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Genetic basis of low-temperature-sensitive sterility in indica-japonica hybrids of rice as determined by RFLP analysis

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Abstract Low-temperature-sensitive sterility (LTSS) has become one of the major obstacles in indica-japonica hybrid rice breeding. In this study, we determined, using RFLP markers, the genetic basis of LTSS in two populations derived from crosses between indica and japonica parents, the BC_1F_1 of 3037/02428//3037 and the F_2 of 3037/02428. The fertility segregation in the two populations under low-temperature conditions was used as a measurement of the temperature sensitivity of the various genotypes in the populations. A RFLP survey of bulked extremes from the BC_1F_1 population identified three genomic regions, two on chromosome 1 and one on chromosome 12, that were likely to contain genes for LTSS (or *Ste* loci). One-way ANOVA and QTL analysis using a total of 19 markers from these three genomic regions resolved three *Ste* loci in the BC_1F_1 population and two *Ste* loci in the F_2 population. On the basis of chromosomal location these loci were distinct from those governing wide-compatibility identified in previous studies. Two- and three-way ANOVA showed that these loci acted essentially independent of each other in conditioning LTSS. The main mode of gene action was an interaction between the indica and the japonica alleles within each locus. For each respective locus this resulted in a drastic fertility reduction in the heterozygote state relative to the homozygote state. The results have significant implications in indica-japonica hybrid rice breeding programs.

Key words *Oryza sativa* · Hybrid sterility · *Ste* loci · Molecular markers · Hybrid rice breeding

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Introduction

The practices of exploiting the strong heterosis in crosses between indica (*Oryza sativa* ssp. *indica*) and japonica (*O. sativa* ssp. *japonica*) rice have confronted a number of biological constraints that have been imposed as a consequence of the extensive genetic differentiation between the two rice groups, which are frequently referred to as two subspecies (Oka 1988; Zhang et al. 1992, 1997). One of the most important constraints is the poor fertility of the hybrids that has been observed in the majority of the indica-japonica crosses (Kato et al. 1928; Oka 1988). This constraint has now been at least partly overcome with the finding of wide-compatibility varieties (WCVs) that are able to produce fertile hybrids when crossed to both indica and japonica varieties (Ikehashi and Araki 1984; Ikehashi and Araki 1986). This finding has enabled the development of a large number of indica-japonica hybrids in recent years, promising significant yield advantages over intra-subspecific hybrids.

However, it was soon realized that the fertility of many indica-japonica hybrids is very sensitive to temperature fluctuations; hybrids containing the wide-compatibility gene (the S_5^n allele at the S_5 locus) have normal fertility under high-temperature conditions, but the fertility may be greatly reduced under somewhat lower temperature conditions which do not affect the fertility of the parents. For example, significant yield loss was observed in a newly released indica-japonica hybrid, Yayou 2, due to the cool summer of 1989 in Jiangsu Province, China (Li and Zhang 1993). Spikelet

fertility below 50% was observed in indica-japonica hybrids in Japan as a consequence of several consecutive cool days in the summer of 1993 (Ikeda 1994). It was shown that an average daily temperature that falls below 24°C at the booting stage can result in a significant fertility reduction in some indica-japonica hybrids (Hong et al. 1993; Li and Zhang 1993; Qi 1993).

Recently, we (Li et al. 1996) conducted a genetic analysis of the low-temperature-sensitive sterility (abbreviated as LTSS) of indica-japonica hybrids by evaluating the fertility segregation of two F_2 and one BC_1F_1 populations under both low- and high-temperature conditions. The fertility distribution in the segregating populations suggested two possible models as the genetic basis of the LTSS. The first was a two-locus complementary model in which the low-temperature sensitivity of the hybrids is due to complementary action of dominant alleles at two loci, with the indica and japonica parents each homozygous for a dominant allele at a different locus. The alternative was a "heterozygote disadvantage" model in which heterozygotes at certain loci cause fertility reduction under low-temperature conditions. However, because of the frequent occurrence of distorted segregation in indica-japonica crosses, which has been observed in many studies (e.g. Lin et al. 1992; Wang et al. 1994), it is not reliable to test genetic models based on the phenotypic ratios in indica-japonica crosses.

In the study reported in this paper, we used molecular markers to characterize the genetic basis of LTSS in indica-japonica hybrids as a follow-up of our previous work. The specific objectives were: (1) to determine the number and chromosomal locations of loci involved in LTSS; (2) to identify the modes of inter- and intra-locus gene actions and (3) to evaluate the genetic effects of the genes at each locus based on the model determined by the data analysis of the present study.

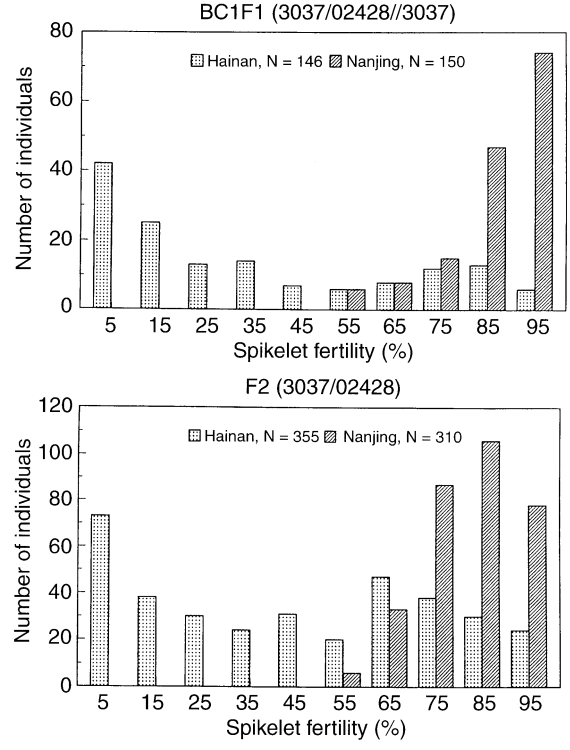

Fig. 1 Distribution of spikelet fertility in the BC_1F_1 and F_2 under two different plantings. Nanjing planting provided high-temperature conditions, and Hainan planting provided low-temperature conditions (see Li et al. 1996 for details)

Materials and methods

The experimental populations

Two populations were used in this study: the BC_1F_1 of 3037/02428//3037 and the F_2 of 3037/02428. One parent, '3037', is an indica variety and the other parent, '02428', a WCV of japonica type. These two varieties are the parents of Yayou 2, one of the first indica-japonica hybrids released for commercial production in China. The details of field planting and fertility examinations were described by Li et al. (1996). Briefly, the temperature sensitivity of individuals in the segregating populations was determined by examining the fertility under both high- and low-temperature conditions. Because hybrid fertility is sensitive only to low-temperature conditions, fertility in the BC_1F_1 and the F_2 populations did not segregate under high-temperature (permissive) conditions but segregated under low-temperature (restricted) conditions (Fig. 1). Thus, the fertility scores in the BC_1F_1 and F_2 under low-temperature conditions provided a measurement for low-temperature sensitivity of hybrid fertility that was used in the genetic analysis of LTSS in this study.

RFLP assay

DNA was extracted from fresh leaves harvested from each plant using the method described previously (Zhang et al. 1992). Southern blot hybridization followed the procedures of Liu et al. (1997).

Using bulked extremes to identify chromosomal regions containing *Ste* genes

The detection of markers possibly linked to LTSS genes (abbreviated as *Ste* hereafter) followed essentially the procedures described by Zhang et al. (1994). Two bulks, bulk F and bulk S, were made by pooling equal amounts of DNA from 30 highly fertile and 30 highly sterile individuals, respectively, from the BC_1F_1 population grown under low-temperate conditions. The two bulks and the two parents were surveyed for DNA restriction fragment length polymorphisms (RFLPs) by being digested with six restriction enzymes and probed with a total of 114 cloned DNA fragments from the Cornell group and the Japanese Rice Genome Mapping Project (Causse et al. 1994; Kurata et al. 1994). Selection of these probes was based on the results of a previous survey (Liu et al. 1997); these polymorphic markers covered at least 93% of the rice genome based on a measurement that the distances between adjacent markers on the linkage maps were not to be greater than 30 cM.

Although the genetic basis of LTSS had not been characterized prior to the data analysis of the present study, it could still be expected that the banding patterns of the two bulks would be the same if a marker was not linked to an *Ste* locus and that banding

patterns would differ between the two bulks if a marker is linked to the targeted *Ste* locus (positive marker).

Determining the modes of gene actions

Samples of 121 and 161 individuals from the BC_1F_1 and the F_2 populations, respectively, grown under low-temperature conditions were assayed with each of the positive markers. More markers were added to the assay until the targeted genomic regions had reasonably good coverage by molecular markers. The presence of a *Ste* locus in each of the regions represented by positive markers was assessed with one-way ANOVA using marker genotypes as the groups. Local linkage maps of the targeted genomic regions were constructed using MAPMAKER (Lincoln et al. 1992a). The approximate location of the *Ste* locus was determined using MAPMAKER/QTL (Lincoln et al. 1992b). Further analysis of gene actions and genetic effects of the *Ste* loci was based on the marker that was most closely linked to the targeted *Ste* locus in each region.

Results

The chromosomal locations of the *Ste* loci

A survey of the bulked extremes detected banding pattern differences between the two bulks by 5 markers from three chromosomal regions. Two markers (RZ566 and RG236) were from the distal end of the long arm of chromosome 1 according to the map of Singh et al. (1996), which we referred to as region A for ease of description. One marker (RZ154) was from the middle of the long arm of chromosome 1 (region B). The remaining 2 markers (RG98 and RZ816) were from the short arm of chromosome 12. These positive markers indicated the possibility that each of the three genomic regions contains a gene for LTSS.

These 5 positive markers together with 14 additional markers from the three genomic regions were used for RFLP assay of the 121 random individuals from the BC_1F_1 and 161 individuals from the F_2. Results of one-way ANOVA of the fertility scores in both the BC_1F_1 and the F_2 for some of the representative markers are given in Table 1. Significant effects were detected by markers from all three regions in the BC_1F_1 population, whereas in the F_2 population only two regions showed significant effects, and no effect was detected by markers from region A of chromosome 1.

To confirm the presence and also to determine the locations of the *Ste* loci, we conducted quantitative trait loci (QTL) analysis based on the local linkage maps of the three genomic regions constructed using MAPMAKER analysis (Fig. 2). QTL analysis of the BC_1F_1 population revealed three peaks that corresponded well with genomic regions A and B on chromosome 1 and the third region on chromosome 12 (Fig. 2). The highest peak (LOD score 9.80) appeared in the 6.1-cM interval between RZ154 and R2635, corresponding to region B on chromosome 1, followed by the peak (LOD 5.19) in the 6.1-cM interval between RZ566 and RG236, corresponding to region A of chromosome 1. The peak on chromosome 12, located in the 3.4-cM interval between C732 and RG98, was lower (LOD 4.20). These three loci individually explained 32.1%, 19.4% and 16.9% of the phenotype difference and jointly accounted for 64.9% of the fertility variation in the BC_1F_1 population.

In the F_2 population, only two peaks were detected by MAPMAKER/QTL, similar to the results of the one-way ANOVA. One peak, with a LOD score of 5.92, was in the 6.1-cM interval between RZ154 and R2635 on chromosome 1, corresponding very well with the locus resolved in the BC_1F_1. The other peak, with LOD 4.90, was located in the 3.3-cM interval between C732 and RG98, and also coincided with the locus detected in the BC_1F_1. These two loci, explaining 17.0% and 14.0% of the phenotypic variance, respectively, accounted for 30.4% of the total fertility variation in this F_2 population.

Thus, our analyses identified three distinct loci for LTSS of the indica-japonica hybrids. We tentatively designate these three loci as *Ste1* for the locus in region B of chromosome 1, *Ste2* for the locus on chromosome 12 and *Ste3* for the locus in region A of chromosome 1, in a descending order of effect.

Table 1 One-way ANOVA of hybrid fertility in BC_1F_1 of 02428/3037//3037 and F_2 of 02428/3037 using representative markers from each of the three genomic regions

Locus	Chromosomes	MS effect[a]		MS error		*F*		*P*	
		BC_1F_1	F_2	BC_1F_1	F_2	BC_1F_1	F_2	BC_1F_1	F_2
RZ566	1 (A)	19 912	22	809	759	24.59	0.02	0.00	0.98
C225	1 (A)	13 949	160	860	955	16.22	0.17	0.00	0.85
RZ154	1 (B)	35 972	6 360	675	877	53.30	7.25	0.00	0.01
R2635	1 (B)	32 612	11 782	703	808	46.38	14.58	0.00	0.00
C362	12	15 901	7 552	844	862	18.85	8.76	0.00	0.00
C732	12	15 901	9 485	844	837	18.85	11.40	0.00	0.00
C104	12	15 901	9 390	844	839	18.85	11.20	0.00	0.00
RG98	12	17 667	9 029	829	843	21.32	10.71	0.00	0.00

[a] Error degrees of freedom are 119 and 158 for BC_1F_1 and F_2, respectively

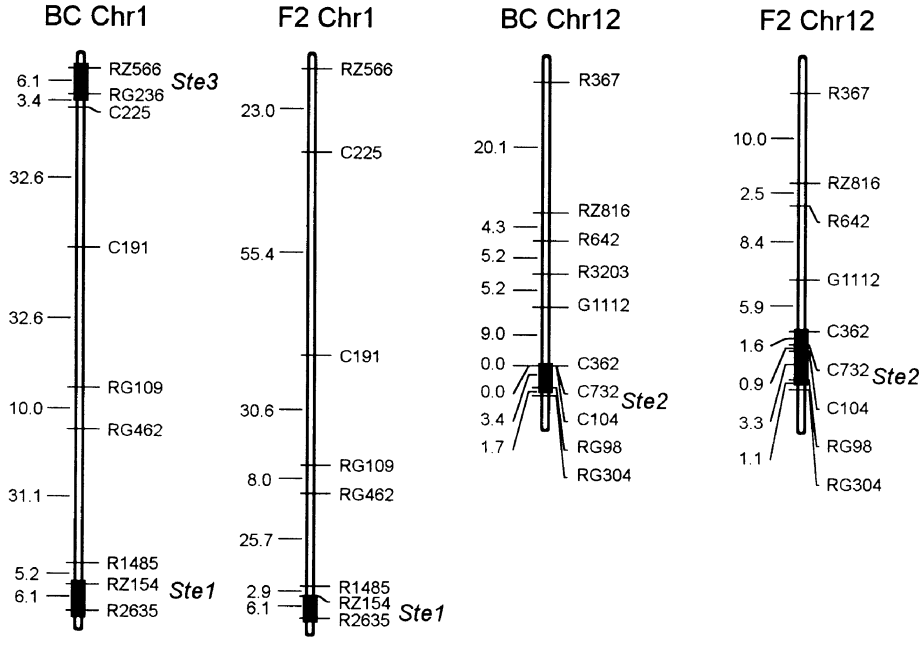

Fig. 2 The locations of the *Ste* loci in the local linkage maps of three genomic regions on chromosomes 1 and 12

The modes of interactions between loci

The marker closest to the *Ste* locus from each region was used for characterizing the modes of gene actions and genetic effects. A three-way ANOVA of the BC_1F_1 (Table 2) detected a significant interaction between *Ste1* as marked by R2635 and *Ste2* as marked by RG98. However, this interaction effect was very small relative to the main effects of the three loci. A two-way ANOVA of the F_2 (Table 3) using the same two marker loci did not detect interaction between loci. Thus, these three loci acted essentially independent of each other in conditioning LTSS.

The modes and effects of allelic interactions within loci

The average seed setting rates based on marker genotypes are given in Table 4 for the three *Ste* loci detected in the BC_1F_1, and in Table 5 for the two *Ste* loci detected in the F_2. It is clear that, in both populations, the heterozygote at each locus had a lower fertility than the homozygote regardless of the genotypes of the other loci. Thus, LTSS is mainly the result of

Table 2 A three-way ANOVA of hybrid fertility in BC_1F_1 based on genotypes of the marker locus that is most closely linked to the *Ste* locus from each of the three genomic regions

Effect	MS	*df*	*F*	*P*
1(R2635)	21639	1	46.8	0.00
2(RG98)	12568	1	27.2	0.00
3(RZ566)	14690	1	31.8	0.00
1 × 2	2810	1	6.1	0.02
1 × 3	1216	1	2.6	0.11
2 × 3	716	1	1.5	0.22
1 × 2 × 3	168	1	0.4	0.55
Error	462	113		

Table 3 A two-way ANOVA of hybrid fertility in the F_2 based on genotypes of the marker locus that is most closely linked to the *Ste* locus from each of the two genomic regions detected in this population

Effect	MS	*df*	*F*	*P*
1(R2635)	7680	2	10.3	0.00
2(RG98)	6091	2	8.2	0.00
1 × 2	849	4	1.1	0.34
Error	744	152		

Table 4 Seed-setting rate for each of the eight three-locus genotypes based on molecular markers in the BC_1F_1 population

RZ566		11		12		Average
RG98		11	12	11	12	
R2635	11[a]	79.38 (18)[b]	47.76 (16)	46.07 (14)	19.85 (11)	51.75 (59)
	12	38.05 (11)	14.85 (13)	19.65 (17)	10.97 (21)	18.91 (62)
Average		63.70 (29)	32.88 (29)	31.58 (31)	13.93 (32)	

[a] 11, homozygote for the allele from '3037' (indica parent); 12, heterozygote for the alleles from '3037' and '02428' (japonica type of WCV)
[b] The number in parenthesis is the number of individuals in each genotypic class

Table 5 Seed-setting rate for each of the nine two-locus genotypes based on molecular markers in the F_2 population

		RG98			
		11	12	22	Average
R2635	11[a]	51.62 (29)[b]	50.49 (20)	67.81 (10)	53.98 (59)
	12	36.95 (21)	22.44 (48)	43.11 (11)	29.09 (80)
	22	63.91 (7)	32.28 (10)	71.16 (5)	51.18 (22)
Average		47.73 (57)	30.89 (78)	58.00 (26)	

[a] 11, homozygote for the allele from '3037' (indica parent); 22, homozygote for the allele from '02428' (japonica type of WCV); 12, heterozygote for the alleles from the two parents
[b] The number in parenthesis is the number of individuals in each genotypic class

interaction between the indica and japonica alleles within each locus.

The amounts of fertility reduction caused by heterozygosity varied with the *Ste* loci and also with the populations. In the BC_1F_1 population, the fertility of heterozygote at the *Ste1* locus was 32.84% lower than that of the homozygote; approximately a 25% fertility difference was detected between homozygotes and heterozygotes at the other two loci. In the F_2 population, approximately 24% and 20% fertility reduction was detected in the heterozygotes as compared to homozygotes at *Ste1* and *Ste2*, respectively.

Discussion

Using molecular markers that covered almost the entire rice genome, we identified three major loci, designated as *Ste1*, *Ste2* and *Ste3*, respectively, that conditioned LTSS of indica-japonica hybrids. This is clearly a set of loci different from those governing wide compatibility based on their genomic locations (Ikahashi and Araki 1986; Liu et al. 1997), which also corroborates the results of our previous analysis based on morphological markers (Li et al. 1996). There is little interlocus interaction between the loci resolved in each population; the loci acted more or less independently of each other in conditioning hybrid fertility in both populations. We further determined that the main mode of gene actions at all three loci is a drastic fertility reduction of the genotype heterozygous for the indica and japonica alleles at each locus as compared to homozygotes of the respective loci. These modes of inter- and intra-locus interactions are similar to those causing indica-japonica hybrid sterility in general (Ikehashi and Araki 1986; Wan et al. 1996; Liu et al. 1997).

The results of the present study have significant implications in indica-japonica hybrid rice breeding programs. It was once hoped that the finding of the wide-compatibility gene would break up the fertility barrier between the two subspecies, hence enabling the development of intersubspecific hybrids. However, it was soon realized that there is a large variation in the fertility level of progenies from the same WCV when crossed to different indica or japonica varieties (Gu et al. 1993; Liu et al. 1996). This fertility variation was most likely due to the involvement of additional minor loci for indica-japonica hybrid sterility, which jointly could cause partial sterility even in the presence of the WCG (Liu et al. 1997). The results of the present study revealed on additional complexity for breeding indica-japonica hybrids in that the LTSS is also conditioned by multiple genes which are variable with respect to their presence and numbers in different populations.

Taken together, it is clear that in order to obtain normal fertility the indica-japonica hybrids need to have the WCG at the S_5 locus, the "right" genotypes at the loci for indica-japonica hybrid fertility under high-temperature conditions and also the "right" genotypes at the LTSS loci as identified in the present study. Thus, for fertility alone, breeders have to manipulate potentially a large number of loci whose genotypes are difficult to deduce based on the phenotypes. Apparently, it is necessary to reconsider the breeding strategy and selection methods to take into account the genetic complexity of the hybrid sterility for the development of indica-japonica hybrids.

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