



Non-target effects of *Bt* transgenes on grain yield and related traits of an elite restorer rice line in response to nitrogen and potassium applications



Yang Jiang^a, Jingjiao Meng^a, Lingli Zhang^a, Mingli Cai^a, Chengfang Li^a, Ming Zhan^a,
Jinping Wang^a, Benfu Wang^{a,b}, Ibrahim Mohamed^{a,c}, Cougui Cao^{a,*}

^a MOA Key Laboratory of Crop Physiology, Ecology and Cultivation (The Middle Reaches of Yangtze River), Huazhong Agricultural University, Wuhan 430070, Hubei, China

^b Hubei Key Laboratory of Food Crop Germplasm and Genetic Improvement, Wuhan 430064, Hubei, China

^c Soil Science Department, Faculty of Agriculture, Benha University, Moshtohor, Toukh 13736, Kalyoubia, Egypt

ARTICLE INFO

Article history:

Received 5 September 2014

Received in revised form

15 September 2014

Accepted 16 September 2014

Available online 30 September 2014

Keywords:

Bio-burden

Bt protein

Bt rice

Grain yield

Leaf senescence

Matter transport

ABSTRACT

Using a transgenic *Bacillus thuringiensis* (*Bt*) crop is reportedly one of the most effective ways to improve insect resistance; however, the insertion of external *Bt* genes may affect the non-target growth characteristics of crop. Field experiments were conducted to investigate the yield performance and physiological traits in an elite restorer rice line, Minghui63 (MH63) with *Cry1C*^{*}, *Cry2A*^{*} or *Cry1Ab/Ac* genes under three fertilizer applications [zero nitrogen fertilizer (N0), zero potassium fertilizer (K0) and recommended nitrogen and potassium fertilizer (NK)]. In comparison with MH63, MH63 (*Cry1C*^{*}) experienced declining grain yields of 15.3–31.0% under different fertilizer applications because of the decreased setting rates associated with the poorer matter transport-related traits. The grain yield of MH63 (*Cry2A*^{*}) was averagely 18.4% lower than MH63 in only the N0 treatment due to the accelerated leaf senescence, which was correlated with the higher ratios of the *Bt* protein content to the soluble protein content (BTC/SPC) in the MH63 (*Cry2A*^{*}) leaves at the filling stage (FS). The yield performance of MH63 (*Cry1Ab/Ac*) was identical to MH63. In addition, no significant differences were recorded between *Bt*-MH63 and MH63 regarding N and K assimilation. Hence, our results suggest that the *Cry1C*^{*} and *Cry2A*^{*} transgenes affect the yield performance and related traits of MH63 in response to nitrogen and/or potassium applications.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Lepidopteran pests cause great damage to rice (Pandi et al., 2009). Until recently, lepidopteran pests have primarily been controlled by chemical pesticides in China, which are not only harmful to the environment but also not sufficiently effective. A transgenic *Bt* rice strain with high resistance to lepidopteran pests has been developed over the last two decades (Fujimoto et al., 1993; Datta et al., 1998; Tu et al., 2000a,b; Chen et al., 2005; Tang et al., 2006). For example, the *Cry1Ab/Ac*, *Cry1C*^{*} and *Cry2A*^{*} genes (which can be effectively expressed in rice) were successfully transferred into MH63, a widely used *indica* cytoplasm male sterile (CMS) restorer line in China (Tu et al., 2000a; Chen et al., 2005; Tang et al., 2006). In 2009, MH63 (*Cry1Ab/Ac*) and SY63 (*Cry1Ab/Ac*)

[Shanyou63 (*Cry1Ab/Ac*) were produced by crossbreeding MH63 (*Cry1Ab/Ac*) with Zhenshan97A (an elite CMS line)] were granted biosafety certificates for commercial production in China (Chen et al., 2011).

To date, *Bt* rice studies have primarily focused on breeding new lines (Tu et al., 2000a,b; Chen et al., 2005; Tang et al., 2006), pest resistance (Han et al., 2011; Jiang et al., 2013a) and environmental safety (Clark et al., 2005; Chen et al., 2006). Transgenic *Bt* crops were reported to have obvious yield advantages compared to their non-*Bt* counterparts under severe infestation by target pests (Mungai et al., 2005; Brookes and Barfoot, 2009; James, 2009; Wang et al., 2012a; Jiang et al., 2013a). Relatively less information is available regarding the growth characteristics of *Bt* rice in comparison to their non-*Bt* counterparts under no pest infestation. Notably, several authors found variations in *Bt* rice such as reduced plant heights and root lengths (Shu et al., 2002), fewer grains per panicle (Kim et al., 2008) and decreased setting rates (Tu et al., 2000b; Shu et al., 2002; Kim et al., 2008; Xia et al., 2010; Wang et al., 2012b),

* Corresponding author. Tel.: +86 27 87283775; fax: +86 27 87282131.
E-mail address: cgui@mail.hzau.edu.cn (C. Cao).

which commonly led to reductions in grain yield. Variations in *Bt* rice may result from the following causes: (1) somaclonal variation (Shu et al., 2002), (2) the disruption of native genes (Feldmann et al., 1989) and (3) the added burden of alien transgenes (Gurr and Rushton, 2005). Yield losses caused by variations in *Bt* rice were often masked by the great advantages of *Bt* rice over their non-*Bt* counterparts during target pest infestation (Jiang et al., 2013a). Therefore, comparing the important traits of *Bt* rice with their non-*Bt* counterparts under strict pest control is essential to ascertain the non-target effects of alien transgenes on the growth characteristics of rice.

Nitrogen (N) and potassium (K) are essential nutrient elements for the photosynthesis and yield formation of crops (Steven, 1985; Kropff et al., 1993; Lawlor, 2002). *Bt* protein synthesis consumes extra N in transgenic *Bt* crops; therefore, scientists have become interested in determining whether transgenic *Bt* crops change in terms of N metabolism. In maize, Bruns and Abel (2003) found no differences in the total N uptake between *Bt* maize and their non-*Bt* counterparts. However, Ma and Subedi (2005) demonstrated that several *Bt* hybrid maize strains had higher N accumulation in stovers but lower N accumulation in grains compared to their non-*Bt* counterparts. Moreover, a *Bt* hybrid maize called Pioneer 38W36*Bt* reportedly accumulated more N in its kernels and whole-plant base in comparison to a conventional hybrid maize strain (Pioneer 38W36), although they had similar leaf chlorophyll contents, N concentrations and N contents at the silking and maturity stages (Subedi and Ma, 2007). In cotton, Chen et al. (2004) indicated that *Bt* cotton had more vigorous N metabolism, which was reflected by higher total leaf N concentration, soluble protein content, and free amino acids, in addition to higher activity in nitrate reductase and glutamic-pyruvic transaminase during the reproductive phase in comparison with its non-*Bt* counterpart. In other studies, *Bt* cotton was found to be more sensitive to K deficiency than non-*Bt* cotton. Zhang et al. (2007) demonstrated that *Bt* cotton had lower total K uptake and biomass in comparison to non-*Bt* cotton under low K application. Yukui et al. (2009) studied K distributions in different parts of *Bt* cotton, and they found lower K contents in all the organs than non-*Bt* cotton. Previous studies showed that the incorporation of *Bt* genes may affect N and K uptake and metabolism of transgenic crops. Thus, it is important to investigate the responses of new *Bt* rice lines to different N and K applications.

Bt protein is a soluble protein, and the *Bt* protein concentration was found to be significantly correlated with the N and total soluble protein concentrations in leaves (Bruns and Abel, 2003; Dong and Li, 2007; Wang et al., 2012a). Moreover, spatial and temporal differences were observed in *Bt* protein synthesis. Fully expanded leaves usually had the highest amount of *Bt* protein among different plant parts (Fearing et al., 1997; Adamczyk et al., 2001; Kranthi et al., 2005; Siebert et al., 2009). The *Bt* protein content was generally maintained at a high level during the early growth stage and then declined (Greenplate, 1999; Kranthi et al., 2005; Olsen et al., 2005). However, Llewellyn et al. (2007) found no dramatic reduction in the *vip3A* protein content during the maturation stage of *Bt* cotton. Although several studies have addressed the difference in *Bt* gene expression in transgenic crops, little attention was paid to its effect on the physiological mechanisms of these crops.

Bt-MH63 with *Cry1C**, *Cry2A** or *Cry1Ab/Ac* genes were important CMS restorer lines that have been screened by breeders in China. The growth characteristics of these lines must be meticulously evaluated before they are commercialized. The objectives of the present study were to (1) compare the important growth characteristics of *Bt*-MH63 and MH63 under different N and K applications (2) and to examine the physiological reasons for the differences in the growth characteristics.

Table 1

Mean monthly air temperatures and mean daily radiation during the rice growing season in 2011 and 2012.

Time	Temperature (°C)	Solar radiation (MJ m ⁻² d ⁻¹)
2011		
June	27.2	16.8
July	31.3	20.8
August	29.8	18.5
September	25.6	16.4
October	21.5	14.3
Mean	27.1	17.4
2012		
June	25.6	14.7
July	29.4	18.9
August	28.3	16.7
September	23.2	14.8
October	20.1	12.5
Mean	25.3	15.5

2. Materials and methods

2.1. Plant materials

Three *Bt* rice lines, MH63 (*Cry1C**), MH63 (*Cry2A**) and MH63 (*Cry1Ab/Ac*), and their non-*Bt* counterpart MH63 were used in this study. The *Cry1C** and *Cry2A** genes were synthesized on the basis of the wild-type *Cry1Ca5* and *Cry2Aa* genes of *Bt*, respectively (Chen et al., 2005; Tang et al., 2006). The *Cry1Ab/Ac* gene is a hybrid *Bt* gene derived from *Cry1Ab* and *Cry1Ac* (Tu et al., 1998). The seeds from these varieties were provided by the National Key Laboratory of Crop Genetic Improvement, Wuhan, China.

2.2. Experimental design

Field experiments were conducted from June to October in 2011 and 2012 at Junchuang town (31°69'N 115°33'E), Suizhou city, Hubei Province, China. The mean monthly air temperature and mean daily radiation during the rice growing season are shown in Table 1. The treatments were arranged in a split-plot design with three fertilizer applications [N0 (0 kg N ha⁻¹ and 135 kg K₂O ha⁻¹), K0 (150 kg N ha⁻¹ and 0 kg K₂O ha⁻¹) and NK (150 kg N ha⁻¹ and 135 kg K₂O ha⁻¹)] as the main plots and four varieties [MH63 (*Cry1C**), MH63 (*Cry2A**), MH63 (*Cry1Ab/Ac*) and MH63] as the subplots. Each treatment was performed in four replicates. Size of each plot was 40 m². Twenty-day-old seedlings were transplanted at a density of 20 cm × 20 cm with one seedling per hill. The soil type of the experimental site was reddish-yellow clay-like paddy soil. The soil chemical properties were as follows: pH, 6.13; organic C, 14.66 g kg⁻¹; total N, 0.88 g kg⁻¹; NO₃⁻-N, 4.92 mg kg⁻¹; NH₄⁺-N, 1.76 mg kg⁻¹; available P, 6.73 mg kg⁻¹; and available K, 87 mg kg⁻¹. For K0 and NK, N fertilizer (urea, 46% N) was applied at a rate of 150 kg N ha⁻¹ with 50% at the basal stage, 20% at the mid-tillering stage (MT) and 30% at the panicle initiation stage (PI). For N0 and NK, K fertilizer (potassium chloride, 60% K₂O) was added at a rate of 135 kg K₂O ha⁻¹ with 50% at the basal stage and 50% at the PI. Phosphorus fertilizer (calcium superphosphate, 15% P₂O₅) was applied at a rate of 90 kg P₂O₅ ha⁻¹ at the basal stage. The experimental field was kept flooded from transplanting until 7 days before maturity. Pests, diseases and weeds were intensively controlled for all treatments to avoid yield losses.

2.3. Data collection and analysis

At the MT, PI, heading stage (HD), FS (15 days after the flowering stage) and plant maturity stage (PM), 10 topmost fully expanded leaves per plot were sampled and frozen with liquid nitrogen,

then stored at -80°C for *Bt* protein content (BTC) analysis. The *Cry1C*^{*}, *Cry2A*^{*} and *Cry1Ab/Ac* protein contents in the leaves were determined using an enzyme-linked immunosorbent assay kit by Enviro-Logix (Portland, ME) and Envirologix (Envirologix, USA) and following the protocol provided by the manufacturer. The soluble protein contents (SPCs) of the leaves were determined using the Coomassie blue dye-binding assay (Bradford, 1976). The BTC/SPC was calculated as the ratio of BTC to SPC. At the FS before sampling, the SPAD values and net photosynthetic rates of the 10 topmost fully expanded leaves were measured using a chlorophyll meter [SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan], a Li-Cor 6400XT portable photosynthesis system (Li-Cor, Lincoln, NE, USA), respectively. The glutamine synthetase (GS) activities and free amino acid contents of the sampled leaves were then determined. The GS activity was expressed as the enzyme activity that catalyzed the formation of $1\ \mu\text{mol}\ \gamma\text{-glutamylhydroxamate}\ \text{h}^{-1}$ at 37°C as determined using the Zhang et al. (1997) method. The free amino acid content was measured by the ninhydrin assay (Yemm and Cocking, 1955), and the results were converted to micrograms of amino acids per gram of leaf fresh weight using a glycine standard curve. The green leaf areas of 8 plants per plot were measured by a leaf area meter (Li-Cor 3100, LI-COR Inc., Lincoln, NE, USA) and leaf area per m^2 (leaf area index) was calculated. Large vascular bundles (LVB) and small vascular bundles (SVB) of approximately 5-cm-long sections near the panicle neck node of the primary tiller of the 8 plants were counted under the microscope. Transverse hand sections were made at identical positions with a razor blade and stained with safranin solution. The tillers of 30 plants in each plot were counted. The LVB and SVB per m^2 were calculated as the products of the vascular bundle numbers per tiller and tiller numbers per m^2 .

Mature flowers were collected at the flowering stage from various parts of different panicles found in the plot for pollen fertility examination. The pollen was stained with $\text{I}_2\text{-KI}$ solution and observed under a microscope (SA3000, Beijing Tech Instrument Co., Ltd.). A total of 1000 pollen grains per plot were investigated. The aboveground parts of 8 plants in each plot were taken at the flowering stage and PM. The plants were separated into panicles, leaves and stems, and then oven-dried at 70°C to a constant weight to determine the dry weight. The ratio of dry matter translocation to the grain (R_{DM}) was calculated using the following Ntanos and Koutroubas (2002) method:

$$R_{\text{DM}}(\%) = \frac{(F_{\text{DM}} - M_{\text{VDM}})}{F_{\text{DM}}} \times 100$$

where F_{DM} is the total aboveground dry matter of the plant at the flowering stage and M_{VDM} is the dry matter of the vegetative part of the plant at the PM. The non-structural carbohydrates (NSC, soluble sugars and starch) concentration of the stem was measured according to Yoshida et al. (1976). The total NSC mass in the stem was calculated by multiplying the stem dry weight by the NSC concentration. The apparent ratio of transferred NSC from stems to grain (AR_{NSC}) was calculated according to the following equation:

$$AR_{\text{NSC}}(\%) = \frac{(F_{\text{NSC}} - PM_{\text{NSC}})}{H_{\text{NSC}}} \times 100$$

where F_{NSC} and M_{NSC} are the total masses of NSC in the stem at the flowering stage and the PM, respectively.

The N concentrations of the different plant tissues were measured by micro-Kjeldahl digestion, distillation, and titration (Bremner and Mulvaney, 1982), and the K concentrations were estimated by flame photometry (Digital Flame Analyzer, Cole Parmer, Illinois) according to Yoshida et al. (1976). The total N and K uptake were the products of the dry weights and N and K concentrations.

At maturity, grain yield was determined from a 10-m^2 sampling area within each plot and adjusted to a moisture content of

14%. Twelve hills were sampled diagonally from the 10-m^2 harvest area to investigate yield components. The panicles were hand-threshed and oven-dried at 70°C to a constant weight. Filled and unfilled spikelets were separated and counted. The oven-dried-filled spikelets were weighed to determine grain weights. The setting rate was calculated as the ratio of the filled spikelets to the total spikelets.

The data were analyzed by an analysis of variance (SAS Institute, 2003), and the means were compared on the basis of the least significant difference (LSD) test. Correlations among the growth characteristics of *Bt* rice were determined by using the CORR model in SAS.

3. Results

3.1. Phenology

The mean monthly air temperature and mean daily radiation ranged from approximately 20°C to 31°C and from approximately $13\ \text{MJ}\ \text{m}^{-2}\ \text{d}^{-1}$ to $21\ \text{MJ}\ \text{m}^{-2}\ \text{d}^{-1}$, respectively, during the rice growing season (Table 1). The mean monthly air temperature and mean daily radiation (means of the five months) during the rice growing season in 2011 were higher by 1.8°C and $1.9\ \text{MJ}\ \text{m}^{-2}\ \text{d}^{-1}$ than those in 2012, respectively.

3.2. Grain yield and yield components

The grain yields of MH63 (*Cry1C*^{*}) were significantly lower than MH63 under all fertilizer applications (Table 2). MH63 (*Cry1C*^{*}) reduced the grain yields by 27.3%, 22.0% and 15.3% in 2011 and 31.0%, 20.7% and 18.0% in 2012 in comparison to MH63 for the N0, K0 and NK treatments, respectively. For N0, the MH63 (*Cry2A*^{*}) grain yields were 24.0% in 2011 and 12.8% in 2012 lower than the MH63 yields. However, no significant differences were observed in the grain yields recorded between MH63 (*Cry2A*^{*}) and MH63 for K0 and NK. In comparison with MH63, the lower setting rates were the primary reasons for reduced grain yields for MH63 (*Cry1C*^{*}) and MH63 (*Cry2A*^{*}). The yield performances of MH63 (*Cry1Ab/Ac*) and MH63 were identical. The responses of *Bt*-MH63 and MH63 to the N and K applications were inconsistent. The grain yields of MH63 (*Cry1C*^{*}), MH63(*Cry2A*^{*}), MH63 (*Cry1Ab/Ac*) and MH63 were 33.0%, 41.8%, 26.5% and 21.9% in 2011 and 28.3%, 25.8%, 17.7% and 14.7% in 2012 lower for N0 than for NK, and they were 17.7%, 14.7%, 15.0% and 10.7% in 2011 and 9.9%, 8.0%, 7.1% and 6.8% in 2012 lower for K0 than for NK, respectively. The fertilizer application significantly affected the grain yield and yield components with the exception of the grain weight. Both the variety and fertilizer application \times variety had significant effects on the grain yield and the setting rate.

3.3. N and K uptakes

No differences in the total N and K uptake levels were found among the four varieties under each fertilizer application (Fig. 1). The total N and K uptake levels were much lower ($P < 0.05$) for N0 than they were for K0 and NK. For NK, the four varieties had the highest levels of N and K uptake.

3.4. Pollen fertility

Fig. 2 shows no differences in pollen fertility between *Bt*-MH63 and MH63 with the exception of MH63 (*Cry1Ab/Ac*) for N0 in 2011. The mean pollen fertility values (means of all fertilizer applications) of MH63 (*Cry1C*^{*}), MH63(*Cry2A*^{*}), MH63 (*Cry1Ab/Ac*) and MH63

Table 2
Grain yields and components of *Bt*-MH63 and MH63 under different N and K applications in 2011 and 2012.

Fertilizer application	Variety	Panicles (m ⁻²)	Spikelet (panicle ⁻¹)	Setting rate (%)	grain weight (mg)	Grain yield (t ha ⁻¹)
2011						
N0	MH63 (<i>Cry1C*</i>)	254 a	84 a	65 b	28 a	4.51 b
	MH63 (<i>Cry2A*</i>)	261 a	84 a	68 b	27 a	4.72 b
	MH63 (<i>Cry1Ab/Ac</i>)	267 a	85 a	82 a	27 a	5.93 a
	MH63	277 a	89 a	83 a	27 a	6.21 a
K0	MH63 (<i>Cry1C*</i>)	316 a	99 a	54 b	28 a	5.54 b
	MH63 (<i>Cry2A*</i>)	312 a	104 a	66 a	28 a	6.92 a
	MH63 (<i>Cry1Ab/Ac</i>)	323 a	98 a	69 a	27 a	6.86 a
	MH63	318 a	107 a	67 a	27 a	7.10 a
NK	MH63 (<i>Cry1C*</i>)	313 a	111 a	58 b	28 a	6.73 b
	MH63 (<i>Cry2A*</i>)	318 a	109 a	72 a	28 a	8.11 a
	MH63 (<i>Cry1Ab/Ac</i>)	310 a	116 a	73 a	27 a	8.07 a
	MH63	312 a	114 a	70 a	28 a	7.95 a
Analysis of variance						
F	**	*	**	NS	**	
V	NS	NS	**	NS	**	
F × V	NS	NS	*	NS	*	
2012						
N0	MH63 (<i>Cry1C*</i>)	262 a	91 a	67 b	27 a	4.95 c
	MH63 (<i>Cry2A*</i>)	273 a	93 a	71 b	28 a	6.25 b
	MH63 (<i>Cry1Ab/Ac</i>)	276 a	94 a	84 a	26 a	6.76 ab
	MH63	284 a	95 a	84 a	27 a	7.17 a
K0	MH63 (<i>Cry1C*</i>)	322 a	103 a	58 b	28 a	6.22 b
	MH63 (<i>Cry2A*</i>)	325 a	99 a	73 a	27 a	7.75 a
	MH63 (<i>Cry1Ab/Ac</i>)	316 a	105 a	74 a	27 a	7.63 a
	MH63	331 a	104 a	76 a	27 a	7.84 a
NK	MH63 (<i>Cry1C*</i>)	326 a	109 a	62 b	27 a	6.90 b
	MH63 (<i>Cry2A*</i>)	321 a	106 a	76 a	28 a	8.42 a
	MH63 (<i>Cry1Ab/Ac</i>)	319 a	110 a	76 a	27 a	8.21 a
	MH63	328 a	106 a	77 a	27 a	8.41 a
Analysis of variance						
F	**	*	*	NS	*	
V	NS	NS	**	NS	**	
F × V	NS	*	*	NS	*	

Within a column for each fertilizer application, the means followed by different letters are significantly different according to the LSD (0.05). F, fertilizer application; V, variety; NS, not significant.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

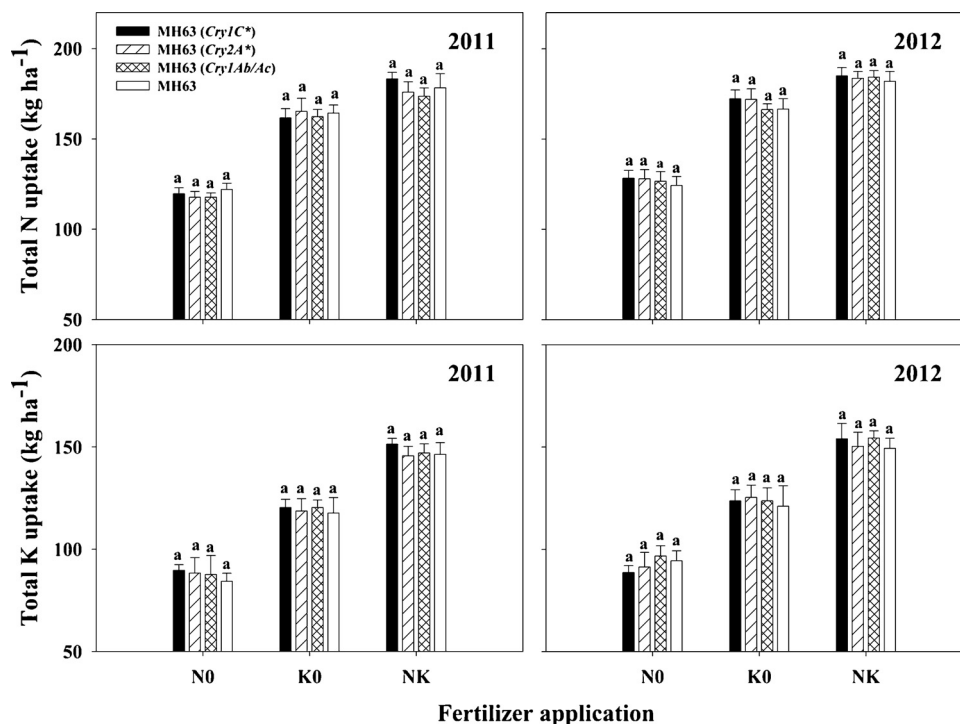


Fig. 1. Total N and K uptake levels of *Bt*-MH63 and MH63 under different N and K applications in 2011 and 2012. The same letters in different columns under each fertilizer application are not significantly different according to the LSD (0.05). The vertical bars indicate standard errors.

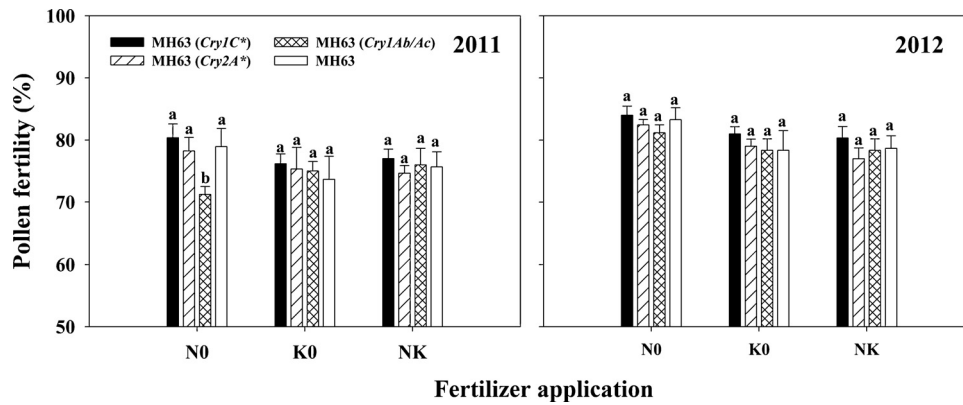


Fig. 2. Pollen fertility of *Bt*-MH63 and MH63 under different N and K applications in 2011 and 2012. The different letters in different columns under each fertilizer application are significantly different according to the LSD (0.05). The vertical bars indicate standard errors.

were 77.8%, 76.1%, 74.1% and 76.1% in 2011 and 81.8%, 79.5%, 79.3% and 80.1% in 2012, respectively.

3.5. Matter transport

The R_{DM} and AR_{NSC} reflect the efficiency of matter translocation to grain, and they were lower in MH63 (*Cry1C**) than in MH63 under all fertilizer applications (Table 3). The mean R_{DM} and AR_{NSC} (means of two years under all fertilizer applications) of MH63 (*Cry1C**) were

16.3% and 26.5%, whereas these mean values of MH63 were 31.2% and 46.0%, respectively. Moreover, MH63 (*Cry1C**) had a reduced SVB in the panicle neck node in comparison with MH63 under all fertilizer applications. The R_{DM} of MH63 (*Cry2A**) was lower than MH63 for N0. Apart from these findings, no differences were observed in the R_{DM} , AR_{NSC} , LVB and SVB among MH63 (*Cry2A**), MH63 (*Cry1Ab/Ac*) and MH63. Fertilizer applications had significant effects on the four traits. All traits except the LVB of the panicle neck node were significantly affected by the variety.

Table 3
Matter transport-related traits of *Bt*-MH63 and MH63 under different N and K applications in 2011 and 2012.

Fertilizer application	Variety	R_{DM} (%)	AR_{NSC} (%)	LVB ($m^{-2} \times 10^3$)	SVB ($m^{-2} \times 10^3$)	
2011	N0	MH63 (<i>Cry1C*</i>)	21 c	42 b	3.86 a	4.65 b
		MH63 (<i>Cry2A*</i>)	26 bc	55 a	3.56 a	5.56 a
		MH63 (<i>Cry1Ab/Ac</i>)	31 ab	58 a	3.77 a	5.31 a
		MH63	34 a	63 a	3.99 a	5.78 a
	K0	MH63 (<i>Cry1C*</i>)	15 b	27 b	4.34 a	5.18 b
		MH63 (<i>Cry2A*</i>)	21 a	38 a	4.23 a	6.37 a
		MH63 (<i>Cry1Ab/Ac</i>)	20 a	35 a	4.18 a	6.28 a
		MH63	23 a	37 a	4.17 a	6.48 a
	NK	MH63 (<i>Cry1C*</i>)	17 b	30 b	4.59 a	5.58 b
		MH63 (<i>Cry2A*</i>)	24 a	44 a	4.88 a	6.82 a
		MH63 (<i>Cry1Ab/Ac</i>)	22 a	45 a	4.49 a	6.72 a
		MH63	24 a	41 a	4.72 a	6.66 a
Analysis of variance						
F	**	**	**	**		
V	**	**	NS	**		
F × V	*	NS	NS	NS		
2012	N0	MH63 (<i>Cry1C*</i>)	18 c	37 b	3.93 a	4.78 b
		MH63 (<i>Cry2A*</i>)	24 b	49 a	3.87 a	5.73 a
		MH63 (<i>Cry1Ab/Ac</i>)	30 a	54 a	3.92 a	5.64 a
		MH63	32 a	53 a	4.10 a	5.92 a
	K0	MH63 (<i>Cry1C*</i>)	13 b	23 b	4.38 a	5.33 b
		MH63 (<i>Cry2A*</i>)	19 a	34 a	4.33 a	6.47 a
		MH63 (<i>Cry1Ab/Ac</i>)	22 a	31 a	4.29 a	6.62 a
		MH63	21 a	36 a	4.27 a	6.72 a
	NK	MH63 (<i>Cry1C*</i>)	14 b	28 b	4.71 a	5.72 b
		MH63 (<i>Cry2A*</i>)	25 a	42 a	4.91 a	6.98 a
		MH63 (<i>Cry1Ab/Ac</i>)	26 a	41 a	4.69 a	6.92 a
		MH63	25 a	46 a	4.77 a	6.83 a
Analysis of variance						
F	*	*	**	**		
V	**	**	NS	**		
F × V	*	*	NS	NS		

Within the column for each fertilizer application, the means followed by different letters are significantly different according to the LSD (0.05). R_{DM} , ratio of dry matter translocation to grain; AR_{NSC} , apparent ratio of transferred non-structural carbohydrates from stems to grain; LVB, large vascular bundles; SVB, small vascular bundles; F, fertilizer application; V, variety; NS, not significant.

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

Table 4
The leaf senescence-related traits of *Bt*-MH63 and MH63 at the FS under different N and K applications in 2011 and 2012.

Fertilizer application	Variety	Leaf area (m ² m ⁻²)	SPAD	Net photosynthetic rate (μmol CO ₂ m ⁻² s ⁻¹)	GS activity (μmol g ⁻¹ protein h ⁻¹)	Free amino acid content (μg g ⁻¹ FW)	SPC (mg g ⁻¹ FW)
2011							
N0	MH63 (<i>Cry1C</i> [*])	5.65 a	27 a	7.4 a	9.4 a	166 ab	5.9 a
	MH63 (<i>Cry2A</i> [*])	5.24 a	21 b	6.2 b	9.2 a	182 a	4.4 b
	MH63 (<i>Cry1Ab/Ac</i>)	5.58 a	26 a	7.8 a	9.7 a	146 b	5.3 a
	MH63	5.28 a	27 a	8.1 a	10.3 a	148 b	5.7 a
K0	MH63 (<i>Cry1C</i> [*])	7.12 a	34 a	11.1 a	12.5 a	231 a	9.5 a
	MH63 (<i>Cry2A</i> [*])	7.15 a	32 a	12.2 a	12.0 a	248 a	9.4 a
	MH63 (<i>Cry1Ab/Ac</i>)	6.89 a	34 a	11.4 a	13.5 a	240 a	9.8 a
	MH63	7.35 a	33 a	11.8 a	13.2 a	229 a	9.5 a
NK	MH63 (<i>Cry1C</i> [*])	7.36 a	35 a	13.4 ab	13.2 a	278 ab	10.5 a
	MH63 (<i>Cry2A</i> [*])	7.24 a	36 a	14.1 a	14.8 a	261 b	10.8 a
	MH63 (<i>Cry1Ab/Ac</i>)	7.48 a	35 a	12.6 b	13.6 a	296 a	11.5 a
	MH63	7.70 a	34 a	13.8 a	14.3 a	255 b	10.6 a
Analysis of variance							
F	**	**	**	**	**	**	**
V	NS	*	*	NS	*	**	**
F × V	NS	*	*	NS	**	*	*
2012							
N0	MH63 (<i>Cry1C</i> [*])	5.75 a	29 a	8.2 a	10.3 a	139 b	6.7 a
	MH63 (<i>Cry2A</i> [*])	5.46 a	23 b	6.4 b	10.2 a	167 a	5.1 b
	MH63 (<i>Cry1Ab/Ac</i>)	5.66 a	29 a	7.8 a	9.9 a	137 b	6.8 a
	MH63	5.78 a	28 a	8.3 a	10.8 a	140 b	7.1 a
K0	MH63 (<i>Cry1C</i> [*])	7.33 a	35 a	13.3 a	13.5 a	207 a	10.2 a
	MH63 (<i>Cry2A</i> [*])	7.43 a	33 a	14.5 a	13.0 a	213 a	10.6 a
	MH63 (<i>Cry1Ab/Ac</i>)	7.51 a	36 a	12.7 a	12.8 a	224 a	10.8 a
	MH63	7.54 a	35 a	14.8 a	14.1 a	221 a	11.1 a
NK	MH63 (<i>Cry1C</i> [*])	7.52 a	37 a	15.4 a	15.0 a	232 a	12.8 a
	MH63 (<i>Cry2A</i> [*])	7.64 a	38 a	14.9 a	15.4 a	238 a	12.5 a
	MH63 (<i>Cry1Ab/Ac</i>)	7.72 a	38 a	14.6 a	14.7 a	248 a	11.9 a
	MH63	7.84 a	39 a	15.2 a	14.6 a	240 a	11.4 a
Analysis of variance							
F	**	**	*	*	**	**	**
V	NS	*	*	NS	*	**	**
F × V	NS	*	*	NS	*	*	*

Within the column for each fertilizer treatment, the means followed by different letters are significantly different according to the LSD (0.05). FS, filling stage; GS, glutamine synthetase; SPC, soluble protein content; FW, fresh weight; F, fertilizer application; V, variety; NS, not significant.

* significant at the 0.05 probability level.

** significant at the 0.01 probability level.

3.6. Leaf senescence

No differences were observed in the leaf areas between *Bt*-MH63 and MH63 (Table 4). However, MH63 (*Cry2A*^{*}) had a lower leaf SPAD and net photosynthetic rate than MH63 for N0. Moreover, a lower SPC and higher free amino acid content were found in the leaves of MH63 (*Cry2A*^{*}) relative to MH63 for N0, whereas no significant difference in the GS activity was found between MH63 (*Cry2A*^{*}) and MH63. None of the leaf senescence-related traits of MH63 (*Cry1C*^{*}) and MH63 (*Cry1Ab/Ac*) were different from MH63, except the net photosynthetic rate and free amino acid content for NK in 2011. Fertilizer applications exerted significant effects on all six traits. The SPAD, net photosynthetic rate, free amino acid content and SPC were significantly affected by both the variety and the fertilizer application × variety.

3.7. *Bt* protein and soluble protein

The BTCs and BTC/SPCs in MH63 (*Cry2A*^{*}) and MH63 (*Cry1Ab/Ac*) leaves were much higher than in MH63 (*Cry1C*^{*}) (Fig. 3 and Table 5). The mean BTCs (means of two years under all fertilizer applications) in the MH63 (*Cry1C*^{*}), MH63 (*Cry2A*^{*}) and MH63 (*Cry1Ab/Ac*) leaves were 1.52 μg g⁻¹ FW, 18.44 μg g⁻¹ FW and 13.19 μg g⁻¹ FW, respectively. The BTCs in the leaves of the three varieties gradually increased from the MT to the HD and then decreased (Fig. 3). The BTCs in the MH63 (*Cry1C*^{*}), MH63 (*Cry2A*^{*}) and MH63 (*Cry1Ab/Ac*) leaves decreased from the HD to the FS by averages

of 33.8%, 11.6% and 43.5% (means of two years under all fertilizer applications), respectively. The BTCs and BTC/SPCs in the MH63 (*Cry2A*^{*}) leaves were maintained at higher levels ($P < 0.05$) than the other two *Bt* varieties from the HD to the PM (Fig. 3 and Table 5). Both the fertilizer application and the variety significantly affected the BTC/SPC (Table 5). Notably, MH63 (*Cry2A*^{*}) had the maximum BTC/SPC (approximately 4.3‰) for N0 at the FS (Table 5). Variance analysis indicated that the SPCs in the leaves changed more in response to the fertilizer application at the FS in comparison to the BTC (Table 6). The BTCs of MH63 (*Cry1C*^{*}) and MH63 (*Cry2A*^{*}) in the leaves changed less in response to the fertilizer application than that of MH63 (*Cry1Ab/Ac*). Moreover, the SPC of MH63 (*Cry2A*^{*}) changed more in response to the fertilizer application than those of the other two *Bt* rice lines.

3.8. Correlations among the growth characteristics of *Bt* rice

The grain yield and setting rate were positively correlated with the R_{DM} , AR_{NSC} and SVB of the panicle neck node under different fertilizer applications (Table 7). Both the R_{DM} and AR_{NSC} showed significant positive correlations with the SVB of the panicle neck node under different fertilizer applications. Positive correlations were observed among the BTC, SPC, SPAD, net photosynthetic rate and grain yield of MH63 (*Cry2A*^{*}) at the FS under different fertilizer applications (Table 8). However, the SPAD, net photosynthetic rate and grain yield were negatively related to the BTC/SPCs in the MH63 (*Cry2A*^{*}) leaves.

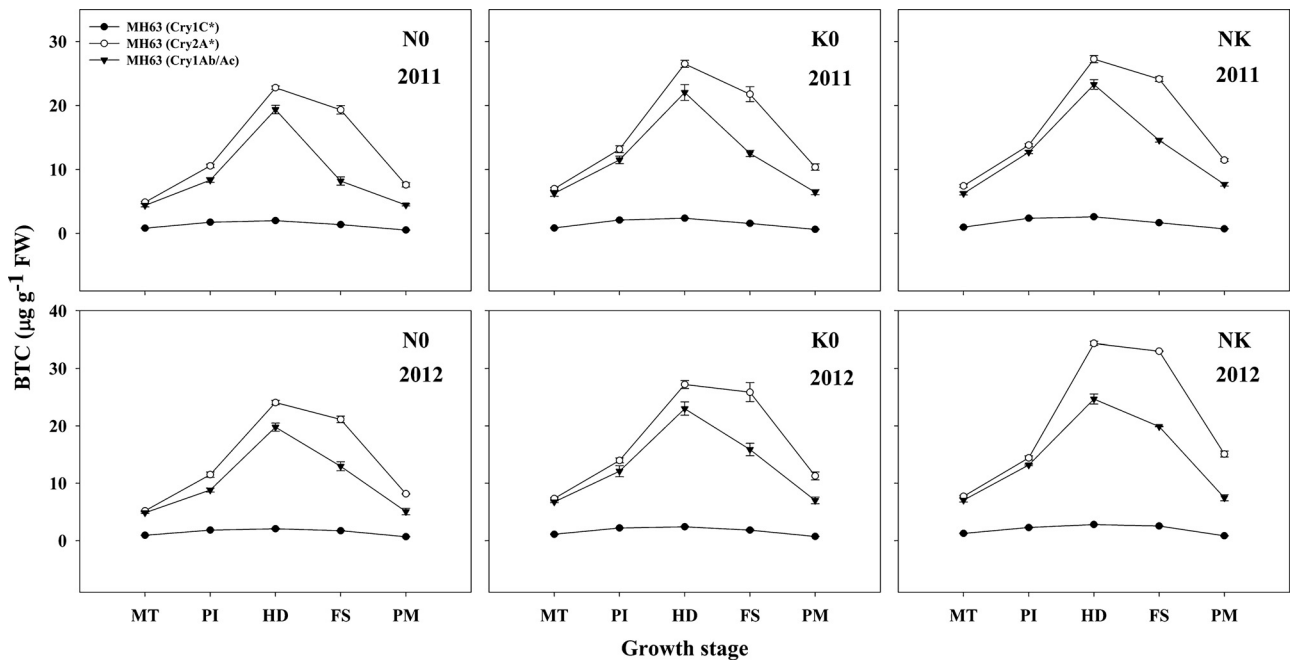


Fig. 3. Changes in the BTC of *Bt*-MH63 leaves during the rice growing seasons under different N and K applications in 2011 and 2012. BTC, *Bt* protein content; FW, fresh weight; MT, mid-tillering stage; PI, panicle initiation stage; HD, heading stage; PM, plant maturity stage. The vertical bars indicate standard errors.

Table 5
Changes in the BTC/SPCs (%) in *Bt*-MH63 leaves during the rice growing season under different N and K applications in 2011 and 2012.

Fertilizer application	Variety	MT	PI	HD	FS	PM	
2011	N0	MH63 (<i>Cry1C*</i>)	0.11 b	0.16 c	0.22 c	0.23 c	0.14 c
		MH63 (<i>Cry2A*</i>)	0.63 a	1.02 a	2.68 a	4.39 a	2.71 a
		MH63 (<i>Cry1Ab/Ac</i>)	0.56 a	0.76 b	2.20 b	1.54 b	1.43 b
	K0	MH63 (<i>Cry1C*</i>)	0.07 b	0.14 b	0.20 c	0.16 c	0.12 c
		MH63 (<i>Cry2A*</i>)	0.53 a	0.87 a	2.35 a	2.32 a	2.36 a
		MH63 (<i>Cry1Ab/Ac</i>)	0.48 a	0.77 a	1.87 b	1.28 b	1.40 b
	NK	MH63 (<i>Cry1C*</i>)	0.07 b	0.14 b	0.21 b	0.16 c	0.13 c
		MH63 (<i>Cry2A*</i>)	0.55 a	0.84 a	2.13 a	2.23 a	2.25 a
		MH63 (<i>Cry1Ab/Ac</i>)	0.46 a	0.76 a	1.79 a	1.27 b	1.47 b
Analysis of variance							
F	*	*	**	*			
V	**	**	**	**			
F × V	NS	NS	*	NS			
2012	N0	MH63 (<i>Cry1C*</i>)	0.09 b	0.12 b	0.18 b	0.26 c	0.11 c
		MH63 (<i>Cry2A*</i>)	0.55 a	0.91 a	2.44 a	4.14 a	2.52 a
		MH63 (<i>Cry1Ab/Ac</i>)	0.46 a	0.83 a	2.33 a	1.88 b	1.39 b
	K0	MH63 (<i>Cry1C*</i>)	0.06 b	0.08 c	0.12 b	0.18 c	0.13 c
		MH63 (<i>Cry2A*</i>)	0.54 a	0.68 a	2.15 a	2.44 a	2.34 a
		MH63 (<i>Cry1Ab/Ac</i>)	0.49 a	0.54 b	2.11 a	1.47 b	1.53 b
	NK	MH63 (<i>Cry1C*</i>)	0.07 b	0.09 b	0.14 c	0.20 c	0.14 c
		MH63 (<i>Cry2A*</i>)	0.61 a	0.93 a	2.33 a	2.64 a	2.13 a
		MH63 (<i>Cry1Ab/Ac</i>)	0.51 a	0.82 a	1.97 b	1.67 b	1.41 b
Analysis of variance							
F	*	**	**	*	*		
V	**	**	**	**	**		
F × V	NS	NS	*	*			

Within the column for each fertilizer treatment, the means followed by different letters are significantly different according to the LSD (0.05). BTC/SPC, ratio of *Bt* protein content to soluble protein content; MT, mid-tillering stage; PI, panicle initiation stage; HD, heading stage; PM, plant maturity stage; F, fertilizer application; V, variety; NS, not significant.

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

4. Discussion

In our study, the MH63 (*Cry1C**) grain yields under different fertilizer applications and in MH63 (*Cry2A**) for N0 were lower than in their MH63 counterpart (Table 2). An analysis of the yield components revealed that decreases in the setting rates were the reasons

for reduced grain yields in the two *Bt* varieties (Table 2). Similarly, lower setting rates were reported in several other *Bt* rice lines with the *Xa21* (Tu et al., 2000b), *Cry1Ac* (Shu et al., 2002), *Cry1Ab* (Shu et al., 2002; Kim et al., 2008) or *Bt/CpTI* (Xia et al., 2010) genes. As a common variation in *Bt* rice, the lower setting rate should be of concern in *Bt* rice breeding. However, Wang et al. (2012b) found

Table 6
Changes in the BTCs and SPCs in *Bt*-MH63 leaves at the FS under different N and K applications in 2011 and 2012.

Variety	BTC				SPC			
	Mean ($\mu\text{g g}^{-1}$ FW)	Min. ($\mu\text{g g}^{-1}$ FW)	Max. ($\mu\text{g g}^{-1}$ FW)	C.V. (%)	Mean (mg g^{-1} FW)	Min. (mg g^{-1} FW)	Max. (mg g^{-1} FW)	C.V. (%)
2011								
MH63 (<i>Cry1C*</i>)	1.5	1.3	1.7	10.4	8.6	5.2	10.8	32.2
MH63 (<i>Cry2A*</i>)	21.7	18.1	24.8	11.1	8.2	4.3	11.1	41.0
MH63 (<i>Cry1Ab/Ac</i>)	11.8	7.3	15.0	27.7	8.9	5.1	11.7	34.8
2012								
MH63 (<i>Cry1C*</i>)	2.1	1.6	2.6	18.2	9.9	6.5	12.9	29.4
MH63 (<i>Cry2A*</i>)	28.9	20.1	33.1	16.4	9.4	4.8	12.8	44.3
MH63 (<i>Cry1Ab/Ac</i>)	16.4	10.4	19.8	25.1	9.8	6.5	12.3	32.8

BTC, *Bt* protein content; SPC, soluble protein content; FS, filling stage; FW, fresh weight; Min., minimum; Max., maximum; C.V., coefficient of variation.

Table 7
Correlations among the matter transport-related traits, grain yield and setting rate under different N and K applications in 2011 and 2012.

Fertilizer application		R_{DM}	AR_{NSC}	LVB	SVB	Setting rate
N0	AR_{NSC}	0.66**				
	LVB	0.38*	0.36*			
	SVB	0.43*	0.65**	0.32*		
	Setting rate	0.69**	0.64**	0.38*	0.40*	
	Grain yield	0.59**	0.67**	0.38*	0.57**	0.59**
K0	AR_{NSC}	0.61**				
	LVB	0.06	0.13			
	SVB	0.53**	0.61**	-0.11		
	Setting rate	0.38*	0.53**	-0.03	0.67**	
	Grain yield	0.54**	0.52**	-0.14	0.50**	0.59**
NK	AR_{NSC}	0.61**				
	LVB	0.21	0.11			
	SVB	0.47**	0.53**	0.22		
	Setting rate	0.59**	0.60**	0.17	0.53**	
	Grain yield	0.66**	0.69**	0.18	0.50**	0.64**

R_{DM} , ratio of dry matter translocation to grain; AR_{NSC} , apparent ratio of transferred non-structural carbohydrates from stems to grain; LVB, large vascular bundles; SVB, small vascular bundles.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

Table 8
Correlations among the leaf senescence-related traits, BTC, grain yield and setting rate of MH63 (*Cry2A**) at the FS under different N and K applications in 2011 and 2012.

	BTC	SPC	BTC/SPC	SPAD	Net photosynthetic rate	Setting rate
SPC	0.68**					
BTC/SPC	-0.31*	-0.57**				
SPAD	0.58**	0.68**	-0.49**			
Net photosynthetic rate	0.71**	0.62**	-0.32*	0.56**		
Setting rate	0.24	0.28	-0.19	0.23	0.24	
Grain yield	0.39*	0.46**	-0.66**	0.55**	0.35*	0.13

FS, filling stage; BTC, *Bt* protein content; SPC, soluble protein content; BTC/SPC, ratio of *Bt* protein content to soluble protein content.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

no reduction in the setting rate of SY63 (*Cry1C**), which was produced by crossbreeding MH63 (*Cry1C**) with Zhenshan97A (an elite CMS line). Thus, the *Cry1C** gene may not always cause decreases in the setting rates in different rice lines. Interestingly, MH63 (*Cry2A**) only had a lower setting rate for N0, suggesting that the variation in MH63 caused by the *Cry2A** gene occurred under a specific condition. In contrast, the variation caused by the *Cry1C** gene was not eliminated by the fertilizer applications in our experiment. Similar variations in the grain yields of MH63 (*Cry1C**) and MH63 (*Cry2A**) were also observed in a previous two-year field experiment (Jiang et al., 2013b). Based on these phenomena, we can infer that reductions in the setting rates of MH63 (*Cry1C**) and MH63 (*Cry2A**) were caused by dissimilar reasons.

Yield formation is determined by the combined effects of the source, sink and flow of a crop (Wada et al., 1993). No differences were found in important source-related traits such as the

biomass, plant height, leaf area index and SPAD value between MH63 (*Cry1C**) and MH63 in our previous study (Jiang et al., 2013a). In this study, we observed no differences in sink-related traits such as the panicle number, spikelets per panicle, grain weight and pollen fertility between MH63 (*Cry1C**) and MH63 (Table 2 and Fig. 2). However, Wang et al. (2012b) examined the sink activity of MH63 (*Cry1C**) and found that MH63 (*Cry1C**) had lower contents of growth-promoting phytohormones in superior spikelets than MH63. The flow-related traits, R_{DM} and translocation efficiency of NSC have been shown to be closely connected to yield formation (Samonte et al., 2001; Ntanos and Koutroubas, 2002; Yang and Zhang, 2010). Pan et al. (2011) studied the relationships between NSC translocation and yield formation in 46 rice recombinant inbred lines and indicated that SVB under a low N level had positive direct effects on AR_{NSC} . In our study, we found that the R_{DM} , AR_{NSC} and SVB of MH63 (*Cry1C**) were lower than MH63, and positive

correlations were observed among these traits and the setting rate (Table 3 and Table 7). Therefore, the decreased grain yield of MH63 (*Cry1C**) was most likely attributed to the weaker flow in physiological function and morphology in comparison to MH63.

Photosynthesis after HD reportedly produced 60–90% of the total carbon in rice panicles at the maturation stage (Mae, 1997). Late leaf senescence sustains the photosynthetic activity in rice. In contrast, rapid leaf senescence will decrease the rice yield if the grains have been not completely filled (IRRI, 1996). In our study, accelerating leaf senescence at the FS, which was reflected by the lower SPAD value, net photosynthetic rate, and SPC along with the higher free amino acid content compared to MH63, was found in MH63 (*Cry2A**) for N0 (Table 4). Moreover, positive correlations were observed among the SPC, SPAD, net photosynthetic rate and grain yield of MH63 (*Cry2A**) (Table 8). Consequently, MH63 (*Cry2A**) had a lower grain yield than MH63 for the N0 treatment (Table 2).

Gurr and Rushton (2005) indicated that transgene expression may bring added burden to a crop because it needs more energy and matter to synthesize resistant protein. Expressing transgenes only when and where they are needed is an effective way to limit the cost of resistance (Hammond-Kosack and Parker, 2003; Michelmor, 2003). Generally, BTC would be dramatically reduced during the late crop stage because of transcriptional regulation and plant aging (Greenplate, 1999; Kranthi et al., 2005; Olsen et al., 2005; Poongothai et al., 2010). Our study showed that MH63 (*Cry2A**) had the highest average BTCs in leaves, and this strain maintained relatively high levels in BTCs during the maturation stage among the three *Bt* lines (Fig. 3). The higher BTC and lower SPC and the relative stability of BTC in response to fertilizer applications led to the maximum BTC/SPC in MH63 (*Cry2A**) at the FS for N0 (Tables 4–6 and Fig. 3). A high BTC/SPC reportedly caused developmental and morphological defects such as sterility and stunted growth in transgenic rice lines with *Cry1Ac* and *Cry2A Bt* genes (Gahakwa et al., 2000). We found that the BTC/SPC was negatively associated with SPAD and the net photosynthetic rate in MH63 (*Cry2A**) at the FS (Table 8), which indicated that the accelerated leaf senescence was possibly related to the high BTC/SPC in MH63 (*Cry2A**) for N0. We hypothesize that the bio-burden is heavier when the N supply is inadequate for varieties with high *Bt* protein expression, and BTC/SPC can be an important indicator in assessing the bio-burden.

N metabolism plays important roles in determining the leaf senescence rate and rice productivity (Yamaya et al., 2002). No differences in N uptake were found between *Bt*-MH63 and MH63 in our study (Fig. 1). However, leaf senescence differed between MH63 (*Cry2A**) and MH63 for N0 (Table 4). This finding suggests that the N metabolism of MH63 (*Cry2A**) changed in relation to MH63 when the N supply was inadequate. Alterations in N metabolism were also reported in several *Bt* cotton strains (Chen et al., 2004; Sun et al., 2007; Poongothai et al., 2010). However, no consistent N metabolism variations were found in different studies. The results may have varied because of differences in the variety and *Bt* gene. As a K-favoring crop, several *Bt* cotton plants were found to have lower K uptake than non-*Bt* cotton (Zhang et al., 2007; Yukui et al., 2009). In our study, N and K uptake levels in *Bt*-MH63 were identical to MH63 (Fig. 1). However, the grain yields of MH63 (*Cry1C**) and MH63 (*Cry2A**) were more sensitive to N and K applications than MH63 (Table 2). In addition, the N and K applications significantly affected *Bt* protein expression in *Bt*-MH63 (Fig. 3 and Table 5). Therefore, *Bt*-MH63 with *Cry1C** or *Cry2A** genes responded differently to N and/or K applications relative to MH63.

In conclusion, yield reductions were found in MH63 (*Cry1C**) under different N and K applications and in MH63 (*Cry2A**) under zero N application. Although the yield reductions in the two *Bt* rice crops were caused by decreased setting rates, declines in the setting rates of the two *Bt* rice types were caused for different reasons. For

MH63 (*Cry1C**), the yield reduction was most likely attributed to a weaker flow reflected by a lower R_{DM} , AR_{NSC} and reduced SVB in the panicle neck node. In the case of MH63 (*Cry2A**), accelerated leaf senescence primarily accounted for the yield reduction under zero N application, which was closely related to the relatively higher BTC/SPCs in the MH63 (*Cry2A**) leaves. Moreover, the grain yields of MH63 (*Cry1C**) and MH63 (*Cry2A**) were more sensitive to N and K applications than MH63, although the two *Bt* rice types were identical to MH63 in N and K uptake. No differences were found in the yield performance between MH63 (*Cry1Ab/Ac*) and MH63. Therefore, it is essential to evaluate the performance and physiological characteristics of new *Bt* rice and to choose a variety with no yield reduction and good adaptability to nutrients.

Acknowledgements

We gratefully acknowledge the National Key Laboratory of Crop Genetic Improvement laboratory for providing the *Bt* rice lines used in this study. We are grateful for grants from the National Natural Science Foundation of China (No. 31371570 and 31171492). We are grateful to J.M. Lenné, Co-Editor-in-Chief of Field Crops Research, for valuable suggestions. We also sincerely thank the two anonymous referees for their critical comments on the original manuscript.

References

- Adamczyk, J.J., Hardee, D.D., Adams, L.C., Sumerford, D.V., 2001. Correlating differences in larval survival and development of bollworm (*Lepidoptera: Noctuidae*) and fall armyworm (*Lepidoptera: Noctuidae*) to differential expression of *Cry1A(c)* δ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.* 94, 284–290.
- Bradford, M.M., 1976. A rapid method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen-total. In: Page, A.L., et al. (Eds.), *Methods of Soil Analysis, Part 2*. Am. Soc. Agron., Madison, WI, pp. 595–624.
- Brookes, G., Barfoot, P., 2009. Global impact of biotech crops: income and production effects, 1996–2007. *AgBioForum* 12, 184–208. Available: (<http://www.pgeconomics.co.uk/pdf/2009socioeconimpactsagbioforumpaper.pdf>).
- Bruns, H.A., Abel, C.A., 2003. Nitrogen fertility effects on *Bt*-endotoxin and nitrogen concentrations of maize during early growth. *Agron. J.* 95, 207–211.
- Chen, D., Ye, G., Yang, C., Chen, Y., Wu, Y., 2004. Effect after introducing *Bacillus thuringiensis* gene on nitrogen metabolism in cotton. *Field Crops Res.* 87, 235–244.
- Chen, H., Tang, W., Xu, C., Li, X., Lin, Y., Zhang, Q., 2005. Transgenic *indica* rice plants harboring a synthetic *cry2A** gene of *Bacillus thuringiensis* exhibit enhanced resistance against lepidopteran rice pests. *Theor. Appl. Genet.* 111, 1330–1337.
- Chen, L., Snow, A.A., Wang, F., Lu, B., 2006. Effects of insect-resistance genes on fecundity in rice (*Oryza sativa*, Poaceae): a test for underlying cost. *Am. J. Bot.* 93, 94–101.
- Chen, M., Shelton, A., Ye, G.Y., 2011. Insect-resistant genetically modified rice in China: from research to commercialization. *Annu. Rev. Entomol.* 56, 81–101.
- Clark, B.W., Phillips, T.A., Coats, J.R., 2005. Environmental fate and effects of *Bacillus thuringiensis* (*Bt*) proteins from transgenic crops: a review. *J. Agric. Food Chem.* 53, 4643–4653.
- Datta, K., Vasquez, A., Tu, J., Torrizo, L.M., Alam, F., Oliva, N., Abrigo, E., Khush, G.S., Datta, S.K., 1998. Constitutive and tissue-specific differential expression of the *cryIA(b)* gene in transgenic rice plants conferring resistance to rice insect pest. *Theor. Appl. Genet.* 97, 20–30.
- Dong, H.Z., Li, W.J., 2007. Variability of endotoxin expression in *Bt* transgenic cotton. *J. Agron. Crop Sci.* 193, 21–29.
- Fearing, P.L., Brown, D., Vlachos, D., Meghji, M., Privalle, L., 1997. Quantitative analysis of *CryIA(b)* expression in *Bt* maize plants, tissues, and silage and stability of expression over successive generations. *Mol. Breed.* 3, 169–176.
- Feldmann, K.A., Marks, M.D., Christianson, M.L., Quatrano, R.S., 1989. A dwarf mutant of *Arabidopsis* generated by T-DNA insertion mutagenesis. *Science* 243, 1351–1354.
- Fujimoto, H., Itoh, K., Yamamoto, M., Kyoizuka, J., Shimamoto, K., 1993. Insect resistant rice generated by introduction of a modified δ -endotoxin gene of *Bacillus thuringiensis*. *Nat. Biotechnol.* 11, 1151–1155.
- Gahakwa, D., Maqbool, S.B., Fu, X., Sudhakar, D., Christou, P., Koli, A., 2000. Transgenic rice as a system to study the stability of transgene expression: multiple heterologous transgenes show similar behaviour in diverse genetic backgrounds. *Theor. Appl. Genet.* 101, 388–399.
- Greenplate, J.T., 1999. Quantification of *Bacillus thuringiensis* insect control protein *Cry1Ac* over time in Bollgard cotton fruit and terminals. *J. Econ. Entomol.* 92, 1377–1383.

- Gurr, S., Rushton, P., 2005. Engineering plants with increased disease resistance: how are we going to express it? *Trends Biotechnol.* 23, 283–290.
- Hammond-Kosack, K.E., Parker, J.E., 2003. Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. *Curr. Opin. Biotechnol.* 14, 177–193.
- Han, Y., Xu, X.L., Ma, W.H., Yuan, B.Q., Wang, H., Liu, F.Z., Wang, M.Q., Wu, G., Hua, H.X., 2011. The influence of transgenic *cry1Ab/cry1Ac*, *cry1C* and *cry2A* rice on non-target planthoppers and their main predators under field conditions. *Agric. Sci. China* 10, 1739–1747.
- IRRI, 1996. Standard Evaluation System for Rice. International Rice Research Institute, Los Baños, Philippines.
- James, C., 2009. Global Status of Commercialized Biotech/GM Crops: 2009. ISAAA Brief No. 41, ISAAA, Ithaca, NY.
- Jiang, Y., Huang, S.Q., Cai, M.L., Li, C.F., Kong, X., Zhang, F., Ibrahim, M., Cao, C.G., 2013b. Yield changes of *Bt*-MH63 with *cry1C* or *cry2A* genes compared with MH63 (*Oryza sativa*) under different nitrogen levels. *Field Crops Res.* 151, 101–106.
- Jiang, Y., Pan, S.G., Cai, M.L., Li, C.F., Zhan, M., Wang, J.P., Ibrahim, M., Cao, C.G., 2013a. Assessment of yield advantages of *Bt*-MH63 with *cry1C* or *cry2A* genes over MH63 (*Oryza sativa* L.) under different pest control modes. *Field Crops Res.* 155, 153–158.
- Kim, S., Kim, C., Li, W., Kim, T., Li, Y., Zaidi, M.A., Altosaar, I., 2008. Inheritance and field performance of transgenic Korean *Bt* rice lines resistant to rice yellow stem borer. *Euphytica* 164, 829–839.
- Kranthi, K.R., Naidu, S., Dhawad, C.S., Tatwawadi, A., Mate, K., Patil, E., Bharose, A.A., Behere, G.T., Wadaskar, R.M., Kranthi, S., 2005. Temporal and intra-plant variability of *Cry1Ac* expression in *Bt*-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera). *Curr. Sci. India* 89, 291–298.
- Kropff, M.J., Cassman, K.G., Van Laar, H.H., Peng, S., 1993. Nitrogen and yield potential of irrigated rice. *Plant Soil* 155/156, 391–394.
- Lawlor, D.W., 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *J. Exp. Bot.* 53, 773–787.
- Llewellyn, D., Mares, C.L., Fitt, G.P., 2007. Field performance and seasonal changes in the efficacy against *Helicoverpa armigera* (Hübner) of transgenic cotton expressing the insecticidal protein vip3A. *Agric. For. Entomol.* 9, 93–101.
- Ma, B.L., Subedi, K.D., 2005. Development, yield, grain moisture and nitrogen uptake of *Bt* corn hybrids and their conventional near-isolines. *Field Crops Res.* 93, 199–211.
- Mae, T., 1997. Physiological nitrogen efficiency in rice: nitrogen utilization, photosynthesis, and yield potential. *Plant Soil* 196, 201–210.
- Michelmore, R.W., 2003. The impact zone: genomics and breeding for durable disease resistance. *Curr. Opin. Plant Biol.* 6, 397–404.
- Mungai, N.W., Motavalli, P.P., Nelson, K.A., Kremer, R.J., 2005. Differences in yields, residue composition and N mineralization dynamics of *Bt* and non-*Bt* maize. *Nutr. Cycl. Agroecosyst.* 73, 101–109.
- Ntanos, D.A., Koutroubas, S.D., 2002. Dry matter and N accumulation and translocation for *Indica* and *Japonica* rice under Mediterranean conditions. *Field Crops Res.* 74, 93–101.
- Olsen, K.M., Daly, J.C., Holt, H.E., Finnegan, E.J., 2005. Season-long variation in expression of *Cry1Ac* gene and efficacy of *Bacillus thuringiensis* toxin in transgenic cotton against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 98, 1007–1017.
- Pan, J., Cui, K., Wei, D., Huang, J., Xiang, J., Nie, L., 2011. Relationships of non-structural carbohydrates accumulation and translocation with yield formation in rice recombinant inbred lines under two nitrogen levels. *Physiol. Plant.* 141, 321–331.
- Pandi, V., Sundara Babu, P.C., Kailasam, C., 2009. Prediction of damage and yield caused by rice leaffolder at different crop periods in a susceptible rice cultivar (IR50). *J. Appl. Entomol.* 122, 595–599.
- Poongothai, S., Ilavarasan, R., Karrunakaran, C.M., 2010. *Cry1Ac* levels and biochemical variations in *Bt* cotton as influenced by tissue maturity and senescence. *J. Plant Breed. Crop Sci.* 2, 96–103.
- Samonte, S.O., Wilson, L.T., McClung, A.M., Tarpley, L., 2001. Seasonal dynamics of nonstructural carbohydrate partitioning in 15 diverse rice genotypes. *Crop Sci.* 41, 902–909.
- SAS Institute, 2003. SAS/STAT User's Guide for Personal Computers. Version 9.1. SAS Institute, Cary, NC.
- Shu, Q., Cui, H., Ye, G., Wu, D., Xia, Y., Gao, M., Altosaar, I., 2002. Agronomic and morphological characterization of *Agrobacterium*-transformed *Bt* rice plants. *Euphytica* 127, 345–352.
- Siebert, M.W., Patterson, T.G., Gilles, G.J., Nolting, S.P., Braxton, L.B., Leonard, B.R., Van Duyn, J.W., Lassiter, R.B., 2009. Quantification of *Cry1Ac* and *Cry1F Bacillus thuringiensis* insecticidal proteins in selected transgenic cotton plant tissue types. *J. Econ. Entomol.* 102, 1301–1308.
- Steven, H.C., 1985. Role of potassium in photosynthesis and respiration. In: Potassium in Agriculture (potassiuminagri), pp. 369–396.
- Subedi, K.D., Ma, B.L., 2007. Dry matter and nitrogen partitioning patterns in *Bt* and non-*Bt* near-isoline maize hybrids. *Crop Sci.* 47, 1186–1192.
- Sun, C.X., Zhang, L.L., Wu, Q., Miao, L., Wang, G.W., Li, S.J., 2007. Nitrogen metabolism of transgenic *Bt* cotton and transgenic *Bt + CpTI* cotton at seedling stage. *Chin. J. Ecol.* 26, 187–191 (in Chinese).
- Tang, W., Chen, H., Xu, C., Li, X., Lin, Y., Zhang, Q., 2006. Development of insect-resistant transgenic *indica* rice with a synthetic *cry1C* gene. *Mol. Breed.* 18, 1–10.
- Tu, J., Datta, K., Khush, G.S., Zhang, Q., Datta, S.K., 2000b. Field performance of *Xa21* transgenic *indica* rice (*Oryza sativa* L.), IR72. *Theor. Appl. Genet.* 101, 15–20.
- Tu, J., Zhang, G., Datta, K., Xu, C., He, Y., Zhang, Q., Khush, G.S., Datta, S.K., 2000a. Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ -endotoxin. *Nat. Biotechnol.* 18, 1101–1104.
- Tu, J.M., Datta, K., Alam, M.F., Fan, Y.L., Khush, G.S., Datta, S.K., 1998. Expression and function of a hybrid *Bt* toxin gene in transgenic rice conferring resistance to insect pest. *Plant Biotechnol.* 15, 195–203.
- Wada, Y., Miura, K., Watanabe, K., 1993. Effects of source-to-sink ratio on carbohydrate production and senescence of rice flag leaves during the ripening period. *Jpn. J. Crop Sci.* 62, 547–553.
- Wang, F., Jian, Z.P., Nie, N.X., Cui, K.H., Peng, S.B., Lin, Y.J., Huang, J.L., 2012a. Effects of N treatments on the yield advantage of *Bt*-SY63 over SY63 (*Oryza sativa*) and the concentration of *Bt* protein. *Field Crops Res.* 129, 39–45.
- Wang, F., Ye, C., Zhu, L.Y., Nie, L.X., Cui, K.H., Peng, S.B., Lin, Y.J., Huang, J.L., 2012b. Yield differences between *Bt* transgenic rice lines and their non-*Bt* counterparts, and its possible mechanism. *Field Crops Res.* 126, 8–15.
- Xia, H., Chen, L., Wang, F., Lu, B., 2010. Yield benefit and underlying cost of insect-resistance transgenic rice: implication in breeding and deployment of transgenic crops. *Field Crops Res.* 118, 215–220.
- Yamaya, T., Obara, M., Nakajima, H., Sasaki, S., Hayakawa, T., Sato, T., 2002. Genetic manipulation and quantitative-trait loci mapping for nitrogen recycling in rice. *J. Exp. Bot.* 53, 917–925.
- Yang, J., Zhang, J., 2010. Crop management techniques to enhance harvest index in rice. *J. Exp. Bot.* 61, 3177–3189.
- Yemm, E.W., Cocking, E.C., 1955. Determination of amino acid with ninhydrin. *Analyst* 80, 209–213 (in Chinese).
- Yoshida, S., Forno, D.A., Cock, J.H., Gomez, K.A., 1976. Laboratory manual for physical studies of rice. International Rice Research Institute Press, Manila, Philippines.
- Yukui, R., Wenya, W., Pinghui, L., Fusuo, Z., 2009. Mineral element distribution in organs of dual-toxin transgenic (*Bt + CpTI*) cotton seedling. *Plant Biosyst.* 143, 137–139.
- Zhang, C., Peng, S., Peng, X., Chavez, A.Q., Bennett, J., 1997. Response of glutamine synthetase isoforms to nitrogen sources in rice (*Oryza sativa* L.) roots. *Plant Sci.* 125, 163–170.
- Zhang, Z., Tian, X., Duan, L., Wang, B., He, Z., Li, Z., 2007. Differential responses of conventional and *Bt*-transgenic cotton to potassium deficiency. *J. Plant Nutr.* 30, 659–670.