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Genetic analysis of rice CMS-WA fertility restoration based on QTL mapping

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Abstract The inheritance of fertility restoration of rice cytoplasmic male sterility of the wild abortive type was studied by means of QTL mapping. The two segregating populations examined showed high frequencies of highly sterile and highly fertile progenies, but a low frequency of partially sterile and partially fertile progenies. The distributions suggested that fertility restoration was mainly controlled by major genes. Based on a linkage map constructed with 57 RFLP and 61 AFLP markers on a B_1F_1 population, composite interval mapping (CIM) revealed that the fertility was restored by the additive effects of two restorer loci located on chromosome 10. One QTL, tightly linked to RFLP marker C1361 in the middle of the long arm of chromosome 10, explained 71.5% of the phenotypic variance. The second QTL was located between RFLP markers R2309 and RG257 on the short arm and explained 27.3% of the phenotypic variance. Similar results were obtained using the simple interval mapping (SIM) methods.

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Introduction

Rice cytoplasmic male sterility of the wild abortive type (CMS–WA) has been extensively used in the commercial production of rice hybrid seeds. The fertility is restored by the dominant and sporophytic restorer genes (*Rf*) (Li and Yuan 1986; Virmani 1996). However, reports regarding the number, positions, and the effects of these *Rf* genes are inconsistent. A variable number of restorer genes have been proposed in various studies, such as one gene (Wang 1980; Shen et al. 1996), two linked genes (Li et al. 1982), two independent genes (Li and Yuan 1986; Virmani et al. 1986; Raj and Virmani 1988; Bharaj et al. 1991; Teng and Shen 1994), and polygenes (Pei 1980). Furthermore, seven chromosomal locations for such genes have been reported: one on chromosome 1 (Zhang et al. 1997), two on chromosomes 7 and 10 (Bharaj et al. 1995), and four QTLs on chromosomes 2, 3, 4, and 5 (Zhu et al. 1996).

The availability of saturated molecular maps of the rice genome (Causse et al. 1994; Kurata et al. 1994; Tanksley 1994) and efficient techniques for DNA marker detection, such as the amplified fragment length polymorphism (AFLP) method, have made it possible to estimate gene position and effect precisely by using appropriate analytic methods of quantitative trait loci (QTLs) mapping based on the linkage to markers. Using the simple interval mapping (SIM) methods (Lander and Botstein 1989; Haley and Knott 1992) and the composite interval mapping (CIM) method (Zeng 1993; Zeng 1994; Jansen 1996), we studied the inheritance of fertility restoration of rice CMS–WA in a backcross population and analyzed the frequency distribution of fertility segregation in two segregating populations.

Materials and methods

Plant materials and restriction fragment length polymorphism (RFLP) clones

Two segregating populations, a B_1F_1 and an F_2, which were generated by the crosses of RD21A//RD21A/IR24 and RD21A/IR13419–113–1, consisted of 122 and 197 progenies, respectively. The three parents are tropical *indica* lines, with RD21A being a CMS line possessing CMS–WA cytoplasm and the two male parents being restorer lines for CMS–WA (Li and Yuan 1986; Yuan and Virmani 1988). The B_1F_1 population was used for QTL mapping.

The RFLP clones prefixed by RG, RZ, or CDO were obtained from Cornell University in the USA, and the clones prefixed by G, C, L, S were obtained from the Rice Genome Program (RGP) in Japan.

Phenotype scoring

The segregating populations were grown under regular rice production conditions in fields at Kasetsart University, Kampaengsaen, Thailand. Fertility restoration was initially determined from the percentage of viable pollen by staining with 1% I_2–KI and verified by checking the anthers as described by Li and Yuan (1986). Progenies of the two populations were classified into highly sterile (S), highly fertile (F), and intermediate (M) groups based on their profile of frequency distribution of fertility segregation.

Genomic DNA isolation, RFLP and AFLP analysis

Total genomic DNA was isolated using the urea–phenol method as described by McCouch et al. (1988) with a modified extraction buffer (7.0 *M* urea, 0.3 *M* NaCl, 0.05 *M* TRIS–Cl, 0.04 *M* EDTA, 2% sarcosyl, 10% phenol reagent; pH 8.0).

The DNA was digested separately by seven enzymes, namely, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Nco*I. DNA hybridization and detection were carried out with the DIG system of Boehringer Mannheim according to the manufacturer's recommendations.

AFLPs were detected using a similar method as that described by Mackill et al. (1996). Briefly, genomic DNA double-digested by restriction enzymes *Eco*RI and *Mse*I was ligated with *Eco*RI- and *Mse*I-adapter. The resultant DNA fragments were preamplified with primers of *Eco*RI + 1 and *Mse*I + 1 (one selective nucleotide added) and reamplified using the primers *Eco*RI + 3 and *Mse*I + 3 (three selective nucleotides added). The AFLP products were detected by silver staining according to the Promega technical manual. The AFLP markers were prefixed with AF followed by numbers.

Linkage map construction and QTL analysis

The linkage map was constructed with MAPMAKER/EXP 3.0 on a personal computer (Lincoln et al. 1992a). Map distances were based on the Kosambi function (Kosambi 1944). The linkage groups were assigned to the corresponding chromosome based on RFLP markers mapped by Kurata et al. (1994) and Tanksley (1994). The chromosomal arms of the linkage groups were determined according to the map published by Khush et al. (1996). QTL positions and their effects were determined by the maximum-likelihood interval mapping approach (Lander and Botstein 1989) through MAPMAKER/QTL 1.1 (Lincoln et al. 1992b). The results were verified by the regression approach (Haley and Knott 1992) using QGENE 2.27d on Macintosh Classic II (Nelson 1997), and confirmed by CIM analysis with PLABQTL on IBM/PC (Utz and Melchinger 1996).

For single-marker analysis, squares of correlation coefficients (R^2) of phenotype on the markers, phenotypic means of marker classes, and the *t*-test for significance of the difference between the phenotypic means were calculated.

Results

Segregation of fertility restoration

F_1 pollen and spikelet fertility of the two crosses were approximately 90%. Pollen fertility ranged from 0.0% to 98.5% in the two segregating populations, and the distribution profiles were like U types. The two groups of highly sterile (S) and highly fertile (F) progenies were present in much higher frequencies than the intermediate group (M) composed of partially sterile and partially fertile progenies (Fig. 1). In the B_1F_1 population, the S, M, and F groups (pollen fertility ranging from 0.0% to 10%, 10.1% to 75%, and 75.1% to 97.6%, respectively) contained 58, 18, and 46 progenies, respectively. The number of progenies in the S group to those in the F group conformed to a 1 : 1 ratio. The proportion of progeny of the M group in the whole population was only 14.75% (Table 1). Similarly, the progeny ratio of the F group to the S in the F_2 population fitted a 3 : 1 ratio, and the proportion of the M group in the whole F_2 population was 20.3% (Table 1). Similar results were obtained by checking the anthers in both populations (data not shown).

Marker segregation and linkage map construction

Among the 395 RFLP clones screened only 57 (14.8%) showed polymorphism in the B_1F_1 population. The AFLP markers showed a similar level of polymorphism with 61 (14.0%) polymorphic bands from 437 total bands. Although the population showed a low level of polymorphism, most RFLP and AFLP markers segregated in 1 : 1 ratios. Out of the 118 polymorphic markers, only 12.5% and 3.4% of the markers segregated with distortion at significant levels of $P \leq 0.05$ and $P \leq 0.01$, respectively.

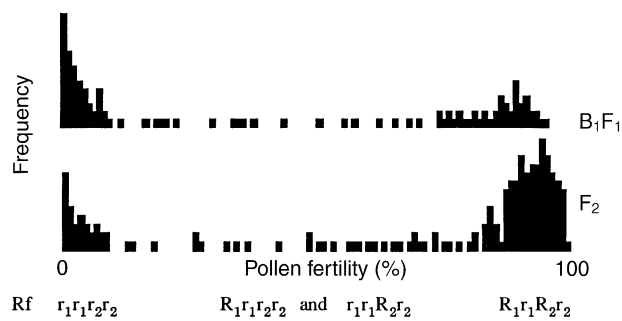

Fig. 1 Pollen fertility frequency distribution. *Rf* is the genotype supposed for each group

Table 1 Pollen fertility segregation of the B_1F_1 and the F_2 populations

Cross	Number	S:M:F[a]	M (%)[b]	S:F	Chi-test (S:F)
RD21 A//RD21 A/IR24	122	58:18:46	14.75	1:1	$P > 0.25$
RD21 A/IR13419–113–1	197	33:40:124	20.30	1:3	$P > 0.25$

[a] Progenies in S, M, and F groups
[b] Proportion of the intermediate progeny in the whole population

Table 2 QTL detection using SIM and CIM methods

Detector	Chromosome	Marker interval	LOD	V%[a]	W[b]	rr[c]	Rr[c]
MAPMAKER	10	S11148–S10019[d]	32.1	70.2	0.667	12.0	78.2
	10	R2309–RG257	7.5	25.8	0.326	25.3	62.3
PLABQTL	10	S11148–C1361	33.0	71.5	59.4	12.0	78.2
	10	R2309–RG257	8.4	27.3	24.4	25.3	62.3

[a] The phenotypic variances explained by the QTLs
[b] QTL weights and additive effects measured by MAPMAKER and PLABQTL, respectively
[c] Pollen fertility means (%) of marker C1361 at homozygous (rr) and heterozygous (Rr) phases
[d] The interval including markers S11148, C1 and R2309361, and S10019

Twelve linkage groups corresponding to the 12 rice chromosomes were constructed with these 118 markers. The total map length was 1419.7 cM, and the average interval length was 13.4 cM. Of all intervals, only two regions on chromosome 5 were larger than 30 cM. The distorted markers were concentrated in the middle of chromosome 3 and the long arm of chromosome 12.

QTL detection

Among the 118 polymorphic RFLP and AFLP markers, only the markers on chromosome 10 showed association with fertility restoration. Using the CIM method, we detected two major QTLs (Table 2) with high LOD scores in two separated regions of chromosome 10 (Fig. 2). One sharp peak of the LOD score was close to marker C1361 in the middle of the long arm of the chromosome; another smaller peak was close to marker R2309 in the short arm of the chromosome. The two QTLs had additive effects. Using the maximum-likelihood approaches, we were able to also detect these two QTLs with similar values of LOD scores, explain phenotypic variances, and map positions (Table 2). Very similar results were also given by the regression approach analysis.

The single-marker analysis also suggested the existence of two QTLs on chromosome 10. With marker C1361, all the 58 highly sterile progenies were homozygous, same as that of the CMS parent. In contrast, of all the fertility-restored progenies, 45 were heterozygous, containing two alleles of both parents (Fig. 3). Only one of the fertility-restored progenies showed the band possessed by the sterile progenies. Of all the markers, only C1361 and S10019, which were linked with a 0.9-cM distance, showed a very high correlation

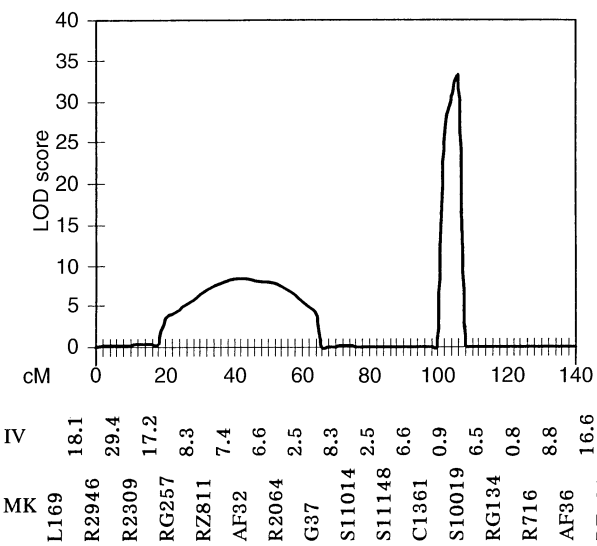

Fig. 2 Fertility restorer QTLs detected by the CIM method. The *IV* represents intervals between markers, and *MK* indicates markers mapped on chromosome 10

with fertility restoration. These two markers had the largest values of R squares (R^2) on the fertility, as high as 0.7, the largest values of the differences between the two phenotypic means of the marker classes, and the largest *t*-test values for significance of the differences. Although other markers had smaller values for these calculations, R2309 and RG257, two markers on the short arm of the chromosome, had larger values than their flanking markers (Table 3).

Discussion

Possibly due to the parents belonging to the same subspecies and ecotype, the mapping population in this

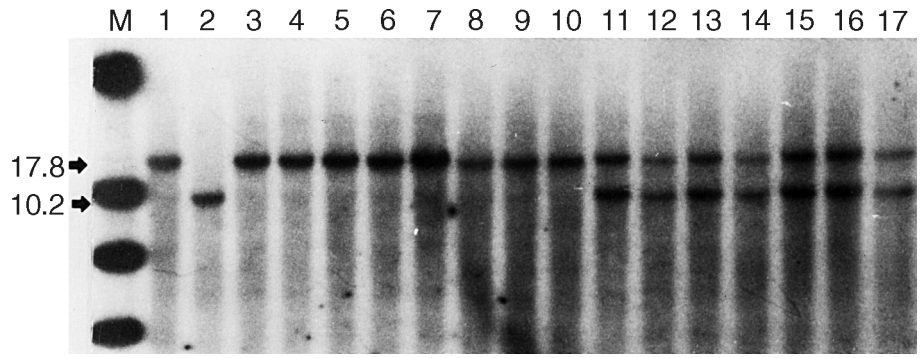

Fig. 3 Cosegregation of marker C1361 with fertility restoration. *Lanes 1* and *2* parents of CMS line and restorer, respectively, *lanes* 3–10 and *11–17* are sterile and fertile individual progenies, respectively

Table 3 Partial data of single-marker analysis on chromosome 10

Marker	R2946	R2309	RG257	RZ811	AF35	S11148	C1361	S10019	RG134	RZ421
t-test[a]	3.62	7.92	6.86	5.54	9.28	22.46	32.17	31.56	19.44	2.64
Rsq	0.108	0.311	0.227	0.197	0.328	0.66	0.697	0.706	0.613	0.076

[a] The values were converted by the 10-base logarithm (Log_{10})

study showed low polymorphism and low distortion of segregation compared with other rice mapping populations generated from highly diverse parents (Wang et al. 1994; Redoña and Mackill 1996; Xiao et al. 1996; Zhu et al. 1996). Such a population can avoid the sterility caused by genetic diversity between the parents, and reduce the false linkage that results from the distortion (Lorieux et al. 1995). The linkages constructed on this population were consistent with those of previously published maps (Tanksley 1994; Kurata et al. 1994; Causse et al. 1994). As the fertility trait is controlled by dominant genes, a fact that has been demonstrated by this and other studies (Li and Yuan 1986; Li and Zhu 1988; Virmani 1996), the backcross population of the CMS line as the recurrent parent was used for the QTL detection in this study, although an F_2 population was also used for phenotypic analysis. As this type of backcross population can increase the frequency of the recessive genotype of the sterile progenies, its use can enhance the power of QTL detection.

We detected two loci on chromosome 10 playing main roles in fertility restoration. These two QTLs were not only detected by single-marker and the SIM analyses but also by the CIM method, which is more suitable than the other two for detecting linked QTLs (Zeng 1994; Jansen 1996).

The two-linked-gene model is also indicated by the distribution of fertility segregation in this and other studies. High frequencies of sterile and fertile progenies were contributed by genotypes of $r_1r_2r_1r_2$ and $R_1r_1R_2r_2$, respectively, while the low frequency of the intermediate progenies having genotypes of $R_1r_1r_2r_2$ or $r_1r_1R_2r_2$ resulted from crossovers of the two linked genes. However, with such a U-like distribution only Li et al. (1982) suggested the model of two linked genes; other researchers have proposed models of one or two independent genes, which could be because of the different approaches used for fertility classification (Wang 1980; Young and Virmani 1984; Li and Yuan 1986; Teng and Shen 1994). The chromosomal location of the *Rf* genes in this study is consistent with that observed in a trisomic analysis (Bharaj et al. 1995)—only on chromosome 10 but not on chromosome 7. An *Rf* gene was also reported on chromosome 7 in the trisomic analysis (Bharaj et al. 1995), but Khush et al. (1984) reported that the trisomic for chromosome 7 was a partial-sterile line. Therefore, the detection of an *Rf* gene on chromosome 7 may be misled by the partial sterility of the trisomic. Although an *Rf* gene of rice CMS–WA was detected by random amplified polymorphic DNA (RAPD) markers that were mapped closely to RFLP marker RG532 on chromosome 1 (Zhang et al. 1997), we did not identify the association between fertility restoration with this and the other RFLP markers on chromosome 1.

As marker C1361 is tightly linked to a major restorer QTL, this marker could be used for marker-assisted selection of the restorer and maintainer lines with the supplement of marker R2309.

Interestingly, a major QTL mapped in this study was the same region as *Rf-1*, a restorer gene for rice CMS–BT (borotype). *Rf-1* was found to be tightly linked to marker G2155, which was only 3.9 cM away from marker C1361 (Kurata et al. 1994; Ichikawa et al. 1997). This cannot be a random coincidence. The restorer genes for both CMS–WA and CMS–BT cytoplasm have mainly been identified in tropical *indica* cultivars, not in *japonica* cultivars, although restorer lines of CMS–BT possess the *japonica* nuclear genomic background (Shinjyo 1975; Li and Zhu 1988; Virmani

1996). Moreover, *Rf-1* is a fertility restorer for most rice CMS cytoplasms (Shinjyo and Sato 1994). For example, C57, an elite *japonica* restorer line of CMS–BT, partially restored the fertility of CMS–WA (Shen and Tang 1994). The *Rf* gene of this restorer line was conferred by tropical *indica* line IR8, which is also the donor of a major *Rf* gene of CMS–WA restorer lines such as IR24 (Li et al. 1982; Li and Yuan 1986). On the other hand, IR24 can also restore many different types of rice CMS including CMS–BT (Li et al. 1982). Even *Rf-2*, a chromosome-2-located *Rf* gene for rice CMS–LD (lead rice) (Shinjyo and Sato 1994), possibly originated from *Rf-1* (S. Sato, personal communication). Therefore, it is possible that the major restorer gene for CMS–WA and *Rf-1* for CMS–BT have some revolutionary relationship.

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