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Mapping QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.)

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Abstract The amplified fragment length polymorphism (AFLP) technique combined with selective genotyping was used to map quantitative trait loci (QTLs) associated with tolerance for phosphorus (P) deficiency in rice. P deficiency tolerant cultivar IR20 was crossed to IR55178-3B-9-3 (sensitive to P-deficiency) and 285 recombinant inbred lines (RILs) were produced by single-seed descent. The RILs were phenotyped for the trait by growing them in P-sufficient (10.0 mg/l) and P-deficient (0.5 mg/l) nutrient solution and determining their relative tillering ability at 28 days after seeding, and relative shoot dry weight and relative root dry weight at 42 days after seeding. Forty two of each of the extreme RILs (sensitive and tolerant) and the parents were subjected to AFLP analysis. A map consisting of 217 AFLP markers was constructed. Its length was 1371.8 cM with an average interval size of 7.62 cM. To assign linkage groups to chromosomes, 30 AFLP and 26 RFLP markers distributed over the 12 chromosomes were employed as anchor markers. Based on the constructed map, a major QTL for P-deficiency tolerance, designated PHO, was located on chromosome 12 and confirmed by RFLP markers RG9 and RG241 on the same chromosome. Several minor QTLs were mapped on chromosomes 1, 6, and 9.

Key words *Oryza sativa* L. · AFLP markers · RFLP markers · Phosphorus deficiency tolerance · QTL analysis

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Introduction

Phosphorus (P) deficiency occurs widely in rice soils with a high native P-fixing capacity, such as acid soils, acid sulphate soils, alkaline soils and acidic soils. There are also soils that are P deficient because of their inherently low P content and not due to fixation. P deficiency is widespread in Bangladesh, India, Indonesia, Nepal, Pakistan, South China and Vietnam. Exact figures on the extent of the rice area affected is not known but our estimate is about 35 million ha. The existence of significant differences among rice cultivars for tolerance to P deficiency (IRRI 1971, 1976; Katyal et al. 1980; Gunawardena et al. 1978; Ikehashi and Ponnamperuma 1978; Senanayake 1984; Fageria et al. 1988), offers opportunities of developing high-yielding cultivars for P-deficient soils. Such cultivars will be ideal for both types of soil, namely high soil P but low availability due to fixation and inherently low P and no fixation. In the latter type, addition of P fertilizers is the most suitable, but in rainfed areas it is difficult to convince farmers to apply P fertilizers until such time that cultivars which could produce reasonably high yields under existing conditions and respond significantly to added P fertilizers become available.

Development of P-deficiency tolerant rice cultivars is constrained by the lack of screening methods suitable for use in breeding programs. We believe that tolerance for P deficiency should be a selection criterion in any rice breeding program. Although not specifically selected for the trait, cultivars such as IR8, IR20, IR36, IR64, Mahsuri, TCNJ1, H4 and Bg90-2 are highly P-efficient and their wide adaptability and acceptability at the time of release cannot be explained by any other trait. The screening method currently used relies on tillering ability (Hung 1985; IRRI 1996) where the test genotypes have to be grown under both P-adequate and P-deficient conditions and the level of tolerance is determined by relative tillering ability. Criteria

based on relative values are difficult to use in the evaluation and selection of breeding populations. Therefore, we initiated a research program to develop a molecule marker-assisted selection (MAS) technique for P-deficiency tolerance in Rice.

The advent of molecular-marker technology has led to the development of genetic maps that make it possible to identify and locate genes controlling quantitative characters. Majumder et al. (1989) postulated that P-deficiency tolerance in rice is a quantitative trait with high heterotic effects. A study by Chaubey et al. (1994) revealed somewhat different results. Determination of the number, location, and magnitude of effects through molecular-marker linkages of quantitative trait loci (QTLs) could also elucidate the genetic basis of the trait, leading to improved breeding and selection efficiency.

P-deficiency tolerance in rice can be attributed to two mechanisms, namely, internal efficiency and external efficiency. Internal efficiency involves low P requirement and external efficiency is the ability to capture unavailable soil P. In the greenhouse method of screening for P efficiency, in which cultivars are tested for relative tillering ability using P-deficient (0.5 mg/l) and P-adequate (10.0 mg/l) culture solution (Yoshida et al. 1976), the mechanism detected is internal efficiency because supplied P, independent of the amount, is freely available. Although the extent of rice soils with high P-fixation capacity is much larger than those with inherently low but available P, we believe that internal efficiency is more important. Because, even in P-fixed soils, there is certain amount of available P. Therefore, in the first phase of our research program, gene mapping and QTL identification was undertaken by amplified fragment length polymorphism (AFLP) analysis and selective genotyping (Vos et al. 1995; Thomas et al. 1995; Maheswaran et al. 1997; Nandi et al. 1997) with the objective of developing a MAS technique for internal P efficiency in rice.

Materials and methods

Development of recombinant inbred lines (RILs)

An F_8 recombinant inbred line population consisting of 285 lines was developed by single-seed descent from a cross between IR20 × IR55178-3B-9-3. IR20 is a tolerant variety to P deficiency, whereas IR55178-3B-9-3 (hereinafter called IR55178) is sensitive. In the F_7 generation, each line consisted of 25 plants and one was harvested as a representative plant. The others were bulk harvested for any future use. Seeds harvested from single plants were used both for phenotyping and for DNA isolation for AFLP analysis.

Phenotyping RILs

A hydroponic culture experiment was conducted at the International Rice Research Institute (IRRI) in 1996 and 1997 using a Phytotron glasshouse with a 29/21°C day/night temperature, 70% relative humidity and natural daylight. Four pre-germinated seeds of each RIL were shown in plastic pots containing 4 l of either P-deficient (P = 0.5 mg/l) or P-sufficient (P = 10 mg/l) nutrient solution (Yoshida et al. 1976). Eighty plants of each parent were also used with the two different P levels. The pH of the nutrient solution was adjusted daily to 5.0 using 1 N NaOH or HCl. The solution was replaced every week and nitrogen concentration was increased from 40 to 60 mg/l at 21 days after seeding. At 28 days after seeding, the number of tillers of each plant was counted and the relative tillering ability (RTA) of each line and the parents was calculated as: RTA (%) = (mean tiller number at 0.5 mg/l/mean tiller number at 10.0 mg/l) × 100. At 42 days after sowing, the roots and shoot of each plant were taken and oven dried at 65°C for 3 days. As RTA, the relative shoot dry weight (RSDW) and relative root dry weight (RRDW) were determined for each RIL and the parents. The forty two most-tolerant and 42 most-sensitive RILs (extreme genotype) were selected based on RTA, RSDW and RRDW and were re-tested under the same conditions as in the first test with all RILs.

AFLP analysis

Genomic DNA of the selected genotypes and the parents was isolated, and AFLP analysis was conducted following the method of Vos et al. (1995), with minor modifications employed by Maheswaran et al. (1997). Both *Pst*I/*Mse*I and *Eco*RI/*Mse*I systems were used to generate polymorphic AFLP markers, and a total of 58 primer pair combinations were employed (see Tables 2 and 3). A set of AFLP markers mapped in a IR64/Azucena doubled-haploid population (Maheswaran et al. 1997) and in a IR74/FR13A recombinant inbred population (Nandi et al. 1997) were used as anchor markers for each linkge group. All detected AFLP markers were assigned to the 12 linkage groups at LOD > 3 based on their linkage to the anchor markers using MAPMAKER (Lander et al. 1987). The assigned markers were ordered using MAPMAKER, and the ripple command was used to verify the order of markers on each chromosome. The map distance (cM) was derived based on the Kosambi function (Kosambi 1944). To verify the map, the parents were surveyed for restricted fragment length polymophism (RFLP) and polymorphic markers were hybridized against Southern blots for restriction fragments of DNA isolated from selected RILs and mapped on chromosomes.

Mapping QTLs for P-deficiency tolerance

To identify the major gene for P-deficiency tolerance, the quantitative data of RTA collected from selected lines were converted to qualitative data. The 42 RILs with high RTA were considered tolerant, similar to IR20, while the 42 RILs with low RTA were treated as sensitive, similar to IR55178. The qualitative data were analyzed using MAPMAKER to locate any major genes for P-deficiency tolerance.

Data for RTA, RSDW, and RRDW were used for QTL analysis after being normalized by log transformation. Single-marker locus analysis (SAS GLM, SAS Institute 1988) at a probability of less than 0.005 for error I and interval mapping with a LOD value > 2.4 (Lander 1993) were used for detecting marker loci associated with the variations in the parameters measured. The mean comparisons between marker genotypes for each trait were conducted using *t*-test analysis. The proportion of the phenotypic variance explained by the marker loci linked to QTLs detected was investigated by regression analysis.

Results

Phenotypic performance

The means of the RTA, RSDW and RRDW of parents, the whole population, and the selected lines are shown

Table 1 P-efficiency parameters and their mean and range for the IR20 × IR55178 population, selected genotypes and the parents

Parameters	Unit	Population			Selected genotypes		Parents	
		Mean	SD	Range	Tolerant	Sensitive	IR20	IR55178
Relative tillering ability (RTA)	%	77.5	17.3	44.8–127.3	108.9	57.0	96.1	65.5
Relative shoot dry weight (RSDW)	%	57.3	11.8	36.4–97.7	76.8	43.9	61.0	49.9
Relative root dry weight (RRDW)	%	112.9	28.7	60.2–204.4	142.2	88.3	138.4	85.3

in Table 1. IR20 has a higher RTA, RSDW, and RRDW than IR55178 indicating its tolerance. This result was consistent with earlier reports (Ni et al. 1996, 1998). Segregation among the 285 RILs for the three tolerance parameters was observed and the distributions of the parameters were normal after log-transformation. The results obtained from the second experiment with the 84 selected lines were similar to those from the first experiment. Transgressive variations in all three parameters were observed. For RTA, approximately 16% of the 285 RI lines had a RTA higher than IR20, and 22% lower than IR55178. Data for the 84 selected lines from the second experiment showed highly significant correlation among the three parameters [r(RTA/RSDW) = 0.867, r(RTA/RRDW) = 0.789, r(RSDW/RRDW) = 0.879].

Construction and validation of AFLP map

A total of 2116 unambiguous bands were generated using 58 primer combinations. These 283 polymorphic bands were detected and 217 were mapped. The number of clear bands ranged from 4 to 68 (mean = 36.5) and the number of polymorphic bands was 0–14 per primer pair. The level of polymorphism for individual primer combinations varied from 0 to 26.8% (Tables 2 and 3). Overall, the polymorphism of AFLP markers in this RI population was only 13.3% with *Pst*I/*Mse*I and 12.9% with *Eco*RI/*Mse*I. The low polymorphism indicated that the parents of the mapping population used in this study were genetically related. Among the 283 polymorphic AFLP markers, about 50% (146) was from IR20. The markers from some primer combinations covered as much as eight chromosomes, suggesting that markers from each primer combination could be distributed on different chromosomes. Most AFLP markers identified followed Mendelian segregation. Twenty four markers (8.5%) deviated from a 1 : 1 ratio. From these markers, 6.4% were biased towards IR20 and 2.1% towards IR55178 (Table 4). These skewed marker loci were distributed on different chromosomes.

Thirty AFLP markers which were polymorphic in the population employed, as well as in other reference populations reported (Maheswaran et al. 1997; Nandi et al. 1997), were used as anchor markers. The number of anchors per chromosome varied from 1 to 5 (Table 4). With the 30 anchored markers, 187 AFLP markers were assigned to linkage groups via their linkage to the anchor markers. The number of AFLP markers per linkage group varied from 7 to 38 (Table 4).

The parental survey revealed 26 polymorphic RFLP markers. All the RFLP markers were mapped to the expected location, verifying the AFLP map. The 26 RFLP markers and 30 AFLP markers were used as anchor markers to generate the linkage map (Fig. 1). For chromosomes 4 and 10, it was difficult to determine the orientation of the map because there was only one anchor on each chromosome. For other chromosomes, the order of anchor markers on each chromosome showed a parallel position and was in agreement with the order in other populations studied previously (Maheswaran et al. 1997; Nandi et al. 1997). The linkage map had a total map length of 1371.8 cM. The average interval size was 7.62 cM with the smallest on chromosome 4 (2.07 cM) and largest on chromosome 3 (11.49 cM) (Table 4). There were seven gaps larger than 30 cM, two with more than 50 cM on chromosomes 1 and 2.

QTLs for P-deficiency tolerance

Based on the qualitative data, a major gene for P-deficiency tolerance, designated PHO, was detected near two AFLP markers, P1/M7-7 and E1/M5-7, and flanked by RFLP markers RG9 and RG241 on chromosome 12 (Fig. 1). In the same region, one common major QTL for RTA, RSDW and RRDW was identified by both single-marker analysis and interval mapping. It explained about 54.0%, 60.8%, and 44.2% of the total variations across the 84 selective lines for RTA, RSDW and RRDW, respectively.

In addition to the presence of a major QTL, two common QTLs for RTA, RSDW and RRDW with relatively small effects were detected from single-marker analysis and interval mapping. One common QTL flanked by marker loci E3/M8-3 and E1/M7-7 on chromosome 6, explained about 33.6%, 34.0% and 25.2% of the total variation in RTA, RSDW and RRDW. The other common QTL flanked by marker loci E4/M1-8 and E4/M3-8 on chromosome 1, explained about 9.9%, 10.8% and 8.9% of the total

Table 2 Polymorphism, origin and distribution of AFLP markers in the 84 F_8 RI lines of the cross IR20 × IR55178 (*Pst*I/*Mse*I)

Primer pair	Visible bands	Polymorphic bands	Polymorphism (%)	Origin of amplification		Polymorphic bands incorporated	Chromosomes covered	Anchor marker
				IR20	IR55178			
*P1/M1	27	5	18.5	3	2	3	1, 2, 12	1
*P1/M2	22	3	13.6	0	3	3	1, 5, 7	1
*P1/M3	39	4	10.3	2	2	4	1, 8, 9	3
*P1/M4	38	4	10.5	2	2	4	2, 6, 12	0
*P1/M5	41	11	26.8	6	5	7	1, 5, 6, 7, 11	4
*P1/M6	32	5	15.6	2	2	4	1, 5, 6, 11	4
*P1/M7	47	12	25.5	8	4	10	6, 7, 9, 11, 12	1
*P1/M8	17	4	23.5	2	2	3	2, 5, 6	2
*P1/M9	17	3	17.6	1	2	3	3, 4, 12	2
*P1/M10	39	3	7.7	1	2	3	2, 3, 12	1
*P2/M1	27	4	14.8	2	2	4	1, 6, 11, 12	0
*P2/M2	22	2	9.1	2	0	2	10, 11	2
*P2/M3	28	0	0.0	0	0	0	0	0
*P2/M4	34	8	23.5	3	5	6	1, 2, 6, 7, 11, 12	2
*P2/M5	23	4	17.4	2	2	3	4, 8	1
*P2/M6	30	5	16.7	3	2	4	2, 3, 7	3
*P2/M7	31	1	3.2	0	1	1	9	1
*P2/M8	16	2	12.5	1	1	2	1, 2	2
*P2/M9	44	8	18.2	3	5	5	1, 6, 7, 8, 12	0
*P2/M10	25	0	0.0	0	0	0	0	0
*P3/M1	40	5	12.5	2	3	3	1, 2	0
*P3/M2	33	7	21.2	4	3	6	1, 6, 10, 11	0
*P3/M3	43	4	9.3	2	2	0	0	0
*P3/M4	19	4	21.1	2	2	3	1, 3	0
*P3/M5	43	4	9.3	2	2	3	6, 8, 10	0
*P3/M6	33	5	15.2	2	3	5	1, 2, 3, 7	0
*P3/M7	18	3	16.7	1	2	1	6	0
*P3/M8	4	0	0.0	0	0	0	0	0
*P3/M9	42	6	14.3	2	4	5	1, 2, 10, 11	0
*P3/M10	50	4	8.0	1	3	2	5, 8	0
*P4/M1	20	3	15.0	2	1	1	10	0
*P4/M2	11	0	0.0	0	0	0	0	0
*P4/M3	15	1	6.7	0	1	1	1	0
*P4/M4	26	3	11.5	2	1	3	6, 11	0
*P4/M7	32	8	25.0	4	4	6	1, 2, 6, 7	0
*P4/M8	13	2	15.4	1	1	1	2	0
*P4/M9	28	2	7.1	2	0	1	7	0
*P4/M10	18	2	11.1	1	1	2	2, 9	0
Total	1087	151		73	78	114	12	30
Mean	28.6	4.0	13.3	1.9	2.1	3.0	2.6	0.9
Range	4–50	0–12	0–26.8	0–8	0–5	0–10	0–8	0–7

variation in RTA, RSDW and RRDW, respectively. For RSDW, one specific QTL flanked by marker loci E1/M7-5 and RG451 on chromosome 9 was identified, and it explained 14.9% of the total variation (Table 5).

Discussion

Phosphorus (P)-deficiency tolerance is unequivocally considered as an important trait to improve rice productivity. In maize, the mean total dry weight has been used as parameter to evaluate P-deficiency tolerance (Reiter et al. 1991). However, relative tillering ability has been considered as the most reliable marker of P-deficiency tolerance in rice (Hung 1985; Chaubey et al. 1994). In the present study, RTA, RSDW and RRDW were used to evaluate P-deficiency tolerance. The tolerant and sensitive parents of the studied population had contrasting expression of the three parameters, which was in accordance with previous results (Ni et al. 1996, 1998). Close correlations were found among the three parameters. However, RTA is easier to determine than the others and could be used as an index for evaluating rice for P-deficiency tolerance.

It has not escaped our notice that most QTLs detected for the three parameters were clustered and linked to RG9, E3/M8-3 and E4/M1-8 on chromosomes 12, 6 and 1, respectively. These loci could be multiple allelic loci. It has been reported in tomato that compared to

Table 3 Polymorphism, origin and distribution of AFLP markers in the 84 F_8 RI lines of the cross IR20 × IR55178 (*Eco*RI/*Mse*I)

Primer pair	Visible bands	Polymorphic bands	Polymorphism (%)	Origin of amplification		Polymorphic bands incorporated	Chromosomes covered	Anchor marker
				IR20	IR55178			
*E1/M3	51	10	19.6	6	4	5	1, 2, 6, 9	0
*E1/M5	56	10	17.9	6	4	8	1, 2, 3, 7, 8, 12	0
*E1/M7	56	10	17.9	5	5	10	1, 2, 4, 5, 6, 8, 9, 12	0
*E3/M3	46	8	17.4	5	3	8	1, 2, 3, 5, 6	0
*E3/M5	59	7	11.9	3	4	5	1, 3, 4, 8, 12	0
*E3/M6	46	6	13.0	4	2	5	2, 4, 7, 12	0
*E3/M7	38	5	13.2	5	0	4	1, 2, 5, 7	0
*E3/M8	57	7	12.3	3	4	4	2, 6, 12	0
*E4/M1	58	7	12.1	4	3	5	1, 4, 6, 7	0
*E4/M2	40	8	20.0	5	3	5	2, 3, 6, 11, 12	0
*E4/M3	63	7	11.1	4	3	5	1, 2, 11	0
*E4/M6	68	6	8.8	2	4	5	1, 2, 3, 6, 11	0
*E4/M7	41	4	9.8	0	4	4	1, 2, 3, 6	0
*E5/M1	40	2	5.0	1	1	2	1, 9	0
*E5/M4	64	14	21.9	7	7	10	1, 2, 3, 6, 7, 11, 12	0
*E5/M6	41	4	9.8	2	2	3	1, 12	0
*E6/M1	53	5	9.4	4	1	5	2, 3, 6, 8	0
*E6/M5	52	2	3.8	1	1	2	5, 7	0
*E7/M5	65	4	6.2	3	1	2	9	0
*E7/M6	35	6	17.1	3	3	6	2, 3, 4, 6, 8, 10	0
Total	1029	132		73	59	103	12	0
Mean	51.5	6.6	12.9	3.7	3.0	5.2	4.2	0
Range	35–68	2–14	3.8–21.7	0–7	0–7	2–10	1–8	0

Table 4 Distribution of markers on each chromosome, number of anchors used to construct the RIL map, and the number of skewed markers on each chromosome

Chromosome		Number of markers				Average distance (cM)	Anchor used[a]	Skewed markers	
No.	Length (cM)	Total	Framework map	AFLP	RFLP			IR20	IR55178
1	209.1	42	31	38	4	6.75	3(7)	2	1
2	157.3	27	22	27	0	7.15	5(5)	6	1
3	183.8	23	16	19	4	11.49	3(7)	1	0
4	18.6	10	9	10	0	2.07	1(1)	0	0
5	92.2	13	9	9	4	10.24	3(7)	2	1
6	214.1	30	21	28	2	10.20	2(4)	1	1
7	118.2	19	12	18	1	9.85	2(3)	1	0
8	57.0	11	11	10	1	5.18	2(3)	0	1
9	115.7	15	15	12	3	7.71	3(6)	3	1
10	40.9	7	7	7	0	5.84	1(1)	0	0
11	65.1	16	10	15	1	6.51	3(4)	0	0
12	99.8	31	16	24	6	5.87	2(8)	2	0
Total	1371.8	244	179	217	26	7.62	30(56)	18	6

[a] The anchor number in parenthesis includes RFLP markers

traits with a low genetic correlation, traits with a high genetic correlation had more genomic regions in common, (Paterson et al. 1990; Witsenboer et al. 1995; Yadav et al. 1997). Correlated traits have been hypothesized to have a common genetic basis (Albert et al. 1991; Paterson et al. 1991; Lebreton et al. 1995; Xu 1997).

Transgression in both directions was observed for all three parameters measured, indicating that neither parents carried all positive or all negative alleles. Three common QTLs were detected on three chromosomes for the three parameters. One positive QTL allele among them was from the sensitive parent. QTLs with effects opposite to those predicted from phenotype has

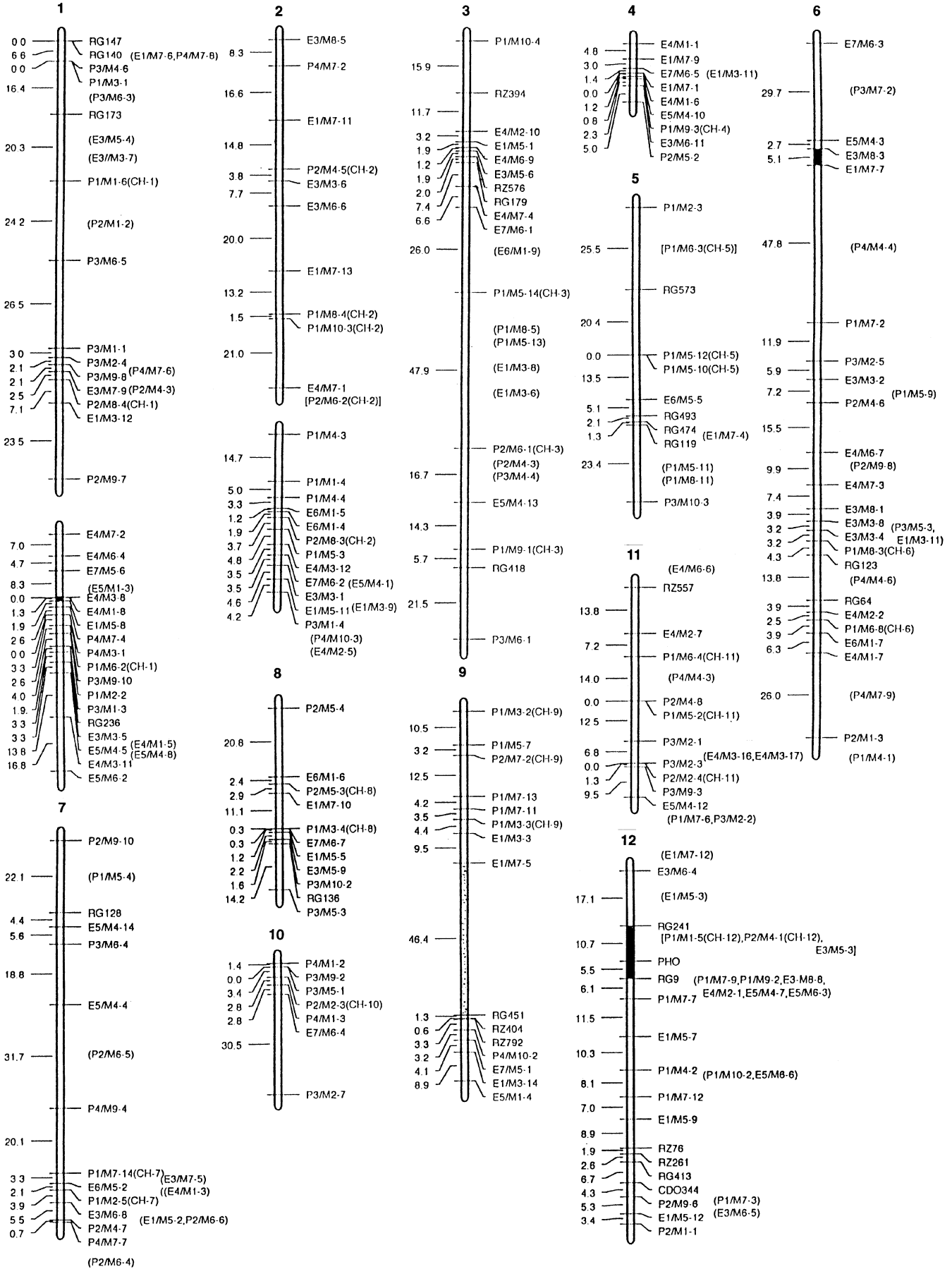
1
0.0 RG147
6.6 RG140 (E1/M7-6,P4/M7-8)
0.0 P3/M4-6
16.4 P1/M3-1
(P3/M6-3)
RG173
20.3 (E3/M5-4)
(E3/M3-7)
P1/M1-6(CH-1)
24.2 (P2/M1-2)
P3/M6-5
26.5
3.0 P3/M1-1
2.1 P3/M2-4
2.1 P3/M9-8 (P4/M7-6)
2.5 E3/M7-9 (P2/M4-3)
7.1 P2/M8-4(CH-1)
E1/M3-12
23.5
P2/M9-7
2
8.3 E3/M8-5
P4/M7-2
16.6
E1/M7-11
14.8
3.8 P2/M4-5(CH-2)
7.7 E3/M3-6
E3/M6-6
20.0
E1/M7-13
13.2
1.5 P1/M8-4(CH-2)
P1/M10-3(CH-2)
21.0
E4/M7-1
[P2/M6-2(CH-2)]
P1/M4-3
14.7
P1/M1-4
5.0
3.3 P1/M4-4
1.2 E6/M1-5
1.9 E6/M1-4
3.7 P2/M8-3(CH-2)
4.8 P1/M5-3
3.5 E4/M3-12
3.5 E7/M6-2 (E5/M4-1)
4.6 E3/M3-1
4.2 E1/M5-11 (E1/M3-9)
P3/M1-4
(P4/M10-3)
(E4/M2-5)
3
P1/M10-4
15.9
RZ394
11.7
3.2 E4/M2-10
1.9 E1/M5-1
1.2 E4/M6-9
1.9 E3/M5-6
2.0 RZ576
7.4 RG179
6.6 E4/M7-4
E7/M6-1
26.0 (E6/M1-9)
P1/M5-14(CH-3)
(P1/M8-5)
(P1/M5-13)
47.9 (E1/M3-8)
(E1/M3-6)
P2/M6-1(CH-3)
(P2/M4-3)
16.7 (P3/M4-4)
E5/M4-13
14.3
P1/M9-1(CH-3)
5.7 RG418
21.5
P3/M6-1
4
4.8 E4/M1-1
3.0 E1/M7-9
1.4 E7/M6-5 (E1/M3-11)
0.0 E1/M7-1
1.2 E4/M1-6
0.8 E5/M4-10
2.3 P1/M9-3(CH-4)
5.0 E3/M6-11
P2/M5-2
5
P1/M2-3
25.5 [P1/M6-3(CH-5)]
RG573
20.4
0.0 P1/M5-12(CH-5)
P1/M5-10(CH-5)
13.5
E6/M5-5
5.1 RG493
2.1 RG474 (E1/M7-4)
1.3 RG119
23.4 (P1/M5-11)
(P1/M8-11)
P3/M10-3
6
E7/M6-3
29.7 (P3/M7-2)
2.7 E5/M4-3
5.1 E3/M8-3
E1/M7-7
47.8 (P4/M4-4)
P1/M7-2
11.9
P3/M2-5
5.9 E3/M3-2
7.2 P2/M4-6 (P1/M5-9)
15.5
E4/M6-7
9.9 (P2/M9-8)
E4/M7-3
7.4
3.9 E3/M8-1
3.2 E3/M3-8 (P3/M5-3,
3.2 E3/M3-4 E1/M3-11)
4.3 P1/M8-3(CH-6)
RG123
13.8 (P4/M4-6)
3.9 RG64
2.5 E4/M2-2
3.9 P1/M6-8(CH-6)
6.3 E6/M1-7
E4/M1-7
26.0 (P4/M7-9)
P2/M1-3
(P1/M4-1)
7
P2/M9-10
22.1 (P1/M5-4)
4.4 RG128
5.6 E5/M4-14
P3/M6-4
18.8
E5/M4-4
31.7 (P2/M6-5)
P4/M9-4
20.1
3.3 P1/M7-14(CH-7) (E3/M7-5)
2.1 E6/M5-2 ((E4/M1-3)
3.9 P1/M2-5(CH-7)
5.5 E3/M6-8 (E1/M5-2,P2/M6-6)
0.7 P2/M4-7
P4/M7-7
(P2/M6-4)
8
P2/M5-4
20.8
2.4 E6/M1-6
2.9 P2/M5-3(CH-8)
11.1 E1/M7-10
0.3 P1/M3-4(CH-8)
0.3 E7/M6-7
1.2 E1/M5-5
2.2 E3/M5-9
1.6 P3/M10-2
14.2 RG136
P3/M5-3
9
P1/M3-2(CH-9)
10.5
3.2 P1/M5-7
P2/M7-2(CH-9)
12.5
4.2 P1/M7-13
3.5 P1/M7-11
4.4 P1/M3-3(CH-9)
9.5 E1/M3-3
E1/M7-5
46.4
1.3 RG451
0.6 RZ404
3.3 RZ792
3.2 P4/M10-2
4.1 E7/M5-1
8.9 E1/M3-14
E5/M1-4
10
1.4 P4/M1-2
0.0 P3/M9-2
3.4 P3/M5-1
2.8 P2/M2-3(CH-10)
2.8 P4/M1-3
E7/M6-4
30.5
P3/M2-7
11
(E4/M6-6)
RZ557
13.8
E4/M2-7
7.2
P1/M6-4(CH-11)
14.0 (P4/M4-3)
0.0 P2/M4-8
12.5 P1/M5-2(CH-11)
P3/M2-1
6.8
0.0 P3/M2-3 (E4/M3-16,E4/M3-17)
1.3 P2/M2-4(CH-11)
9.5 P3/M9-3
E5/M4-12
(P1/M7-6,P3/M2-2)
12
(E1/M7-12)
E3/M6-4
17.1 (E1/M5-3)
RG241
[P1/M1-5(CH-12),P2/M4-1(CH-12),
10.7 E3/M5-3]
PHO
5.5 RG9 (P1/M7-9,P1/M9-2,E3-M8-8,
6.1 P1/M7-7 E4/M2-1,E5/M4-7,E5/M6-3)
11.5
E1/M5-7
10.3
P1/M4-2 (P1/M10-2,E5/M8-6)
8.1
P1/M7-12
7.0
E1/M5-9
8.9
1.9 RZ76
2.6 RZ261
6.7 RG413
4.3 CDO344
5.3 P2/M9-6 (P1/M7-3)
3.4 E1/M5-12 (E3/M6-5)
P2/M1-1

Table 5 QTLs associated with the P-efficiency parameters RTA, RSDW and RRDW as indicated by single-marker analysis at the 0.005 threshold ($F > 8.2$) and interval-mapping analysis

Phenotypic parameters	QTLs	Marker interval	Chromosome	LOD	Marker	Allele means		Pe[a]	F[b]	R^{2c}
						IR20	IR55178			
RTA	1	E4/M1-8-E4/M3-8	1	2.42	E4/M1-8	93.1	73.7	27.0	9.8	0.099
	2	E3/M8-3-E1/M7-7	6	7.82	E3/M8-3	65.2	99.6	− 34.4	38.9	0.336
					E5/M4-3	68.8	99.8	− 31.0	30.6	0.275
					E1/M7-7	71.0	98.8	− 27.8	24.4	0.231
	3	RG9--RG241	12	16.51	RG9	104.6	61.7	42.9	91.3	0.540
					P1/M7-7	100.8	64.5	36.3	45.9	0.377
					RG241	102.1	66.9	35.2	43.5	0.356
					E1/M5-7	97.5	64.5	33.0	37.6	0.317
					E3/M6-4	96.6	67.2	29.4	29.2	0.254
RSDW	1	E4/M1-8--E4/M3-8	1	2.66	E4/M1-8	68.3	54.1	14.3	11.6	0.108
	2	E3/M8-3--E1/M7-7	6	7.90	E3/M8-3	48.4	70.7	− 22.4	36.1	0.340
					E5/M4-3	50.4	71.5	− 21.1	32.6	0.307
					E1/M7-7	52.7	70.8	− 18.1	22.1	0.227
	3	E1/M7-75--RG451	9	2.47	E1/M7-5	68.6	52.5	16.1	15.5	0.149
	4	RG9--RG241	12	16.98	RG9	76.7	45.4	31.3	120.2	0.608
					RG241	74.3	49.2	25.1	51.6	0.404
					P1/M7-7	72.8	48.9	23.9	38.6	0.357
					E3/M6-4	70.6	49.8	20.8	30.1	0.280
					E1/M5-7	69.3	50.6	18.7	20.8	0.233
RRDW	1	E4/M1-8 ~ E4/M3-8	1	2.42	E4/M1-8	126.2	104.9	21.3	8.8	0.089
	2	E4/M8-3 ~ ~ E1/M7-7	6	5.80	E3/M8-3	97.9	130.2	− 32.3	23.4	0.252
					E5/M4-3	100.4	132.1	− 31.7	22.5	0.234
					E1/M7-7	103.2	131.6	− 28.4	16.8	0.189
	3	RG9 ~ ~ RG241	12	12.05	RG9	139.9	92.9	47.0	62.1	0.442
					RG241	138.9	96.8	42.1	45.4	0.374
					E3/M6-4	131.8	98.7	33.1	23.7	0.242
					P1/M7-7	131.1	99.3	81.8	22.5	0.246
					E1/M5-7	128.6	101.5	27.1	13.6	0.168

[a] Phenotypic effects
[b] The *F*-statistic as determined by the PROC GLM procedure of SAS
[c] Coefficient of determination, the percent of phenotypic variation explained by individual markers as determined from the PROC GLM procedure of SAS

also been reported for several quantitative traits in other crop species (De Vicente and Tanksley 1993; Asins et al. 1994; Veldboom et al. 1994) and has been suggested to be associated to some degree with transgressive segregation (De Vicente and Tanksley 1993). This implies that transgressive lines that have higher tolerance than their parents could be obtained and that breeding could rely on parents with moderate tolerance.

Fig. 1 AFLP/RFLP linkage map of the IR20/IR55178 recombinant inbred population showing the location of quantitative trait loci associated with RTA, RSDW and RRDW. The *solid-filled* areas on chromosomes 12, 1 and 6 represent supporting intervals containing common QTLs underlying the three parameters. The *stippled* areas on chromosome 9 show supporting intervals containing specific QTLs underlying RSDW

The population used in present study had a extremely low RFLP ratio (less than 20% for eight enzymes) because it is derived from a cross between two indica cultivars. Comparisons with RFLP, AFLP (Vos et al. 1995) and Microsatellites (Chen et al. 1997; McCouch et al. 1997) permit the rapid generation of numerous and reproducible markers. Therefore, AFLPs are commonly used for the establishment of molecular maps. In this study, with 58 primer combinations, 217 AFLP markers were detected on all 12 chromosomes. The overall mean distance between markers, as well as the mean distance between markers per chromosome in the present map, satisfied the 20-cM requirement for gene tagging with a 99% selection fidelity (Tanksley 1993). The genomic coverage based on the map developed was about 92.0% of the published rice map (Causse et al. 1994). A few linkage groups contained large intervals between markers. QTLs in these

intervals might not have been detected. Since the map was developed from an intrasubspecific cross, the negative effects of recombination shrinkge and aberrant segregation associated with interspecific and intersubspecific crosses in rice (McCouch et al. 1988; Pham et al. 1990; Lin et al. 1992; Causse et al. 1994; Xu et al. 1997) are minimized. As a consequence, the potential usefulness of the map in plant breeding applications is increased.

One major QTL on chromosome 12 was detected for the three parameters. It was interesting that Acp-1 and Acp-2, which are related to phosphorous uptake and use efficiency in plants, are also on chromosome 12 (Causse et al. 1994). Rice (*Oryza sativa* L.) is a member of the very large and diverse family Gramineae. Comparative mapping has indicated a genetic relationship among cereals including wheat, maize and barley. The discovery of a major QTL for P-deficiency tolerance in rice may lead to the identification of the same orthologous QTL in other species. Fine mapping of major orthologous QTLs may aid selection for high P-efficient genotypes, and ultimately lead to molecular cloning of this key locus.

As stated in the introduction of this paper, we believe that the QTLs identified relate to internal efficiency. Chaubey et al. (1994) classified IR20 (the tolerant parent of our RIL population) as tolerant to P deficiency based on the results of both nutrient solution and P-deficient field tests. The soil they used for the field test contained about 700 mg/kg of total P and about 2 mg/kg of available P. Apparently, the available P was sufficient for IR20 to express tolerance through its internal efficiency. Experiments conducted recently by IRRI in the science field (data not published) have shown that there are genotypes that outperform IR20 with respect to RTA and relative grain yield. The superior types identified, such as IR64, probably possess external efficiency or both types of efficiencies. While fine mapping of the QTL identified for internal efficiency we plan to isolate genotypes with external efficiency, and examine whether the QTLs for the two efficiency types are the same or different.

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