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Effects of cytoplasm on the fertility of thermo-sensitive genetic male sterile (TGMS) lines of rice

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Abstract

To study the effect of cytoplasm on the fertility of thermo-sensitive genetic male sterile (TGMS) lines, eight rice genotypes with different kind of donor cytoplasm were used. The genotypes Guanglu'ai-4, R402, 93-11, Nipponbare, Brazil upland rice, Lemont, V20A, Chaling wild rice (*Oryza rufipogon* griff) were used as female parents in crosses with 2 TGMS lines, Zhu-1S and ZhunS (as males). These nuclear genomes were substituted into the eight cytoplasms via 12 backcrosses using the male as recurrent parent. Sixteen combinations of $BC_{12}F_2$ and their parents were sown from April to October in 2011. The pollen fertility changing patterns of the mononuclear-heteroplasmic TGMS lines were studied under natural condition and low temperature treatments (22.0 ºC, 23.5 ºC) at the formation stage of pollen mother cells (PMCs). The results showed that the effect of cytoplasm on the pollen fertility of TGMS lines was significant. In the two nuclear backgrounds (Zhu-1S and ZhunS), the substitution lines with the cytoplasms of R402 and V20A had low critical sterility temperature points (CSTP), highly stable sterilities, and most safe hybrid seed production. In the nuclear background of Zhu-1S, the Zhu-1-6S genotype had the least stable pollen sterility, and its CSTP was above 23.5 ºC. The Zhu-1-2S and Zhu-1-3S genotypes showed more stable pollen sterilities than Zhu-1S, but their fecundities were lower than that of Zhu-1S. In the nuclear background of ZhunS, the Zhun-7S genotypes exhibited a higher CSTP and the least stable pollen sterility. These results showed that the cytoplasm of R402 was the most suitable cytoplasm for breeding TGMS lines. This study suggests that the role of cytoplasm should be considered in breeding of the TGMS lines in the future programs.

Keywords: rice (*Oryza sativa* L.); cytoplasm; nucleu; thermo-sensitive genic male sterile line; pollen fertility.
Abbreviations: TGMS- Thermo-sensitive Genetic Male Sterility, CSTP- Critical Sterility Temperature Point, CMS- Cytoplasmic Male Sterile.

Introduction

The rice thermo-sensitive genetic male sterile (TGMS) lines are valuable germplasm resources for developing two-line hybrid rice. A number of TGMS genotypes have been discovered and some of these materials have been applied in two-line hybrid rice breeding (Yuan, 1998). Zhu-1S is an *indica* TGMS rice line with *indica*-type cytoplasm (Liu, 2008). The sterility of Zhu-1S continues to be the most stable among the applied TGMS lines. Breeders have developed 26 hybrid combinations for large-scale production using Zhu-1S. The CSTP of Zhu-1S is below 22.0 ºC (Li, 2003). ZhunS is an *indica* TGMS rice line with *japonica*-type cytoplasm (Liu, 2008). ZhunS has a CSTP of approximately 24.0 ºC and not very stable sterility. During hybrid seed production at high-temperature sterility-inducing locations, a sudden drop in temperature can be disastrous to this plant because of the reversion to fertility phase, resulting in selfed seeds. The CSTP of the TGMS line must be characterized to enhance the safety of hybrid seed production. A 6-day, low-temperature (23.5 ºC) treatment should be applied (from the formation stage of the pollen mother cell to the meiotic division of the pollen mother cell) (Chen, 1993). The ratio of the sterile pollen of the TGMS must be over 99.9% in the ecological region in the middle and lower reaches of the Yangtze River. The safety of seed production of the two-line hybrid rice has been enhanced significantly by the aforementioned technological standard. However, breeders have tried to obtain TGMS lines with low CSTP and insensitivity to the low temperatures.

In a three-line hybrid rice system, a cytoplasmic male sterility (CMS) source, a maintainer and a restorer get involved, which is frequently used in the production of commercial rice hybrids in many hybrid rice-growing countries (Virmani et al., 1997). A CMS line is always multiplied by crossing it with its maintainer line. A restorer line, with dominant fertility restorer genes, restores fertility in the derived hybrid when crossed with a CMS line. A three-line hybrid rice system results from the interaction between the specific sterility-inducing cytoplasm and the nuclear genes. The sterile cytoplasm and recessive nuclear genes are required to obtain male sterility expression. A combination of sterile cytoplasm and dominant nuclear genes results in fertile plants (Yuan, 1986). Several previous reports have indicated that different nuclei and different cytoplasms result in varied interactive effects. The selection of the special nucleus that can cause the ratio of the sterile pollen of a CMS line to exceed 99.9% is the key to breeding a CMS line, whereas the selection of the special nucleus can cause the derived hybrid to restore pollen fertility. This is the

Table 1. The materials of cytoplasmic donor.

Cultivar	Origin of cytoplasm	Cultivar	Origin of cytoplasm
Guanglu'ai-4	Guangdong Province in China	R402	The Philippines
V20A	Hainan Province in China	9311	Jiangsu Province in China
Nipponbare	Japan	Brazil upland rice	South America
Lemont	North America	Chaling wild rice	Hunan Province in China

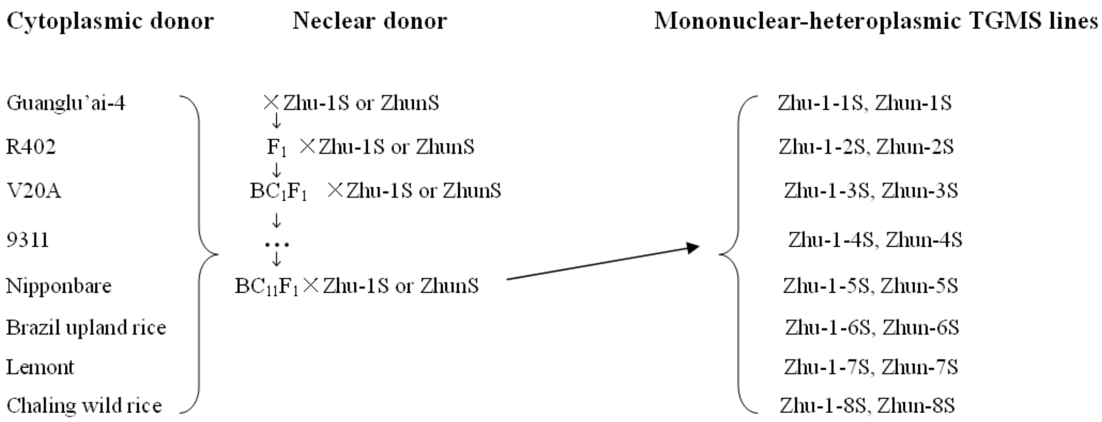

Fig 1. The preparation of the mononuclear-heteroplasmic TGMS lines.

Table 2. Thermo-sensitive time range of fertility alternation and average temperature under the treatment of the condition of natural air temperature in Changsha.

Thermo-sensitive time range of fertility alternation (m/d)	Average temperature (℃)		
	T_{avg}	T_{min}	T_{max}
6/16-8/5	28.6	25.7	35.2
8/6-8/8	24.2	23.2	27.4
8/9-8/22	30.7	26.6	35.5
8/23-8/25	23.9	21.6	27.3
8/26-9/7	28.1	24.7	33.2
9/8-9/11	22.5	21.0	25.3
9/12-9/17	29.3	24.8	35.3

key to breeding a restorer line. The difference between the two groups of mononuclear-heteroplasmic hybridized combinations occurs when the CMS and maintainer lines cross with the same restorer line, including agronomic traits (Chandraratna, 1960; Young, 1990; Shi, 1996), quality (Somrith, 1979; Chang, 1974; Shi, 1995; Hariprasanna, 2006), and resistance (Liu, 2003; Yang, 1989; Ratho, 1992). Previous studies have indicated that the male sterile cytoplasm has negative effects on the heterosis in rice (Zhu, 1979; Tan, 2003). Two-line hybrid rice system is more advantageous than three-line hybrid rice system because of the absence of any cytoplasmic restriction on breeding a TGMS line and hybrid seed production. However, information on the cytoplasmic effects of TGMS lines on pollen fertility is not available. We analyzed and established the *indica-japonica* character and molecular fingerprint of the cytoplasms of the experimental materials using nine cpDNA sequences, including ORF100, ORF29-*TrnC*^{GCA}, *TrnT*^{UGU}-*TrnL*^{UAA}, *rps16*, among others, and 11 mtDNA fragments (Liu, 2008). The cytoplasms include 4 *indica*-type (Guanglu'ai-4, R402, 93-11, and Zhu-1S), 2 *japonica*-type (Niponbare, ZhunS), 1 upland rice (Brazil upland rice), 1 *javanica*-type (Lemont), 1 wild abortive sterile line (V20A), and 1 common wild rice (Chaling wild rice) (Liu, 2008). Previously, we had characterized the nuclear molecular fingerprints of Zhu-1S and ZhunS using simple sequence repeats, inter-simple sequence repeat, and target region amplification polymorphism molecular markers (Liu, 2008). The mononuclear-heteroplasmic TGMS lines were confirmed to have cytoplasms and genotypes identical with their respective donors by the established molecular fingerprint of the cytoplasm and nucleus. Meanwhile, no segregation of characters was found in BC_8F_1-$BC_{12}F_1$ generations of the same substitution line in the field experiment. Based on these findings, the effect of different cytoplasms on the pollen fertility of the TGMS lines was explored under different low-temperature conditions to further comprehend the changing fertility patterns of the TGMS lines and provide a theoretical basis for breeding the excellent TGMS lines.

Results

Comparison of the changing fertility patterns of the mononuclear-heteroplasmic TGMS lines of rice under natural conditions

Zhu-1S has the most stable sterility in the applied TGMS lines in China (Li, 2008; Li, 2003). In the current study, Zhu-1S showed no stages of fertile pollen from June 28 to September 30 (Table 3), and Zhu-1-2S, Zhu-1-3S, Zhu-1-4S, Zhu-1-5S and Zhu-1-7S exhibited stable sterility during the same period. However, fertile pollen and several selfed seeds appeared in Zhu-1-6S during heading time (September 5 to 7 and 21 to 24). According to the natural temperature record (Table 2), the diurnal average temperature from August 23 to 25 was 23.9 ºC, and the diurnal average temperature from September 8 to 11 was 22.5 ºC. So, the low temperature of 22.5-23.9 ºC could induce pollen fertility in Zhu-1-6S at the sensitive phase of fertility alteration. The diurnal average temperature of 22.5 ºC from September 8 to 11 induced Zhu-1-1S and Zhu-1-8S pollen fertility and several selfed seeds. These results indicate the highly significant effect of different genetic cytoplasms on the fertility of TGMS lines. The cytoplasm of Brazil upland rice causes the fertility of TGMS lines to be more sensitive to transient low temperature during the sensitive phase of fertility alteration, weakening the stability of TGMS line sterility and reducing the safety of hybrid seed production.

The sterile gene of ZhunS is derived from the first *indica* TGMS line, Annong S-1 (Wu, 2004). The safety of hybrid seed production using ZhunS as the female parent is reduced because the CSTP of ZhunS is higher than that of Zhu-1S. ZhunS showed two stages of fertile pollen from June 28 to September 30 (Table 4). In the nuclear background of ZhunS, Zhun-2S and Zhun-3S had the most stable sterility and exhibited no stages of fertile pollen from June 28 to September 30. Zhun-5S, Zhun-6S and Zhun-8S showed one stage of fertile pollen from June 28 to September 30, exhibiting a more stable sterility than ZhunS. Zhun-7S showed three stages of fertile pollen from June 28 to September 30. So, the 3-day low temperature of 24.2 ºC could induce pollen fertility in this sterile line at the sensitive phase of fertility alteration.

Comparison of the CSTPs of the changing patterns of mononuclear-heteroplasmic TGMS lines of rice

A TGMS line that remains sterile under the 6-day low temperature treatment (23.5 ºC) can be applied in the ecological region in the middle to lower reaches of the Yangtze River (including the province of Hunan and Hubei, among others). The TGMS with a CSTP below 23.5 ºC has a safe time range and region of hybrid seed production in the ecological region in the middle to lower reaches of the Yangtze River. This condition is an authoritative technology index provided by the authors' institution (DB43/ T405-2008). In the present study, Zhu-1S exhibited stable sterility under the 6- and 10-day conditions at 23.5 ºC (Table 5). Zhu-1-6S was fertile in the nuclear background of Zhu-1S. However, all substitution lines with other cytoplasms had stable sterilities. Their CSTPs were lower than 23.5 ºC and suitable for production in the ecological region in the middle and lower reaches of the Yangtze River. ZhunS was fertile at 23.5 ºC under both 6- and 10-day treatment periods (Table 6). In the nuclear background of ZhunS, only the CSTPs of Zhun-2S and Zhun-3S were lower than 23.5 ºC. These two sterile lines can be applied safely in the ecological region in the middle and lower reaches of the Yangtze River. Zhun-7S had the highest fertile pollen ratio, highest filled-grain ratio, and highest CSTP, but with the least stable sterility.

Cytoplasmic effects on the fecundities of the TGMS lines of rice

Irrigation with low-temperature, 21.0 ºC to 22.0 ºC, water is a basic technique in large-scale multiplication of TGMS lines during the sensitive phase of fertility alteration (Li 2008). In the current study, 6- and 10-day treatments of 22.0 ºC water during the sensitive phase of fertility alteration were applied to study the effect of the cytoplasm on the fecundities of the TGMS lines. Zhu-1S remained sterile under the 6-day treatment at 22.0 ºC (Table 5), which indicated that Zhu-1S has a low CSTP and stable sterility. Under the 10-day treatment at 22.0 ºC, the filled-grain ratio of Zhu-1S was only 4.31%, which indicated that Zhu-1S would have a higher filled-grain ratio if the irrigation water had a temperature below 22.0 ºC during the sensitive phase of fertility alteration. The filled-grain ratios of Zhu-1-1S, Zhu-1-6S and Zhu-1-8S were higher than those of Zhu-1S under the 10-day treatment at 22.0 ºC, whereas under the 6-day treatment at 22.0 ºC, pollen fertility was observed. These results indicate that the three TGMS lines have higher CSTPs, better fecundities, and less stable sterilities, compared with Zhu-1S. However, Zhu-1-2S and Zhu-1-3S had more stable sterilities and worse fecundities compared with those of Zhu-1S.

ZhunS demonstrated a higher pollen fertility and filled-grain ratio under the 10-day treatment at 22.0 ºC. The pollen fertilities and filled-grain ratios of Zhun-4S and Zhun-7S were both higher than those of ZhunS (Table 6). The pollen fertilities and the filled-grain ratios of Zhun-2S, Zhun-3S, Zhun-5S and Zhun-8S were significantly lower than those with ZhunS. Zhun-3S had the lowest filled-grain ratio and lowest fecundity.

Discussion

The cytoplasmic effects on the agronomic traits of rice, especially heterosis in rice, have been paid increasing attention by rice breeders. Cytoplasm influences the different traits of rice and produce different genetic effects (Zhu, 1979; Cai, 1997; Chen, 1992; Shen, 1997; Tan, 2003; Zhan, 2003; Tao, 2004, 2011). The cytoplasmic effect on the fertility of the TGMS lines of rice deserves further study. The current study focuses on the changing fertility patterns of mononuclear-heteroplasmic TGMS lines of rice derived from two TGMS lines. The results showed a significant cytoplasmic effect on the fertility of TGMS lines of rice, but the same pattern of effect was not observed when different cytoplasms were in different nuclear backgrounds. In the nuclear background of Zhu-1S, the least stable pollen sterility was observed in Zhu-1-6S, with a CSTP above 23.5 ºC. Hence, the cytoplasm of Brazil upland rice is unsuitable in the ecological region in the middle and lower reaches of the Yangtze River. Zhu-1-1S and Zhu-1-8S had less stable pollen sterilities than Zhu-1S, but their fecundities were higher than that of Zhu-1S. Zhu-1-2S and Zhu-1-3S had more stable pollen sterilities than Zhu-1S, but their fecundities were lower than that of Zhu-1S. In the nuclear background of ZhunS, Zhun-7S had a higher CSTP and the least stable pollen sterility. Zhun-2S and Zhun-3S had the most stable pollen sterility and were suitable for application in the ecological region in the middle to lower reaches of the Yangtze River.

In general, the stability of sterility of the substitution lines in Zhu-1S nuclear background (excluding Zhu-1-6S) were better than those of the substitution lines in the nuclear background of ZhunS. This condition showed that the stabilities of fertility of the TGMS lines were dominated by the nuclear sterile gene, but the cytoplasmic effect on the fertilities of the TGMS lines was also significant.

Table 3. Fertility of substitution lines of Zhu-1S in the condition of natural air temperature in Changsha.

Substitution lines of Zhu-1S	Pollen fertility (%)							Spikelet fertility (%)						
	Time range of heading (m/d)							Time range of heading (m/d)						
	6/28-8/18	8/19-8/21	8/22-9/4	9/5-9/7	9/8-9/20	9/21-9/24	9/25-9/30	6/28-8/18	8/19-8/21	8/22-9/4	9/5-9/7	9/8-9/20	9/21-9/24	9/25-9/30
Zhu-1S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zhu-1-1S	0.00	0.00	0.00	0.00	0.00	5.26	0.00	0.00	0.00	0.00	0.00	0.00	1.02	0.00
Zhu-1-2S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zhu-1-3S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zhu-1-4S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zhu-1-5S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zhu-1-6S	0.00	0.00	0.00	1.45	0.00	6.02	0.00	0.00	0.00	0.00	0.00	0.00	1.61	0.00
Zhu-1-7S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zhu-1-8S	0.00	0.00	0.00	0.00	0.00	5.56	0.00	0.00	0.00	0.00	0.00	0.00	1.37	0.00

Table 4. Fertility of substitution lines of ZhunS in the condition of natural air temperature in Changsha.

Substitution lines of ZhunS	Pollen fertility (%)							Spikelet fertility (%)						
	Time range of heading (m/d)							Time range of heading (m/d)						
	6/28-8/18	8/19-8/21	8/22-9/4	9/5-9/7	9/8-9/20	9/21-9/24	9/25-9/30	6/28-8/18	8/19-8/21	8/22-9/4	9/5-9/7	9/8-9/20	9/21-9/24	9/25-9/30
ZhunS	0.00	0.00	0.00	2.12	0.00	8.56	0.00	0.00	0.00	0.00	0.00	0.00	1.83	0.00
Zhun-1S	0.00	0.00	0.00	0.00	0.00	5.36	0.00	0.00	0.00	0.00	0.00	0.00	1.22	0.00
Zhun-2S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zhun-3S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zhun-4S	0.00	0.00	0.00	2.36	0.00	5.32	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00
Zhun-5S	0.00	0.00	0.00	0.00	0.00	6.64	0.00	0.00	0.00	0.00	0.00	0.00	1.01	0.00
Zhun-6S	0.00	0.00	0.00	0.00	0.00	3.54	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00
Zhun-7S	0.00	10.23	0.00	20.32	0.00	38.57	0.00	0.00	2.83	0.00	8.63	0.00	18.34	0.00
Zhun-8S	0.00	0.00	0.00	0.00	0.00	5.31	0.00	0.00	0.00	0.00	0.00	0.00	1.32	0.00

Table 5. The effect of 6 days or 10 days of low temperature on the fertility of substitution lines of Zhu-1S during the sensitive fertility period.

Substitution lines of Zhu-1S	22.0℃/6 d		22.0℃/10 d		23.5℃/6 d		23.5℃/10 d	
	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)	Spikelet fertility (%)
Zhu-1S	0.00c	0.00c	15.91d	4.31d	0.00b	0.00b	0.00b	0.00b
Zhu-1-1S	13.29a	4.67a	54.06a	34.56a	0.00b	0.00b	0.00b	0.00b
Zhu-1-2S	0.00c	0.00c	11.68e	2.93e	0.00b	0.00b	0.00b	0.00b
Zhu-1-3S	0.00c	0.00c	2.05f	0.00f	0.00b	0.00b	0.00b	0.00b
Zhu-1-4S	0.00c	0.00c	28.51c	14.15b	0.00b	0.00b	0.00b	0.00b
Zhu-1-5S	0.00c	0.00c	16.65d	4.84d	0.00b	0.00b	0.00b	0.00b
Zhu-1-6S	7.64b	1.92b	28.02c	11.66c	3.95a	0.72a	10.51a	3.15a
Zhu-1-7S	0.00c	0.00c	26.22c	12.34c	0.00b	0.00b	0.00b	0.00b
Zhu-1-8S	11.95a	4.70a	36.57b	14.72b	0.00b	0.00b	0.00b	0.00b

Values followed by the same letter are not significantly different. Lower-case letters indicate significant differences at P = 0.05, up-case at 0.01.

Table 6. The effect of 6 days or 10 days of low temperature on the fertility of substitution lines of ZhunS during the sensitive fertility period.

Substitution lines of ZhunS	22.0℃/6 d		22.0℃/10 d		23.5℃/6 d		23.5℃/10 d	
	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)	Spikelet fertility (%)	Pollen fertility (%)	Spikelet fertility (%)
ZhunS	25.98b	10.44b	76.57b	61.18c	10.22b	2.92b	65.09b	44.55c
Zhun-1S	18.19d	6.39d	71.45c	55.83d	6.46d	1.92c	38.12c	15.94d
Zhun-2S	3.14f	0.00f	70.43c	50.91e	0.00f	0.00d	36.95c	14.86d
Zhun-3S	0.00g	0.00f	18.53e	6.79h	0.00f	0.00d	0.00g	0.00h
Zhun-4S	23.80c	10.33b	89.69a	81.43a	11.56b	3.24b	63.25b	54.38b
Zhun-5S	11.44e	3.19e	22.35e	9.73g	5.03c	1.08c	18.96e	8.57f
Zhun-6S	13.96e	3.63e	61.00d	50.11e	5.37d	1.21c	15.75f	5.21g
Zhun-7S	64.55a	49.82a	87.48a	72.43b	55.21a	34.88a	75.54a	61.17a
Zhun-8S	22.13c	8.31c	69.67c	31.72f	6.22d	1.58c	24.89d	10.37e

Values followed by the same letter are not significantly different. Lower-case letters indicate significant differences at P = 0.05, up-case at 0.01

In the two nuclear backgrounds, Zhu-1-2S, Zhu-1-3S, Zhun-2S and Zhun-3S showed low CSTPs, highly stable sterilities, and most safe hybrid seed production. Hence, the cytoplasms of R402 and V20A are the most suitable cytoplasm for breeding TGMS lines. However, the substitution line with the cytoplasm of V20A has a lower fecundity. Whether the appearance is associated with the restoring-maintaining relationship or not, must be examined. Therefore, the cytoplasm of V20A is unsuitable for the optional cytoplasms in breeding the TGMS lines because of the negative effect of the restoring-maintaining relationship of wild abortive cytoplasm on the pollen fertility of the hybridized combination. This study suggests that the role of cytoplasm should be considered in breeding of the TGMS lines in the future programs.

Materials and Methods

Plant materials

Eight rice genotypes were used as the cytoplasmic donors (Table 1). Two TGMS lines, one Zhu-1S with low CSTP, stable sterility and safe hybrid seed production, and the other ZhunS with high CSTP, unstable sterility, unsafe hybrid seed production, were used as the nuclear donors.

The preparation of the mononuclear-heteroplasmic TGMS lines

The cytoplasmic donors were used as female parents in crosses with the nuclear donors, as males. Then, 12 backcrosses using the male parent as recurrent parent were done (Fig 1). The nuclear donors reproduced through the ratoon plants. The mononuclear-heteroplasmic TGMS lines were confirmed to have nuclei identical with their respective donors by the established molecular fingerprint of the nucleus. Meanwhile, no segregation of characters was found in BC_8F_1-$BC_{12}F_1$ generations of the same substitution line in field experiment.

Natural air temperature treatments

Sixteen combinations of $BC_{12}F_2$ and their parents were sown by stages in Hunan Normal University experimental farm, Hunan Province, P. P. China (28°45′N) in 2011. From April 15 to July 5, plant materials were sown for 6 batches, so plant materials tasseled every day from June 28 to September 30. The materials grown under the natural condition were evaluated for their pollen fertility and spikelet fertility (seed set percent after bagging). The diurnal average temperature was recorded by Temperature Date Recorder (LogTag). Pollen grains were stained with 2% Iodine-Potassium Iodide (I-KI) solution. Round, fully developed and uniformly–stained pollen were considered as normal fertile and irregular –shaped, yellowish, partly stained pollen were scored as sterile (Liang, 2008). Pollen staining ratio, which was used as an indication of the pollen fertility of the materials, investigated from 10 individuals for each of materials. Five panicles per plant were evaluated for spikelet fertility. Panicles emerging from the sheath were bagged with glassine paper bags prior to anthesis to prevent cross-pollination. Bagged panicles were harvested 25-30 days after pollination with panicles inside for seed counting to determine spikelet fertility.

Low-temperature treatments

Sixteen combinations of $BC_{12}F_2$ and their parents were treated respectively in two pool poured into 22.0 ºC, 23.5 ºC water for 6 , 10 days at the formation stage of pollen mother cell at Hunan Normal University experimental farm, Hunan Province, P. P. China (Li, 2008; Li, 2003). The materials were evaluated for their pollen (Liang, 2008) and spikelet fertility (seed set percent after bagging). The experiment treatments had three replications.

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References

Cai SX (1997) Effeets of male sterile cytoplasms of Yegong (*Oryza sativa* L. subsp. *indica*) and abortive wild rice on agronomic characters of hybrid rice. Chin J Rice Sci. 11(4): 211-214.

Chang TT, Lin FH (1974) Diallel analysis of protein content in rice. Agron Abstracts. 65.

Chandraratna MF, Sakai KI (1960) A biometrical analysis of matroclinous inheritance of grain weight in rice. Heredity. 14: 365-373.

Chen LB, Li XZ, Zhou GQ (1993) Effects of temperature on the sterility of rice in photo-sensitive and thermo-sensitive male sterile lines. Acta Agron Sinica. 19: 47-54.

Chen P (1992) Astudy on effect of different cytoplasms on heserosis. Hybrid Rice. 3: 42-44.

Hariprasanna K, Zaman FU, Singh AK (2006) Influence of male sterile cytoplasms on the physico-chemical grain quality traits in hybrid rice (*Oryza sativa* L.). Euphytica. 149: 273-280.

Li BH, Wu HX, Xu ML (2003) Comparative studies on the sensitivity of fertility of TGMS rice lines to low temperature of consecutive time. Acta Agron Sinica. 34(6): 930-936.

Li H, Yang YZ, Yao C (2008) Studies on fertility characteristics of sensitive to photoperiod and temperature of TGMS lines Zhu-1S and conditions for it's multiplication. Life Sci Res. 12(2): 158-162.

Liang MZ, Deng LX, Liu JF, He A, Chen LB (2008) Interaction between the eui gene and thermo-sensitive genic male sterility in rice. Euphytica. 164: 637-643.

Liu GJ, Zhan XD, Shen JH (2003) Preliminary study on the resistance of mononuclear-heteroplasmic CMS lines of rice to the Whitebacked Planthopper,Sogatella furcifera. Chin J Rice Sci. 17(1): 89-90.

Liu P, Dai XJ, Yang YZ (2008) Indica-Japonica Characteristics in Thermo-Sensitive Genic Male Sterile Rice Line Zhu-1S. Acta Agron Sinica. 34: 2112-2120.

Ratho SN, Pradhan SB (1992) Cytoplasmically controlled cold tolerance in a cytoplasmic-genetic male sterile line of rice. Euphytica. 58: 241-244.

Somrith B, Chang TT, Jackson BR (1979) Genetic analysis of traits related to grain characteristics and quality in two crosses of rice. In: IRRI Research Paper Series 35 International Rice Research Institute, Los Banos, Philippines.

Shen SQ, Xue QZ (1997) Effects of different cytoplasms on some agronomic traits of indica-japonica hybrids. Chin J Rice Sci.11(1): 6-10.

Shi CH, Xue JM, Yu YG (1996) Analysis of genetic effects on nutrient quality traits in indica rice. Theor Appl Genet. 92: 1099-1102.

Shi CH, Zhu J (1995) Analysis of seed, cytoplasmic, and maternal genetic effects on rice quality traits. IRRI Newslett. 20(2):4.

Tan SZ, Zhang YD, Sun HQ (2003) Effects of CMS cytoplasm in isonuclear alloplasmic CMS lines with wide compatibility in rice. Acta Agron Sinica. 29(3): 202-207.

Tao DY, Hu F, Yang J, Yang G (2004) Cytoplasm and cytoplasm-nucleus interactions affect agronomic traits in *japonica* rice. Euphytica. 135(1): 129-134.

Tao DY, Xu P (2011) Cytoplasm affects grain weight and filled-grain ratio in *indica* rice. BMC Genetics. 12(53): 1-21.

Virmani SS, BC Viraktamath, CL Casal, RS Toledo, MT Lopez and JO Manalo (1997) Hybrid rice breeding manual, International Rice Research Institute, Philippines.

Wu XJ,Yuan LP (2004) Develop thermo-sensitive genic male sterile(TGMS) rice line by means of population improvement. Acta Agron Sinica. 30(4): 589-592.

Yang RC, Lu HR (1989) A study on effects of nucleu-cytoplasmic interaction in Oryza L. Scientia Agron Sinica. 22 (2): 56-63.

Young JB, Virmani SS (1990) Effects of cytoplasm on heterosis and combining ability for agronomic traits in rice (*Oryza sativa* L.). Euphytica. 48: 177-188.

Yuan LP (1986) Hybrid rice in China. Chin J Rice Sci. 1(1): 8-18.

Yuan LP (1998) Exploiting crop heterosis by two-line system hybrids: current status and future prospects. High Tech Lett. 4(1): 90-95.

Zhu YG (1979) Studies on male sterile lines of rice with different cytoplasms. Acta Agron Sinica. 5(4): 29-38.

Zhan XD, Cao LY, Zhai HQ (2003) Development of isonucleus-allocytoplasmic CMS lines with wide compatibility in rice and their performance in main agronomic characters. Acta Agron Sinica. 29(2): 241-244.