Omonrice 14 1-9 (2006)

MAPPING QTLs FOR PHOSPHORUS DEFICIENCY TOLERANCE IN RICE (*Oryza sativa* . L)

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ABSTRACT

A BC_2F_3 line population was developed by single-seed descent from a cross between OM2395 x AS996. The tolerant variety AS996 to P deficiency is one derivative of Oryza rufipogon whereas high yielding variety OM2395 is sensitive. SSR linkage map consisting of 116 polymorphic SSR markers showed the location of quantitative trait loci associated with relative shoot length, relative root length, relative shoot dry weight, relative root dry weight. SSR technique combined with selective genotyping was used to map quantitative trait loci associated with P-deficiency tolerance in rice. A map consisting of 116 SSR markers was constructed. Its length was 2,905.5cM with an average interval size of 23.05cM. Based on the constructed map, a major QTL for P-deficiency tolerance was located on chromosome 12. Several minor QTLs were mapped on chromosome 1, 2, 5 and 9. This study indicated the candidate gene linked to RM235 and RM247 on chromosome 12, with the interval distance of 0.2 cM.

Key words: P-deficiency tolerance, quantitative trait loci (QTL)

INTRODUCTION

Rice yield are often limited by the low availability of phosphorus (P) in acid sulfate soils in Mekong Delta Vietnam. P deficiency has been identified as a main factor in preventing the realization of high yield potentials of modern varieties in lowland rice production (De Datta et al. 1990). Deficiency can be alleviated by fertilizer application, but farmers are constantly facing financial difficulties when increasing higher input. To develop rice cultivars with an improved tolerance to P-deficiency may therefore be a cost-effective solution to this problem. The existence of significant differences among rice cultivars for tolerance to P deficiency offers opportunities of developing high-yielding cultivars for P-deficiency soils (IRRI 1971, 1976; Katyal et al. 1980; Gunawardena et al. 1978; Ikehashi and Ponnamperuma 1978; Senanayake 1984; Fageria et al. 1988). 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. Development of P-defiency tolerant rice cultivars is constrained by the lack of screening methods suitable for use in breeding program. The screening method currently used relies on tillering ability (Hung 1985; IRRI 1996) where the test genotypes have no to be grown under both P-adequate and P-deficiency conditions and the level of tolerance is determined by relative tillering ability.

Quantitative trait loci (QTLs) for P defiency tolerance had been identified in a rice population derived from a cross of the intolerant japonica cultivar Nipponbare and the tolerant indica landrace Kasalath (Wissuwa et al. 1998), and the cross between tolerant indica cultivar IR20 and indica sensitive variety to P-deficiency IR55178-3B-9-3 (Ni et al. 1998). 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 (Ni et al. 1998).

A major QTL for P uptake had previously been mapped to a 13-cM marker interval on the long arm of chromosome 12 (Wissuwa et al. 2002). *Pup-1* gene was placed in a 3-cM interval flanked by markers S14025 and S13126, which is within 1 cM of the position

identified in the original QTL mapping experiment. (Wissuwa et al. 2002).

These studies showed that only chromosome 12 contributed to differences P-uptake. P deficiency tolerance in rice can be attributed to two mechanisms, namely, internal efficiency and external efficiency (Ni et al. 1998)

MATERIALS AND METHODS

Plant materials

A BC_2F_3 line population was developed by single-seed descent from a cross between OM2395 x AS996. A tolerant variety AS996 to P deficiency is one derivative of *Oryza rufipogon* whereas high yielding variety OM2395 is sensitive. A molecular genetic survey was conducted to find PCR-based markers that detected polymorphism between DNAs from the cross OM2395 / AS996.

Rapid isolation of rice DNAs

The young leaf was ground using a polished glass rod in a well of a Spot Test plate (Thomas Scientific) after adding 400 µl of extraction buffer (50 mM Tris-HCl pH 8.0, 25 mM EDTA, 300mM NaCl and 1% SDS). Ginding was done until the buffer turned green which is an indication of cell breakage and release of chloroplasts and cell contents . Another 400µl of the extraction buffer was added and mixed into the well by pipetting. 400µl of the lysate was transferred to the original tube of the leaf sample. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA precipitated using absolute ethanol. DNA was air-dried and resuspended in 50µ l of TE buffer (10mM Tris- HCl pH 8.0, 1mM EDTA pH 8.0).An aliquot of 1µ l is sufficient for PCR analysis. DNA quality and quantity were spectrophotometrically determined.

PCR amplification:

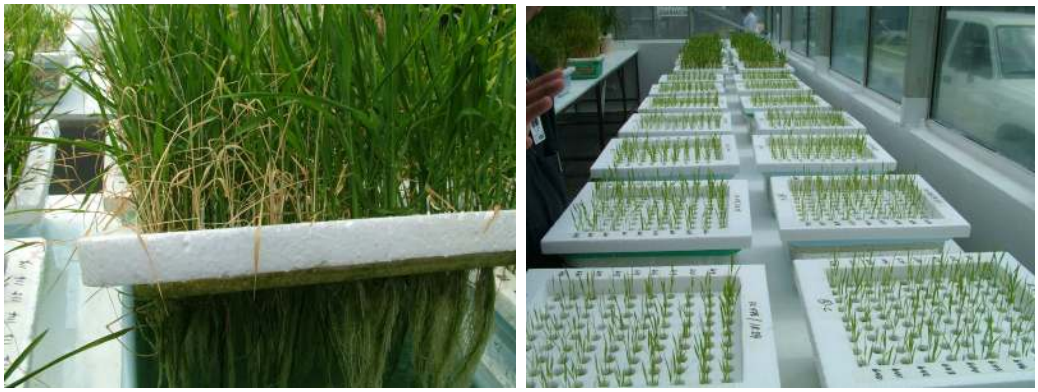
The genomic DNA from both of the BC_2F_3 plants and the parents were subjected to PCR amplification using the synthesized primers. The PCR buffer consisted of : Tris pH 8.4[10mM], KCl[50mM], $MgCl_2$[1.8mM] 0.01mg/ml gelatin. *Taq* [5 unit of *Taq* polymerase in a volume of 25µl]. Template DNA were initially denatured at 94^{o}C for 5 min, followed by 30 cycles of PCR amplification under the following parameters, 1 min denaturation at 94^{o}C, 1 min primer annealing at 55^{o}C and 2 min primer extension at 72^{o}C. Final 5 min incubation at 72^{o}C was allowed for completion of primer extension on a 480-thermal cycler. The amplified products were electrophoretically resolved on 1.2 % agarose and using 1X TAE buffer.

QTL analysis

QTL analysis was performed with software package Q-GEN 1994 from Cornell University and MapL 1995 from Japan University. MapMarker/QTL(IRRI) was use to fine the location of major and minor genes. QTL detection was performed by single marker analysis (SMA) and interval mapping (IM). One way ANOVA was performed for each single marker and each combination of two markers to be identified as putatively associated with salt tolerance. This was done to confirm the association between the marker and P-deficiency tolerance loci. Log of odds (LOD) peaks for each significant QTL were use to position the QTL on the map. The threshold for declaring a QTL for P-deficiency tolerance was LOD>3. All markers were tested for the expected 1:1. To identify the mode of inheritance, reexamination of putative QTL regions was carried out by three constrained genetics, such as dominant, recessive, and additive using MAPMAKER/QTL software. Likelihood ratio (LRs) were calculated at 1cM interval along the mapped genome. The proportion of phenotypic variation explained by significant marker was estimated as a coefficient of determination (R^2) for the single locus model.

RESULTS AND DISCUSSION

Screening for P efficiency, in which cultivars are tested for relative tillering ability using P-deficiency (0,5mg/l) and P-adequate (10.0mg/l) culture solution (Yoshida et al. 1976).



P-deficiency (0,5mg/l) and P-adequate (10.0mg/l)

Figure 1: Screening for P deficiency in greenhouse

Phenotypic variation in salinity tolerance

The mean of relative root length (RRL), relative shoot length (RSL), relative root dry weight (RRDW), relative shoot dry weight (RSDW) of parents, the whole population, and the selected lines are shown in figure 2. Segregation among the BC_2F_3 lines for the parameters was observed and the distributions of them were normal.

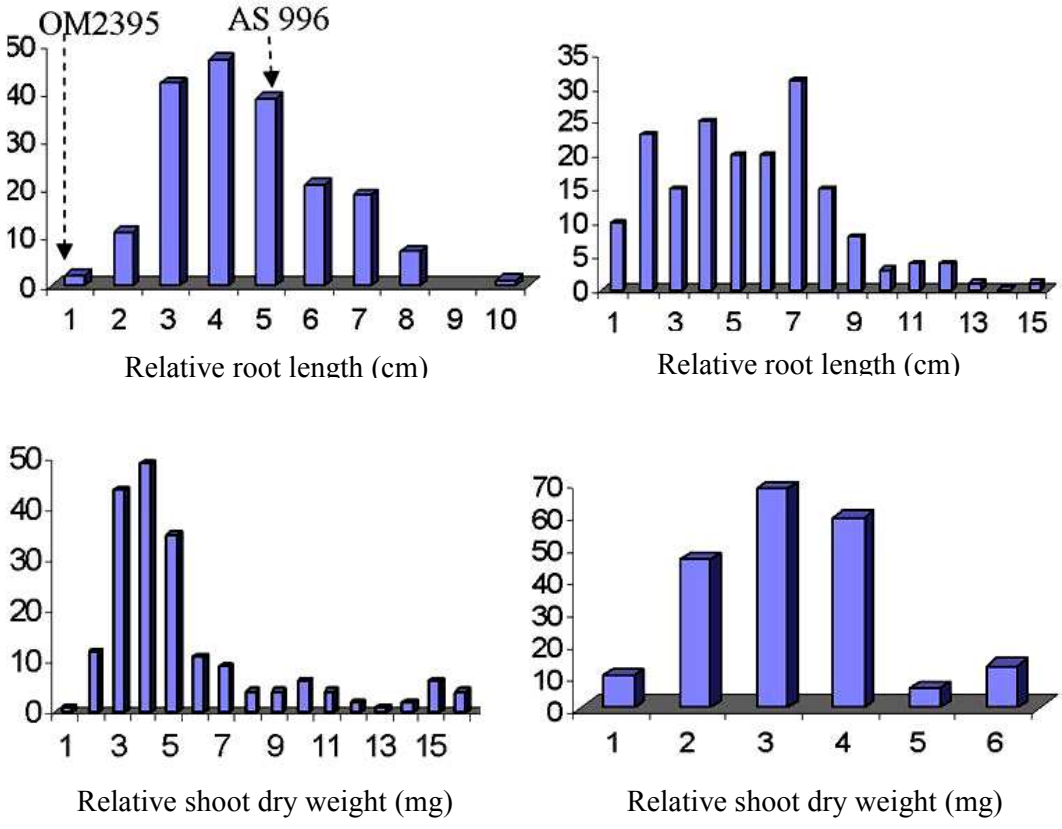


Figure 2: Phenotypic distribution for root length (RL) shoot length(SL) and relative shoot dry weight (RSDW), relative root dry weight(RRDW)

Construction of a linkage map

Linkage analysis was performed with microsatellite mapping data using MapMarker version 3.0 (Lander et al. 1987).

A molecular map was constructed according to published microsatellites from Cornell University and from Japan. The 116 microsatellite markers, were assigned to linkage group. MapMarker was used to generate microsatellite. Figure 3 show the linkage map for 116 SSR markers employed in this study. Although there are a few gaps of more than 50cM, the linkage map had a total map length of 2,905.50 cM. The average interval size was 23.05cM, the smallest size in chromosome 12 and chromosome 9 (12.50cM) (Table 1) and the largest in chromosome 3. There are a few gaps larger than 50 cM. It indicated that the genetically related parents caused the low turn of polymorphism for microsatellite markers.

Table 1: Distribution of markers on each chromosome among 225 individuals of BC2F3 population of OM2395 / AS996

Chromosome	cM	Number of SSRs	Mean of genetic distance between 2 markers
1	507.5	18	28.19
2	206.7	14	14.76
3	795.9	12	66.33
4	216.7	13	16.70
5	196.6	11	17.87
6	101.4	6	16.90
7	319.2	13	24.55
8	115.7	7	16.52
9	99.9	8	12.48
10	79.9	5	15.98
11	115.9	7	16.55
12	150.1	12	12.50
Total	**126**	**23.05**	

Mapping QTLs for salinity tolerance

A mapping population of 225 BC_2F_3 lines derived from a cross between OM2395 / AS996 by single seed descent method, was used to detect quantitative trait loci (QTLs) for traits associated with P deficiency tolerance.

Table 2 showed the results of one way ANOVA analysis. The association between relative shoot length's indices of RM306, RM322, RM291, RM316, RM247, RM235 was significant ($P < 0.05$). Six markers displayed a significant deviation from expected 1:1 allele frequency ratio in the selective genotyping experiment.

From the random sample and SMA, F-tests were significant, indicating markers associated with P-deficiency tolerant. Table 2 showed that individual putative QTL explained the average of phenotypic variation $11.01 \leq R^2 \leq 11.67\%$ for relative shoot length. RM247, RM235 showed the highest F-value ($P<0.001$) and therefore are most likely to be linked to P-deficiency tolerant trait. Four QTLs were detected for relative shooth length in chromosome 1, 2, 5, 9 and 12.

Table 2: QTLs identified by single marker analysis for relative shoot length (RSL)

Marker	Chr.	G-type	Allelic Mean ±SE	F-value	P-value	R^2 (%)	DPE
RM306	1	A B	16.50±0.44	5.80	0.001	11.21	B
RM322	2	A B	22.40±0.45	3.16	0.006	11.28	B
RM291	5	A B	22.40±0.45	2.57	0.054	11.67	B
RM316	9	A B	16.50±0.45	3.32	0.020	11.56	A
RM247	12	A B	16.5±0.44	5.80	0.001	11.21	B
RM235	12	A B	16.50±0.44	5.80	0.001	11.01	A

DPE: (Direction of phenotypic effect) A: AS996, B: OM 2395

Two markers RM297 and RM322 were detected to be linked to relative root length (RRL) in chromosome 1 and 2. They explained 2.37%, and 9.19 % of the phenotypic variation, respectively.

Table 3: QTL identified by single marker analysis for relative root length (RRL)

Marker	Chr.	G-type	Allelic Mean ±SE	F-value	P-value	R^2 (%)	DPE
RM297	1	A B	9.85±0.44	2.83	0.039	2.37	B
RM322	2	A B	10.36± 0.52	2.25	0.039	9.19	B

DPE: (Direction of phenotypic effect) A: AS996, B: OM 2395

Four markers RM302, RM322, RM291 and RM247 were detected to be linked to relative dry shoot weight (RDSW) in chromosome 3 and 9. They explained 9.04, 9.19, 8.64 and 9.13% of the phenotypic variation, respectively

Bảng 4. QTL identified by single marker analysis for relative dry shoot weight (RDSW)

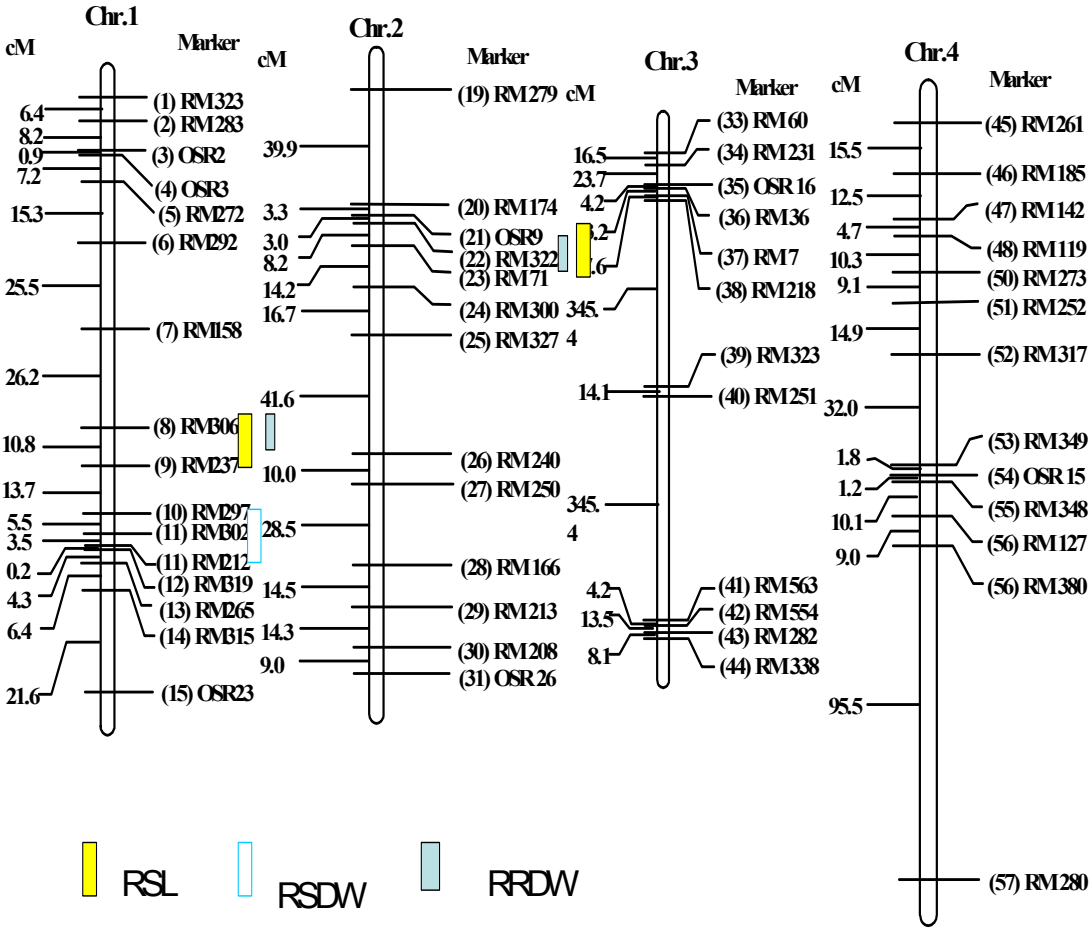
Marker	Chr.	G-type	Allelic Mean ±SE	F-value	P-value	R^2 (%)	DPE
RM302	1	A B	9.85±0.44	3.87	0.005	9.04	B
RM322	2	A B	10.36± 0.52	2.25	0.039	9.19	B
RM291	5	A B	10.13±0.38	8.42	0.000	8.64	B
RM247	12	A B	12.72±0.400	3.99	0.009	9.13	B

DPE: (Direction of phenotypic effect) A: AS996, B: OM2395

Composite interval mapping was implemented using QTL MapMarker software, with a threshold of LOD > 3.0. A total three putative QTLs were detected with percentage of variance explained running between 11.2% - 9.13% for RSL, RSL and RSDW.

Table 4: Interval mapping analysis of the target characters

Index	Interval marker	Chromosome	P-value	centi-Morgan
8-9 (RSL)	RM306-RM237	1	0.001	10.8
63-64 (RSDW)	RM291-RM261	5	0.000	12.9
125-126 (RSDW) (RSL)	RM235-RM247	12	0.001	0.2



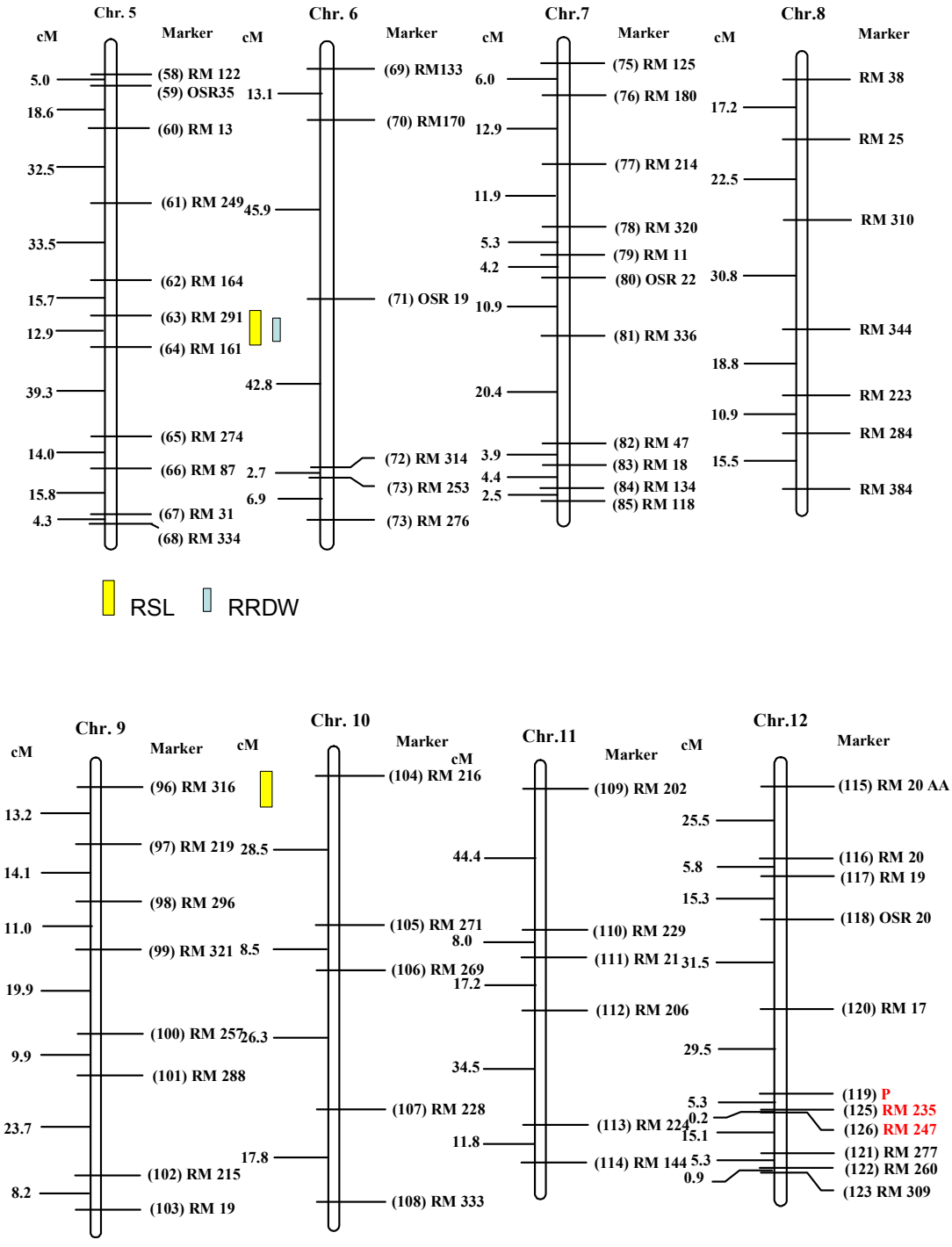


Figure 3: SSR linkage map of the OM2395 / AS996 BC_2F_3 population showing the location of quantitative trait loci associated with relative shoot length, relative root length, relative shoot dry weight, relative root dry weight

Relative shoot dry weight (RSDW) and relative shoot length (RSL) showed as indicators of P-deficiency tolerance. Dry weight production under P-deficient conditions largely depended on P uptake (Fageria et al. 1988). Absolute and relative tiller numbers have been suggested as indirect indicators of P-deficiency tolerance in rice (Hung 1985; Chaubey et al. 1994). QTLs on chromosome 12 are good candidates because positive allele in each case come from the parent, namely *Pup-1* linked to S14025 and S13126 (Wissuwa et al. 1998, 2002), *PHO* linked to RG9 and RG241 (Ni et al. 1998); and this study indicated the candidate gene linked to RM235 and RM247 with the interval distance of 0.2 cM (table 4).

CONCLUSION

1. SSR technique combined with selective genotyping was used to map quantitative trait loci associated with P-deficiency tolerance in rice
2. A map consisting of 116 SSR markers was constructed. Its length was 2,905.5cM with an average interval size of 23.05cM
3. Based on the constructed map, a major QTL for P-deficiency tolerance was located on chromosome 12 linked to RM235 and RM247
4. Several minor QTLs were mapped on chromosome 1, 2, 5 and 9.

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Bản đồ QTL tính trạng chống chịu thiếu lân

Thiếu lân là hạn chế năng suất chính, đặc biệt trong vụ Hè thu ở đồng bằng sông Cửu Long trên đất phèn. Giống chống chịu thiếu lân là mục tiêu chính của nhà chọn giống trong trường hợp này. Bản đồ di truyền tính trạng số lượng (QTL) điều khiển chống chịu thiếu lân được thực hiện trên quần thể BC_2F_3 của tổ hợp lai OM2395 x AS996. Giống AS996 là dẫn xuất của lúa hoang *Oryza rufipogon* chống chịu thiếu lân. Giống OM2395 là giống cao sản, nhạy cảm thiếu lân. Bản đồ được phủ bởi 116 SSR markers, với chiều dài tổng số 2.905,5 cM, trung bình quãng giữa hai marker là 23,05 cM. Bản đồ tập trung vào kết qủa đánh giá kiểu hình của 4 tính trạng có liên quan: chiều dài rễ tương đối (RRL), chiều dài thân tương đối (RSL), trọng lượng khô rễ tương đối (RRDW), trọng lượng khô thân tương đối (RSDW). Trên cơ sở phân tích bản đồ, QTL chủ lực được xác định trên nhiễm sắc thể 12, và nhiều QTL thứ yếu được xác định trên nhiễm sắc thể 1, 2, 5, và 9. Gen ứng cử viên định vị ở vùng có liên kết rất chặt chẽ với hai marker RM235 và RM247 trên nhiễm sắc thể số 12, với khoảng cách của quãng giữa hai marker là 0.2 cM.