

SSR ANALYSIS OF RICE NEAR-ISOGENIC LINES (NILs) FOR P-DEFICIENCY TOLERANCE

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SUMMARY

Analysis of five near-isogenic lines (NILs) differing in tolerance of phosphorus (P) deficiency was conducted using simple sequence repeat (SSR) markers. These NILs were segregating for a major QTL on chromosome 12, called *Pup1*, which explains about 80% of the phenotypic variance for P-deficiency tolerance. By analyzing the graphical genotypes of the NILs, a relatively small, ~ 5 cM chromosomal segment containing *Pup1* was identified. SSR markers that were linked to the *Pup1* locus were tested in a sample of accessions in order to determine whether these markers could be useful for marker-assisted selection. The four polymorphic markers identified, clearly discriminated between the Kasalath donor parent and the Kasalath x Nipponbare-derived NILs, and could be useful for monitoring *Pup1* introgression in breeding programs. Band sizes varied in other accessions including other potential phosphorus deficiency tolerance donors and elite genotypes, which might suggest that markers need to be individually selected for a particular cross.

Key words: Phosphorus deficiency, near-isogenic lines, graphical genotypes, SSR, marker assisted selection.

The low level of plant available phosphorus (P) in the soil is an important factor limiting rice production in many regions of the world, especially in upland areas. This is because many of these rice soils are characterized by high P-fixing ability. Therefore, exploiting the existing genetic variation for tolerance of P-deficiency is an important breeding objective (Wissuwa and Ae, 2001a). Genetic analysis of tolerance of P-deficiency has indicated quantitative inheritance (Ni *et al.*, 1998; Wissuwa *et al.*, 1998). Research in mapping quantitative trait loci (QTLs) associated with P-deficiency tolerance has identified several regions that were associated with this trait using a Kasalath (tolerant of P-deficiency) x Nipponbare (sensitive) population (Wissuwa *et al.*, 1998). A major QTL designated as *Pup1* (P uptake) was identified in the proximal region of chromosome 12 (Wissuwa *et al.*, 1998, 2002). This QTL offers much promise for breeding for enhanced tolerance of P-deficiency because *Pup1* was detected with a very high LOD score (10.5-11.6) and explained ~ 78% of the phenotypic variance for P uptake, tiller number, and dry weight, which are traits used to assess P-deficiency tolerance (Wissuwa *et al.*, 2001b, 2002). Another minor QTL on the proximal region of chromosome 6 was also found to

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significantly enhance P uptake (Wissuwa and Ae, 2001b). A transcription factor gene, *OsPTF1*, has recently been cloned from this region and was shown to increase tolerance of P-deficiency (Yi *et al.*, 2005).

Further characterization of near-isogenic lines (NILs) derived from the original mapping population has confirmed the preliminary mapping of these two QTLs (Wissuwa and Ae, 2001b; Wissuwa *et al.*, 2002). Since previous QTL maps were constructed with restriction fragment length polymorphism (RFLP) markers, the aim of this research was to use simple sequence repeat (SSR or 'microsatellite') markers to further characterize the NILs targeting the *Pup1* locus on chromosome 12 and also the distal region on chromosome 6 with the minor QTL for P-deficiency tolerance. Both QTLs were derived from the Kasalath parent.

MATERIALS AND METHODS

Plant material and DNA extraction

Parental lines Kasalath and Nipponbare and five selected NILs (Nipponbare background) with well-characterized phenotypes to P-deficiency called NIL C443, NIL 6-3, NIL 6-4, NIL 14-4, and NIL 14-6, were used for SSR analysis of *Pup1*. A selection of 13 additional accessions, most with known phenotypes for P-deficiency tolerance, was used for marker validation (Table 1; Wissuwa and Ae, 2001a). DNA was isolated from seedling leaf tissue using the sodium hydroxide-Tris method (Wang *et al.*, 1993).

PCR and gel electrophoresis

PCR mixtures contained PCR buffer (Promega), 2 mM of MgCl₂, 0.2 mM dNTPs, 1 U of *Taq* polymerase (Promega), 0.5 μM of each SSR primer, and 2 μl (5 μM) of DNA template. SSR primer sequences were obtained from Chen *et al.* (1997), McCouch *et al.* (2002), and Temnykh *et al.* (2000). The total reaction volume was 10 μl. The PCR cycle consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min, and a final extension step of 72°C for 3 min. PCR products were resolved using polyacrylamide gels: 6% gels were run for 1 hr 30 min, whereas 8% gels were run for 2 hr 15 min at 100 V. Gels were stained with ethidium bromide and visualized under UV light.

Data analysis

SSR marker positions flanking the *Pup1* QTL on chromosome 12 and the minor QTL on chromosome 6 were determined from the IRMI 2003 genetic map and the rice physical map using the Gramene Web site (www.gramene.org). SSR marker alleles were scored and used to produce 'graphical genotypes' of the NILs using the software program GGT (Van Berloo, 1999). BLAST searches were used to determine the positions of tightly-linked RFLP markers with *Pup1* from Wissuwa *et al.* (2002) on the physical map and to correlate marker positions in relation to the SSR map.

RESULTS

A total of 41 SSR primers, including 28 from chromosome 12 and 13 from chromosome 6, were tested for polymorphism using DNA samples from Kasalath and Nipponbare. Sixteen primers from chromosome 12 and 11 primers from chromosome 6

Table 1. Analysis of a representative set of accessions with tightly linked SSR markers for *Pup1*. Allele sizes (base pairs) were estimated from polyacrylamide gels. Kasalath alleles are indicated in bold.

Line or accession	Phenotype ¹	SSR marker			
		Physical map (Mb)		IRMI 2003 map (cM)	
		RM28102	RM1261	RM277	RM519
		16.0	17.6	18.2	20.0
		-	61.6	57.2	62.6
Kasalath	T	174	198	128	163
Nipponbare	S	180	200	123	148
NIL C443	T	174	198	128	163
NIL 6-3	S	180	200	123	148
NIL 6-4	T	174	198	128	163
NIL 14-4	T	174	198	128	163
NIL 14-6	S	180	200	123	148
IR64	I	171	202	128	161
IR71525	U	171	200	128	161
IR74371	U	171	200	128	163
IAC25	T	171	192	123	148
IAC47	T	178	192	123	148
Dular	T	180	250	123	149
Jalmagna	T	178	200	123	148
IR66	I	171	205	128	161
IR72	I	190	220	133	157
Lemont	S	md ²	210	123	148
YS27	I	md ²	205	123	148
Way Rarem	U	171	205	127	161
IR36	I	190	200	133	157

¹Phenotypes were classified from Wissuwa and Ae (2001) based on P uptake from a P-deficient soil or from field observations based on unpublished data of M. Wissuwa: T = tolerant; I = Intermediate; S = Sensitive; U = unknown.

²md = missing data. PCR using DNA from these accessions was unsuccessful after four repetitions (even after fresh DNA extractions), which suggests that PCR conditions need to be specifically optimized for these templates or null alleles are present.

produced clear polymorphisms and these primers were used to genotype the NILs. Analysis of the graphical genotypes indicated that several recombination events had occurred on chromosome 12 (Figure 1). The rice physical map was used to determine the correct order of the SSR markers. When compared to the IRMI 2003 genetic map, the order was almost identical with the exception of the position of marker RM277. Comparison of both maps clearly indicated the differences between the physical and genetic distances. For the NILs that were tolerant of P-deficiency, the sizes of the Kasalath donor segment ranged from approximately 30 to 65 cM when genetic distances were used. By deduction from the phenotypic data and genotypes of the NILs, the interval in which *Pup1* was located was delineated to approximately 14 to 20 Mb (or 57 to 62 cM based on the IRMI 2003 genetic map). This is consistent with the physical map position of two tightly linked RFLP markers from Wissuwa *et al.* (2002): S14025 (13 Mb) and S13752 (15.8 Mb).

Analysis of the graphical genotypes for chromosome 6 indicated that, for all of the NILs used in this study, this region was inherited from the Nipponbare recurrent parent (data not shown). This indicated that it was extremely unlikely that any QTLs on chromosome 6 were contributing to the phenotype, and is consistent with the fact that background markers were used to select against Kasalath during the development of NIL C443 (Wissuwa and Ae, 2001a, 2001b). Based on the analysis of the graphical genotypes, four markers were identified as being tightly linked to *Pup1* and these markers were used to genotype the 13 selected accessions (Figure 2). The SSR allele sizes are indicated in Table 1.

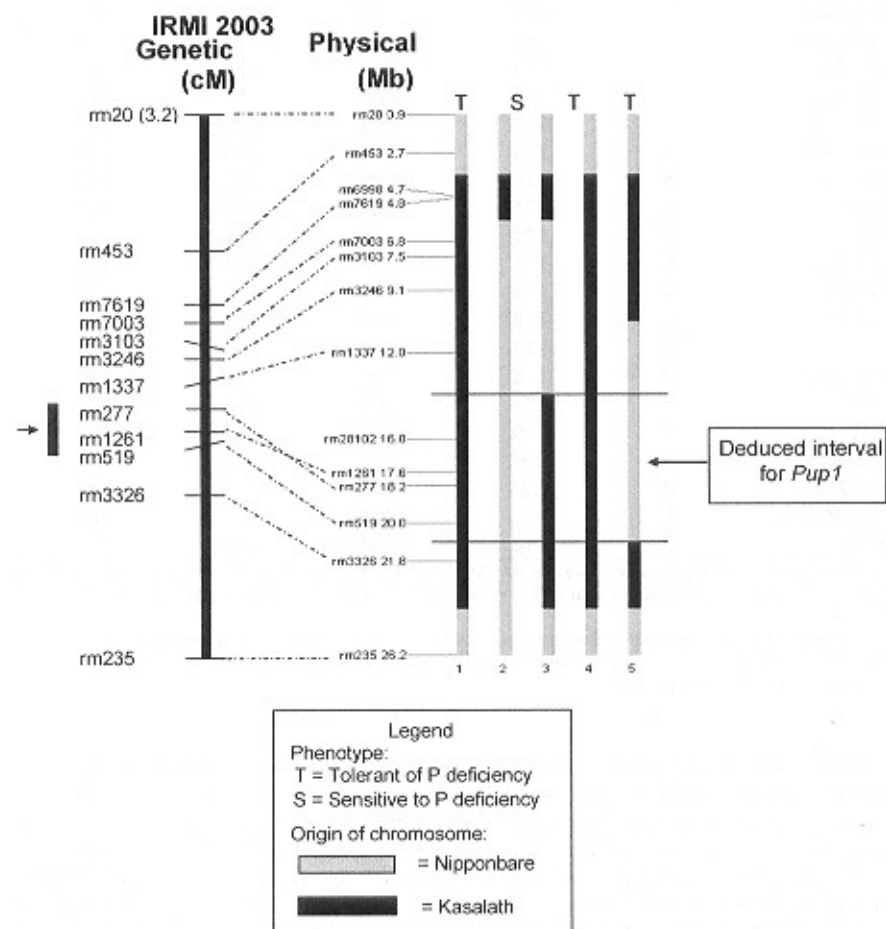


Figure 1. Graphical genotypes of chromosome 12 for P-deficiency-tolerant and -sensitive NILs based on the SSR positions located on the rice physical map. The deduced region for *Pup1* is indicated on both maps with arrows. The IRMI 2003 genetic map is shown for comparison. Two markers (RM6998 and 28102) were not mapped in the IRMI 2003 population. 1= NIL C443, 2= NIL 6-3, 3= NIL 6-4, 4= NIL 14-4, 5= NIL 14-6.

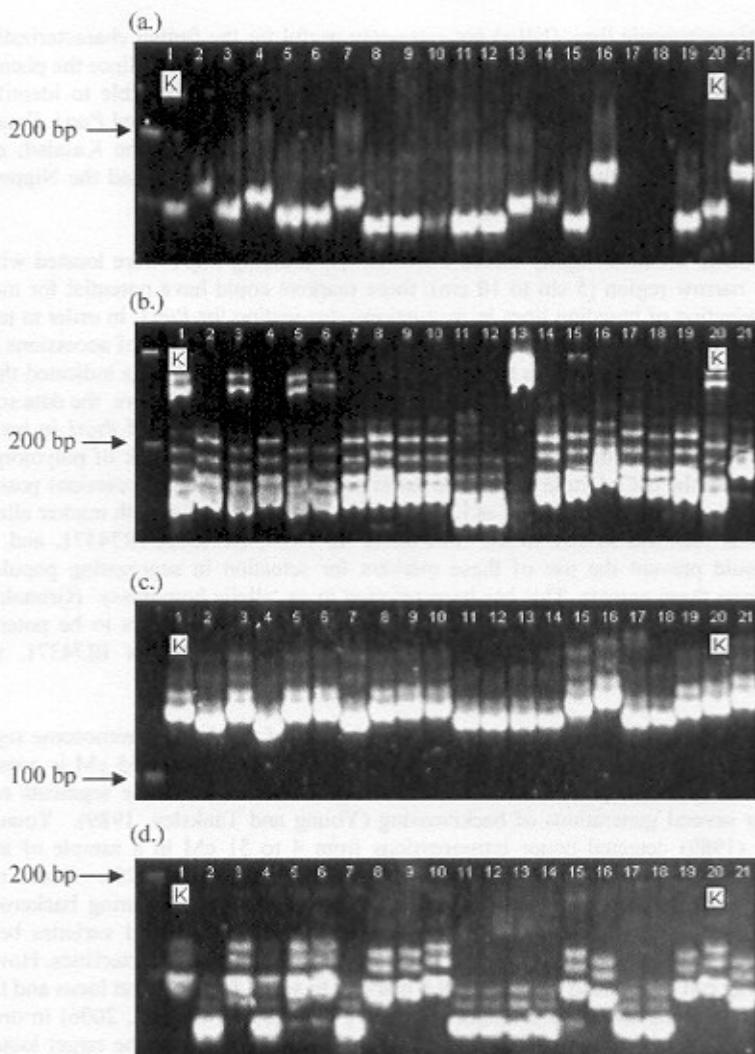


Figure 2. Polyacrylamide gel photos of *Pup1* SSR markers screened on a set of accessions. a) marker RM28102, b) RM1261, c) RM277, and d) RM519. For marker allele size estimations, Kasalath was run twice (marked with 'K'). Lanes: 1- Kasalath; 2- Nipponbare; 3- NIL C443; 4- NIL 6-3; 5- NIL 6-4; 6- NIL 14-4; 7- NIL 14-6; 8- IR64; 9- IR71525; 10- IR74371; 11- IAC25; 12- IAC47; 13- Dular; 14- Jalmagna; 15- IR66; 16- IR72; 17- VL701; 18- Lemont; 19- Way rarem; 20- Kasalath; and 21- IR36. Molecular weight markers are indicated with arrows.

DISCUSSION

Near-isogenic lines (NILs) are extremely useful for the further characterization of a single QTL because the effects of genetic background are eliminated. Since the phenotype for these selected NILs had been previously determined, it was possible to identify the chromosomal region associated with P-deficiency tolerance that contained *Pup1*. Four SSR markers (RM28102, RM1261, RM277, and RM519) indicated that the Kasalath alleles were present in all of the tolerant NILs, whereas the sensitive NILs had the Nipponbare alleles at these marker loci.

Since the most tightly linked SSR markers flanking *Pup1* were located within a relatively narrow region (5 cm to 10 cm), these markers could have potential for marker-assisted selection of breeding lines in populations segregating for *Pup1*. In order to test the level of polymorphism of these four SSR markers, a representative set of accessions (most of which had known phenotypes to P-deficiency) was tested. The results indicated that the Kasalath SSR marker haplotype was unique. From a breeding perspective, the data suggest that the four SSRs could be useful for monitoring the introgression of *Pup1* in breeding material. However, with some primer/accession combinations, the lack of polymorphism would prevent the use of these markers to assist selection, since some accessions possessed the same size of SSR marker allele as Kasalath. For example, the Kasalath marker allele for RM277 was identical in size to the SSR allele for IR64, IR71525, IR74371, and IR66, which would prevent the use of these markers for selection in segregating populations derived from these parents. This has been referred to as 'allelic homoplasy' (Grimaldi and Crouau-Roy, 1997; Hayden *et al.*, 2004). The marker RM519 appears to be potentially useful with the exception of populations derived from Kasalath x IR74371, which possessed the same allele as Kasalath.

SSR analysis of the NILs also enabled the size of the donor chromosome segment from Kasalath to be monitored. The approximate size range of 30 to 65 cM is consistent with theoretical and experimental results that large donor chromosome segments remain even after several generations of backcrossing (Young and Tanksley, 1989). Young and Tanksley (1989) detected donor introgressions from 4 to 51 cM in a sample of tomato backcross populations even after numerous backcross generations (>20). These results clearly demonstrate the extent of linkage drag that usually occurs during backcrossing. This could be a potential problem for transferring *Pup1* into adapted varieties because Kasalath is a landrace and possesses many undesirable agronomic characteristics. However, linkage drag can be reduced by using DNA markers to select for the target locus and tightly linked flanking markers ('recombinant selection'; Collard and Mackill, 2006) in order to identify lines in which a recombination event has occurred between the target locus and flanking SSR markers (Chen *et al.*, 2000; Frisch *et al.*, 1999; Young and Tanksley, 1989).

In conclusion, the SSR markers identified in the *Pup1* region in this study could be useful for the introgression of P-deficiency tolerance from Kasalath into specific genetic backgrounds, and some of the less tightly-linked markers could be useful for recombinant selection, in order to minimize linkage drag.

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