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Yield changes of *Bt*-MH63 with *cry1C** or *cry2A** genes compared with MH63 (*Oryza sativa*) under different nitrogen levels

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ABSTRACT

Field performance of insect-resistant transgenic rice needs to be meticulously evaluated before it is commercialized. To our knowledge, little information are available about the field performance of *Bt* rice with *cry1C** or *cry2A** genes under different nitrogen (N) levels. Field experiments were conducted to investigate the yield performance and yield-related traits of *Bt*-MH63 under three N levels (0, 150 and 195 kg N ha^{-1}). The results showed that MH63 (*cry1C**) had lower grain yield than MH63 at all N levels due to the reduced grain filling percentage. Furthermore, MH63 (*cry1C**) as compared with MH63 had lower dry matter translocation efficiency and higher reservation of soluble sugar in stem and sheath at mature at all N levels. At 0 kg N ha^{-1}, grain yield and internal N use efficiency (IE_N) of MH63 (*cry2A**) were significantly lower than those of MH63 possibly due to the leaf premature aging of MH63 (*cry2A**). In contrast, there were no significant differences in the grain yield and leaf senescence between MH63 (*cry2A**) and MH63 at 150 and 195 kg N ha^{-1}. The results indicated that the incorporation of *cry1C** or *cry2A** caused varying degrees of yield reduction in rice due to different agronomic reasons.

1. Introduction

Recently, breeding of insect-resistant transgenic *Bt* rice has made great progress. Many insect-resistant transgenic rice lines were successfully cultivated (Fujimoto et al., 1993; Datta et al., 1998; Tu et al., 2000; Chen et al., 2004, 2005; Tang et al., 2006). In 2009, two *Bt* rice lines, MH63 (*cry1Ab/c*) and SY63 (*cry1Ab/c*), were granted biosafety certificates by the Ministry of Agriculture in China. The *Bacillus thuringiensis* (*Bt*) genes were transferred into crops to increase their resistance against pests. The *cry1C** and *cry2A** genes were synthesized on the basis of wild-type *cry1Ca5* and *cry2Aa* genes of *Bt*, respectively, which could be effectively expressed in rice (Chen et al., 2005; Tang et al., 2006). These genes were transferred into MH63 (Minghui63), an *indica* CMS (cytoplasm male sterile) restorer line in China. *Bt*-MH63 with *cry1C** or *cry2A** genes may become important breeding materials for insect-resistant transgenic rice in China.

Bt transgenic cultivars could have higher yields by 13–23% as compared with their non-*Bt* counterparts under severe insect infestation (Mungai et al., 2005). Wang et al. (2012a) found that SY63 (*cry1C**) and SY63 (*cry2A**) increased yields by near 20% compared with their counterparts SY63 [Shanyou63, produced by crossbreeding MH63 with Zhenshan97A (an elite CMS line)] when no pesticides were applied against target pests. However, under low insect conditions, no yield advantages for *Bt* transgenic cultivars were reported (Lauer and Wedberg, 1999; Ma and Subedi, 2005). For example, no yield differences were observed in *Bt*-SY63 with *cry1Ab/c*, *cry1C** or *cry2A** compared with their non-*Bt* counterparts under moderate insect conditions (Tu et al., 2000; Wang et al., 2010, 2012b). On the other hand, some authors found decreases in the grain yields of *Bt* rice. Chen et al. (2006) and Xia et al. (2010) observed a yield loss in *Bt/CPTI* transgenic MH86 (a restorer line) under low insect pressure. Cost in grain yield brought by transgenes may not be easily detected under severe insect infestation, and therefore studying yield performance of *Bt* rice under no pest infestation is an essential process to evaluate the effects of incorporation of external genes.

The insertion of external genes usually caused variations in rice, such as, reduced plant height, root length, grains per panicle and grain filling percentage, which commonly led to reductions in grain yield (Shu et al., 2002; Jiang et al., 2004; Kim et al., 2008; Xia et al., 2010; Wang et al., 2012b). Although some studies were conducted to evaluate the phenotypic variations of *Bt* transgenic crops, few studies have been done to examine the agronomic and physiological mechanisms of the variations.

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Nitrogen (N) is essential to the formation of grain yield (Kropff et al., 1993; Lawlor, 2002), and is indispensable to the *Bt* protein synthesis. It has been demonstrated that concentrations of *Bt* protein in plant tissues were significantly correlated with concentrations of overall N (Bruns and Abel, 2003; Dong and Li, 2007; Wang et al., 2012a). Subedi and Ma (2007) found that there were no significant differences in N partitioning in different tissues of *Bt* transgenic maize and its non-*Bt* counterpart. However, Chen et al. (2004) found that *Bt* cotton had more vigorous N metabolism in the vegetative stage compared with its non-*Bt* counterpart, resulting in a reduction in boll size. Previous studies showed that the incorporation of *Bt* genes might affect the N metabolism of transgenic crops. However, researches about N utilization and metabolism of *Bt* rice were relatively few so far.

The objectives of this study were (1) to investigate yield performance of *Bt*-MH63 with *cry1C** or *cry2A** genes compared with MH63 under different N levels and (2) to find out the main agronomic and physiological mechanisms of the yield changes.

2. Materials and methods

Three varieties, MH63 (*cry1C**), MH63 (*cry2A**) and their non-transgenic counterpart MH63 were used in the study. The *cry1C** and *cry2A** protein contents were about 2.0 and 14.0 μg g^{-1} leaf fresh weight in *Bt* rice, respectively (Wang et al., 2012a).

Field experiments were conducted from May to October in 2009 and 2010 at Junchuang village, Suizhou city (31°69′N 115°33′E), Hubei Province, China. The main soil properties of the experimental site were as follows: pH, 6.09; organic C, 15.93 g kg^{-1}; total N, 0.91 g kg^{-1}; NO_3^-–N, 5.07 mg Kg^{-1}; NH_4^+–N, 1.88 mg kg^{-1}. The treatments were arranged in a split-plot design with N levels as the main plots and varieties as the subplots. N levels included three N levels (0, 150 and 195 kg N ha^{-1}). Size of each plot was 30 m^2. Twenty-five-day old seedlings were transplanted at a density of 20 cm × 20 cm with one seedling per hill. Nitrogen (urea, 46% N) was applied with 50% at basal, 20% at mid-tillering stage and 30% at panicle initiation stage. P_2O_5 (calcium superphosphate) and K_2O (potassium chloride) were applied with rates of 90 and 135 kg ha^{-1}, respectively. Pests, diseases and weeds were intensively controlled for all treatments to avoid yield loss.

A chlorophyll meter [SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan] was used to obtain SPAD values on 10 topmost fully expanded leaves per plot. SPAD readings were acquired at the mid-tillering, panicle initiation, flowering stage and 21 days after flowering stage. A corrected method of Abdelkhalik et al. (2005) was used to estimate leaf senescence. Reduction in SPAD was estimated as an indicator of the leaf senescence through calculating the difference between the SPAD at flowering stage and at 21 days after flowering stage.

Twelve hills (0.48 m^2) were taken at the flowering and maturity stage. Plants were separated into leaves, panicles, stems and sheaths. Samples were oven-dried at 70 °C to constant weight for determination of biomass and total N uptake. The dried samples of stems and sheaths were then ground to measure the content of soluble sugar following the method of Yoshida et al. (1976). The N concentrations of different tissues were measured by micro Kjeldahl digestion, distillation, and titration (Bremner and Mulvaney, 1982). Aboveground total N uptake was the product of the biomass and N concentration. Total aboveground dry matter at the flowering stage and dry matter of vegetative parts at the maturity were weighed. Dry matter translocation was calculated as the difference between the total aboveground dry matter at the flowering stage and dry matter of vegetative parts at the maturity, and dry matter translocation efficiency was calculated as the ratio of the dry matter translocation to the total aboveground dry matter at the flowering stage (Ntanos and Koutroubas, 2002).

Table 1
Definitions of fertilizer-N use efficiency.

Term	Equation	Unit
Recovery efficiency (RE_N)	$100 \times (TN_{+N} - TN_{-N})/FN$	%
Physiological efficiency (PE_N)	$(GY_{+N} - GY_{-N})/(TN_{+N} - TN_{-N})$	kg kg^{-1}
Agronomic efficiency (AE_N)	$(GY_{+N} - GY_{-N})/FN$	kg kg^{-1}
Partial factor productivity of applied N (PFP_N)	GY_{+N}/FN	kg kg^{-1}
Internal N use efficiency (IE_N)	GY/TN	kg kg^{-1}

TN = total aboveground plant N; TN_{+N} = total aboveground plant N accumulation in the plot received N fertilizer; TN_{-N} = total aboveground plant N accumulation in the zero-N control; FN = the amount of N fertilizer applied; GY = grain yield; GY_{+N} = grain yield in the plot received N fertilizer; GY_{-N} = grain yield in the zero-N control.

At maturity, grain yield was determined from a 5-m^2 sampling area within each plot and adjusted to a moisture content of 14%. Twelve hills were sampled diagonally from the 5-m^2 harvest area to investigate yield components. Panicle number per m^2 was determined by counting the panicle numbers of each hill. Panicles were hand-threshed and the unfilled spikelets were separated from filled spikelets by submerging them in tap water. Three subsamples each of 5 g of unfilled spikelets and 30 g of filled spikelets were taken to calculate the spikelets per panicle and grain filling percentage. Filled spikelets were oven-dried at 70 °C to constant weight to determine the grain weight. Fertilizer-N use efficiency (FNUE) was calculated following the methods of Novoa and Loomis (1981) and Peng et al. (2006) (Table 1).

Data were analyzed following analysis of variance (SAS Institute, 2003) and means were compared based on the least significant difference (LSD) test at the 0.05 probability level.

3. Results

3.1. Grain yield and yield components

Grain yields of MH63 (*cry1C**) were significantly lower than those of MH63 (*cry2A**) and MH63 at all N levels in 2009 and 2010, respectively (Table 2). MH63 (*cry1C**) reduced grain yields by 16.9 and 21.0% (means of all N levels) compared with MH63 in 2009 and 2010, respectively. The lower grain yield of MH63 (*cry1C**) was mainly due to the significant lower grain filling percentage compared with MH63 (Table 2).

In both two years at 0 kg N ha^{-1}, grain yields and grain filling percentages of MH63 (*cry2A**) were significantly lower than those of MH63, respectively. In 2009, MH63 (*cry1C**), MH63 (*cry2A**) and MH63 reduced grain yields by 25.0, 33.1 and 19.1% at 0 kg N ha^{-1} compared with 195 kg N ha^{-1}, and by 20.8, 32.0 and 23.1% in 2010, respectively (Table 2). There were no significant differences in grain yields between MH63 (*cry2A**) and MH63 at 150 and 195 kg N ha^{-1} (Table 2).

3.2. Dry matter translocation efficiency and soluble sugar concentration

Dry matter translocation efficiency of MH63 (*cry1C**) was significantly lower than those of MH63 (*cry2A**) and MH63 at all N levels in 2009 and 2010, respectively (Fig. 1). Maximum and minimum values of dry matter translocation efficiency of MH63 (*cry1C**) were 22.8% at 0 kg N ha^{-1} in 2009 and 14.2% at 150 kg N ha^{-1} in 2010, respectively. In 2009 and 2010, dry matter translocation efficiency of MH63 (*cry2A**) was significantly lower than that of MH63 at 0 kg N ha^{-1} (Fig. 1). MH63 (*cry1C**) had significantly higher concentrations of soluble sugar in stem and sheath at mature than MH63 at all N levels in both two years (Table 3). Concentrations

Table 2
Grain yields and components of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 at different N levels in 2009 and 2010.

Nitrogen level (kg ha^{-1})	Variety	Biomass (t ha^{-1})	Panicle number (m^{-2})	Spikelets per panicle	Grain filling percentage (%)	Grain weight (mg)	Grain yield (t ha^{-1})
2009							
0	MH63 (*cry1C**)	11.0 a	274 a	84 a	71.8 b	28.4 a	4.68 c
	MH63 (*cry2A**)	10.2 b	272 a	83 a	74.6 b	28.2 a	4.94 b
	MH63	11.5 a	285 a	84 a	83.5 a	27.9 a	5.94 a
	Mean	10.9	277	84	76.6	28.2	5.19
150	MH63 (*cry1C**)	15.0 a	324 a	112 a	69.0 b	27.9 a	6.17 b
	MH63 (*cry2A**)	15.0 a	317 a	109 a	76.2 a	27.8 a	7.19 a
	MH63	15.1 a	314 a	118 a	79.4 a	28.0 a	7.28 a
	Mean	15.0	318	113	74.8	27.9	6.88
195	MH63 (*cry1C**)	15.4 a	324 a	117 a	66.0 b	27.8 a	6.24 b
	MH63 (*cry2A**)	15.8 a	320 a	118 a	73.6 a	27.7 a	7.38 a
	MH63	15.6 a	326 a	115 a	73.8 a	27.1 b	7.34 a
	Mean	15.6	323	117	71.1	27.5	6.99
2010							
0	MH63 (*cry1C**)	9.0 ab	250 ab	78 a	69.0 c	27.9 a	4.08 c
	MH63 (*cry2A**)	8.8 b	242 b	78 a	74.9 b	27.9 a	4.35 b
	MH63	9.1 a	260 a	80 a	82.4 a	27.1 b	5.04 a
	Mean	9.0	250	79	75.4	27.6	4.49
150	MH63 (*cry1C**)	13.0 a	296 a	92 a	73.8 b	27.9 ab	4.97 b
	MH63 (*cry2A**)	13.0 a	301 a	84 b	79.5 a	28.3 a	6.30 a
	MH63	13.1 a	308 a	92 a	82.4 a	26.9 b	6.38 a
	Mean	13.1	302	89	78.6	27.7	5.88
195	MH63 (*cry1C**)	13.1 a	320 a	96 a	68.1 b	27.7 ab	5.15 b
	MH63 (*cry2A**)	13.4 a	325 a	99 a	77.4 a	28.0 a	6.40 a
	MH63	13.5 a	319 a	97 a	78.3 a	27.4 b	6.55 a
	Mean	13.3	321	97	74.6	27.7	6.03

Within a column for each year at the same N level, means followed by different letters are significantly different according to LSD (0.05). MH63: Minghui63, MH63 (*cry1C**) and MH63 (*cry2A**) contain a *Bt* gene of *cry1C** and *cry2A**, respectively.

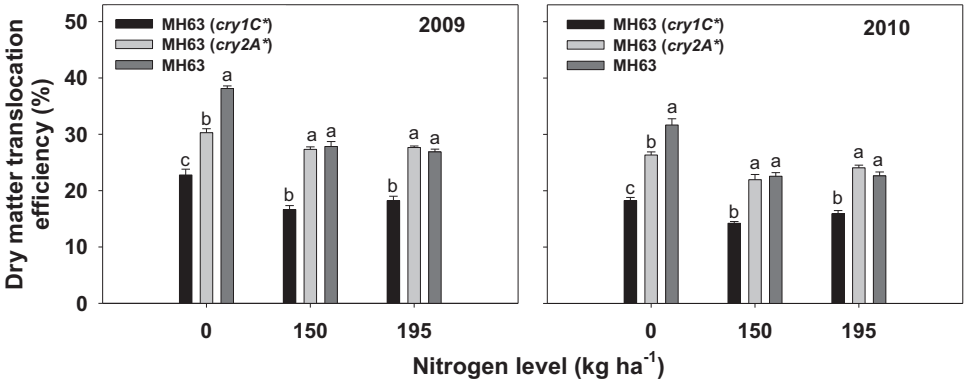

Fig. 1. Dry matter translocation efficiency of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 at different N levels in 2009 and 2010. MH63: Minghui63, MH63 (*cry1C**) and MH63 (*cry2A**) contain a *Bt* gene of *cry1C** and *cry2A**, respectively. Columns with different letters at the same N level are significantly different according to LSD (0.05). Vertical bars indicate standard errors.

Table 3
Soluble sugar concentrations (%) in stem and sheath of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 at different N levels in 2009 and 2010.

Nitrogen level (kg ha^{-1})	Variety	2009		2010	
		Flowering stage	Maturity stage	Flowering stage	Maturity stage
0	MH63 (*cry1C**)	10.5 a	7.3 a	9.2 a	6.3 a
	MH63 (*cry2A**)	10.2 a	3.7 b	9.2 a	3.3 b
	MH63	10.6 a	4.2 b	9.9 a	3.9 b
	Mean	10.4	5.1	9.4	4.5
150	MH63 (*cry1C**)	15.3 a	9.6 a	13.3 b	7.9 a
	MH63 (*cry2A**)	15.1 a	6.4 b	14.4 ab	5.7 b
	MH63	17.7 a	5.2 b	15.4 a	5.2 b
	Mean	16.0	7.1	14.4	6.3
195	MH63 (*cry1C**)	16.4 a	10.9 a	15.4 a	8.7 a
	MH63 (*cry2A**)	16.2 a	7.1 b	16.1 a	6.5 b
	MH63	18.5 a	6.1 b	15.6 a	6.9 b
	Mean	17.0	8.0	15.7	7.4

Within a column for each N level, means followed by different letters are significantly different according to LSD (0.05). MH63: Minghui63, MH63 (*cry1C**) and MH63 (*cry2A**) contain a *Bt* gene of *cry1C** and *cry2A**, respectively.

of soluble sugar in stem and sheath of MH63 (*cry1C**) and MH63 at mature were 8.5 and 5.3% (means of two years at all N levels), respectively. There were no significant differences in concentrations of soluble sugar in stem and sheath between MH63 (*cry1C**) and MH63 at the flowering stage except at 150 kg N ha^{-1} in 2010 (Table 3).

3.3. SPAD and leaf senescence

In 2009 and 2010, reductions in SPAD of MH63 (*cry2A**) were significantly higher than those of MH63 at 0 kg N ha^{-1} (Table 4). Reductions in SPAD of MH63 (*cry2A**) and MH63 were 13.3 and 8.3 (means of two years at 0 kg N ha^{-1}), respectively. The higher reduction in SPAD of MH63 (*cry2A**) was mainly due to the lower SPAD value at 21 days after flowering stage compared with MH63 (Table 4). There were no significant differences in reductions in SPAD between MH63 (*cry2A**) and MH63 at 150 and 195 kg N ha^{-1} (Table 4). No significant differences in SPAD values were existed between MH63 (*cry2A**) and MH63 at the mid-tillering, panicle initiation and flowering stage in both two years (Table 4). On the other hand, the grain yield was negatively correlated with the reduction in SPAD of MH63 (*cry2A**) at different N levels in 2009 and 2010 (Fig. 2).

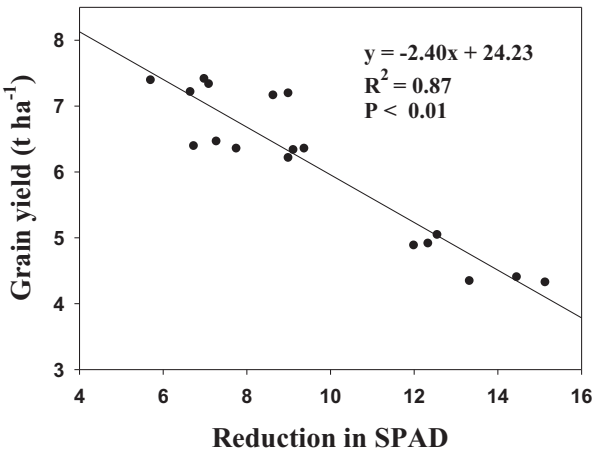

Fig. 2. Correlation between reduction in SPAD and grain yield of MH63 (*cry2A**) at different N levels in 2009 and 2010. MH63 (*cry2A**) contains a *Bt* gene of *cry2A**.

3.4. Fertilizer-N use efficiency

In 2009 and 2010, IE_N of MH63 (*cry2A**) significantly decreased by 17.9 and 15.6% compared with MH63 at 0 kg N ha^{-1} (Table 5). MH63 (*cry2A**) had the highest PE_N and AE_N among the three varieties (Table 5). The lower IE_N, higher PE_N and AE_N of MH63 (*cry2A**) were mainly due to the lower grain yield compared with MH63 at 0 kg N ha^{-1} (Table 2). IE_N and PFP_N of MH63 (*cry1C**) were significantly lower than those of MH63 at all N levels in both two years (Table 5). The lower IE_N and PFP_N of MH63 (*cry1C**) were mainly due to the lower grain yield compared with MH63 (Table 2). There were no significant differences in total N uptake among MH63 (*cry1C**), MH63 (*cry2A**) and MH63 except at 195 kg N ha^{-1} in 2009 (Table 5).

4. Discussion

In our study, MH63 (*cry1C**) had lower grain yield than MH63, and the yield reduction was mainly ascribed to the reduced grain filling percentage (Table 2). Similarly, reduction in grain filling percentage leading to yield loss was reported in *Bt* rice with some other different *Bt* genes (Shu et al., 2002; Jiang et al., 2004; Kim et al., 2008; Xia et al., 2010). However, Wang et al. (2012b) found that *Bt*-SY63, produced by crossbreeding MH63 (*cry1C**) with Zhenshan97A (an elite CMS line), showed no yield cost. Reduction in grain filling percentage caused by *cry1C** was eliminated by hybridization in *Bt*-SY63. So, *cry1C**

Table 4
SPAD values and reductions in SPAD of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 at different N levels in 2009 and 2010.

Nitrogen level (kg ha^{-1})	Variety	Mid-tillering stage	Panicle initiation stage	Flowering stage	21 days after flowering stage	Reduction in SPAD
2009						
0	MH63 (*cry1C**)	32.4 a	33.9 a	35.5 a	28.5 a	7.0 b
	MH63 (*cry2A**)	32.0 ab	33.4 ab	34.7 a	22.4 b	12.3 a
	MH63	31.4 b	33.0 b	36.2 a	28.2 a	8.0 b
	Mean	32.0	33.4	35.5	26.4	9.1
150	MH63 (*cry1C**)	36.1 a	38.4 a	40.3 a	32.5 a	7.8 a
	MH63 (*cry2A**)	36.3 a	38.5 a	40.0 a	31.9 a	8.1 a
	MH63	36.2 a	38.3 a	40.2 a	31.9 a	8.3 a
	Mean	36.2	38.4	40.1	32.1	8.0
195	MH63 (*cry1C**)	36.9 a	39.7 a	42.2 a	35.9 a	6.3 a
	MH63 (*cry2A**)	36.9 a	39.0 a	41.4 a	34.8 a	6.6 a
	MH63	37.6 a	40.1 a	42.4 a	35.7 a	6.7 a
	Mean	37.1	39.6	42.0	35.5	6.5
2010						
0	MH63 (*cry1C**)	27.5 a	30.1 a	32.6 a	23.3 a	9.3 b
	MH63 (*cry2A**)	27.5 a	29.2 a	31.1 a	16.8 b	14.3 a
	MH63	27.3 a	29.0 a	31.5 a	23.0 a	8.5 b
	Mean	27.4	29.5	31.7	21.0	10.7
150	MH63 (*cry1C**)	33.6 a	35.7 a	38.2 a	29.2 a	9.0 a
	MH63 (*cry2A**)	32.4 a	35.7 a	37.6 a	28.5 a	9.1 a
	MH63	33.1 a	36.3 a	38.6 a	29.4 a	9.2 a
	Mean	33.0	35.9	38.1	29.0	9.1
195	MH63 (*cry1C**)	37.8 a	39.0 a	41.8 a	34.3 a	7.5 a
	MH63 (*cry2A**)	37.1 a	39.0 a	41.2 a	34.0 a	7.2 a
	MH63	38.0 a	39.7 a	42.2 a	35.0 a	7.2 a
	Mean	37.6	39.3	41.7	34.4	7.3

Within a column for each year at the same N level, means followed by different letters are significantly different according to LSD (0.05). MH63: Minghui63, MH63 (*cry1C**) and MH63 (*cry2A**) contain a *Bt* gene of *cry1C** and *cry2A**, respectively.

Table 5
Total N uptake, recovery efficiency (RE_N), physiological efficiency (PE_N), agronomic efficiency (AE_N), partial factor productivity of applied N (PFP_N) and internal N use efficiency (IE_N) of MH63 (*cry1C**), MH63 (*cry2A**) and MH63 at different N levels in 2009 and 2010.

Nitrogen level (kg ha^{-1})	Variety	Total N uptake (kg ha^{-1})	RE_N (%)	PE_N (kg kg^{-1})	AE_N (kg kg^{-1})	PFP_N (kg kg^{-1})	IE_N (kg kg^{-1})
2009							
0	MH63 (*cry1C**)	111 a	–	–	–	–	42.3 b
	MH63 (*cry2A**)	115 a	–	–	–	–	43.1 b
	MH63	113 a	–	–	–	–	52.5 a
	Mean	113	–	–	–	–	45.9
150	MH63 (*cry1C**)	174 a	42.4 a	23.3 b	9.9 b	41.1 b	35.4 b
	MH63 (*cry2A**)	178 a	42.1 a	35.5 a	15.0 a	47.9 a	40.4 a
	MH63	177 a	42.9 a	21.1 b	9.0 b	48.5 a	41.2 a
	Mean	176	42.5	26.7	11.3	45.9	39.0
195	MH63 (*cry1C**)	182 b	36.7 a	21.7 b	8.0 b	32.0 b	34.2 b
	MH63 (*cry2A**)	187 a	36.8 a	34.1 a	12.5 a	37.8 a	39.6 a
	MH63	185 ab	37.1 a	19.7 b	7.2 b	37.7 a	39.7 a
	Mean	185	36.9	25.2	9.2	35.8	37.8
2010							
0	MH63 (*cry1C**)	87 a	–	–	–	–	46.8 c
	MH63 (*cry2A**)	88 a	–	–	–	–	49.4 b
	MH63	86 a	–	–	–	–	58.5 a
	Mean	87	–	–	–	–	51.6
150	MH63 (*cry1C**)	142 a	36.9 a	16.2 c	6.0 c	33.1 b	34.9 b
	MH63 (*cry2A**)	145 a	37.8 a	34.4 a	13.0 a	42.0 a	43.5 a
	MH63	147 a	40.7 a	22.0 b	8.9 b	42.5 a	43.4 a
	Mean	145	38.5	24.2	9.3	39.2	40.6
195	MH63 (*cry1C**)	155 a	34.6 a	16.1 c	5.5 c	26.4 c	33.4 b
	MH63 (*cry2A**)	155 a	34.2 a	30.7 a	10.5 a	32.8 b	41.3 a
	MH63	157 a	36.2 a	21.5 b	7.8 b	33.6 a	41.9 a
	Mean	155	35.0	22.8	7.9	31.0	38.9

Within a column for each year at the same N level, means followed by different letters are significantly different according to LSD (0.05). MH63: Minghui63, MH63 (*cry1C**) and MH63 (*cry2A**) contain a *Bt* gene of *cry1C** and *cry2A**, respectively.

gene would not always cause yield reductions in different rice lines. However, as a common variation in *Bt* rice, the lower grain filling percentage should be concerned in transgenic breeding.

Although no significant differences were observed in biomass, panicle number per square meter and spikelets per panicle between MH63 (*cry1C**) and MH63 (Table 2), MH63 (*cry1C**) had lower dry matter translocation efficiency and higher reservation of soluble sugar in stem and sheath at mature (Fig. 1 and Table 3), possibly reflecting that MH63 *(cry1C**) had weaker flow compared with MH63. Wang et al. (2012b) found that the incorporation of *cry1C** into MH63 brought a significant reduction in the content of growth-promoting phytohormones in the superior spikelets, and indicated that MH63 (*cry1C**) had differential sink activity compared with MH63. Yield formation depends on the combined effects of source, sink and flow (Wada et al., 1993). It can be generalized that the main agronomic mechanisms of the reduction in grain yield of MH63 (*cry1C**) were the poor flow and lower sink activity.

MH63 (*cry2A**) as compared with MH63 provides additional N for the synthesis of *Bt* protein, and the *cry2A** protein concentration in leaf of *Bt* rice was several times higher than that of *cry1C** (Wang et al., 2012a). Gurr and Rushton (2005) suggested that the insertion of external *Bt* genes into rice might bring added burden to rice. When the *Bt* protein content was higher than 1% of total soluble protein, it might be harmful to rice as reported by Gahakwa et al. (2000). In our study, reductions in SPAD of MH63 (*cry2A**) were significantly higher than those of MH63 at 0 kg N ha^{-1} (Table 4), reflecting that MH63 (*cry2A**) had leaf premature aging compared with MH63. It was reported that 60–90% of the total carbon in rice panicles at maturity was produced by photosynthesis after heading (Mae, 1997). Late leaf senescence can increase photosynthetic activity in rice. However, the rapid leaf senescence can decrease the rice yield if the grains have not completely filled (IRRI, 1996). Nitrogen metabolism is an important factor in determining the leaf senescence rate and then the rice productivity (Yamaya et al., 2002). The variations of *Bt* protein concentrations were usually associated with the alterations in N metabolism as reported in some researches (Wu et al., 2002; Chen et al., 2004, 2005; Llewellyn et al., 2007; Badea et al., 2010; Poongothai et al., 2010). In our study, grain yields and IE_N of MH63 (*cry2A**) were significantly lower than those of MH63 at 0 kg N ha^{-1} (Table 2 and Table 5), but no significant differences were observed in total N uptake (Table 5), indicating that there were alterations in nitrogen utilization and metabolism in MH63 (*cry2A**) compared with MH63 when N supply was inadequate.

Variations in transgenic plants might result from the following sources: (1) somaclonal variation (Larkin and Scowcroft, 1981), (2) insertion mutagensis (Van Lijsebetens et al., 1991), (3) multiple effects on apparently unrelated genes, (4) endogene silencing (Matzke et al., 2000) and (5) effects of the transgenes products on plant growth. Shu et al. (2002) suggested that somaclonal variation was the main genetic mechanism of the agronomic and morphological changes in *Bt* rice. The somaclonal variations in producing transgenic plants were relatively frequent (Dale and McPartlan, 1992; Kaeppler et al., 2000), and somaclonal variations caused by *Agrobacterium*-mediated transformation in rice could be to a large extent eliminated by continuous backcrossing. Our results showed that yield reduction of MH63 (*cry1C**) existed at all nitrogen levels (Table 2). However, yield reduction of MH63 (*cry2A**) was only observed at 0 kg N ha^{-1} (Table 1), which meant that variations in MH63 (*cry2A**) could be eliminated by appropriate N management practice. It is emphasized that the genetic metabolisms of the variations of *Bt*-MH63 need to be investigated by more experiments.

Yield performance of two *Bt* transgenic lines, MH63 (*cry1C**) and MH63 (*cry2A**) were investigated under no pest infestation. We found that MH63 (*cry1C**) had lower grain yield compared with MH63. When no nitrogen fertilizer was applied, MH63 (*cry2A**) showed a reduction in grain yield compared with MH63. Lower

grain filling percentage could be the main reason for the yield reduction of MH63 (*cry1C**), which was associated with lower dry matter translocation efficiency. Leaf premature aging and lower internal N use efficiency (IE_N) were chiefly the agronomic reasons of yield decline of MH63 (*cry2A**) when N supply was inadequate. More endeavors are encouraged to clarify the following issues based on our study: (1) the physiological and genetic mechanism of reduction in grain filling percentage of *Bt* rice and (2) relation between the leaf premature aging and the relatively high *Bt* protein synthesis of *Bt* rice when N supply is inadequate. Furthermore, more *Bt* transgenic rice lines should be studied to evaluate the effects of the insertion of external *Bt* genes on yield formation.

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