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Genetic bases of instability of male sterility and fertility reversibility in photoperiod-sensitive genic male-sterile rice

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Abstract Photoperiod-sensitive genetic male-sterile (PSGMS) rice, with its male fertility regulated by photoperiod length, is very useful for hybrid rice development. However, breeding for new PSGMS lines has faced two major difficulties – the stability of male sterility and the reversibility of male fertility. In this study we assessed the genetic bases of stability of sterility and fertility reversibility using a molecular marker-based approach. A cross was made between two newly bred PSGMS lines: Peiai 64S, which has a stable sterility but is difficult to reverse to fertility, and 8902S, which has a unstable sterility but is easy to reverse to fertility. The fertility of the parents and of the F_1 and F_2 populations was repeatedly examined under 11 different long-day and short-day conditions. The genetic effects were assayed by interval mapping and two-way analyses of variance using the F_2 data of 128 polymorphic loci representing all the 12 rice chromosomes. The analyses resolved a number of single-locus QTLs and two-locus interactions under both long-day and short day conditions. The interactions involved a large number of loci, most of which were not detectable on a single-locus basis. The results showed that the genetic bases of both stability of sterility and reversibility of fertility are the joint effects of the additive effects of the QTLs and additive-by-additive components of two-locus interactions. The implications of these findings in hybrid rice development are also discussed.

Key words Genetic analysis · Molecular marker · Quantitative trait locus (QTL) · Epistasis · Hybrid rice

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Introduction

A photoperiod-sensitive genic male-sterile (PSGMS) rice was found as a spontaneous mutant in a japonica (*Oryza sativa* ssp. *japonica*) rice cultivar (‘Nongken 58’) grown in Hubei Province, China (Shi 1985). Large numbers of studies conducted in the past have established that this novel mutant (referred to as Nongken 58S) possess a number of desirable characteristics useful for hybrid rice development (Yuan et al. 1993; Z.G. Zhang et al. 1994), providing a good opportunity to replace the widely used ‘three-line’ system with a ‘two-line’ system in hybrid rice production. Utilization of PSGMS rice for the development of two-line hybrids has thus become a major goal in many rice breeding programs in China (Yuan 1992). Many new PSGMS lines have been developed in the last decade by crossing the original Nongken 58S to various indica and japonica varieties.

However, the practice of developing new PSGMS lines has faced two major difficulties: i.e. the instability of sterility and low reversibility of fertility, especially in breeding indica-type PSGMS lines. As a result, the majority of the newly developed indica PSGMS lines can be divided into two classes based on the stability of the male sterility under natural long-day conditions and the reversibility of the fertility under natural short-day conditions. In the first class of PSGMS lines, the male sterility is complete (or nearly complete) and highly stable across a range of environmental conditions, including those with very different temperature regimes. However, these lines are difficult to reverse to fertility, and only a very low seed-setting percentage can be obtained under natural short-day conditions, thus causing a major difficulty in propagating the male-sterile lines. The reverse is the case for PSGMS lines in the second class. These lines are easy to reverse to normal fertility under natural short-day conditions, but the male sterility is unstable and fluctuates with temperature changes under natural long-day conditions. Very high rates (e.g. >40%) of seed-setting have been observed in many male-sterile lines grown under natural long-day conditions when the temperature falls below certain thresholds during the period of panicle development; however, this would cause problems in hybrid seed production. These two problems have severely hindered the development of ‘two-line’ hybrid rice.

The study reported in this paper was undertaken to characterize, using a molecular marker-based approach, the genetic components underlying both the stability of male sterility under long-day conditions and the reversibility of male fertility under short-day conditions. It is expected that an enhanced understanding of the genetic bases related to the stability of male sterility and reversibility of male fertility will provide a knowledge base for rational strategies for overcoming the difficulties encountered in breeding PSGMS lines.

Materials and methods

The genetic materials

The genetic materials used in this study were two indica-type PSGMS lines, Peiai 64S and 8902S, and progenies from a cross between these two lines including five F_1 plants and 240 plants of an F_2 population. Both Peiai 64S and 8902S were developed by transferring PSGMS gene(s) from the original PSGMS mutant Nongken 58S to respective varieties. These two lines respectively represent the two classes of PSGMS lines described in the previous section. Peiai 64S has highly stable male sterility under long-day conditions but is difficult to reverse to fertility under short-day conditions, while 8902S has unstable sterility under natural long-day conditions but is easy to reverse to fertility under short-day conditions.

The photoperiod and temperature treatments

All the plants of the parents and of the F_1 and F_2 population were maintained vegetatively over-winter by ratooning of Hainan (South China Sea) Island or in a greenhouse in Wuhan. Young shoots obtained the following spring were separated and collected as the source of materials for various treatments.

The phytotron long-day/high-temperature treatment

The long-day conditions were created by exposing the phytotron unit (glass-walled, day-light type) to natural long days in the summer at Wuhan (daylength ≥14.5 h). The high-temperature conditions were created by setting the average daily temperature in the chamber at 30°C with a 24 h temperature cycle, this simulated a typical hot summer day in Wuhan.

The phyotron long-day/low-temperature treatment

The long-day conditions were created in the same way as described in the previous paragraph. The low-temperature conditions were obtained by setting the average daily temperature in the chamber at 24°C with a 24-h temperature cycle, which similated a typical occasional cool summer day in Wuhan.

The phytotron short-day/high-temperature treatment

The short-day conditions were created by exposing the phytotron units to the natural conditions of Wuhan during the rice growing season for 10 h each day and covering the units with several layers of black cloths in the mornings and evenings for the remaining 14 h. The average temperature was set at 28°C with a daily temperature cycle simulating the temperatures of the late summer and early fall in Wuhan

The natural long-day treatment

Two sites were selected for the natural long-day treatment. The first site was located at the Experimental Farm of Huazhong Agricultural University at Wuhan China (latitude 30.5° N, 15 m above sea level) where the summer is typically hot and humid. However, cool days occur occasionally during the summer time in certain years. The second site was located in Yaohe Village, near Gouya Town in Yuan’an County (the same latitude as Wuhan), Hubei Province (elevation of 540 m above sea level), where the summers are usually cool. Fertility was examined for tillers that headed before August 25, which would presumably place the critical stage for photoperiod induction of male fertility under long-day conditions. Experiments for collecting fertility data under the natural long-day conditions in Wuhan were repeated for 3 consecutive years (1994–1996), and fertility data under Yuan’an natural long-day conditions were collected in for 2 years (1995 and 1996).

The natural short-day treatment

Two sites, Wuhan and Hainan Island (Lingshui County, latitude 18.9° N), were chosen for examining the fertility under natural short-day conditions. Fertility was examined on tillers that headed on September 10 or later in Wuhan and on panicles that headed between April 1 and April 5 in Hainan. These heading dates would ensure that the panicles had passed through the critical stages for photoperiod induction of male fertility under short-day conditions. Fertility data under natural short-day conditions in Wuhan were collected in 1994 and 1995, and fertility, and fertility under natural short-day conditions in Hainan was scored in 1996.

Theoretically, a good PSGMS line should be stably sterile under long-day conditions and fertile under short-day conditions regardless of the temperature. Thus, the long-day treatments under various temperature profiles provided conditions for testing the stability of the male sterility and the short-day treatments for testing the reversibility of male fertility.

Fertility examination

Fertility of the plants under various photoperiod and temperature treatments was assessed using two criteria: pollen fertility and spikelet fertility. Pollen fertility was scored as the proportion of darkly stained pollen grains in all of the pollen counted (at least 500 pollen grains per floret; samples taken from three to five florets in the upper parts of the panicles. Spikelet fertility was scored as the seed-setting rates of three to five panicles per plant.

Restriction fragment length polymorphism (RFLP) markers and assay

DNA was extracted from fresh leaf tissue of field-grown plants of each of the parents and the F_2 population. A total of 337 RFLP probes from the Cornell University and the RGP (Japanese Rice Genome Research Program) linkage maps (Causse et al. 1994; Kurata et al. 1994), in combinations with six restriction endonucleases, were selected for surveying the polymorphisms between the parents. Digestion, electrophoresis, blotting, hybridization followed by methods described previously (Liu et al. 1997).

Data processing and statistical analysis

A molecular linkage map based on the F_2 data for the RFLP markers that wee polymorphic between the parents was constructed using MAPMAKER/EXP 3.0 (Lincoln et al. 1992a). Quantitative trait

Table 1 The fertility (%) of Peiai 64S, 8902S and the F_1 under various long-day (LD) conditions (%)

Material	Phytotron LD (>14.5 h/30°C)	Phytotron LD (>14.5 h/24°C)		Natural LD (Yuan'an)			Natural LD (Wuhan)	
	Spikelet fertility	Pollen fertility	Spikelet fertility	Spikelet fertility (1995)	Spikelet fertility (1996)	Spikelet fertiligy (1994)	Spikelet fertility (1995)	Spikelet fertility (1996)
Peiai 64S	0	4.03	0	11.46	4.05	10.75	10.07	7.31
8902S	0	53.51	32.93	67.43	55.37	66.67	35.3	58.42
F_1	0	11.62	0	28.24	38.23	36.87	34.6	28.43

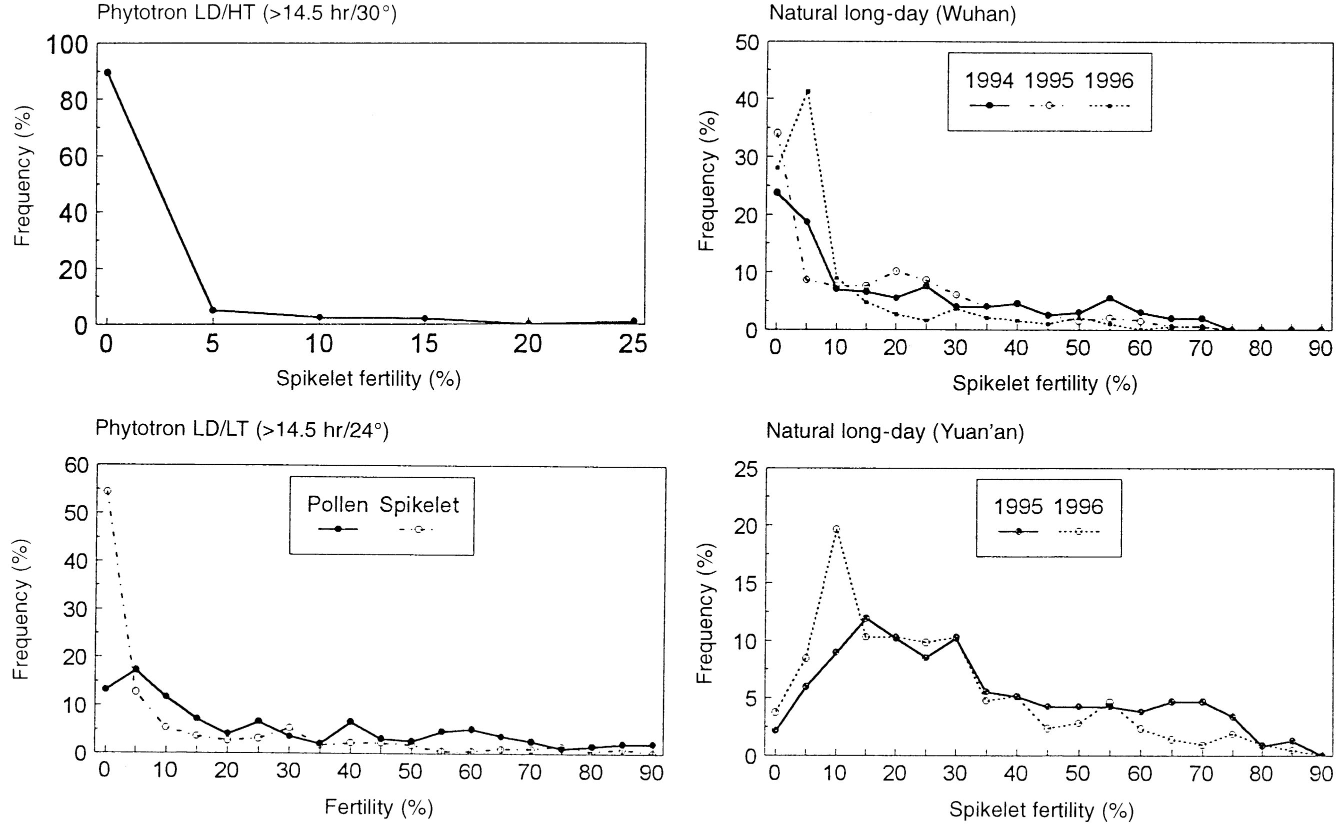


Fig. 1 Fertility distribution of the F_2 population under various long-day (LD) conditions. *HT* and *LT* high and low temperature, respectively

loci (QTLs) that have significant effects on fertility under various conditions were determined using MAPMAKER/QTL 1.1 (Lincoln et al. 1992b) at a LOD threshold 2.0. Digenic interactions were assayed with two-way analyses of variance using all possible two-locus combinations of marker genotypes. The calculation was based on unweighted cell means (Snedcor and Cochran 1980), and the sums of squares were multiplied by the harmonic means of the cell sizes to form the test criteria. There are eight degrees of freedom among the nine genotypes formed of two codominant loci, two degrees of freedom for the additive and dominance effects within each locus, and four degrees of freedom for interaction between loci. The interaction, often referred to as epistasis, can be further partitioned into four terms each specified by a single degree of freedom: additive (first locus) ×additive (second locus) (AA), additive×dominance (AD), dominance×additive (DA) and dominance×dominance (DD). Statistical significance for each term was assessed using an orthogonal contrast test provided by the statistical package Statistica (StatSoft 1995).

Results

Fertility segregation under long-day conditions

The fertilities of the two parents, Peiai 64S and 8902S, and of the F_1 plants undergoing various long-day treatments are given in Table 1. Under the phytotron long-day/high-temperature (14.5 h/30°C) conditions, both parents and the F_1 were completely sterile; the F_2 population was also highly sterile (Fig. 1). Under the phytotron long-day/low-temperature conditions (14.5 h/24°C), Peiai 64S and the F_1 were still sterile, but 8902S became partly fertile. The fertility distribution of the F_2 population ranged from completely sterile to fully fertile with the modal located in the area of high stability (Fig. 1).

The fertilities of the parents and F_1 plants under the natural long-day conditions of Wuhan and Yuan'an ap-

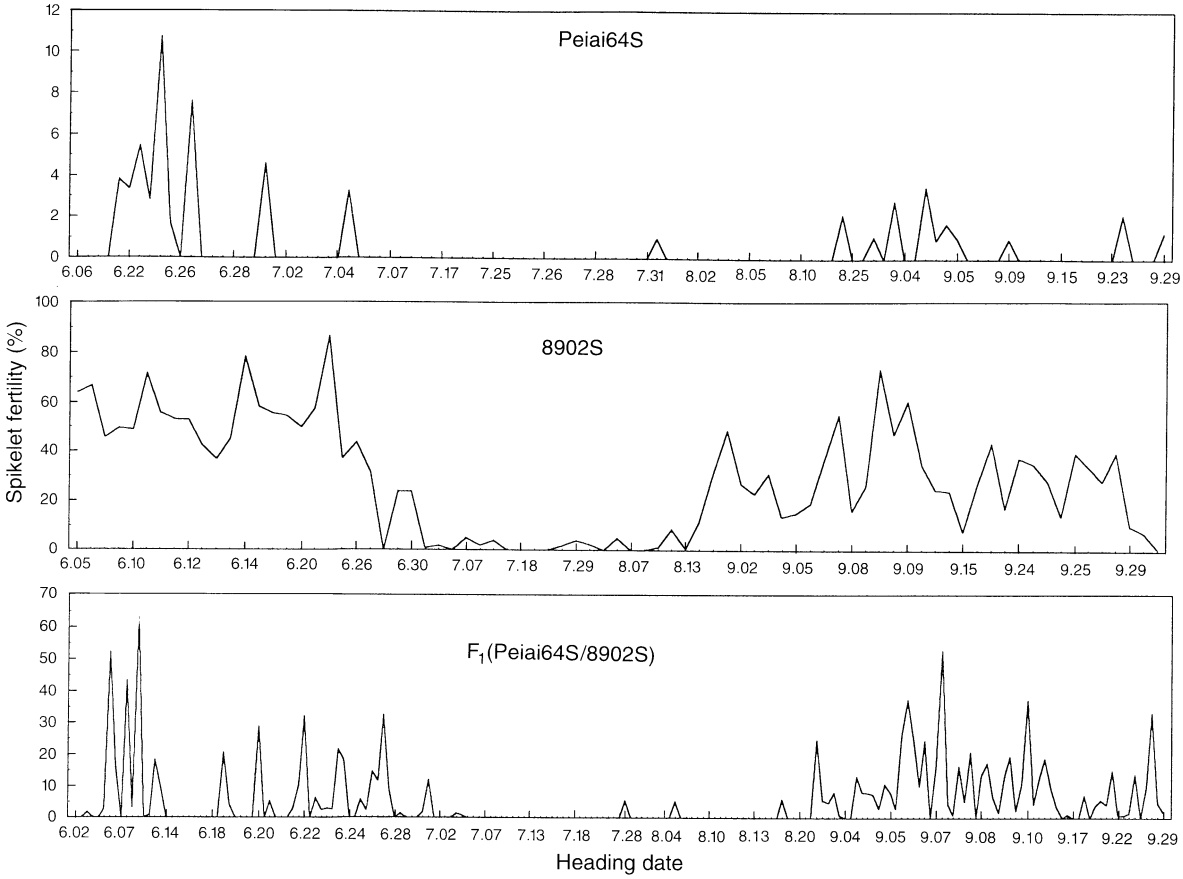


Fig. 2 Spikelet fertility of the two parents, 'Peiai 64S' and '8902S', and the F_1 under natural long-day conditions in Wuhan for panicles headed from June to September in 1994. The *numbers* along the *horizontal axis* are heading dates of the panicles (not proportional in scale)

peared to be similar (Table 1). In all the cases, Peiai 64S was highly sterile, while 8902S varied from partly sterile to sub-fertile, with the F_1 being intermediate. However, the fertility of the F_2 population behaved very differently between Wuhan and Yuan'an under natural long-day conditions (Fig. 1). In all 3 years tested in Wuhan, although a full range of fertility distribution was observed, the modal was located between 0 and 10%. In comparison, the fertility of the F_2 population in both, of the years tested was much higher on average in Yuan'an than in Wuhan; much larger numbers of individuals showed higher fertility, and the modal was also much higher.

To gain a better view of the fertility changes in response to environment fluctuations, we followed the dynamic changes in fertility of the F_1 and the parents under the natural long-day conditions by labeling the panicles that headed during the period from June 1 to September 30 in 1994. The results (Fig. 2) clearly showed that Peiai 64S was highly sterile when it headed during this period, except for a few days in late June, during which time the panicles that headed expressed slightly higher fertility (close to 10%). For 8902S, there was also a period from early July to early August when the panicles that headed showed relatively low sterility (<10% seed-setting). The F_1 was highly sterile when it headed during the 2 months of July and August.

Fertility segretation under short-day conditions

Fertilities of the two parents and the F_1 under various short-day conditions are given in Table 2. Peiai 64S remained highly sterile in all the environments tested, and the fertility of 8902S varied from partly sterile to fully fertile; the F_1 again appeared to be intermediate.

The fertility of the F_2 population under various experimental conditions (Fig. 3) was again distributed continuously from completely sterile to fully fertile, as under long-day conditions. However, a comparison of Figs. 1 and 3 clearly shows that overall fertility was much higher under short-day conditions than under long-day conditions.

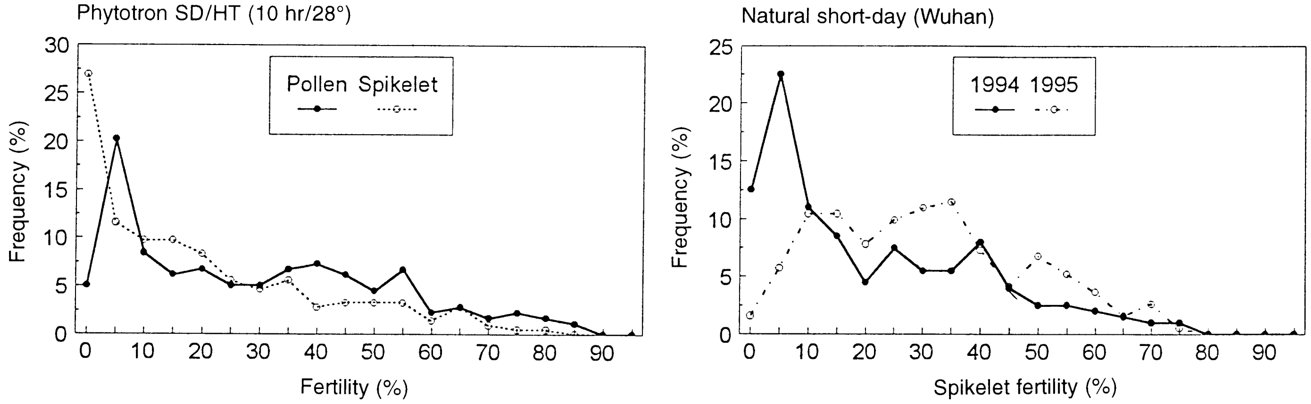
Molecular marker polymorphism and the linkage map

Of the 337 RFLP probes selected from the two high-density maps, 117 detected polymorphisms between the parents with at least one restriction enzyme. Analysis of the 240 F_2 individuals using these 117 probes resolved a total of 129 RFLP loci, of which 119 loci showed co-dominant segregation and the remaining 10 displayed a dominant type of segregation.

Table 2 The fertility (%) of Peiai 64S, 8902S and the F_1 and the F_1 under various short-day (SD) conditions

Material	Phytotron SD (10 h/28°C)		Natural SD[a]		
	Pollen fertility	Spikelet fertility	Hainan (1996)	Wuhan (1994)	Wuhan (1995)
Peiai 64S	13.25	0	13.58	2.03	1.83
8902S	67.89	32.26	72.83	67.82	40.30
F_1	45.09	18.61	59.66	49.81	32.42

[a] Spikelet fertilitly



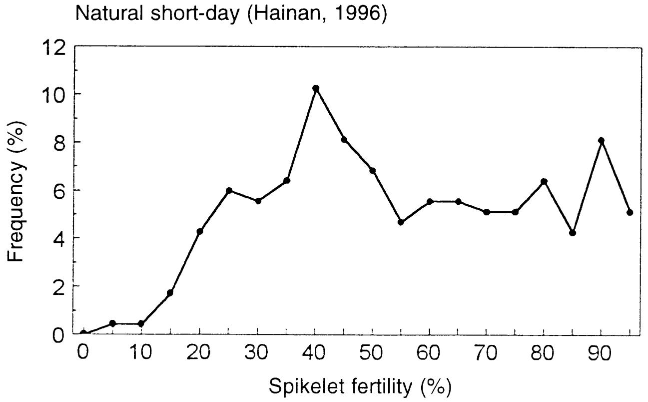


Fig. 3 Fertility distribution of the F_2 population under various short-day (SD) conditions. *HT* High temperature

Overall, 24.7% of the individuals/loci were homozygous for the Peiai 64S genotypes, 24.6% of the individuals/loci were homozygous for the 8902S genotypes and the remaining 50.7% were heterozygous, thereby conforming to the expected 1:2:1 proportions. Further examinations showed that 9 of the 119 loci deviated significantly from the expected 1:2:1 segregation ratio, and alleles at 3 of these 9 loci also showed significant deviation from 0.5:0.5 allelic frequencies. This level of segregation distortion is much lower than those observed in a number of previous studies (e.g. Causse et al. 1994; Kurata et al. 1994; Yu et al. 1997).

A linkage map, 1448 cM in length, was constructed based on the segregation data of the F_2 population using MAPMAKER/EXP 3.0, in which 128 of the 129 marker loci were incorporated into 14 linkage groups, with an average interval of 11.2 cM between adjacent marker loci (map not shown). Comparison of the resulting map with the Cornell University (Causse et al. 1994) and RGP (Kurata et al. 1994) maps showed that this map had a reasonably good representation of the rice genome. The locations of the marker loci are essentially the same as those in the Cornell University and RGP maps except for a few cases: (1) an additional locus was detected by each of the 10 probes (postfixed with ”b”, except R1014b); (2) the locations of several loci were different from those in the Cornell University or RGP maps; (3) the locations of a few marker loci from the same chromosomes were exchanged compared with the Cornell and RGP maps, which is likely the result of different resolutions of the mapping populations used in the studies. The map also had poorly represented regions of the genome due to the lack of polymorphism in those chromosomal segments. The main reason for this lack of polymorphisms is that both of these parental lines had Nongken 58S as their parent in the development of PSGMS lines and they shared the major PSGMS genes (see Discussion section).

Dissecting the genetic effects of stability of the male sterility

The fertility levels of a genotype under different long-day treatments were used for measuring stability of male sterility. We analyzed both single-locus QTLs and two-locus interactions to assess the genetic components influencing stability.

QTLs on stability of the male sterility

Table 3 lists the QTLs that were detected by interval mapping under various long-day conditions. Two to four QTLs were resolved for each of the long-day treatments (Table 3), and a total of 7 distinct QTLs were identified

Table 3 QTLs on fertility detected in the F_2 population of (Peiai 64S×8902S) under various long-day (LD) conditions

Environment [a]	QTL	Chromosome	Interval	Additive effect[b]	Dominance effect	Percentage variance explained	LOD
PH-LD-LT (pollen)	*L3a*	3	R1927-C217	8.70	−2.01	5.6	2.29
	L3b	3	C1135-CDO38	−10.30	1.75	8.0	3.39
	L6	6	C235-RZ612	−13.50	−3.72	13.6	5.44
	L7	7	RZ696b-R2677	12.62	−5.50	10.8	4.38
PH-LD-LT (spikelet)	L3b	3	CDO38-RG191	−7.61	−4.78	9.1	2.71
	L5	5	RZ182a-RG480	6.94	−7.45	11.3	3.12
	L6	6	C235-RZ612	−5.68	−1.11	4.4	2.14
	L7	7	RZ696b-R2677	8.42	−3.27	9.5	4.86
Natural LD (YA95)	*L6*	6	C235-RZ612	−13.68	−1.10	18.8	9.59
	L7	7	RZ696b-R2677	8.95	−2.65	8.0	3.84
Natural LD (YA96)	*L2*	2	RG152-RG83	3.76	6.79	4.9	2.17
	L6	6	C235-RZ612	−12.32	−4.19	19.7	5.44
	L7	7	C909a-RZ626	6.39	−0.06	5.3	2.36
	L10	10	RZ561-F1738a	−4.49	3.57	5.4	2.68
Natural LD (WH94)	*L3a*	3	C595-R321	6.88	−0.80	5.8	2.75
	L6	6	C235-RZ612	−11.45	−4.45	16.3	8.00
	L7	7	RZ721-RZ696b	8.39	−2.37	7.7	3.46
Natural LD (WH95)	*L6*	6	RG64-C235	−8.17	−0.47	11.9	5.67
	L7	7	RZ721-RZ696b	9.04	−2.13	8.9	3.36
Natural LD (WH96)	*L6*	6	C235-RZ612	−7.57	−3.73	16.9	6.37
	L7	7	RZ721-RZ696b	5.49	−3.57	8.4	3.62

[a] PH-LD-LT: Phytotron long day/low temperature (>14.5 h/24°C)

[b] Additive effect: a positive number indicates the 8902S genotype to be in the direction of increasing fertility and a negative number indicates the Peiai 64S genotype to be in the direction of increasing fertility

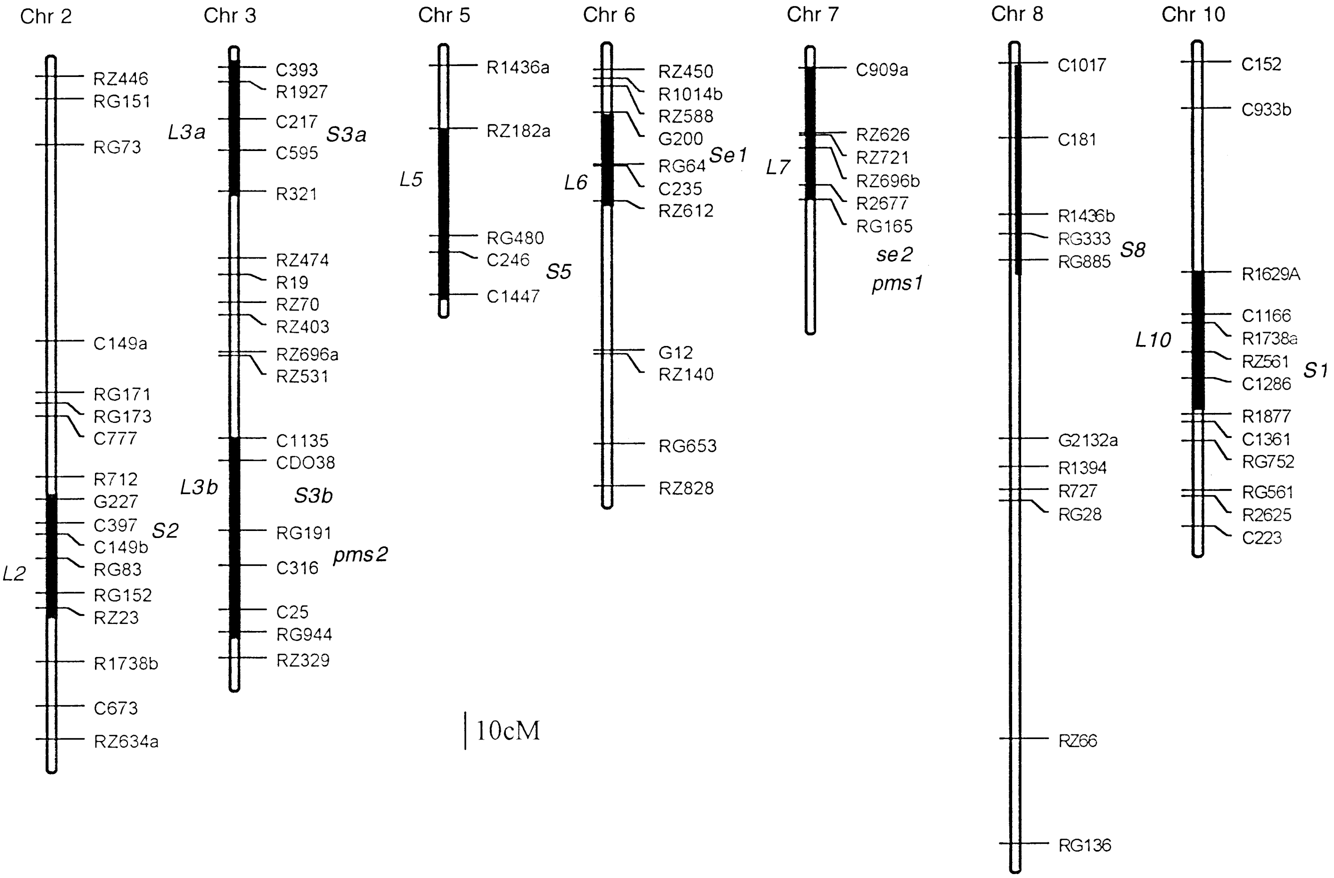


Fig. 4 Distribution of the QTLs on fertility detected under various long-day conditions (ones labeled *L*) und under various short-day conditions (ones labeled *S*). The *black bars* represent the 1-LOD-supporting intervals for the QTLs, which are approximate since the confidence intervals are slightly different for data collected in different environments

based on the 1-LOD-supporting confidence intervals (Fig. 4). Two QTLs, *L6* amd *L7*, were detected in all the environments; *L6* showed consistently a larger effect than *L7* except in the case of seed-setting in the phytotron long-day/low-temperature treatment. Two QTLs, *L3a* and *L3b*, were each detected twice, and the other 3

QTLs, *L2*, *L5* and *L10* were each detected only once. Alleles from 8902S at QTLs *L2*, *L3a*, *L5* and *L7* contributed to the increase in fertility, thereby causing an instability of sterility. Surprisingly, however, the estimated additive effects showed that the Peiai 64S allele at *L6*, an apparent major locus on fertility in this cross, contributed to the increase in fertility in all the long-day treatments. Overall, the proportion of fertility variation explained by the QTLs as a whole was small in all the cases.

Table 4 Numbers of significant digenic interactions (out of 5181 possible two-locus pairs) detected under various long-day (LD) conditions

Environment[a]	Number of interactions	
	$P<0.01$	$0.01<P<0.05$
PH-LD-LT (pollen)	9	57
PH-LD-LT (spikelet)	6	38
Natural LD (YA95)	11	51
Natural LD (YA96)	17	86
Natural LD (WH94)	15	82
Natural LD (WH95)	23	79
Natural LD (WH96)	30	75

[a] PH-LD-LT: Phytotron long day/low temperature (>14.5 h/24°C)

Effects of digenic interactions on stability of male sterility

A search among a total of 5181 possible two-locus combinations resulted in 6–30 pairs of loci that detected significant interaction effects at the 0.01 probability level under various long-day conditions (Table 4). Additionally, 38–86 pairs of loci detected significant interactions at the 0.05 probability level (Tabel 4). Many of the interactions were detected in multiple environments at the 0.01 probability level, among which 9 interactions were repeatedly detected in four or more of the six environmental conditions tested (Table 5). As argued by Yu et al. (1997), simultaneous occurrence of the same interactions under different experimental conditions can be regarded as evidence against false-positives that occurred by chance alone. Thus, these 9 interactions can be regarded as the minimum for the two-locus interactions affecting stability of the male sterility.

The 9 interactions involved two types of two-locus combinations: (1) 2 interactions, each between a QTL and a locus that did not detect significant effect-by-single-locus analysis (non-QTL), RZ182a(5)×RZ561(10) (the numbers in parentheses indicate the chromosomal locations) and C393(3)×RZ612(6), in which RZ612 marked *L6* and RZ561 marked *L10*; (2) 7 interactions,

Table 5 Two-locus combinations that detected significant interaction effects on fertility under various long-day conditions

Interaction	PH-LD-LT		Natural long day				
	Pollen	Spikelet	YA95	YA96	WH94	WH95	WH96
C393×RZ612	+[a]	++	++		++	++	++
Type	AA*	AA*	AA*		AA**		AA**
	AD*	AD**	AD**		AD**	AD*	AD**
C112×RZ602	++	++	++	++	+	++	+
Type	AD**	AD**	AD**	AD**	AD**	AD**	AD**
						DD*	
C181×RG103	+	++	++	++		++	++
Type	AA**	AA**	AA*	AA**		AA**	AA*
							DA*
C909a×R1629		+	++	++	+		+
Type		AA*	AA**				
		DA**	DA*	DA**	DA**		DA*
RZ182a×RZ561			++	++			
Type				AA*			
			AD*	AD**			
			DD**				
RG561×RG103				++	+	+	++
Type				AA**	AA*	AA*	AA**
				AD*		AD*	AD**
						DA*	DA**
C393×RG103					++	++	
Type					AA**	AA**	
					AD*		
RG151×R2625	+	+		+	+	++	+
Type				AA*			
	AD**	AD*		AD*	AD*	AD*	AD*
							DA**
RG472×C393	++		++	+	++	++	+
Type	AA**		AA**	AA*	AA**	AA**	AA**
				AD*	AD**		

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

[a] +, Interaction detected at $0.01<P<0.05$; ++, interaction detected at $P<0.01$

Table. 6 QTLs on fertility detected in the F_2 population of (Peiai 64S×8902S) under various short-day (SD) conditions

Environment[a]	QTL	Chromosome	Interval	Additive effect	Dominance effect explained	Percentage variance	LOD
PH-SD-HT	*S8*	8	C181-R1436b	7.42	–5.92	8.0	2.38
(pollen)	*S10*	10	C1286-RZ561	–10.16	8.37	9.9	3.80
PH-SD-HT	*S8*	8	RG333-RG885	9.25	–1.07	12.2	5.01
(spikelet)	*S10*	10	C1286-RZ561	–6.50	3.08	6.6	3.09
Natural (SD)	*S2*	2	C397-C149b	7.47	5.08	5.7	2.79
(HN96)	*S3b*	3	CDO38-RG191	–15.55	–2.05	20.6	8.25
	S8	8	RG333-RG885	10.28	–0.29	9.7	4.46
Natural SD	*S3a*	3	C595-R321	6.69	–4.57	7.0	2.48
(WH94)	*S3b*	3	CDO38-RG191	–7.37	–0.25	7.2	2.34
	S8	8	RG333-RG885	7.92	–1.22	9.2	3.40
Natural SD	*S3a*	3	R1927-C217	7.19	–2.08	8.5	3.71
(WH95)	*S5*	5	C246-C1447	6.67	1.19	6.9	2.96
	S8	8	RG333-RG885	9.04	1.30	13.3	5.39

[a] PH-SD-HT: Phytotron short day/high temperature (10 h/28°C)

Table 7 Numbers of significant digenic interactions (out of 5181 possible two-locus pairs) detected under various short-day (SD) conditions

Environment[a]	Number of interactions	
	$P<0.01$	$0.01<P<0.05$
PH-SD-HT (pollen)	11	62
PH-SD-HT (spikelet)	8	54
Natural SD (HN96)	5	51
Natural SD (WH94)	17	90
Natural SD (WH95)	12	64

[a] PH-SD-HT: Phytotron short day/high temperature (10 h/28°C)

each involving a pair of non-QTLs, C112(1)×RZ602(4), RG472(1)×C393(3), C393(3)×RG103(11), C909a(7)×R1629(10), C181(8)×RG103(11) and RG151(2)×R2625(10). Thus, overall, the 9 interactions involved 14 marker loci and 2 of the loci, C393 and RG103, were each involved in 3 interactions.

Partitioning of the interactions using orthogonal contrasts revealed three types of interactions between loci: additive by additive (AA), additive by dominance (AD or DA) and dominance by dominance (DD), each of which explained from 2.0% to 11.9% of the genetic variation (data not shown), with an average of 4.5%. Some of the two-locus combinations involved two or more types of interactions. It is also clear from Table 5 that the types of interactions were very consistent across the environments for a given pair of interacting loci. The consistency in the types of interactions under different environmental conditions provided further evidence against the possibility of chance events for the detected interactions.

Dissecting the genetic effects of fertility reversibility

The fertility levels of a genotype under different short-day treatments were used as the measurement of reversibility. Single-locus QTLs and digenic interactions were also analyzed to determine the genetic basis of reversibility.

Effects of QTLs on fertility reversibility

QTLs that had significant effects on fertility under various short-day conditions as determined by interval mapping are presented in Table 6. A total of 6 QTLs were identified across all the environments taking into accout the 1-LOD-supporting intervals (Fig. 4), with 2 or 3 QTLs being detected in each of the environments. One QTL, *S8* located on chromosome 8, was detected under all environmental conditions: the additive effect indicated that the allele from 8902S was in the direction of increasing fertility. Two QTLs, *S3a* and *S3b*, were each detected in two environments; the 8902S genotype at the *S3a* locus was in the direction of increasing fertility, while the Peiai 64S genotype at the *S3b* locus was in the direction of increasing fertility. The remaining 3 QTLs, *S2*, *S5* and *S10*, were each detected under only one of the experimental conditions. The Peiai 64S allele contributed to increased fertility at the *S10* locus, while the 8902S alleles were in the direction of increasing fertility at the other 2 QTLs.

The effects of digenic interactions on fertility reversibility

Two-way analyses of variance using all possible two-locus combinations identified 5–17 pairs of loci showing significant interactions on fertility at the 0.01 probability level under the various short-day conditions (Table 7). Another 51–90 two-locus combinations showed significant interactions at the 0.05 probability level (Table 7). Table 8 lists the 8 interactions that were simultaneously detected in two or more environments, which we assumed to be real interactions using the same argument mentioned in the previous case against false-positives.

Table 8 Two-locus combinations that detected significant interaction effects on fertility under various short-day conditions

Interaction	PH-SD-HT[a]		Natural short day		
	Pollen	Spikelet	HN96	WH94	WH95
C217×RZ404	++[b]	+			
Type	AA**	AA**			
	AD**	AD*			
RZ474×C1135	++	+			
	AA**	AA**			
R2638×CDO920	++	++			
Type	AA*	AA**			
	DA*				
	DD*	DD*			
R1738a×RG536	++	++			
Type	AA*	AA**			
	DA**				
	DD**	DD**			
G359×C25				++	++
Type				AA*	AD*
				DA**	DA**
				DD**	
R1436a×RG333			++	++	++
Type			AA**		
					DA*
			DD*	DD**	DD**
C25×RZ696		+	++		
Type		AA**	AA**		
		DA*			
			DD*		
RZ450×RG151	+			+	++
Type	AA**			AA**	AA**

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

[a] PH-SD-HT: Phytotron short day/high temperature (10 h/28°C)

[b] +, Interaction detected at $0.01 < p < 0.05$; ++, interaction detected at $P < 0.01$

Of the 8 interactions 4 were each between at QTL and a non-QTL: R1738a (*S10*)×RG536(1), G359(1)×C25(*S3b*), R1436a(5)×RG333(*S8*) and C25 (*S3b*)×RZ696(7). The outher four interactions occurred between non-QTLs.

Partitioning of the interactions into individual components revealed that most of the two-locus combinations involved two or three types of interactions including AA, AD and DD (Table 8), each of which explained from 2.5% to 10.8% of the genetic variation (data not shown), with an average of approximately 4.2%.

Discussion

The major genes for photoperiod-sensitive male sterility in 8902S and Peiai 64S are allelic

One conclusion that can be reached based on the data of this study is that the major genes for photoperiod-sensitive male sterility in the two parents, Peiai 64S and 8902S, are allelic with each other. This is clearly indicated by the fact that both parents, F$_1$ and F$_2$ are completely sterile under phytotron-controlled long-day/high-temperature conditions (Table 1 and Fig. 1). Complete sterility of both parents and F$_1$ was also observed during specific certain periods under natural long-day conditions in Wuhan (Fig. 2). Additional evidence was provided by the lack of polymorphisms in certain genomic regions where the genes for photoperiod-sensitive male sterility were mapped, i.e. *pms1* on chromosome 7 (Q Zhang et al. 1994; Mei et al. 1999) and *pms3* on chromosome 12 (Mei et al. 1998). Polymorphisms was not detected between the parents on the half of chromosome 7 containing the *pms1* locus, and a large gap was present in the *pms3* region on chromosome 12; in both of these regions the RFLP genotypes of the two parents are the same as that of the PSGMS gene donor parent (Nongken 58S. This suggests the possibility that these are most likely the loci conditioning photoperiod-sensitive male sterility in the two parents.

The genetic basis of unstable male sterility

The analyses of the F$_2$ data obtained over years and locations detected two types of genetic effects influencing the male sterility, i.e. single-locus QTLs and two-locus interactions. The numbers of QTLs detected range from 2 to 4 under various long-day conditions, while the numbers of loci involved in the interactions are much larger, and most of the these were not detectable on a single-locus basis. It should be pointed out that the genetic components resolved in the F$_2$ analyses are much more complex than those present in real PSGMS lines. This is because the dominant type of genetic components including dominance detected by single-locus analysis and interactions involving dominance, such as additive-by-dominance and dominance-by-dominance effects resolved by digenic analyses, would not play any role in homozygous lines. Consequently, only the additive components, including single-locus additive effects and additive-by-additive interactions between loci, are relevant as the genetic basis underlying fertility changes in homozygous lines.

At the single-locus level, additive effects of alleles from Peiai 64S contributed to stable sterility at some loci but to instability at other loci, and the reverse was the case for alleles from 8902S. However, *L6*, the QTL that was detected as having the largest effect on fertility on a single-locus basis, was involved in an interaction with a second locus, in which the tendency of the Peiai 64S genotype at the *L6* locus toward instability was inhibited by the Peiai 64S genotype of the other locus.

At the two-locus level, genotypes homozygous for the Peiai 64S alleles at both loci (11/11) showed low or the lowest fertility among the 9 genotypes in most of the interacting two-locus combinations. In certain cases, however, 11/22 (or even 22/22) genotypes of some two-locus combinations also produced the lowest fertility, indicating that the double homozygote 11/11 is not necessarily the most stable genotype in all two-locus combinations. Conversely, genotypes that expressed the highest fertility (most unstable) are not necessarily the ones that are doubly homozygous for the 8902S alleles. They can be any two-locus allelic combination, such as 11/22 (or 22/11), 22/11), 22/22 or even 11/11, depending on the loci involved, and the complementary homozygotes 11//22 (or 22/11) were more frequently observed than the others to be the most unstable genotypes. It should also be pointed out that there may be higher-order intactions as indicated by the fact that 2 loci (RG103 and C393) were each involved in 3 interactions, and they also interacted with each other. Unfortunately, the data from the present experiment did not allow for assessing high-order interactions. Nonetheless, it is clear, based on this analysis, that the genetic basis of the unstable sterility is a complex interplay of additive effects of single-locus QTLs and additive-by-additive interactions of two-locus combinations.

Genetic basis of fertility revesibility

The analyses of the F_2 data obtained over years and locations also detected 2 or 3 QTLs and a number of two-locus interactions under various short-day conditions. Comparison of Tables 3 and 5 with Tables 6 and 8 revealed that the loci (both single-locus QTLs and two-locus interactions) detected under long-day conditions are more repeatable across environments than those detected under short-day conditions. This is consistent with the extent of relative similarities among the experimental environments in which fertility was examined. For all long-day treatments, the day-length regimes were approximately the same in the phytotron and under natural conditions in Wuhan and Yuan’an, whereas for the short-day treatments, the phytotron and the natural conditions of Wuhan and Hainan each represented a different day-length regime. However, there is still quite a bit of commonality in the genetic components detected under the various experimental conditions, especially under those conditions that are more similar to each other.

The most noticeable commonality is the detection of the QTL *S8* in all of the environments. Another remarkable feature is the prevalence of dominant types of interactions, including AD (or DA) and DD, that played a major role in fertility of the F_2 population, although these types of interactions are not relevant to the performance of the parental lines.

Insofar as the additive genetic components are concerned, the 2 QTLs, *S10* and *S3b*, which displayed “opposite” effects in which the 8902S genotypes showed reduced fertility, were each involved in an interaction. And the most important QTL, *S8*, was also found to be involved in an interaction under all three natural short-day conditions. At the two-locus level, the genotypes that showed the highest fertility were, in the majority of two-locus combinations given in Figs. 6 and 7, the complementary homozygotes either 11/22 or 22/11, and only occasionally did the genotype homozygous for the 8902S alleles at both loci (22/22) show the highest fertility. Thus, the genetic basis of fertility reversibility also appears to be the joint effects of single-locus QTLs and epistatic interactions.

The possible nature of the genes for instability of male sterility and fertility reversibility

Stability of male sterility requires the genotype to be insensitive to temperature fluctuations under long-day conditions, while reversibility of fertility requires the genotype to be responsive to short-day induction Z.G. Zhang et al. (1994) observed that, for every PSGMS line they studied, there is certain range of temperature for effective photoperiod induction, above which the line will be sterile and below which the line will be fertile regardless of the length of photoperiod. They referred to the upper and lower temperature limits for effective photoperiod induction as the critical sterile point (CSP) and critical fertile point (CFP), respectively. In the light of this observation, these two lines have very different temperature regimes for fertility induction. Peiai 64S is low in both CSP and CFP; hence it has a narrow temperature range for photoperiod induction of male fertility, which makes it unable to respond to photoperiod changes. This type of PSGMS line shows stable sterility under natural long-day conditions, but it is difficult to reverse to fertility. In contrast, 8902S has both high CSP and CFP, hence it has also a narrow temperature range for photoperiod induction of male sterility, which makes the sterility of the line very sensitive to temperature fluctuations. This type of male-sterile line would be easy to reverse to normal fertility under natural short-day conditions, but its sterility under long-day conditions is unstable.

Thus, given the same major genes for photoperiod-sensitive male sterility, the genetic components resolved under long-day conditions are, in theory, those governing the temperature response of different genotypes, and the effects detected under various short-day conditions can be viewed as genetic components related to responsiveness of the genotypes to day-lenth changes. The differences in the genetic components detected in the two analyses should be considered as reflections of such distinctions, which included the QTLs with relatively large effects and almost all the interactions. It should be noted that the phenomenon of day-length response controlling the reversibility of fertility is not the same as the photoperiod response that regulates the transfer from vegetative phase to reproductive phase, as pointed out by Yuan et al. (1993), who hypothesized that there are two photoperiodic reactions in PSGMS rice. The commonality in the genetic basis of the two photoperiodic reactions remains to be characterized.

In addition to day-length response, the fertility of the genotypes under short-day conditions is also subject to temperature modifications, which is essentially the same as the response of fertility to temperature fluctuations under long-day conditions. This type of fertility changes should have similar genetic bases for long-day and short-day conditions. This expected similarity may provide the explanation for the QTLs that were detected under both long-day and short-day conditions.

Implications of the findings in PSGMS rice breeding

The present findings may have significant implications for the development of PSGMS lines that would show the desired properties of the PSGMS rice, i.e. completely sterile under long-day conditions and highly fertile under short-day conditions, non-responsive to temperature fluctuations. The complexity of the genetic bases of both sterility instability and fertility reversibility suggests that there is no simple way to improve either the reversibility of Peiai 64S-type PSGMS lines or the stability of 8902S-type PSGMS lines, as in both cases the breeders have to manipulate a large number of loci involving single-locus QTLs and epistatic interactions. A further complication may result from the fact that there is a certain degree of overlapping in the genetic components controlling fertility under short-day and long-day conditions, which means that breeders probably have to sacrifice a certain amount of reversibility of fertility in order to gain more stability of sterility.

In summary, we conclude that the genetic bases are complex for both stability of male sterility under long-day conditions and reversibility of fertility under short-day conditions, both involving single-locus QTLs and epistatic interactions. The cause for the instability of sterility under long-day conditions is the sensitivity of the genotypes to temperature fluctuations, and that for the reversibility of fertility under short-day conditions is the responsiveness to short-day conditions. The complexity of the genetic bases indicates both the difficulty in improving PSGMS lines and also the need for developing strategies for utilizing information from marker-based genetic analyses.

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