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A genome-wide analysis of wide compatibility in rice and the precise location of the S_5 locus in the molecular map

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Abstract The discovery of wide-compatibility varieties (WCVs) that are able to produce normal fertility hybrids when crossed both to indica and japonica rice has enabled the fertility barrier between indica and japonica subspecies to be broken and provided the possibility of developing inter-subspecific hybrids in rice breeding programs. However, a considerable variation in the fertility level of hybrids from the same WCV crossed to different varieties has often been observed. One hypothesis for this variable fertility is that additional genes are involved in hybrid fertility besides the wide-compatibility gene (WCG). To assess such a possibility, we performed a genome-wide analysis by assaying a large population from a three-way cross '02428'/'Nanjing 11'//'Balilla' using a total of 171 RFLP probes detecting 191 polymorphic loci distributed throughout the entire rice linkage map. Our analysis recovered 3 loci conferring significant effects on hybrid fertility. The major locus on chromosome 6 coincided in chromosomal location with the previously identified S_5 locus, and the 2 minor loci that mapped to chromosomes 2 and 12, respectively, were apparently distinct from all previously reported hybrid sterility genes. Interaction between the indica and japonica alleles at each of the loci caused a reduction in hybrid fertility. The joint effect of the 2 minor loci could lead to partial sterility even in the presence of the WCG. The location of the S_5 locus on the molecular marker linkage map was determined to be approximately 1.0 cM from the RFLP locus R2349. This tight linkage will be useful for marker-aided transfer of the WCG in hybrid rice breeding and for map-based cloning.

Key words *Oryza sativa* L · Indica and japonica · Hybrid sterility · Mapping · Rice breeding

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Introduction

Wide-compatibility varieties (WCVs) are a special class of rice germplasm that are able to produce fertile hybrids when crossed to both indica and japonica rice (Ikehashi and Araki 1984). Hybrids between indica and japonica varieties usually show partial sterility (Kato et al. 1928). The discovery of WCVs (Ikehashi and Araki 1984) has brought breeders hope for breaking the fertility barrier between indica and japonica subspecies and provided the possibility of exploiting the very strong heterosis demonstrated in crosses between the two subspecies.

Based on the results of their inheritance studies, Ikehashi and Araki (1986) also proposed a genetic model to account for wide compatibility. According to this model, there are three alleles at the S_5 locus: a neutral allele, S_5^n; an indica allele, S_5^i, and a japonica allele, S_5^j. A zygote formed of the S_5^n allele with either of the other two alleles, $S_5^nS_5^i$ and $S_5^nS_5^j$, would be fully fertile, while a zygote genotypically $S_5^iS_5^j$ would be partly sterile. Ikehashi and Araki (1986) also determined, using morphological markers, that the S_5 locus is located on chromosome 6. This chromosomal location has been confirmed in several studies using isozymes (Li et al. 1991) and RFLP (restriction fragment length polymorphism) markers (Liu et al. 1992; Zheng et al. 1992; Yanagihara et al. 1995).

In fertility analyses of many indica-japonica hybrids, a considerable variation in the fertility level in hybrids from the same WCV crossed to different indica or

japonica varieties has often been observed (Gu et al. 1993; Liu et al. 1996). One hypothesis for this variable fertility is that additional genes are involved that modify hybrid fertility in the presence of the wide-compatibility gene (WCG), since a large number of genes exist that affect hybrid fertility in one way or another (Kinoshita 1995). On the other hand, all of the linkage analyses reported so far have been based only on markers from chromosome 6 and did not provide data for assessing the possibility that additional hybrid sterility loci may occur in other parts of the genome. It is therefore necessary to adopt a genome-wide approach to determine whether or not loci that may significantly influence the effects of WCG are indeed present in the genome. Moreover, the precise map location of the S_5 locus is still not quite certain because of difficulties experienced in previous studies in classifying individual plants into discrete fertile versus sterile classes (e.g. Liu et al. 1992).

The study reported in this paper was undertaken to assess, using a whole-genome approach, the presence of additional loci for hybrid fertility in a three-way cross involving a WCV. We also determined the precise location of the S_5 locus in the RFLP linkage map using data only from highly fertile individuals in the segregating population.

Materials and methods

Experimental population and fertility scoring

A three-way cross, '02428'/'Nanjing11'//'Balilla', was made in the summer rice growing season of 1994 in Nanjing and off season in the winter of 1994–1995 in Hainan Island. '02428' is a japonica WCV widely used in hybrid rice breeding programs in China. 'Nanjing 11' is an indica variety developed by Jiangsu Academy of Agricultural Sciences in Nanjing, and 'Balilla' is a japonica variety introduced from Italy. Both 'Nanjing 11' and 'Balilla' have been used as testers for screening WCVs in Chinese rice breeding programs. This three-way cross is the same as the one used by Liu et al. (1992) for mapping the WCG, except that the order of parents in their cross was '02428'/'Balilla'//'Nanjing 11'. The reason for changing the order of the parents in the cross is that the level of polymorphism between '02428' and 'Nanjing 11' is much higher than that between '02428' and 'Balilla'. Consequently such change greatly increased the level of effective polymorphism in the mapping population because only the polymorphic loci between first two parents would be segregating in the mapping population with the third parent being merely a tester.

Three hundred and fifty-six F_1 plants from this cross were planted in the 1995 rice growing season in the experimental fields of Huazhong Agricultural University in Wuhan. Spikelet fertility was scored as seed setting rate on the upper halves of two to five panicles for each plant. Two-hundred and thirty seven plants were randomly selected for RFLP analysis.

RFLP assay

Exactly 8 g of fresh leaf tissue was harvested from each plant and ground to fine powder under liquid nitrogen. Total cellular DNA was extracted following essentially the method of Murray and Thompson (1980).

As a routine procedure, about 2.5 μg DNA for each sample was digested with 5–8 units of a restriction endonuclease. The digests were separated in 0.6% agarose gels and blotted onto Hybond N^+ membrane (Amersham) with 0.4 N NaOH. Six new blots containing DNA samples of the three parents and 237 F_1 plants were prehybridized for about 10 h in a bag containing 15 ml hybridization buffer and then hybridized for 8–12 h in the same hybridization buffer containing the probe prepared using the random hexamer labeling method (Feinberg and Vogelstein 1983). The prehybridization time was reduced to 0–6 h for reprobing used blots.

For surveying the parental polymorphisms, the DNA samples of the three parents were digested with each of six restriction enzymes: *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII. The probes screened included a total of 400 clones from Cornell University (Causse et al. 1994), the Japanese Rice Genetic Mapping Project (Kurata et al. 1994), the North America Barley Mapping Project (Saghai Maroof et al. 1996), the North America Maize Mapping Project (Coe et al. 1995) and a few randomly amplified polymerase chain restriction (PCR) fragments. Over 300 probes detected polymorphisms between the parents, of which 170 probes detecting 191 segregating loci were used in assaying the mapping population.

RFLP data processing and statistical analysis

For a given locus, only the bands polymorphic between '02428' and 'Nanjing 11' would segregate in the three-way cross population, and the band from the third parent, 'Balilla', would appear in all individuals. Therefore, the RFLP data were scored using the scheme of backcross population for map construction and QTL (quantitative trait locus) analysis; individuals carrying the '02428' allele were scored as homozygotes, and those carrying the 'Nanjing 11' allele were scored as heterozygotes.

A linkage map of the RFLP markers was constructed using MAPMAKER/EXP 3.0 at a LOD score of 3.0 (Lincoln et al. 1992). QTLs controlling hybrid fertility were determined by interval mapping using MAPMAKER/QTL 1.0 at a LOD threshold of 3.0 (Lincoln et al. 1992).

Determining the precise map location of the S_5 locus

Parental polymorphisms were further surveyed by digesting the DNA samples with an additional 17 restriction enzymes (*Bcl*I, *Bgl*I, *Cla*I, *Kpn*I, *Pst*I, *Sca*I, *Sma*I, *Xba*I, *Xho*I, *Alu*I, *Hae*II, *Hae*III, *Hha*I, *Hpa*II, *Rsa*I, *Sau*3AI and *Taq*I) and hybridizing with all available rice probes from the chromosomal block surrounding the S_5 locus in the two high-density maps (Causse et al. 1994; Kurata et al. 1994). Based on the synteny between rice and other cereals according to the results of comparative mapping, 5 probes from maize (Coe et al. 1995) and 2 probes from barley (Saghai Maroof et al. 1996) were also used in screening parental polymorphisms.

Data from highly fertile individuals only were used to determine the map location of the S_5 locus, based on the assumption that every highly fertile individual carried the S_5^n allele, which appeared to be the case according to the results of this study (see next section). This avoided misclassification by dichotomizing the individuals in a continuously distributed population into high-versus low-fertility classes, because low fertility could result from many unfavorable environmental conditions, irrespective of the genotypes at the S_5 locus. The 101 highly fertile plants were assayed individually with all the polymorphic markers surrounding the S_5 locus. The recombination frequency (c) between a marker and the S_5 locus was calculated using a maximum likelihood estimator (Allard 1956).

Results

Segregation of hybrid fertility

Spikelet fertility in the three-way cross population was distributed continuously from a low of 10% to a high of 95% (Fig. 1). The distribution is clearly bimodal with an apparent valley at approximately 60% fertility. The segregation of highly fertile and partly sterile individuals deviated significantly from the expected 1 : 1 ratio, with more individuals in the low-fertility class than in the high-fertility class.

RFLP linkage map

A RFLP linkage map was constructed using 230 truly hybrid plants, as confirmed by both morphological traits and molecular markers. The map, consisting of 191 RFLP loci, spanned a total of 1392 cM with an average interval of 7.2 cM between adjacent markers (map not shown). The 191 loci were placed into 19 linkage groups at LOD 3.0. For rigorous QTL search, we did not reduce the LOD threshold in order to join the unlinked groups, although comparison with the published maps showed that the gaps between markers at the disjoined points were small. The linear order of the markers in the map on the chromosomes was in good agreement with the Cornell and Japanese maps, except that a few probes detecting multiple bands were mapped to chromosomal locations different from where they appeared in the Cornell or Japanese map. This indicated that each of these probes detected a different locus in our mapping population.

Allele distribution in the segregating population

The allele distribution patterns of the polymorphic loci among the three parents fell into one of the three possible categories (data not shown). The first pattern was the most frequent among the loci in which '02428' and 'Balilla' had the same allele at each locus while 'Nanjing 11' had a different allele. The second pattern was observed in 17 loci at which 'Nanjing 11' and 'Balilla' had the same allele while '02428' had a different allele. In the third pattern, observed at 15 loci, alleles of the three parents were all different from each other. Interestingly, the hybridization patterns of 4 adjacent markers (RZ450, R2349, RG138 and RG213) that flank the S_5 locus spanning a distance of 20 cM on chromosome 6 (Fig. 2) displayed the second pattern. The WCV 02428 had a specific allele at these loci, while 'Balilla' and 'Nanjing 11' had a different allele.

Alleles at a majority of the RFLP loci segregated in the expected 1 : 1 ratio. Only a small portion of the loci (16 out of 191) exhibited significant segregation distortion, all of which were in favor of the alleles from the indica parent 'Nanjing 11'. These skewed segregating loci were located on five chromosomal segments including two segments on chromosome 12 and one segment each on chromosomes 2, 7, 9 and 10, respectively.

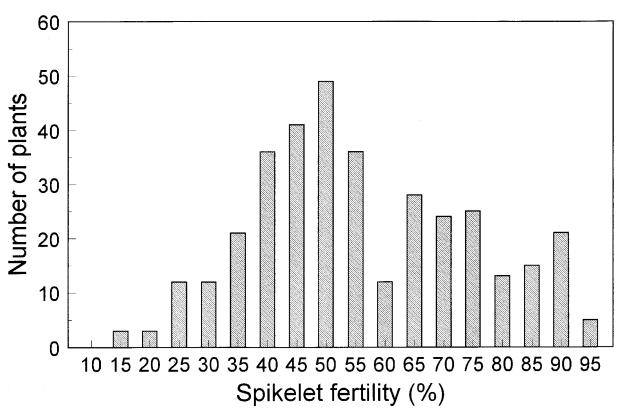

Fig. 1 Distribution of spikelet fertility of 356 plants in the three-way cross '02428'/'Nanjing 11'//'Balilla'

QTL analysis on hybrid fertility

The entire genome, as represented by the RFLP linkage map, was searched for QTLs conferring significant effects on hybrid sterility using the MAPMAKER/QTL analysis. A very high peak with a LOD score of 33.16 was detected on chromosome 6 (Fig 2); the chromosomal location of this peak coincided with the S_5 locus determined in previous studies (Ikehashi and Araki 1986; Liu et al. 1992; Zheng et al. 1992; Yanagihara et al. 1995). Two apparent peaks, with LOD scores of 3.94 and 3.26, were observed within a region of about 20 cM between R1534 and RG98 on chromosome 12 (Fig 2). To test the possibility of 2 linked QTLs, we rescanned this region after allowing for the first putative QTL, which resolved only 1 QTL in this region. The position of the higher peak, which occurred between G1112A and C362A, was regarded to be the chromosomal location of this QTL. One minor peak with a LOD score of 3.16 was detected on chromosome 2 located between C560 and RG324 (Fig. 2). These 3 QTLs individually explained 48.5%, 8.1% and 6.1% of the phenotypic variance, respectively, and collectively accounted for 56.7% of the total phenotypic variance as determined by a multiple QTL analysis (Lincoln et al. 1992).

The genetic effects of these loci

A three-way ANOVA, based on the genotypes of the marker locus lying closest to the peak in each of the three QTL-containing genomic regions, was conducted to characterize the mode of gene action in this three-locus system. The analysis showed that, essentially,

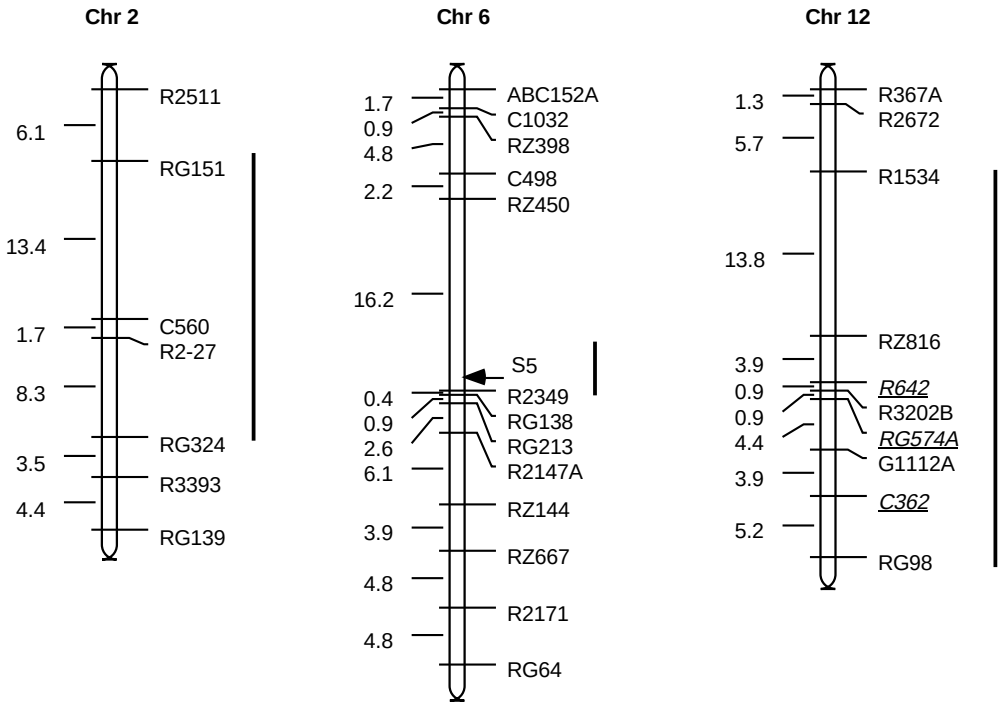

Fig. 2 The local linkage maps of the three genomic regions showing significant effects on hybrid fertility detected by MAPMAKER/QTL at LOD 3.0. The map was constructed using the 230 truly hybrid plants derived from the three-way cross '02428'/'Nanjing 11'//'Balilla'. *Underlined* markers represent loci showing skewed segregation in favor of the indica parent 'Nanjing 11'. The *vertical solid bars* indicate the positions of the loci for hybrid sterility with 1 LOD supporting intervals determined by the QTL analysis

Table 1 A three-way ANOVA using one marker locus from each of the three genomic regions showing effects on hybrid fertility

Effect[a]	MS	F	*P*
1 (R2349, Chrom 6)	3.218	212.76	0.000
2 (G1112A, Chrom 12)	0.520	34.41	0.000
3 (R2-27, Chrom 2)	0.131	8.71	0.004
1×2	0.013	0.90	0.344
1×3	0.036	2.41	0.122
2×3	0.066	4.42	0.037
$1 \times 2 \times 3$	0.043	2.86	0.092

[a] Each one of the effects has only 1 degree of freedom and there were 219 degrees of freedom for the error term

Table 2 Spikelet fertility averaged for each of the three-locus genotypes. Chromosomal locations of the markers are in parentheses

R2349 (6)	G1112A (12)	R2-27 (2)	Spikelet fertility (%)[a]
1[b]	1	1	72.03
1	1	2	70.84
1	2	1	66.97
1	2	2	53.08
2	1	1	46.25
2	1	2	44.60
2	2	1	38.71
2	2	2	35.67

[a] Least significant difference between classes: $LSD_{0.05} = 6.56$ and $LSD_{0.01} = 8.60$

[b] 1, Genotype consisting of the '02428' (WCV) and 'Balilla' (japonica) alleles; 2, genotype composed of the 'Nanjing 11' (indica) and 'Balilla' alleles

these 3 loci acted independently of each other, except for a very small amount of interaction between the 2 minor loci, marked by G1112A and R2-27 on chromosomes 12 and 2, respectively (Table 1). Thus, fertility reduction was mainly a result of allelic interaction within each of the 3 loci, as was the case of a number of hybrid sterility loci identified in previous studies (Ikehashi and Araki 1986; Wan et al. 1996).

All these QTLs affected the hybrid fertility in the same direction. For each locus, the genotype consisting of the '02428' and 'Balilla' alleles exhibited higher fertility than the one of 'Nanjing 11' and 'Balilla' alleles (Table 2). The fertility difference between the 2 genotypes was approximately 24% for the locus on chromosome 6 (S_5), 10% for the locus on chromosome 12 and 5% for the locus on chromosome 2. It is also clear from Table 2 that the joint effect of the two minor loci could result in partial sterility (53.08%) in the presence of the S_5^n allele.

Sixteen loci conferring hybrid sterility have been reported in previous studies (Kinoshita 1995). There has not been a hybrid sterility locus reported on chromosome 2. The location of S_{15} (*t*) on chromosome 12 identified by Wan et al. (1996) appeared to be different from the one detected in this study, as deduced from the linkage map (Causse et al. 1994). Thus, the 2 minor loci detected in the present study were distinct from all those identified previously.

The precise map location of the S_5 locus

It is clear from Table 2 that a highly fertile plant had to carry the S_5^n allele, while a plant carrying the S_5^n allele

Table 3 Recombination frequencies (c) between markers and the S_5 locus on chromosome 6, calculated by assuming that each of the 101 highly fertile individuals carries a S_5^n allele

Locus	Genotype[a]			Map distance (cM)
	1	2	c	
RZ450	86	13	13.13	13.4
R2349	100	1	0.99	1.0
G138	100	1	0.99	1.0
RG213	98	3	2.97	3.0

[a] 1, Genotype consisting of alleles from '02428' and 'Balilla'; 2, genotype consisting of alleles from 'Nanjing 11' and 'Balilla'

was not necessarily highly fertile due to modifications by the minor loci. To determine the precise map location of the S_5 locus, we selected 101 highly fertile plants from the population and assayed these individually using the markers surrounding the S_5 locus. Recombination frequency was calculated between each marker and the S_5 locus (Table 3). No recombination was detected between RG138 and R2349 among the highly fertile individuals. According to the recombination frequencies and also with reference to the linkage map constructed in this study, we determined that the S_5 locus is located between RZ450 and R2349 (Fig. 2) with a map distance of 1.0 cM from R2349 and 13.4 cM from RZ450 (Table 3).

Discussion

An important finding of the present study is that there are indeed minor loci affecting fertility and that the presence of WCG is not sufficient to suppress hybrid sterility in indica-japonica crosses. The marker-based analysis of '02428'/'Nanjing' '11'//'Balilla' showed that heterozygosity for the indica and japonica alleles at the 2 minor loci can collectively cause as much as a 19% reduction in spikelet fertility in the presence of the WCG. The actual amount of fertility reduction may be even greater, since the effects of these loci are certainly underestimated by the marker-based analysis because of recombinations between the markers and the loci for hybrid fertility.

The present results have important implications in inter-subspecific hybrid rice breeding programs. The tight linkage between the RFLP marker R2349 and the S_5 locus should be very useful for transferring the S_5^n allele to different varieties. Meanwhile, adequate attention should also be directed to the genotypes of the 2 minor loci to ensure normal fertility of the hybrids. It should also be pointed out that the existence of a large number of loci conferring hybrid sterility (Kinoshita 1995; Zhang et al. 1997) indicates the possibility that, given the presence of the S_5^n allele, different and/or additional loci may become involved in hybrid fertility in crosses of the same WCV with other varieties. Different pairs of parents may have their own complements of multilocus genotypes for fully fertile hybrids. Should this be the case, breeding for a fully fertile hybrid will be very difficult, and each hybrid has to be investigated individually using molecular marker-based genetic analysis.

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