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Research Paper

Development of photoperiod- and thermo-sensitive male sterility rice expressing transgene *Bacillus thuringiensis*

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Stem borers and leaffolders are the main pests that cause severe damage in rice (*Oryza sativa* L.) production worldwide. We developed the first photoperiod- and thermo-sensitive male sterility (PTSMS) rice 208S with the *cry1Ab/1Ac Bacillus thuringiensis* (*Bt*) gene, through sexual crossing with Huahui 1 (elite line with the *cry1Ab/1Ac* gene). The novel 208S and its hybrids presented high and stable resistance to stem borers and leaffolders, and the content of Cry1Ab/1Ac protein in chlorophyllous tissues achieved the identical level as donor and showed little accumulation in non-chlorophyllous tissue. No dominant dosage effect in the *Bt* gene was observed in 208S and its derived hybrids. An analysis of fertility transition traits indicated that 208S was completely sterile under long day length/high temperature, but partially fertile under short day length/low temperature. With fine grain quality and favorable combining ability, 208S had no observed negative effects on fertility and agronomic traits from *Bt* (*cry1Ab/1Ac*). Additionally, 208S as a male sterile line showed no fertility decrease caused by *Bt* transgenic process, as it is the case in Huahui 1. Thus, 208S has great application value in two-line hybrid production for insect resistance, and can also be used as a bridge material in rice *Bt* transgenic breeding.

Key Words: *cry1Ab/1Ac*, insect resistance, transgenic breeding, dosage effect, photoperiod- and thermo-sensitive male sterility, two-line hybrid, rice (*Oryza sativa* L.).

Introduction

Stem borers and leaffolders are two major groups of lepidopteran pests which cause severe yield loss in most of the rice producing countries. In China alone, more than 10 million tons of rice is lost every year (Chen *et al.* 2008, Wang 2006). For a long time, farmers had to depend on application of large amounts of poisonous chemical insecticides to fight insects, though insecticides contribute to additional costs and cause contamination of the environment and foods. Consequently, alternative methods to control pests have been developed. *Bacillus thuringiensis* (*Bt*), which produces Bt crystal toxin protein, has been applied as an efficient biological insecticide for several decades, and considerable research on Bt transgenic crops has been carried out. By 2012, biotech crops with *Bt* genes alone accounted for more than 15%, or 26.3 million hectares of the total global biotech crop area of 175.2 million hectares. Most research focused on staple crops, such as Bt cotton, Bt corn, and Bt rice (James 2013). The decrease in production losses by Bt crop plantation is estimated to be US$7.5 billion worldwide, and the environmental benefits are even beyond measure (James 2012). Widely used *Bt* genes in rice were *cry1Ab*, *cry1Ac*, and the hybrid gene *cry1Ab/1Ac*, which exhibited a high level of insect resistance (Alam *et al.* 1999, Cheng *et al.* 1998, Datta *et al.* 1998, Fujimoto *et al.* 1993, Ghareyazie *et al.* 1997, Nayak *et al.* 1997, Tang *et al.* 2006, Tu *et al.* 2000, Wang *et al.* 2002, Wu *et al.* 1997, Wünn *et al.* 1996, Yang *et al.* 2011). These research efforts have played significant roles in the replacement of poisonous chemical insecticides and environmental conservation (Ferré and Van Rie 2002, James 2011). There have been few reports on the risk of evolution of Bt resistance in pests in extensive commercial plantation of Bt crops, though some pest species that evolved resistance to Bt crops have been found under laboratory and field conditions (Luttrell *et al.* 2004, Storer *et al.* 2010, Tabashnik 2008, Van Rensburg 2007). Nevertheless, application of Bt transgenic crops is still one of the most effective measures against lepidopteran pests (James 2013).

China is the main producer of hybrid rice. With the discovery of the photoperiod-sensitive genic male sterility line

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Nongken 58S (Shi 1985, Yuan 1990), there has been great progress in two-line hybrid rice breeding in China. Photoperiod- and thermo-sensitive male sterility (PTSMS) has variable fertility features, including sterility under long day length/high temperature and fertility under short day length/low temperature. Compared with three-line sterile lines in a hybrid rice system, PTSMS can maintain sterile line production without using restore lines. Furthermore, a two-line hybrid rice system by application of PTSMS has many advantages, including a wider range of germplasm resources used as breeding parents, higher yields, and simpler procedures for breeding and hybrid seed production (Zhou *et al.* 2012); a series of super high yield varieties which were bred with PTSMS have been widely cultivated in China, such as Liangyoupei 9, Fengliangyou 1, and Yangliangyou 6 (Si *et al.* 2011). According to a report by the Ministry of Agriculture of China (2012), two-line hybrid rice accounted for more than 20% of the total planting area of hybrid rice. However, rice-producing areas in the south of the country, which mainly plant two-line hybrid rice, had suffered great losses from stem borers and leaffolders in recent years (Xiang *et al.* 2011). Therefore, enhancing resistance of two-line hybrid rice is not only central for food security but also for environmental protection. A study by Tu *et al.* (2000) showed that a transgenic plant, Minghui 63, with the *cry1Ab/1Ac* gene provided high protection against lepidopteran pests without reducing the restorer line's combining ability and its hybrid yield potential. Riaz *et al.* (2006) revealed that basmati rice with *Bt* genes (*cry1Ac* and *cry2A*) could efficiently resist insect harm without decreasing rice quality. Liu *et al.* (2010) improved the elite parental line 9311, which has been comprehensively applied for hybrid rice in China, and the improved line through backcross and marker-assisted selection (MAS) breeding with *Bt* (*cry1Ab/1Ac*) helped to achieve desired resistance to lepidopteran pests with no negative effects on agronomic traits. In 2009, the Chinese government authorized the first safety certificate of a genetically modified rice cultivar, Huahui 1 (Tu *et al.* 2000), which can efficiently control the losses caused by lepidopteran pests. This has promoted further development and application of Bt rice in China.

We recently developed a novel PTSMS line, 208S, which harbored the hybrid *Bt* gene *cry1Ab/1Ac* by sexual crossing using Huahui 1 and Guangzhan 63S. Besides high resistance to stem borers and leaffolders, the line and its hybrids have exhibited high grain quality and outstanding combining ability, and have paved the way for breeding "green super rice" (GSR) (Zhang 2007).

Materials and Methods

Parent materials used for breeding 208S

The line PTSMS 329S, which was obtained through sexual crossing of Guangzhan 63S and 2018S, was used as the *Bt* gene recipient in this study. Huahui 1, which was approved for biosafety in China in 2009 and has shown outstanding performance in insect resistance, was used as the gene donor to develop the elite line 208S. The hybrid progeny of 208S, Zheyou 3, was also selected for assaying insect resistance and evaluation of agronomic performance.

Molecular verification of Bt gene

Plant DNA was prepared following Dellaporta *et al.* (1983). Amplification protocols were adapted from Ye *et al.* (2009). Each amplification was performed in 20 µl reaction volumes containing 10 ng template DNA, 0.4 µM of each primer, 5 mM dNTP, 2 µl 10 × Mg^{2+} free buffer, 4 mM $MgCl_2$, and 1.0 U rTaq DNA polymerase (Takara Bio Inc., Japan). The PCR procedure was as follows: initial denaturation at 95°C for 5 min, followed by 34 cycles at 95°C for 30 s, 57°C for 50 s, and 72°C for 90 s, and final extension step at 72°C for 10 min. The primer set used for *Bt* (*cry1Ab/1Ac*) amplification was as follows: Bt-F (5′-tcgagacgttagcgtgtttg-3′) and Bt-R (5′-aagtaaccgaaatcgctgga-3′). The PCR products were evaluated by 3% agarose gel electrophoresis running at 250 V for 30 min. The details of PCR reaction and the detection system followed the procedures described elsewhere (Liu *et al.* 2010).

A fragment the size of 1.2 Kb, which was associated with plants positive for *Bt* (*cry1Ab/1Ac*), was amplified. The fragment and two flanking sequences were sequenced (Invitrogen Company, USA) to confirm the presence of the *cry1Ab/1Ac* gene. Sequencing the 5′ flanking sequence was performed using (5′-cctcatacgaacctggcact-3′) and (5′-ataataccgcgccacatagc-3′) as forward and reverse primers, respectively, while that for the 3′ flanking sequence was performed using (5′-acggaggtcaatggaatcac-3′) and (5′-gcaaacgaacttaaaacccg-3′) as forward and reverse primers, respectively. The flanking sequences of the donor, Huahui 1, were published in 2009 under the Chinese patent number ZL 2005 1 0062980.9.

Quantification of insecticidal Cry1Ab/1Ac protein

The enzyme-linked immunosorbent assay (ELISA) kit AP003 CRBS (EnviroLogix, Inc., Portland, ME) was used to determine the amount of Cry1Ab/1Ac protein. Approximately 20 mg of fresh samples from the leaf (at tillering, heading, and filling stages), stem (at tillering, heading, and filling stages), root (at heading stage), and endosperm (at filling stage) were picked. The details of enzyme-linking reaction followed the manufacturer's instruction. Optical density values of the diluted samples were measured by a microplate reader (M5-SpectraMax; Molecular Devices, USA) at 450 nm wavelength, and the values were used to compute the Cry1Ab/1Ac protein content.

Insect bioassay

Insect bioassays of 208S and its hybrid were performed at the booting stage of the plants by artificial inoculation using yellow stem borers. The experiments were carried out at Zhenjiang University, Hangzhou. After a 5-day incubation period, the plants were examined for growth status of host and survival rate of the insects following the

procedures of Tu *et al.* (2000). Natural infection tests were also carried out in its hybrids in Changxing, China (2011).

Evaluation of agronomic traits

Agronomic performance of 208S and its hybrids were evaluated by randomized block design tests in three replications. Each plot consisted of 22 rows with 12 plants per row, and a spacing of 17.0 cm between plants and 26.0 cm between rows. In each replicate, 10 individuals at the middle of the block were sampled for evaluation of the heading date, panicle length, plant height, number of grains per panicle, and seed set in bagged self-fertility and unbagged panicles. Seeds were also evaluated for percentage of brown rice, milling rice and head rice, length-width ratio, chalkiness rate, alkali spread value (ASV), gel consistency (GC), amylose content (AC), and protein content to determine the levels of quality traits by the Rice Product Quality Inspection and Supervision Testing Center of the China National Rice Research Institute. Hybrid lines Zheyou 3, Zheyou 5, and Zheyou 7, which were produced using 208S as female parent, were used to test for combining abilities and heterosis traits against control elite Fengliangyou 4. Statistical analyses were carried out using SPSS v. 12.0 (IBM Corp., Armonk, NY, USA) to determine the significant differences between the transgenic lines and control variety.

Results

Breeding of 208S

The insect-resistant Bt donor line Huahui 1 and elite PTSMS 329S were crossed, and the hybrid was selfed to the F5 generation using MAS for the *cry1Ab/1Ac* gene and pedigree breeding for PTSMS traits. One F5 line was transferred into a climatic chamber to investigate the PTSMS features and fertility transition temperature at the China National Rice Research Institute, Hangzhou, in 2009. At the heading stage, the seed set rate (%) of bagged main panicles was evaluated, and plants with <0.01% self-fertilities were identified as male sterile lines. Line 208S was identified as suitable material in breeding of hybrid seeds because it had good agronomic traits and preferable fertility features.

208S is the derivative line of Huahui 1

The results of PCR detection of the *Bt* gene (*cry1Ab/1Ac*) in 208S and its hybrids indicated that the lengths of amplified bands were the same as those of the donor, Huahui 1 (**Supplemental Fig. 1**A). Additionally, the genomic sequence flanking exogenous *Bt* gene was amplified and sequenced to identify the source of the transgenic gene. The length of the 5′ amplified fragment was 2005 bp, of which 779 bp was the flanking sequence (**Supplemental Fig. 1**B); the length of the 3′ amplified fragment was 1482 bp, of which 1177 bp was the flanking sequence (**Supplemental Fig. 1**C). Both the 5′ and 3′ flanking sequences differed by only 3 bp and 4 bp, respectively, from those of the donor Muahui 1. More than 99.6% of these flanking sequences showed complete similarity to Huahui 1 (unpublished data). Neither insertion nor deletion caused by integration of *cry1Ab/1Ac* was detected in both 208S and Huahui 1.

208S is highly resistant to stem borers and leaffolders

The novel line 208S with the *Bt* gene showed excellent resistance against lepidopteran insects (**Fig. 1**). In artificial infestation tests, 208S and its hybrid suffered only 0.2% and 1.5% damage to dead heart rate, respectively, compared to 50.3% and 31.5%, respectively, in the negative controls (**Fig. 2**A). The results of natural infestation also showed that 208S and its hybrid achieved an identical level of resistance as that of donor parent Huahui 1, and exhibited far fewer folded leaves than the non-transgenic controls, Minghui 63 and Shanyou 63 (**Fig. 2**B). These results confirmed that 208S originating from cross-breeding (of Huahui 1 and Guangzhan 63S) and its hybrid offspring showed high resistance to stem borers and leaffolders.

208S has stable protein content of Cry1Ab/1Ac

Quantitative analysis of Cry1Ab/1Ac protein showed that the Bt protein contents in 208S and its hybrid Zheyou 3 decreased with plant growth. This was particularly obvious in leaves where Bt protein content fell from 13.6 μg g^{-1} at tillering stage to 6.1 μg g^{-1} at grain filling stage, respectively, in 208S. A similar trend was seen in its hybrid progeny. Bt protein contents varied considerably between different tissues (**Fig. 3**); concentration of Cry1Ab/1Ac protein was obviously higher in the leaf and stem than in the endosperm and root. The Cry1Ab/1Ac protein contents in 208S and its hybrid were identical to the levels in donor parent Huahui 1, and there was no significant difference between the two at each growth stage of different tissues (**Fig. 3**). These results indicated that dosage effect of the gene had little effect on the concentration of Bt protein.

208S has low fertility transition temperature

The results of analysis of PTSMS traits in an artificial climatic chamber showed that the percentage of pollen fertility and self-seed setting rate were both 0.0% at high

Fig. 1. The resistance of control Guangzhan 63S (left) and 208S (right) against natural infection of leaffolders under field conditions without chemical control (Changxing, China, 2011).

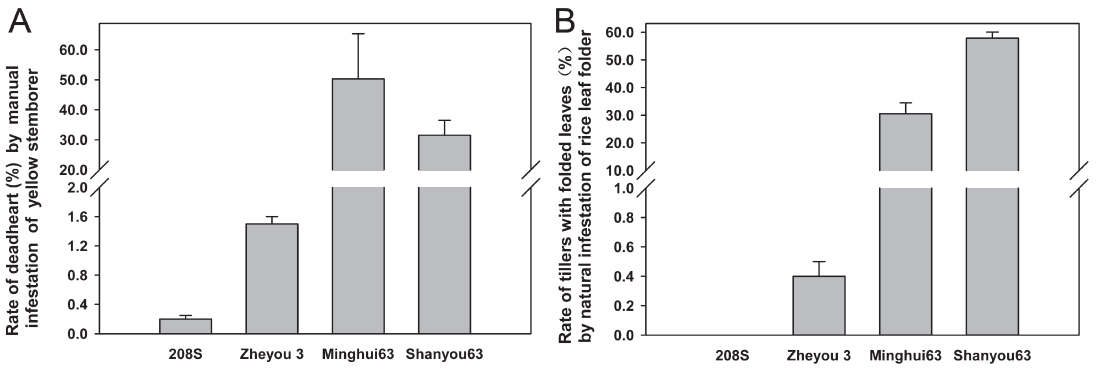

Fig. 2. Resistance of 208S and its hybrid against natural and manual infestation of stem borers and leaffolders under field conditions without chemical control. Minghui 63 and Shanyou 63 are two negative controls (Chanxing, China, 2011).

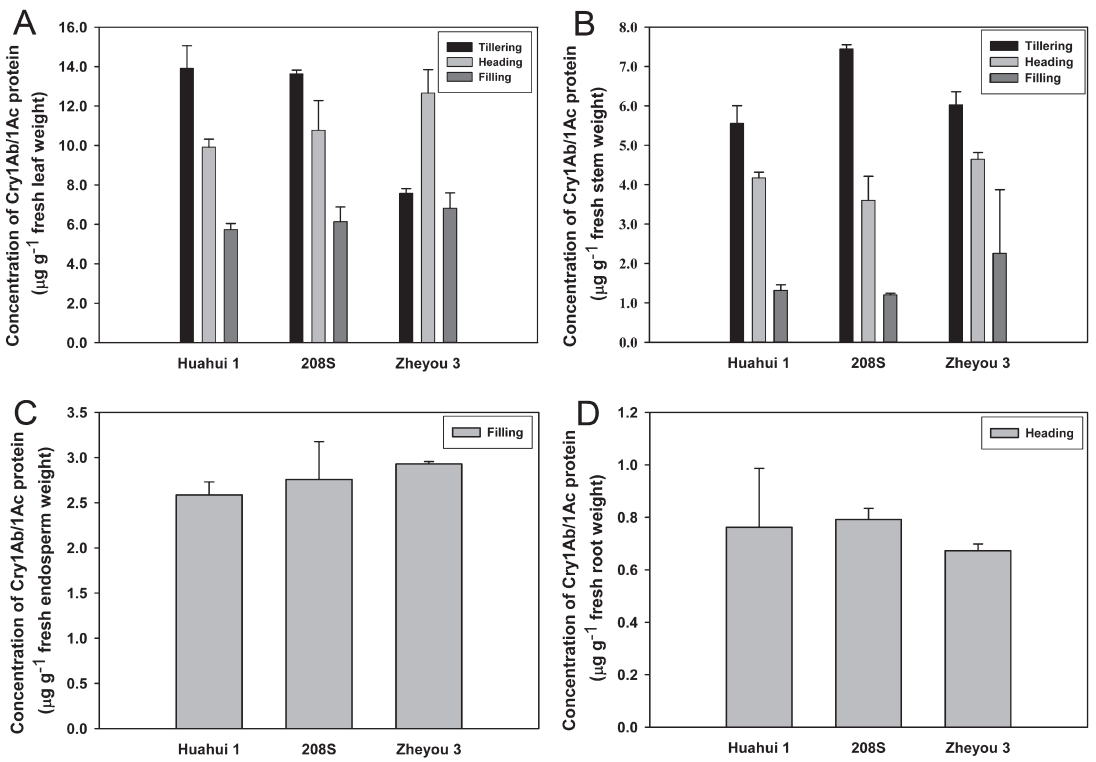

Fig. 3. Cry1Ab/1Ac concentration detected in donor line Huahui 1, 208S and its hybrid Zheyou 3 at tillering, heading and filling stages. (A–D) Cry1Ab/1Ac concentration detected in the leaf, stem, root and endosperm, respectively. Error bars indicate ± SD (n = 3). The x-axis represents corresponding rice lines containing the *Bt* gene.

temperatures (≥28°C) in a wide photoperiod range (11.5–14.5 h). In addition, 208S also presented complete sterility at 24°C and under 13.5 h day length growth condition, while the control, Peiai64S, had partial fertility (0.4% self-seed setting rate) at 24°C and in a 13.5 h photoperiod (**Table 1**). The novel line 208S was partially fertile under 13.5 h and 14.5 h long day length growth conditions when the temperature was set at 23°C and the self-seed setting rates were 8.7% and 8.9%, respectively. Furthermore, 208S had a 7.3% self-seed setting rate at 24°C under short day length (12.5 h) condition. It showed partial fertility under long day length/low temperature growth conditions (13.5 h/23°C and 14.5 h/23°C), but sterility at 24°C or higher temperatures under long day length growth conditions. These results thereby indicated that 23°C was the approximate threshold temperature of fertility transition. These fertility traits were comparable with or even better than those in the elite PTSMS line Peiai64S, since the latter had 4.9% fertile pollens under 12.5 h short day length growth condition at 28°C, indicating that our new breeding line 208S was entirely in accordance with the application standard of PTSMS.

208S has excellent grain quality and high combination ability

The novel line 208S has favorable agronomic traits, which

Table 1. Fertility transition temperature and pollen fertility of 208S and control PTSMS line Peiai64S in artificial climatic chamber (Hangzhou, China, 2010)

Line	Photoperiod (h)	Temperature (°C)	Pollen fertility (%)	Self-seed setting rate (%)
208S	11.5	28.0	0.0	0.0
		24.0	NA	0.0*
	12.5	28.0	0.0*	0.0
		24.0	6.1*	7.3*
	13.5	28.0	NA	0.0
		24.0	0.0	0.0
		23.0	9.4	8.7*
	14.5	28.0	NA	0.0
		24.0	2.0*	0.7
		23.0	10.5**	8.9**
Peiai64S (CK)	11.5	28.0	0.0	0.0
		24.0	10.0	2.4
	12.5	28.0	4.9	0.0
		24.0	0.0	0.8
	13.5	28.0	0.0	0.0
		24.0	0.2	0.4
		23.0	8.3	3.5
	14.5	28.0	0.0	0.0
		24.0	0.0	0.0
		23.0	0.0	0.0

NA, not available because of pollen degeneration.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.

are suitable for PTSMS application. The duration from sowing to heading stages was between 100 and 112 days in different sowing dates, and number of grains per panicle and tillers per plant were 181.0 and 10.0, respectively (**Supplemental Table 1**). In addition, plant growth vigor showed no obvious differences in diverse sowing dates. These results favored producing the hybrid seed in breeding applications.

The self-seed production of 208S in Hainan in winter (short day length/low temperature) reached approximately 3.5 ton ha^{-1}, which was much higher than that of the control elite PTSMS line. In addition, due to the high (>30%) stigma exsertion rate, it was easily cross pollinated to access superior hybrid seed production compared to control in its sterile environment (unpublished data).

Regarding yield performance, all 208S-derived hybrids, including Zheyou 3, Zheyou 5, and Zheyou 7, reached about 8.0 ton ha^{-1}, which was comparable with that of the control line, Fengliangyou 4, an elite two-line hybrid line widely grown in the south part of China (**Table 2**). These results therefore showed the good combining ability of 208S, the male sterile line used in this study. Yield trait analysis revealed that all three 208S-derived hybrids had a significantly lower 1000-grain weight but a remarkably higher number of tillers than the control (**Table 2**). In addition, Zheyou 3 had the tallest plant height while Zheyou 7 had the shortest growth period among the four hybrids (**Table 2**).

The results of quality analysis showed that the quality indexes of 208S and its hybrid Zheyou 3, such as chalkiness rate (2.0%/10%), alkali spread value (7.0/7.0), gel consistency (86.0 mm/88.0 mm), length-width ratio (3.0/3.0), reached the national first grade of quality rice (China national standard) (**Supplemental Table 2**), and 13.8%/11.1% of protein content, were much higher than those of the common lines. In addition, the processing quality of 208S and Zheyou 3, including percentage of brown rice, milling rice and head rice, which reached 80.7%/81%, 71.2%/71.4%, and 65.3%/62.9%, respectively, was also much higher than that of the national standard. The amylose content of 12.5%/14.8% was lower than that of the first grade quality standard, but 208S and its hybrid Zheyou 3 were soft and thus more palatable.

Discussion

With the discovery of Nongken 58S, PTSMS has been broadly applied in rice production. PTSMS hybrids account for approximately 20% of total planting area of hybrid rice in China (Si *et al.* 2011). Through multiple crosses and MAS breeding using Huahui 1 as donor parent of the hybrid *Bt* gene *cry1Ab/1Ac*, the novel line 208S displayed excellent resistance to main field rice pests, namely, stem borers and leaffolders. The 99.6% sequence similarity of the transferred gene clone confirmed that the *Bt* (*cry1Ab/1Ac*) gene derived from Huahui 1 with differences of 3 and 4 bp were indicative of diverse cultivars. The results of insect resistance showed that both homozygous 208S and its heterozygous hybrid demonstrated high insect resistance, and there was no significant difference between homozygotes and heterozygotes. Another desirable feature of 208S was the complete dominance of Bt toxin in *cry1Ab/1Ac* transgenic plants. The Bt protein assays showed that 208S and its derived hybrid had a constant expression of Cry1Ab/1Ac protein and no dosage effects on constitutive expression of the *Bt* gene. In addition, toxin proteins in the leaf and stem were at their highest concentrations throughout the growth period, which was good for defense against pests. During plant

Table 2. Agronomic traits of 208S-derived hybrids with chemical control (Changxing, China, 2014)

Line	Heading date (days)	Plant height (cm)	No. of grains per panicle	No. of tillers per plant	1000-grain weight (g)	Spikelet fertility (%)	Expected yield (ton/ha)	Observed yield (ton/ha)
Fengliangyou 4 (CK)	97.0	114.0	187.1	8.4	29.8	77.2	9.6	8.0
Zheyou 3	97.0	125.6**	199.8	12.0*	27.7**	80.8	10.4	8.0
Zheyou 5	97.0	124.1**	181.8	11.8*	27.6**	76.8	10.1	8.0
Zheyou 7	93.0**	119.7	197.0	12.2**	24.5**	75.1	9.2	7.9

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

growth and development, the content of toxin protein decreased by approximately 50%, thus favoring the energy translocation from a source to sink, which in turn improved the agronomic performance. The presence of toxin protein in non-chlorophyllous tissue was also measured separately in the root (at heading stage) and endosperm (at filling stage) in 208S and its hybrid. The concentration of Cry1Ab/1Ac protein in the endosperm approximated that of the control Huahui 1, which enabled receiving a food safety evaluation certificate. A previous study showed that the concentration of Bt protein in the root of transgenic plants was much lower than that in its residue in the soil after Bt chemical pesticide application (Zaidi *et al.* 2012). Our results of Bt protein content in the foot also conformed with those of the previous study. When using a constitutive promoter, line 208S had an extremely low level of Bt protein expressed in the root, thus decreasing negative effects on the environment and soil microorganisms. The results presented here indicated that heterozygous hybrids effectively resisted pest infestation without adverse influence on the human body and environment, and thus 208S can be used directly for hybrid application for insect resistance.

The features and threshold temperature of fertility transition/conversion are significant in PTSMS and two-line hybrid rice. The results of fertility transition in an artificial climate chamber revealed that 208S was completely sterile under long day length/high temperature, and partially fertile under short day length/low temperature. Additionally, field investigation in a different field environment also confirmed the results in the artificial climate chamber. The self-seed setting rate (%) in Hangzhou in the summer months (May to October) was nearly zero, while the self-seed setting rate (%) in Sanya in winter (December to April) was approximately 80% (unpublished data). Additionally, a temperature of 23°C as a critical sterility/fertility transition point was a better threshold in two-line hybrid rice seed production compared to that in the elite PTSMS Peiai64S.

Gene insertion site of target genes by transgenic technology influences plant character and hence transgenic technology application. Unfavorable insertion sites of exogenous gene fragments in transgenic plants usually contribute to decreased fertility caused by pollen activity decrease. In this study, the insertion site in the transgenic Huahui 1 located in the promoter region of an endogenous gene and the gene regulation variation caused by exogenous DNA insertion tended to decrease pollen activity by 10% (unpublished data). This in turn was beneficial to the fertility features of the novel male sterile line without any adverse effect on agricultural performance. The results also suggested that using a male sterile line as a transgenic receptor may have great value in rice hybrid application because it avoids a fertility problem in transgenic technology.

Other desirable features of the novel male sterile line were the preferable combining ability and agronomic traits that favored self-propagation and hybrid production. Stigma-exsertion is such an important agronomic trait for male sterile lines, which increases the chance to catch more pollen from the paternal parent and overcomes the barrier of flowering asynchronization between maternal and paternal parents, and as a consequence, decreases the cost of hybrid seed production. It is because of these reasons that the stigma exertion trait has got the constant attention of rice researchers (Li *et al.* 2001, Lou *et al.* 2014, Miyata *et al.* 2007, Uga *et al.* 2003, Virmani and Athwal 1974, Yan *et al.* 2009). In our transgenic breeding line 208S, the stigma-exsertion rate was higher than 30%, which was very beneficial for outcrossing and hybrid seed production. Its hybrids, including Zheyou 3, Zheyou 5, and Zheyou 7, also achieved outstanding performance in official transgenic plant production tests conducted by the Ministry of Agriculture of China. While the presently used PTSMS cultivars face quality problems, the novel 208S and its hybrid had significantly improved quality traits, especially chalkiness rate, ASV, and gel consistency. Additionally, its hybrid outperformed commonly used cultivars in competitive quality trait evaluation. These results suggested that the insertion of *cry1Ab/1Ac* in 208S had no undesirable impact, especially on cooking and eating quality. Therefore, the new male sterile line (*cry1Ab/1Ac* receptor variety) has a great future potential in research of transgenic rice breeding.

Sterility assays and field observation showed that the exogenetic *cry1Ab/1Ac* transferred by Huahui 1 did not have a negative effect on fertility traits. To date, some traditional cultivars have been bred by transgenic breeding (Liu *et al.* 2010, Riaz *et al.* 2006, Tu *et al.* 2000). Here, we developed the first PTSMS line harboring a *cry1Ab/1Ac* hybrid gene, line 208S, which has desirable insect resistance, outstanding quality, preferable combining ability, and compatible fertility transition features. Transgenic Bt breeding, which can reduce pesticide application, is in accordance with the strategy of green super rice advanced by Zhang (2007). We can conclude that 208S has great potential in hybrid-producing applications. Other studies have proved that pyramiding *Bt* genes could efficiently increase durable insect resistance (Riaz *et al.* 2006, Yang *et al.* 2011). Therefore, we will continue our research in this field, and expect to develop new PTSMS lines with durable insect resistance in the future.

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