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Fertility Expression of TGMS-Genes in the Backgrounds of indica CMS-lines, B-lines and R-lines of Hybrid Rice

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Abstract: The generation fertility of 51 F_1, 19 F_2 and 6 BC_1 between 3 thermo-sensitive genic male sterile lines (TGMS-lines) Pei'ai 64S, 6311S and 360S, and the three lines of hybrid rice including 7 indica cytoplasmic male sterile lines (CMS-lines) and their corresponding maintainer lines (B-lines) and 3 indica restorer lines (R-lines) were investigated to study the expression of TGMS-genes in the backgrounds of the three lines of hybrid rice. Pei'ai 64S has stronger fertility restoring (*Rf*) genes for CMS-lines and its TGMS trait is governed by 2 pairs of independent recessive genes; The TGMS trait of 6311S is governed by a single recessive gene with weaker *Rf*-gene in 6311S and the TGMS trait of 360S is governed by a single recessive gene with no *Rf*-gene in 360S. The investigation on the fertility of F_1 plants between 5 CMS-lines and 4 TGMS generations selected from F_2 plants of 4 CMS-lines × 6311S confirmed that the expression of TGMS-gene was controlled by *Rf*-gene in the genetic background of cytoplasm of CMS-lines, but not affected by *Rf*-gene in the genetic background of normal fertile cytoplasm. The potential breeding strategies of TGMS-lines with cytoplasm of CMS-lines and CMS-lines with the nucleus of TGMS-genes were discussed.

Key word: thermo-sensitive genic male sterility-gene; three-line system; genetic background; gene expression; restorer gene

The cytoplasmic male sterile (CMS-lines) and photoperiod/thermo-sensitive genic male sterile lines (P/TGMS-lines) are important genetic tools in hybrid rice breeding, which served as the female parents of the three-line and two-line hybrid rice breeding system, respectively. It has been reported that the CMS system possesses complete and stable sterility which is controlled by the interaction of the abortive cytoplasm and nucleus genes from their cognate B-lines [1] but TGMS-lines used to possess high seed purity because 1) TGMS-lines can be self-pollinated without B-lines by avoiding the mixture of the TGMS-lines seed with B-line and 2) a technical procedure can be designed to genetically purify the TGMS-lines in critical sterility-inducing temperature (CSIT)[2]. However, one of big problems is that there might be some parental seeds in commercial hybrids causing low level of genetic purity of hybrids. In general, B-line can be easily mixed with CMS-line by self-crossed, which cause contamination of the hybrid seeds and causes the impurity of F_1 hybrids during three-line hybrid seed production [3]. On the other hand, although some ecological techniques [4] are used to overcome the low purity of seed in two-line hybrid seed production under cool summer conditions, but TGMS-line can restore fertility due to the inherent nature of the TGMS trait at low temperatures, resulting in the impurity of the F_1 seed [5-6]. Therefore, the scientists are more interested in investigating the relations between CMS-line and TGMS-line to recombine a new breeding line [7] by reducing the disadvantages in hybrid rice seed production [8]. The objective of this research work is to study the influence of cytoplasm–nucleus and genes interaction on fertility expression of TGMS-genes in the genetic backgrounds of the three-lines of hybrid rice and to exploit a potential strategies to develop new CMS-lines with TGMS-gene and TGMS-lines with abortive cytoplasm.

MATERIALS AND METHODS

Plant materials

During the experiment, 7 cytoplasmically diverse

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CMS-lines and their corresponding B-lines, 3 R-lines, 3 TGMS-lines and 1 check were used (Table 1). The 51 F_1 hybrids between three female parental lines and TGMS-male parental lines were listed in Table 2 and Table 4. Six backcrosses and 19 F_2 populations were listed in Table 3 and Table 5.

Growth conditions

The plant materials were sown at Jiangpu Experimental Station of Nanjing Agricultural University in May, 2004 and 2005. About 40 plants from F_1, 300 from F_2 and 50-60 from BC_1 were transplanted during the mid June. There was 23.3 cm and 16.7 cm spacing between and within rows. To investigate the fertility expression of BC_1 and F_2 populations, Pei'ai 64S, 6311S, 360S, Zhenshan 97A and II-32A were used as checks and sown on 13 May, 23 May, 5 June, 15 June, 28 June and 8 July, respectively.

Fertility investigation of F_1 hybrids

The pollen fertility and seed setting rate were investigated at heading and harvesting, respectively.

The pollen fertility investigation was performed with a microscope by observation of the pollen from 5 plants of each F_1 at the heading stage. Three spikelets that blossom were collected from the upper, middle and lower positions of a large panicle or a panicle on the main culm, and the pollen grains were stained with 1% (*W*/*V*) I_2-KI solution. Finally, the pollen fertility (number of stained-pollen grains / number of stained- and unstained-pollen grains) was calculated. At maturity, 5 plants of each F_1 were harvested and the seed setting rates were recorded.

Table 1. Rice lines used in this study.

	Parental line	CMS-type	Origin
CMS- and B-line			
1	Longtepu A, Longtepu B	WA-type	ZIAS
2	Xieqingzao A, Xieqingzao B	DA-type	GIAS
3	Gang 46A, Gang 46B	G-type	SAU
4	II-32A, II-32B	ID-type	HHRRC
5	D62A, D62B	D-type	SAU
6	Zhenshan 97A, Zhenshan 97B	W-type	PIAS
7	Xinxie A, Xinxie B	DA-type	NAU
TGMS line			
8	Pei'ai 64S		HHRRC
9	6311S		NAU
10	360S		GAAS
Restorer line			
12	Shuhui 527		SAU
13	Minghui 86		SIAS
14	Minghui 63		SIAS
CK			
	Shanyou 63 (Zhenshan 97A × Minghui 63)		SIAS

ZIAS, Zhangzhou Institute of Agricultural Sciences, Zhangzhou, Fujian; GIAS, Guangde Institute of Agricultural Sciences, Guangde, Anhui; SAU, Sichuan Agricultural University, Chengdu; HHRRC, Hunan Hybrid Rice Research Center; PIAS, Pingxiang Institute of Agricultural Sciences, Pingxiang, Jiangxi; NAU, Nanjing Agricultural University, Nanjing; GAAS, Guizhou Academy of Agricultural Sciences, Guiyang; SAS, Sanming Institute of Agricultural Sciences, Fujian.

Fertility investigation in F_2, BC_1 populations and the F_1 hybrids between CMS-lines and TGMS-plants from F_2 populations

The pollen fertility and seed setting rate of each plant in F_2 and BC_1 populations were investigated under high temperature conditions and long daylength in summer in 2004 and 2005 in Nanjing. The plants of F_2 and BC_1 populations with less than 5% stained pollen grains were considered as sterile.

Eighteen TGMS-progeny selected from F_2 populations of Longtepu A/6311S, II-32A/6311S, D62A/6311S and Xinxie A/6311S were harvested in autumn of 2004, and the seeds (F_1) were sown at Breeding Station of Nanjing Agricultural University in Lingshui, Hainan on 30 November 2004. Only one F_3 population from each combination with uniform agronomic characteristics was selected in March, 2005. The five plants randomly taken from each F_3 population were used as male parent to cross with the 5 CMS-lines, Gang 46A, Zhenshan 97A, II-32A, D62A and Xinxie A, respectively, and 20 F_1 hybrids (Table 6) were harvested in April of 2005 and sown in Nanjing in May, 2005. Fertility of each plant of the 20 F_1 hybrids was investigated in August, 2005.

Breeding and evaluation of near isogenic lines (NILs) 6311S and 6311SB derived from 6311S

By backcrossing the progeny without *Rf*-gene selected from F_2 population of Xinxie B/6311S with recurrent parent '6311S' for 4 generations, we developed NILs 6311B without TGMS-gene and 6311SB with TGMS-gene possessing the same genetic background and agronomic traits but maintaining the

Table 2. Fertile pollen and seed setting rate of F_1 generations between B-lines or R-lines and TGMS-lines.

B-line or R-line	Fertile pollen (%)				Seed setting rate (%)			
	Pei'ai 64S	6311S	360S	Mean	Pei'ai 64S	6311S	360S	Mean
Longtepu B	53.82	59.42	69.16	60.90 Cbc	64.52	77.84	74.33	71.87 Cc
Xieqinzao B	50.42	56.45	58.00	54.97 Dc	62.52	78.72	77.39	73.16 Cbc
Gang 46B	61.83	67.84	70.73	66.85 ABab	71.84	78.82	75.30	75.38 BCbc
II-32B	55.95	64.61	69.64	63.49 BCab	83.88	88.50	67.72	80.74 Aa
D62B	56.15	67.76	64.50	62.87 BCb	80.49	71.89	80.31	77.68 ABab
Zhenshan 97B	58.03	68.44	65.50	64.03 BCab	78.28	79.42	76.51	78.08 ABab
Xinxie B	73.07	65.53	69.32	69.35 Aa	69.18	69.63	75.17	71.28 Cc
Mean of B-lines	58.58 Bb	64.35 Aa	66.74 Aa	63.27	73.32 Bb	77.91 Aa	75.33 Bab	75.54
Shuhui 527	72.85	81.70	76.69	77.18 Aa	77.20	84.20	80.42	80.69 Aa
Minghui 86	66.80	71.16	74.20	70.77 Bb	64.67	84.19	77.68	75.95 Bab
Minghui 63	70.73	79.22	74.33	74.84 Bb	66.64	78.35	74.30	73.23 Bab
Mean of R-lines	70.16 Bb	77.51 Aa	75.08 Aab	74.31	69.66 Cb	82.34 Aa	77.51 Ba	76.71

In a column of mean, the numbers followed by the different uppercase and lowercase letters indicate significant difference at 1% and 5% levels, respectively.

sterility of CMS-lines due to no *Rf*-gene. Furthermore, two new CMS-lines 6311A and 6311SA were developed by using 6311B and 6311SB as B-lines through continuous backcrossing with the CMS-line Xinxie A for 5 generations. Moreover, 6311B and 6311SB were used as male parent to cross with the CMS-lines such as Gang 46A, Zhenshan 97A, II-32A, D62A and Xinxie A to get F_1 hybrids in Lingshui, Hainan in March, 2005. The fertility of two new CMS-lines 6311A and 6311SA and all F_1 hybrids was investigated in Nanjing, Jiangsu in August, 2005.

Data analysis

After being transformed by Arcsin$\sqrt{P}$, the fertility data were analyzed with SPSS11.0.

RESULTS

Fertility expression of TGMS-gene in the genetic background of B-lines and R-lines

The rates of stained pollen and seed setting of F_1 hybrids between 7 B-lines or 3 R-lines and 3 TGMS-lines are listed in Table 2. The results indicated that there were a highly significant difference among B-lines or R-lines or TGMS-lines and a significant difference in the variances of interaction between B-lines and TGMS-lines at 1% and at 5% levels, respectively. In 20 of all the 30 F_1 combinations, the seed setting rate were higher than 75% including the combinations from 4 of 7 B-lines or 2 of 3 R-lines and 3 TGMS-lines, suggesting that only few conventional rice lines including B-lines and R-lines can restore normal fertility of TGMS-lines.

The data on the segregation of sterile and fertile plants from F_2 and BC_1 populations are presented in Table 3. The data on *Chi*-square test indicated that: 1) The F_2 generations derived from F_1 plants between Pei'ai 64S and B-lines or R-lines segregated at a ratio of 15 fertile plants to 1 sterile plant and the BC_1 plants segregated at a ratio of 3 fertile pants to 1 sterile plant, suggesting that TGMS-trait of Pei'ai 64S was controlled by two pairs of dependent recessive genes. 2) The F_2 descendant of F_1 plants between 6311S or 360S and B-lines or R-lines were segregated in the ratio of 3 fertile plants to 1 sterile plant and the BC_1 at a ratio of 1 fertile plant to 1 sterile plant, suggesting that the TGMS-trait of 6311S and 360S was governed by a single recessive gene.

Fertility expression of TGMS-gene under the genetic background of CMS-lines

The seed setting rates of F_1 hybrids between 7 CMS-lines and 3 TGMS-lines are presented in Table 4. The results showed that: 1) F_1 plants between CMS-lines and 360S were sterile but F_1 plants between their corresponding B-lines and 360S

Table 3. Ratios of fertile and sterile plants of F_1 and BCF_1 between R-lines, B-lines and TGMS lines.

	Combination	No. of fertile plant	No. of sterile plant	Expected value	χ^2	*P*
Restorer line						
F_2	Shuhui 527/Pei'ai 64S	273	20	15 : 1	0.0821	0.90-0.75
	Minghui 86/Pei'ai 64S	255	18	15 : 1	0.0120	0.95-0.90
	Shuhui 527/6311S	222	80	3 : 1	0.2826	0.75-0.50
	Minghui 86/6311S	227	67	3 : 1	0.6531	0.50-0.25
	Shuhui 527/360S	149	55	3 : 1	0.3203	0.75-0.50
	Minghui 86/360S	219	69	3 : 1	0.1157	0.75-0.50
BC_1	Shuhui 527/Pei'ai 64S//Pei'ai 64S	49	15	3 : 1	0.0208	0.90-0.75
	Shuhui 527/6311S//6311S	37	33	1 : 1	0.1286	0.75-0.50
	Shuhui 527/360S//360S	40	35	1 : 1	0.2133	0.75-0.50
Maintainer line						
F_2	Xinxie B/Pei'ai 64S	289	14	15 : 1	1.1091	0.50-0.25
	Xinxie B/6311S	153	57	3 : 1	0.4063	0.75-0.50
	Xinxie B/360S	235	70	3 : 1	0.5781	0.50-0.25
BC_1	Xinxie B/Pei'ai 64//Pei'ai 64S	49	13	3 : 1	0.3441	0.75-0.50
	Xinxie B/6311S//6311S	27	33	1 : 1	0.4167	0.75-0.50
	Xinxie B/360S//360S	35	30	1 : 1	0.2462	0.75-0.50

Table 4. Seed setting rate of F_1 generations between CMS-lines and TGMS-lines.

CMS-line	Seed setting rate(%)		
	Pei'ai 64S	6311S	360S
Longtepu A	69.13	35.02	6.78
Xieqinzao A	62.41	30.17	1.03
Gang 46A	68.35	43.37	0.00
II-32A	77.03	36.07	0.33
D62A	80.72	34.75	1.79
Zhenshan 97A	78.17	34.98	0.00
Xinxie A	79.19	35.74	0.00
Mean	72.69 Aa	38.80 Bb	1.42 Cc
CK	75.16		

In a column of mean, the numbers followed by the different uppercase and lowercase letters indicate significant difference at 1% and 5% levels, respectively.

were fertile (67.72%-80.31%) (Table 2), suggesting that cytoplasm suppressed the fertility restoration of TGMS-gene due to absence of *Rf*-gene in CMS-lines of 360S. 2) The seed setting rates of F_1 plants between CMS-lines and Pei'ai 64S were 62.41-80.31%, equal to the F_1 plants between B-lines and Pei'ai 64S (Table 2). Moreover, the F_1 plants between CMS-lines and 6311S were 30.17-43.37%, about half of F_1 plants among B-lines and 6311S (Table 2), demonstrating that Pei'ai 64S and 6311S had different fertility restoring ability to CMS-lines. Obviously, Pei'ai 64S could completely restore the CMS-lines fertility, suggesting that Pei'ai 64S has stronger *Rf*-gene, but 6311S could partially restore the fertility of CMS-lines, suggesting that 6311S has a pair of weaker *Rf*-gene. By comparison with seed setting rate of the elite check 'Zhenshan 97A/Minghui 63', it was proposed that the stronger *Rf*-genes in Pei'ai 64S and the weaker *Rf*-gene in 6311S could be similar to the stronger and weaker *Rf*-gene in Minghui 63, an elite R-line [11-13].

The segregation rate of sterile and fertile plants in the F_2 populations from F_1 between CMS-lines and TGMS-lines are listed in Table 5. The results showed that the F_2 generations from F_1 plants of Pei'ai 64S × CMS-line and 6311S × CMS-line segregated at the ratio of 7.3-9.4 fertile plants to 1 sterile plant and at the ratio of 1.2-1.4 fertile plants to 1 sterile plant, respectively, but the progeny from Xinxie A and TGMS-line 360S was to be sterile with TGMS-line 360S as a pollen donor for continuous backcrossing, suggesting that the inheritance of the TGMS-trait in the background of CMS-lines was significantly distinct from that of the TGMS-trait in the background of B-lines or R-lines. The above results revealed that the genotypes of Pei'ai 64S, 6311S and CMS-lines are hypothesized to be N ($Rf_1Rf_1Rf_2Rf_2ms_1ms_1ms_2ms_2$,

Table 5. Ratios of fertile: sterile plants of F_2 from CMS-lines×TGMS-lines.

Combination	No. of fertile plants	No. of sterile plants	F : S	Expected value	χ^2	*P*
Longtepu A /Pei'ai 64S	255	27	9.44:1	225:31	1.4727	0.25-0.10
II-32A/Pei'ai 64S	273	33	8.27:1	225:31	0.3880	0.75-0.50
D62A/ Pei'ai 64S	272	34	8.00:1	225:31	0.2004	0.75-0.50
Gang 46A/ Pei'ai 64S	268	30	8.93:1	225:31	0.9838	0.50-0.25
Xinxie A/ Pei'ai 64S	276	38	7.26:1	225:31	0.0068	0.95-0.90
Longtepu A/6311S	165	114	1.45:1	9:7	0.8330	0.50-0.25
II-32A/6311S	154	126	1.22:1	9:7	0.1306	0.75-0.50
D62A/6311S	175	122	1.43:1	9:7	0.7568	0.50-0.25
Gang 46A/6311S	163	135	1.21:1	9:7	0.2320	0.75-0.50
Xinxie A/6311S	158	129	1.22:1	9:7	0.1222	0.75-0.50
Xinxie A/360S//360S	0	38	0:1	0:1		

Table 6. Seed setting rate of F_1 generations between CMS-lines and TGMS phenotype progeny in the CMS background.

Origin of TGMS-plants	Female parent				
	Gang 46A	Zhenshan 97A	II-32A	D62A	Xinxie A
Longtepu A/6311S	25F	21F	38F	28F	19F
II-32A/6311S	13F, 15S	30F	19F, 16S	10F, 12S	14F, 16S
D62A/6311S	22F	28F	36F	27F	21F
Xinxie A/6311S	10F, 12S	15F, 17S	20F, 18S	23F	25F

F, S are fertile plants (seed setting rate) and sterile plants (seed setting rate), respectively and the values are numbers of fertile- or sterile-plants.

N（$Rf_2Rf_2ms_1ms_1$） and S（$rf_1rf_1rf_2rf_2Ms_1Ms_1Ms_2Ms_2$）, respectively, while Rf_1 and Rf_2 are stronger and weaker *Rf*-genes, respectively and *ms* is PGMS-gene. Obviously, genotypes of S(————$ms_1ms_1ms_2ms_2$), S($rf_1rf_1rf_2rf_2$————), S(——ms_1ms_1), S(rf_2rf_2——) exhibit sterility under high temperature. The populations derived from Pei'ai 64S×CMS-lines and 6311S×CMS-lines segregated at an expected ratio of 31 sterile plants to 225 fertile plants, and 7 sterile plants to 9 fertile plants, respectively. The *Chi*-square test indicated that the F_2 segregation patterns of sterile to fertile plants are fitted to the expected ratio. Therefore, it can be ascertained that the expression of TGMS-gene in the background of aborted cytoplasm was controlled by *Rf*-gene.

Test of conditions for fertility expression of TGMS-gene in the genetic background of CMS-lines

The fertility of hybrid between 5 CMS-lines and 5 plants in each F_3 PGMS-population derived from the F_2 populations are presented in Table 6. The results showed that 10 F_1 hybrids between 10 plants from 2 of 4 F_3 populations and 5 CMS-lines exhibited fertility (30-50%), while 3 of 10 F_1 hybrids between 10 plants from the rest 2 F_3 populations and 5 CMS-lines exhibited fertility (30-50%) and the rest 7 hybrids segregated at the ratio of 1 sterile plant to 1 fertile plant, suggesting that all the TGMS-individuals of 2 F_3 populations were homozygous for *Rf*-gene and some of the individuals from the rest of the two F_3 populations were homozygous and the other were heterozygous. The results confirmed that PGMS-plants with abortive-cytoplasm had *Rf*-gene.

We noted that the F_1 plants between NILs 6311B/6311SB and the CMS-lines Gang 46A, II-32A, D62A and Xinxie A exhibited complete sterility. Furthermore, a new CMS-line '6311A' and a new CMS-line '6311SA' with TGMS-gene were bred by using 6311B and 6311SB as B-line backcorssed to

Xinxie A for 5 generations, which not only showed a complete sterility but also no TGMS-trait. The results indicated that abortive cytoplasm suppressed sterility expression of TGMS-gene in no *Rf*-gene background.

Thus the above tests confirmed that *Rf*-gene controls the expression of TGMS-gene in abortive cytoplasm background.

DISCUSSION

Influence of cytoplasm and *Rf*-gene on expression of TGMS-gene

The results of the current study indicated that the cytoplasm and *Rf*-gene influenced the expression and inheritance of TGMS-gene. For the TGMS-trait, genetic segregation of F_2 population from CMS-line× TGMS-line was obviously distinct from that of F_2 population from B-line (or R-line)×TGMS-line. On one hand, without *Rf*-gene, abortive cytoplasm could suppress the genetic expression of TGMS-gene, resulting in sterile plants without TGMS-trait. Moreover, with homozygous and heterozygous *Rf*-gene, the plants with homozygous TGMS-gene in abortive-cytoplasm background exhibited sterility under high temperature but fertility under low temperature, resulting in PGMS-plants. To find out from which the stronger *Rf*-genes in Pei'ai 64S and the weaker *Rf*-gene in 6311S derived, we investigated into the pedigrees of Pei'ai 64S and 6311S. (a) According to the pedigree of Pei'ai 64S [14-15] (Paddy/ Huang'aidao //Ce 64/// Nongken 58S), the stronger *Rf*-genes should be derived from strong R-line Ce 64. Previously, we bred a new R-line Zi 66 (Pei'ai 64S/ 9311//9311), which can restore the fertility of CMS-lines. (b) TGMS-line 6311S is a new breeding line derived from Guangzhan 63S/9311. According to the pedigree of TGMS-line Guangzhan 63S [16] [7001S /Lun 422// (Paddy/C57// Luweidao)/// Guangzhan 63], the weaker *Rf*-gene in 6311S should be inherited from japonica R-line C57 [17]. In this experiment, though the used CMS-lines had diverse cytoplasm, but the mechanisms of sterilization and fertility restoration of these CMS-lines were similar to those of wild-abortive CMS-line and distinct from those of BT-type and Honglian-type CMS-lines. Though the *Rf1*-gene for the BT-type CMS-lines has been isolated using map-based cloning [19] and the *Rf5*-gene for Honglian-type CMS-line has been finely located at a genetic distance of 6.1 cM and 2.1 cM from the linked marker G2155 and S10019, respectively, on chromosome 10[20], the expression of TGMS-genes possessing BT- and Honglian-type CMS-lines background is still unclear. To study the inheritance of TGMS-genes is our second goal in the background of BT- and Honglian-types CMS-lines through the above studying strategy.

Breeding strategy for TGMS-lines with abortive cytoplasm and CMS-lines with TGMS-gene

To date, the TGMS-lines used in hybrid breeding program in China are of normal cytoplasm. In this study, two types of new sterile rice lines TGMS-lines with abortive cytoplasm and CMS-lines with TGMS-gene were bred, respectively. TGMS-lines possess abortive cytoplasm and *Rf*-gene but the TGMS-lines, which applied in commercial hybrids, have normal cytoplasm and *Rf*-gene or no *Rf*-gene. The comparison of the new TGMS-lines with 6311S in Lingshui, Hainan and in Nanjing, Jiangsu, indicated that the abortive cytoplasm might be responsible for decrease of the critical sterility inducing temperature of TGMS-lines and increase of hybrids purity. CMS-lines and their corresponding B-lines have TGMS-gene, while the fertile B-lines of the CMS-lines used in hybrid rice breeding haven't TGMS-gene. For example, 360S[18], derived from An'xiang S is an elite TGMS-line, which completely maintains the sterility of CMS-lines. Using 360S as a recurrent parent to backcross with CMS-lines for 5-6 generations, a new CMS-line 360SA with TGMS-gene were bred. It has been found that 6311SB was similar to 360S and could be used in the development of a new CMS-line 6311SA with TGMS-gene. Thus, it can be suggested that the new CMS-lines should be backcrossed with their corresponding B-line exposed to low temperature(≤ 23℃) after panicle initiation during multiplication process. However, during hybrid production the CMS-lines and their corresponding B-lines exhibit sterility under high temperature(≥24℃), resulting in pure hybrids. During this work, our ultimate goal was to use the two types of sterile lines in hybrid breeding production.

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