1} every time to control the target pests. In MPC and MNTP, pymetrozine (50% water-dispersible granule) produced by Syngenta Company (Basel, CH) was applied at a dose of 150 g ha^{-1} every time to control the non-target pests. The chlorantraniliprole and pymetrozine were sprayed every 20 days (5 times in total) from the transplantation to the maturity stage. The two pesticides were applied by conventional spraying with 300 L ha^{-1} spray volume at a time, respectively. The size of each plot was 20 m × 25 m surrounded by 1-m wide unplanted border. The entire experimental field was bordered by ten rows of the non-transgenic counterpart MH63. Twenty-day old seedlings were transplanted with one seedling per hill at a density of 20 cm × 20 cm. Nitrogen fertilize (urea, 46% N) at the rate of 150 kg ha^{-1} were applied with 50% at the basal, 20% at the mid-tillering stage and 30% at the panicle initiation stage. Phosphorus fertilize (calcium superphosphate, 15% P_2O_5) at the rate of 90 kg ha^{-1} was applied at the basal. Potassium fertilize (potassium chloride, 60% K_2O) at the rate of 135 kg ha^{-1} were added with 50% at the basal and 50% at the panicle initiation stage. Diseases and weeds were intensively controlled for all the treatments to avoid yield losses.

2.3. Measurements

At the heading stage, plant heights of 30 plants in each plot were determined. SPAD values on 10 topmost fully expanded leaves per plot were measured using a chlorophyll meter [SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan]. Stem numbers of 12 hills were counted and area of green leaves were measured by a Li-Cor area meter (Li-Cor Model 3100, LI-COR Inc., Lincoln, NE, USA) to calculate leaf area index (LAI). The SunScan Canopy Analysis System (Delta-T Devices Ltd., Burwell, Cambridge, UK) was used to measure canopy photosynthetically active radiation (PAR) between 11:00 and 13:00 h. Radiation transmitting efficiency was calculated as a ratio of below-canopy PAR to above-canopy PAR.

At the heading stage, damage caused by leaffolders (expressed as percentage of white leaves) was examined. The damaged leaves and whole leaves of 30 hills were counted, and then percentage of damaged leaves was calculated. At the panicle initiation and heading stages, leaves of 12 plants were sampled in each plot and stored at −80 °C for *Bt* protein content analysis. The contents of *cry1C** and *cry2A** protein in leaves were determined using the enzyme-linked immunosorbent assay kit by Enviro-Logix (Portland, Me.) and Environlogix (Environlogix, USA).

At the maturity stage, grain yield was determined from a 20-m^2 sampling area within each plot and adjusted to a moisture content of 14%. Twelve hills were sampled from the 20-m^2 harvest area for investigation of yield components. Panicle numbers of each hill were counted to determine the panicle number per m^2. Filled spikelets were separated from unfilled spikelets by submerging them in water. Four replicates each of 30 g of the filled spikelets and 5 g of the unfilled spikelets were counted to calculate the spikelets per panicle and grain filling percentage. The grain weight was determined after oven-drying the filled spikelets at 70 °C to constant weight. Aboveground parts of the 12 plant samples were oven-dried at 70 °C and weighed for determination of the biomass. Harvest index was calculated as a ratio of grain weight to total aboveground dry weight. Yield advantage of *Bt*-MH63 over MH63 was determined as $100 \times (GY_{BT} - GY_{CK})/GY_{CK}$, where GY_{BT} was the grain yield of MH63(*cry1C**) or MH63(*cry2A**) and GY_{CK} was the grain yield of MH63.

2.4. Statistical analysis

Data were analyzed following analysis of variance (SAS Institute, 1999) and means were compared based on the least significant difference (LSD) test at the 0.05 probability level. The relationship between yield advantage of *Bt*-MH63 over MH63 and percentage of white leaves of MH63 was determined by using the CORR model in SAS.

3. Results

3.1. Grain yield and yield advantage

Grain yields of MH63 (*cry1C**) were significantly lower than those of MH63 (*cry2A**) and MH63 under MPC in 2011 and 2012 (Table 1). No significant differences in grain yield between MH63 (*cry2A**) and MH63 were observed under MPC (Table 1). However, under MTP, both MH63 (*cry1C**) and MH63 (*cry2A**) had significantly lower grain yields compared with MH63 (Table 1). There were no marked changes recorded in panicle number, spikelets per panicle and grain weight between MH63 (*cry1C**) and MH63 under MPC (Table 2). MH63 (*cry1C**) decreased grain filling percentages by 16.3 and 15.1% ($P < 0.05$) compared with MH63 under MPC in 2011 and 2012, respectively (Table 2). Yield advantages of *Bt* rice over MH63 were obvious under MNTP and MNPC in 2011, and were higher under MNTP than MNPC. Yield advantages of MH63 (*cry1C**) and MH63 (*cry2A**) over MH63 were 8.4 and 25.4% ($P < 0.05$, means of two years) under MNTP, respectively (Table 3). The effects of pest

Table 1
Grain yields (t ha^{-1}) of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 under different pest control modes in 2011 and 2012 at Junchuang town, Suizhou city, Hubei Province, China.

Genotype	Pest control mode			
	MPC	MNTP	MTP	MNPC
2011				
MH63 (*cry1C**)	6.08 b	6.21 b	5.02 b	5.17 b
MH63 (*cry2A**)	6.94 a	7.03 a	5.31 b	5.94 a
MH63	7.01 a	5.16 c	5.78 a	4.53 c
2012				
MH63 (*cry1C**)	5.48 b	5.63 b	4.04 c	3.84 c
MH63 (*cry2A**)	6.54 a	6.69 a	4.56 b	4.73 a
MH63	6.51 a	5.84 b	4.89 a	4.23 b

Means within a column for each year followed by different letters are significantly different according to LSD (P = 0.05). MPC, chemical control for all the pests; MNTP, no chemical control for the target pests; MTP, chemical control for the target pests; MNPC, no chemical control for all the pests. MH63, Minghui63; MH63 (*cry1C**), Minghui63 with a *Bt* gene of *cry1C**; MH63 (*cry2A**), Minghui63 with a *Bt* gene of *cry2A**.

Table 4
Significant terms (*F*-values) from the effects of pest control mode, genotype and year on grain yield and yield advantage of *Bt* rice over their non-transgenic counterpart MH63 in 2011 and 2012 at Junchuang town, Suizhou city, Hubei Province, China.

Independent variable	Grain yield	Yield advantage over MH63
Pest control mode	257.8***	269.7***
Genotype	57.0***	151.0***
Year	640.5**	282.3**
Pest control mode × genotype	18.5***	4.3*
Pest control mode × year	14.59***	51.3***
Genotype × year	9.2***	NS
Pest control mode × genotype × year	2.9*	NS

NS, not significant.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

control mode, genotype and year on grain yield and yield advantage of *Bt* rice over their non-transgenic counterpart MH63 were noticeable (Table 4).

3.2. Yield-related traits

There were no significant differences in biomass at the maturity stage, and plant height, LAI and SPAD value at the heading stage among MH63 (*cry1C**), MH63 (*cry2A**) and MH63 under MPC in 2011 and 2012 (Fig. 1).

3.3. Resistance to leaffolders

Bt protein contents of MH63 (*cry2A**) were about 10 times higher than those of MH63 (*cry1C**) in the leaves at the panicle initiation and heading stages in 2012 (Fig. 2). Both MH63 (*cry1C**) and MH63 (*cry2A**) exhibited high effective resistances to leaffolders. Percentages of white leaves of MH63 (*cry1C**), MH63 (*cry2A**) under different pest control modes at the heading stage were <10% in 2011 and 2012 (Fig. 3). On the other hand, percentages of white leaves of MH63 were >40% under MNTP and MNPC in 2011, and were about 20% in 2012. Moreover, percentages of white leaves of MH63 were significantly changed (P < 0.05) between 2011 and 2012. The radiation transmitting efficiencies of MH63 were significantly higher than those of MH63 (*cry1C**) and MH63 (*cry2A**) under MNTP and MNPC at the heading stage in 2011 and 2012 (Fig. 4). There was a significant positive correlation between yield advantages of MH63 (*cry1C**) and MH63 (*cry2A**) over MH63 and percentages of white leaves of MH63 (Fig. 5).

4. Discussion

Variations, the fitness cost by the constitutive expression of the transgenes (Jackson et al., 2004; Subedi and Ma, 2007), could frequently happen in *Bt* crops (Romeis et al., 2007; Pasonen et al., 2008). For example, the reductions in plant height, root length, grains per panicle and grain filling percentage, commonly leading to decrease in yield, were reported in some *Bt* rice (Shu et al., 2002; Jiang et al., 2004; Kim et al., 2008; Xia et al., 2010). In our study, no significant differences in biomass, plant height, LAI and SPAD value were observed between MH63 (*cry1C**) and MH63 (Fig. 1).

Table 2
Yield components of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 under MPC in 2011 and 2012 at Junchuang town, Suizhou city, Hubei Province, China.

Genotype	Panicle number (m^{-2})	Spikelets per panicle	Grain filling percentage (%)	Grain weight (mg)	Harvest index (%)
2011					
MH63 (*cry1C**)	319 a	110 a	64.0 b	27.5 a	41.1 b
MH63 (*cry2A**)	306 a	105 a	73.6 a	26.8 a	47.9 a
MH63	302 a	114 a	76.5 a	27.0 a	48.2 a
2012					
MH63 (cry1C*)	276 a	101 a	71.4 b	26.9 a	38.2 b
MH63 (cry2A*)	282 a	85 b	82.5 a	27.3 a	48.5 a
MH63	278 a	94 a	84.1 a	27.4 a	48.7 a

Means within a column for each year followed by different letters are significantly different according to LSD (P = 0.05). MPC, chemical control for all the pests. MH63, Minghui63; MH63 (*cry1C**), Minghui63 with a *Bt* gene of *cry1C**; MH63 (*cry2A**), Minghui63 with a *Bt* gene of *cry2A**.

Table 3
Yield advantages (%) of MH63 (*cry1C**) and MH63 (*cry2A**) over MH63 under different pest control modes in 2011 and 2012 at Junchuang town, Suizhou city, Hubei Province, China.

Pest control mode	Genotype (2011)		Genotype (2012)	
	MH63 (*cry1C**)	MH63 (*cry2A**)	MH63 (*cry1C**)	MH63 (*cry2A**)
MPC	−13.3 c	−1.0 c (NS)	−15.8 bc	0.5 b (NS)
MNTP	20.4 a	36.2 a	−3.6 a (NS)	14.6 a
MTP	−13.2 c	−8.1 d	−17.4 c	−6.8 b
MNPC	14.1 b	31.1 b	−9.2 ab	11.8 a

Means within a column followed by different letters are significantly different according to LSD (P = 0.05). NS, difference in yield between *Bt* rice and MH63 was not significant. MPC, chemical control for all the pests; MNTP, no chemical control for the target pests; MTP, chemical control for the target pests; MNPC, no chemical control for all the pests. MH63 (*cry1C**), Minghui63 with a *Bt* gene of *cry1C**; MH63 (*cry2A**), Minghui63 with a *Bt* gene of *cry2A**.

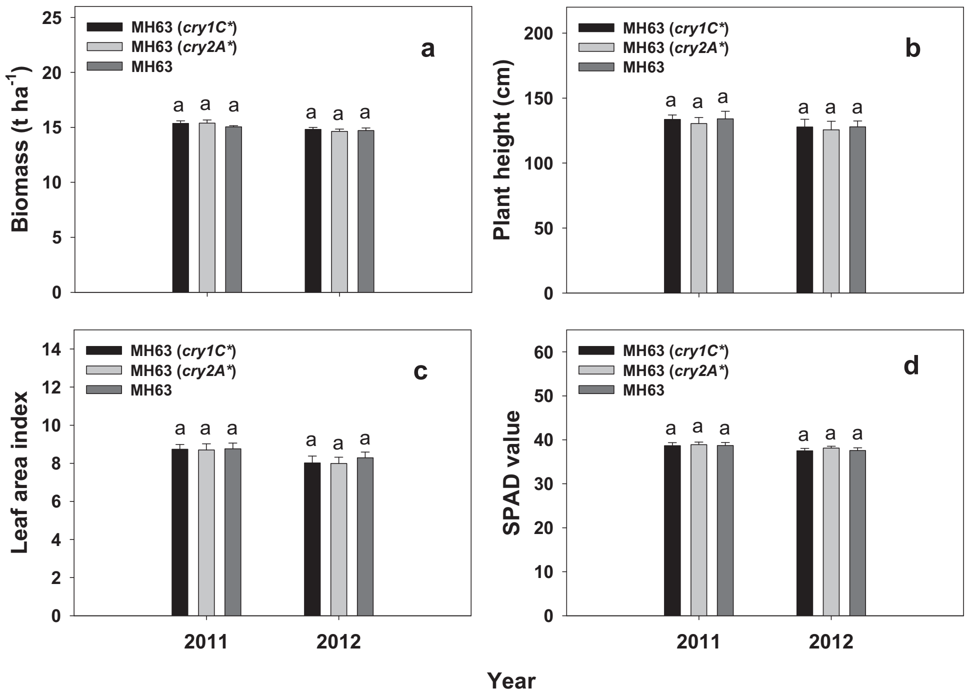

Fig. 1. Biomass (a) at the maturity stage, plant height (b), LAI (c) and SPAD value (d) at the heading stage of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 under MPC in 2011 and 2012 at Junchuang town, Suizhou city, Hubei Province, China. MPC, chemical control for all the pests. MH63, Minghui63; MH63 (*cry1C**), Minghui63 with a *Bt* gene of *cry1C**; MH63 (*cry2A**), Minghui63 with a *Bt* gene of *cry2A**. Same letters in different columns in each year are not significantly different according to LSD ($P = 0.05$). Vertical bars indicate standard errors.

Investigation of yield components revealed that the lower grain filling percentage was the main reason for the reduction in grain yield of MH63 (*cry1C**) under MPC (Tables 1 and 2). Wang et al. (2012b) found that MH63 (*cry1C**) had a differential sink activity compared with MH63. Moreover, there was no insect infestation leading to yield reduction under MPC. Therefore, the yield reduction of MH63 (*cry1C**) was resulted from physiological variations caused by transgenes.

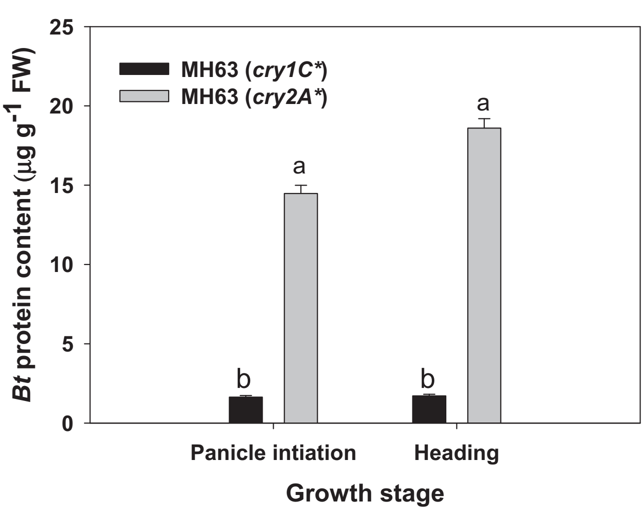

Fig. 2. *Bt* protein contents in the leaves of MH63 (*cry1C**), MH63 (*cry2A**) under MPC at the panicle initiation and heading stages in 2012 at Junchuang town, Suizhou city, Hubei Province, China. MPC, chemical control for all the pests. MH63 (*cry1C**), Minghui63 with a *Bt* gene of *cry1C**; MH63 (*cry2A**), Minghui63 with a *Bt* gene of *cry2A**. FW, fresh weight. Different letters in different columns at each stage are significantly different according to LSD ($P = 0.05$). Vertical bars indicate standard errors.

The resistance advantage of *Bt* rice was masked under MTP. Our results showed that MH63 (*cry2A**) had significantly lower grain yield compared with MH63 under MTP. On the other hand, there were no alterations recorded in their grain yields under MPC (Table 1), suggesting that physiological variations caused by transgenes could not be the main reasons of the yield decline in MH63 (*cry2A**). It was indicated that planting *Bt* crops might changed the densities and species dominance of non-target pests in the field (Marvier et al., 2007; Wolfenbarger et al., 2008; Naranjo, 2009). For example, Schoenly et al. (2003) found variations in the species richness of parasitoids and predators in the fields sprayed with *Bt* toxin. Moreover, Han et al. (2011) observed a higher population density of planthoppers in MH63 (*cry2A**) compared with MH63. Under MTP, damage caused by non-target pests was the main factor leading to reduction in grain yield. So, the yield decline in MH63 (*cry2A**) under MTP was possibly due to the more severe infestation of non-target pests.

The higher yield advantages of *Bt* rice under MNTP and MNPC were probably resulted from the severe damage to MH63 caused by leaffolders (Table 3, Figs. 3 and 4). On the other hand, the lower advantages of *Bt* rice under MTP and MPC were mainly due to the relatively minor damage to MH63 caused by leaffolders (Table 3, Figs. 3 and 4). Moreover, year significantly affected the yield advantages because the occurrence of leaffolders was varied between 2011 and 2012 (Table 4 and Fig. 3). Our results indicated that yield advantages of the *Bt* rice over non-*Bt* rice changed with the dynamic of the target pests. We suggested that the assessment of yield advantage of *Bt* rice should be done under different insect pressures. To implement the proposal, we can (1) control the target and non-target insect pressures by chemical pesticides or (2) conduct the field experiments in different regions with various species dominance of pests.

There was a widespread agreement indicated that the “high-dose/refuge” strategy was the most promising and practical approach to prolong the effectiveness of *Bt* crops (Gould, 1998).

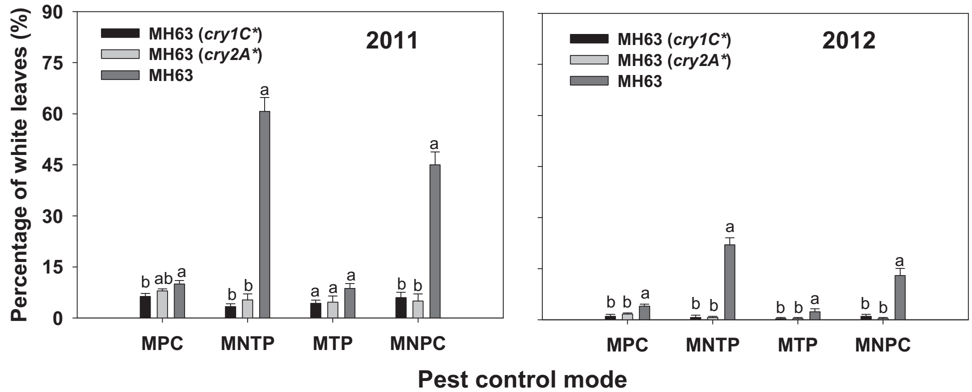

Fig. 3. Percentages of white leaves of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 under different pest control modes at the heading stage in 2011 and 2012 at Junchuang town, Suizhou city, Hubei Province, China. MH63, Minghui63; MH63 (*cry1C**), Minghui63 with a *Bt* gene of *cry1C**; MH63 (*cry2A**), Minghui63 with a *Bt* gene of *cry2A**. MPC, chemical control for all the pests; MNTP, no chemical control for the target pests; MTP, chemical control for the target pests; MNPC, no chemical control for all the pests. Different letters in different columns under each pest control mode are significantly different according to LSD ($P = 0.05$). Vertical bars indicate standard errors.

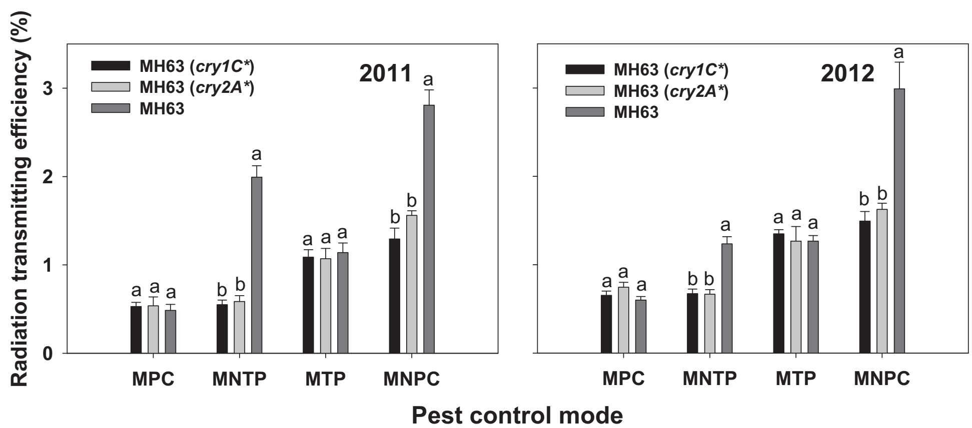

Fig. 4. Radiation transmitting efficiencies of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 under different pest control modes at the heading stage in 2011 and 2012 at Junchuang town, Suizhou city, Hubei Province, China. MH63, Minghui63; MH63 (*cry1C**), Minghui63 with a *Bt* gene of *cry1C**; MH63 (*cry2A**), Minghui63 with a *Bt* gene of *cry2A**. MPC, chemical control for all the pests; MNTP, no chemical control for the target pests; MTP, chemical control for the target pests; MNPC, no chemical control for all the pests. Different letters in different columns under each pest control mode are significantly different according to LSD ($P = 0.05$). Vertical bars indicate standard errors.

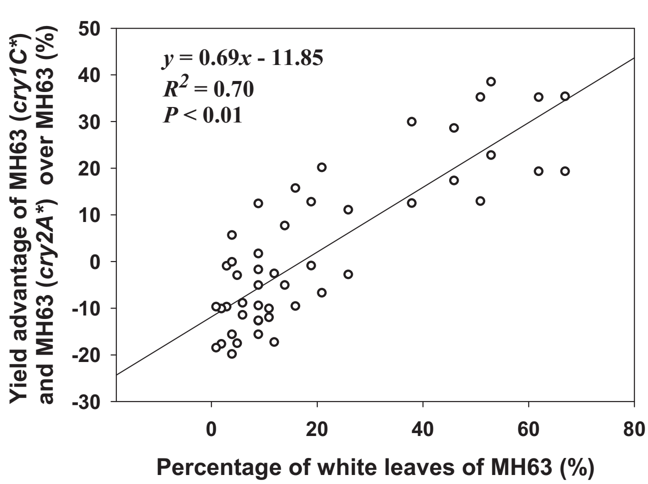

Fig. 5. Correlation between yield advantages of MH63 (*cry1C**) and MH63 (*cry2A**) over MH63 and percentages of white leaves of MH63 under different pest control modes in 2011 and 2012 at Junchuang town, Suizhou city, Hubei Province, China. MH63, Minghui63; MH63 (*cry1C**), Minghui63 with a *Bt* gene of *cry1C**; MH63 (*cry2A**), Minghui63 with a *Bt* gene of *cry2A**.

In the "high-dose/refuge" strategy, it advocated releasing the *Bt* varieties that had a high dose of *Bt* toxin (Cohen et al., 2000). To proceed from this point, MH63 (*cry2A**) was more appropriate than MH63 (*cry1C**), because MH63 (*cry2A**) had several times higher *Bt* protein synthesis than MH63 (*cry1C**) in their leaves (Fig. 2). However, *Bt* rice with high expression of *Bt* protein might had heavier added burden, which would be harmful to rice (Gurr and Rushton, 2005). For example, Jiang et al. (2013) found that MH63 (*cry2A**) had leaf premature aging compared with MH63 when the supply of nitrogen fertilizer was inadequate. Though MH63 (*cry1C**) showed a yield loss compared with MH63 under MPC in our study (Table 1), Wang et al. (2012b) found that there was no difference in grain yield between SY63 (*cry1C**) and its counterpart SY63. So, the *Bt* gene *cry1C** would not always lead to yield reductions in different genotypes of rice. Moreover, the evaluation of *Bt* genes such as *cry1C** and *cry2A** should not be merely based on studies about a certain *Bt* rice line or the content of *Bt* protein.

Yield performance of two *Bt* transgenic lines, MH63 (*cry1C**) and MH63 (*cry2A**) were investigated under four pest control modes. We found that MH63 (*cry1C**) had lower grain filling percentage compared with MH63. This led to yield reduction in MH63 (*cry1C**) under no pest infestation, reflecting that there were adverse physiological variations in MH63 caused by *cry1C**. The lower grain yield of MH63 (*cry2A**) could probably be explained by the more severe infestation of non-target pests compared with MH63 when the target pests were controlled. Under the non-control condition of target pests, MH63 (*cry1C**) and MH63 (*cry2A**) had obvious yield advantages over MH63. The yield advantage of *Bt* rice changed with the dynamic of the target pests. Though the *Bt* protein content of MH63 (*cry1C**) was markedly lower than that of MH63 (*cry2A**), both of them showed high resistances to target pests. Further investigation are required to evaluate the field performances and physiological characteristics of more *Bt* rice lines with *cry1C** or *cry2A** genes under different pest pressures.

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