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Fine mapping of the rice low phytic acid (*Lpa1*) locus

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Abstract Phytic acid is the primary storage form of phosphorus (P) in cereal grains. In addition to being essential for normal seedling growth and development, phytic acid plays an important role in human and animal nutrition. The rice low phytic acid mutation *lpa1* results in a 45% reduction in seed phytic acid with a molar equivalent increase in inorganic P. The *Lpa1* locus was previously mapped to the long arm of chromosome 2. Using microsatellite markers and a recombinant inbred line population, we fine mapped this locus between the markers RM3542 and RM482, which encompass a region of 135 kb. Additional markers were developed from the DNA sequence of this region. Two of these markers further delimited the locus to a 47-kb region containing eight putative open reading frames. Cloning and molecular characterization of the *Lpa1* gene will provide insight into phytic acid biosynthesis in plants. The markers reported here should also be useful in introgressing the low phytic acid phenotype into other rice cultivars.

Introduction

Phytic acid (PA, also known as *myo*-inositol hexa *kis*phosphate) is the primary storage form of phosphorus (P) in seeds, accounting for up to 85% of the seed total P. The negatively charged phosphates in phytic acid strongly bind to metallic cations (e.g. K, Mg, Mn, Fe, Ca, and Zn) to form a mixed salt called phytin or phytate that accumulates in seed protein bodies either dispersed throughout the bodies or in dense inclusions called globoids (Lott et al. 1995). Phytin is broken down following germination and provides the P, *myo*-inositol, and mineral cations that are needed for seedling growth and development (Oatway et al. 2001). PA also plays an important role in animal and human nutrition. Monogastric animals such as pigs, poultry, and fish cannot digest PA. As a result, animal feeds are supplemented with P, and a large fraction of PA is excreted, subsequently accumulating in soil and water which leads to the pollution of waterways (Sharpley et al. 1994). The ability of PA to bind divalent minerals, resulting in the formation of stable complexes that prevent the absorption of these important nutrients from foods or feeds (Torre et al. 1991), can lead to micronutrient deficiencies (e.g., iron and zinc), particularly in Third World countries where diets are primarily seed-based.

Low phytic acid mutants have been isolated in some key crops, including maize, barley, rice, soybean, and wheat (Raboy and Gerbasi 1996; Larson et al. 1998, 2000; Rasmussen and Hatzack 1998; Dorsch et al. 2003; Wilcox et al. 2000; Hitz et al. 2002; Guttieri et al. 2004). The development of low phytate crops may improve phosphate bioavailability in animal feeds and reduce phytic acid excretion, thus lessening some of the negative effects of animal waste on the environment (Raboy 2001). Another potential advantage of such crops is an increase in the availability and uptake of iron and zinc, which would significantly improve human nutrition. Initial studies have indicated that low phytic acid products increase the absorption of several important minerals (Mendoza et al. 1998, 2001; Sugiura 1999; Adams et al. 2002).

In maize, where low PA mutants have been partially characterized, normal levels of seed total P accompany low levels of PA. Two mutations affecting phytic acid

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levels, *lpa1*and *lpa2*, have been identified. Seeds of homozygous *lpa1* plants have reduced PA in comparison to the wild type and a molar increase in inorganic P (Pi). Seeds of homozygous *lpa2* plants have reduced PA and a molar increase in Pi and inositol phosphates (Raboy et al. 2001). The maize *lpa1* mutation maps near one of the seven maize *myo*-inositol phosphate synthase genes, *MIPS*, which encodes MIPS, an enzyme involved in the first step of inositol phosphate biosynthesis. In maize, the *MIPS* gene and protein expression is reduced in *lpa1* seeds compared to the wild type (Raboy et al. 2000; Pilu et al. 2003; Shukla et al. 2004). Sequence analysis of the *MIPS* gene that maps near the *lpa1* mutation did not reveal any lesions within the coding sequence (Shukla et al. 2004), and the nature of the *lpa1* mutation in maize still needs to be determined. More progress has been made with respect to the *lpa2*-type mutation, which has been shown to affect a gene encoding an inositol phosphate kinase, ZmIpk (Shi et al. 2003). This gene is expressed in the maize embryo where phytic acid is known to accumulate (Shi et al. 2003).

A rice low phytic acid mutant, *lpa1*, has recently been isolated from a gamma-irradiation induced mutant population (Larson et al. 2000). This mutant is similar to maize and barley *lpa1* mutants in that it has a low phytic acid content and high Pi. Seeds of homozygous *lpa1* plants have a 45% reduction in seed PA with a molar equivalent increase in Pi. This rice *lpa1* mutant has been shown to be the result of a single recessive mutation mapping to rice chromosome 2L (Larson et al. 2000). As the rice ortholog of *MIPS* has been mapped to chromosome 3, Larson et al. (2000) concluded that the rice *lpa1* mutation is not in the rice *MIPS* gene.

Using a recombinant inbred line (RIL) mapping population and published microsatellite markers, we have constructed a fine genetic map of the rice *Lpa1* locus. Additional markers were developed using publicly available rice DNA sequences, and the locus has been delimited to a 47-kb region containing at least eight putative open reading frames (ORFs).

Materials and methods

Plant materials and DNA extraction

A total of 341 RILs derived from 341 F_2 progeny resulting from a cross between Kaybonnet 1-1 (KB 1-1; *lpa1*/*lpa1*; tropical *japonica*; Larson et al. 2000; Rutger et al. 2004b) and Zhe 733 (*Lpa1*/*Lpa1*; *indica*; Yan and Cai 1991) were used for fine mapping of the locus. Of these 186 RILs were in the F_8 generation and the remaining 155 RILs were in the F_7 generation. The generation difference between the two sets of RILs is due to differences in fertility, heading dates, and other factors that prevented simultaneous advancement of all 341 lines. Kaybonnet 1-1 has a 45% reduction in phytic acid P compared to wild-type Kaybonnet (Larson et al. 2000). The seed phytic acid P content of KB1-1 is approximately 1.39 mg/g; for Zhe 733, it is 2.56 mg/g (K. Peterson and V. Raboy, personal communication). Plants were grown in the greenhouse, and leaf samples were collected and stored frozen at −80°C prior to extraction. Genomic DNA samples were extracted using the method of Tai and Tanksley (1990).

Low phytic acid scoring

Eight seeds per line were indirectly phenotyped for low PA content using the high Pi colorimetric assay as described by Larson et al. (2000) with the following minor modifications. The seeds were soaked in 0.4 *M* HCl overnight, then crushed using a 96-well grinding tool and incubated for 1 h at room temperature (RT). Colorimetric assays were performed using freshly prepared Chen's reagent (Chen et al. 1956). About 10 µl of the supernatant from the ground seeds was mixed with 90 µl water and 100 µl Chen's reagent. Color reactions were observed following incubation of the solution for 1 h at RT. A blue color indicated the presence of relatively high levels of Pi typical of *lpa1*/*lpa1* seeds, whereas colorless samples typified wild-type levels of Pi found in *Lpa1*/*Lpa1* seeds.

Microsatellite analysis and linkage analysis

Microsatellite markers within the region flanked by RM207 and RM208 (as previously reported by Larson et al. 2000) were identified from the Gramene Database (http://www.gramene.org). Microsatellite markers RM207, -208, -138, -535, -250, -266, -498, -482, -3542, -5916, -1092, -1251, -3535, -3692, -3774, -3789a, -3789b, -5421, -5300, -5894, -6307, -5421, -3248, -5894, -1063, -6307, -1255, -2265, -3650, -3850, -6312, -4470, -5643, -7337, and -7388 were obtained from Research Genetics (Huntsville, Ala.). Microsatellite marker analysis was performed as described by Panaud et al. (1996) with modifications to employ automated genotyping. The PCR reactions consisted of 50–100 ng template DNA, 0.25 µ*M* primer, 1× PCR buffer (20 m *M* Tris-HCl pH 8.0, 50 m *M* KCl, 2.5 m *M* $MgCl_2$, 0.1 m *M* EDTA, 1 m *M* DTT, 50% glycerol), 0.9 µ*M* dNTPs, 0.46 µ*M* fluorescein-12 or tetramethylrhodamine-6 labeled dUTPs (Molecular Probes, Eugene, Ore.), and 0.6 U *Taq* polymerase in a final volume of 8 µl. Amplifications were performed using MJ Research PTC-200 thermal cyclers (Waltham, Mass.) and the following PCR profile: 94°C for 2 min, 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, with a final extension of 72°C for 5 min. The PCR products were analyzed on an ABI 377 DNA sequencer using standard protocols and fragment analysis was performed using GENESCAN ver. 3.1.2 (Applied Biosystems, Foster City, Calif.).

Multipoint analysis was performed using the recombinant inbred self model of MAPMAKER 2.0 (Lander et al. 1987; Macintosh version). The GROUP command

was set at LOD = 5.0, and the genetic distances were determined using the Kosambi function.

Development of markers at the *Lpa1* locus

Microsatellites in the region between RM3542 and RM482 were identified by analyzing sequence data of two genomic clones from the Rice Genome Project Database (http://rgp.dna.affrc.go.jp/), accessions AP004878 (PAC clone P0474F11) and AP004048 (BAC clone OJ1202_E07), using the SIMPLE SEQUENCE REPEAT IDENTIFICATION TOOL (SSRIT; http://www.gramene.org/microsat;; Temnykh et al. 2001). Primers were designed using the PRIMER 3 program (http://www.genome.wi.mit.edu) (Table 1) and were obtained from Integrated DNA Technologies (Coralville, Iowa). Microsatellite marker analyses were as described above.

Annotations for the putative genes in the region were based on the TIGR prediction method (http://www.tigr.org) that utilizes both the database search and combinations of gene prediction programs, including FGENESH and GENE SCAN. We also performed homology searches of the putative ORFs' protein sequence using the NCBI BLASTP program (http://www.ncbi.nlm.nih.gov/BLAST/).

Markers were developed based on various putative ORFs in the region (Table 2). Sequence data for these ORFs were obtained and primers were designed to amplify various regions of the putative genes (Table 3) using the program WEBPRIMER (http://www.genome-ww.stanford.edu). The PCR reactions for ORF-based markers consisted of 200–400 ng DNA, 0.30 μ*M* primer pair, 1× PCR buffer (10 m *M* Tris-HCl pH 8.3, 50 m *M* KCl, 1.5 m *M* $MgCl_2$, 0.01% Gelatin), 0.8 μ*M* dNTPs, 3% DMSO, and 2 U *Taq* polymerase in a 20-μl reaction. Amplifications were carried out using the PCR profile: 94°C for 5 min, 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min, with a final extension of 72°C for 7 min. About 10–15 μl of each PCR sample was fractionated on 0.8% agarose/1× TAE gels using the standard protocols (Sambrook et al. 1989). Gels were stained with ethidium bromide and photographed using a Gel Doc 2000 Imaging System (BioRad, Hercules, Calif.).

PCR DIG labeling of probes, Southern blotting, and hybridization

Selected markers (Table 3) were labeled for Southern hybridization using the PCR DIG Probe Synthesis kit (Roche, Indianapolis, Ind.). Parental DNAs (approximately 1 μg) were digested with *Dra*I,*Eco*RI, *Eco*RV, *Hin*dIII, *Mlu*I, *Pst*I, *Sac*II, *Sal*I, *Xba*I, *and Xho*I in 50-μl reactions according to the manufacturer's instructions

Table 1 Microsatellite markers used for fine mapping of the *Lpa1* locus

Markers	Primer sequence		Size (bp) Kaybonnet, Zhe 733
	Forward primer	Reverse primer	
RM138	agcgcaacaaccaatccatccg	aagaagctgcctttgacgctat	233, 235
RM250	ggttcaaaccaagctgatca	gatgaaggccttccacgcag	153, 158
RM266	tagtttaaccaagactctc	ggttgaacccaaatctgca	127, 120
RM498	aatctgggcctgctcttttc	tcctagggtgaagaaagggg	205, 210
RM207	ccattcgtgagaagatctga	cacctcatcctcgtaacgcc	125, 118
RM482	tctgaaagcctgactcatcg	gtcaattgcagtgcccttc	184, 188
RM3542	ctccatggaaaagctagccag	aatcacccttcagtgcctc	90, 92
RM3774	tttgacctcaactcccaagc	gctccctcactcacaactcc	150, 156
RM3789A	ttcccgaattaagcagatata	ctgtagaccattgactggtg	195, 190
RM3789B	attaagggcaggggcatatc	cattgactggtgtggtcagg	120, 115
RM5300	ccaccccatcattattgagg	aagctgaggttggttgcttg	185, 188
CA1	atggtggcaaaagctcaaac	ttccattgtgtcgtgttgct	225
CA2	agttgggggacgggttatac	ctccacctcccagcaacctaa	105
CA3	tcgttgtccaaacatccaaa	tgattggctgctgtcattgt	194
CA4	gctgctgcttcagctcgt	catcaaaggaggaaggaaagg	81
CA5	gtgccctgtcctgatgattt	gacctgctttcttcctgcac	198
CA6	gaattgtgatggggttttgg	cactatcatcgtgcctgcat	202
CA7	gagggagaggtggaggaatc	aaatgggccttatccaatcc	178
CA8	acctgcggttcctcctt	cgcaacgtgagagagagaga	250
CA9	cacctccaaccaaccactct	ccagaaggttcgagagc	245
CA10	gggccagattaggcagaat	cccaagctcctctacattgc	262
CA11	tgatcgaaaagacgaaacca	tcccaatattcgcgtacaca	210, 215
CA12	gaattcgtcgtgggattgac	acacacgcaccacgctataa	185
CA13	ggtatggcccctatggttct	tttctaagccaggagacatgc	180
CA14	gttccgagggaatctgagc	attggggttgatgttgaagg	220
CA15	gtgtgctccttgggaagaaa	gaagagaggggagggagaaa	190
CA16	cgatacaattgaccaatgcaa	gcaggaccagctagacatca	152
CA17	ggggagccttcttcttcttctg	atctggatgggctgtattgg	180
CA18	gggattcggattggatttt	aatctccaaccaaggagcaa	150
CA19	gtagctgcggctgatgagtt	ttgagagtgccgaaagttga	200
CA20	tcgaagtctgatgattgttgtg	tctgagcaaaggctgagatg	240

Table 2 Putative ORFs between RM3542 and RM482

Markers	Gene description (putative)	Transcript length[a] (bp)
RM3542		
	Zinc Finger (DHHC type)[b]	720
	No similarity found[b]	312
	Thioredoxin-related protein[c]	423
	Putative pumilio/Mpt5 family RNA binding protein related[b]	300
	2-Phosphoglycerate kinase[b]	2058
	OTU-like cysteine protease[c]	868
	Putative protein kinase (p-kinase1)[c]	1,353
	Zinc finger (DNL type)[b]	567
KN2	Putative protein kinase (p-kinase 2)[c]	1,200
	Protein phosphatase[c]	1,035
	Zinc finger (KOG, RING)[b]	1,236
	Putative kinesin light chain[c]	1,866
	Transferase[b]	1,362
	DUF260/LOB domain[b]	651
	Unknown protein (expressed in Arabidopsis)[b]	1,242
	DUF296, unknown function[c]	981
	No similarity (hypothetical protein)[b]	285
HK3	Ethylene receptor-like kinase[c]	2,142
	Ribosomal L28e protein family[c]	444
	Zinc finger-superman related[c]	588
	Weak similarity to protein kinase (p-kinase 3)[c]	1,419
	MATT E efflux family/hypothetical protein[b]	1,092
	MATT E efflux family/putative ripening regulator gene[b]	543
	Fibrillarin[b]	921
	Conserved hypothetical protein[b]	972
RM482		

[a]Transcript lengths were obtained from Gramene (http://www.gramene.org)
[b]Gene descriptions were obtained from the BLASTP search of the predicted protein
[c]Gene descriptions were obtained from Gramene

Table 3 ORF-based primers used for fine mapping the *Lpa1* locus

Markers[a]	Primer sequence		Annealing temperature	Polymorphism[b]	Approximate size of fragment (bp) (Kaybonnet, Zhe 733)
	RM482	Reverse primer			
KN1(whole gene)	tcgcccatggttaccctct	tttcaccttcacctgggagc	55	Monomorphic	1,300
KN2 (5′ half)	tcgcccatggttaccctct	tggaggtggacgtagtgga	55	Polymorphic (SCAR)	500, 600
KN3 (3′ half)	tccactacgtccacctcca	tttcaccttcacctgggagc	55	Monomorphic	600
HK1 (whole gene)	tggagcatggacagcatctt	tcagcatcatgtgctctgaa	55	Monomorphic	2,200
HK2 (5′ half)	tggagcatggacagcatctt	acatggaaatcctccgct	55	Monomorphic	1,300
HK3 (3′ half)	tcacgacgtcatggagatgt	tcagcatcatgtgctctgaa	55	Polymorphic (RFLP)	1,100
PPH1 (whole gene)	aagaacttggagagaagggga	tttgtattgatactgccacgc	55	Monomorphic	2,700
PPH2 (5′ half)	aagaacttggagagaagggga	aacccagtatcaggcacatcc	55	Monomorphic	1,500
PPH3 (3′ half)	gattctttgtatgcacggagg	tttgtattgatactgccacgc	55	Monomorphic	1,300
UL1 (whole gene)	aacagtttctgaccacctgca	tcgcatagaacatggccata	55	Monomorphic	2,400
UL2 (5′ half)	aacagtttctgaccacctgca	aacatggaagacatggaggca	55	Monomorphic	1,900
UL3 (3′ half)	aatactggtgctcaccgtcct	tcgcatagaacatggccata	55	Monomorphic	900
PT1 (whole gene)	aagaattctctgcaggggaa	tcgtagcagcaagaaagcaa	60	Monomorphic	1,400
PT2 (5′ half)	aagaattctctgcaggggaa	tgcttgagcttgtcgatgct	62	Monomorphic	800
PT3 (3′ half)	atgtggcctgcgacgatt	tcgtagcagcaagaaagcaa	60	Monomorphic	900

[a]KN, p-Kinase 2; HK, histidine kinase; PPH protein phosphatase homolog; UL, ubiquitin ligase; PT, putative transferase
[b]SCAR, Sequence-characterized amplified region; RFLP, restriction fragment length polymorphism

(New England Biolabs, Beverly, Mass.). Digests were precipitated with 1/10 volume 3 *M* sodium acetate and two volumes 95% ethanol, incubated at −20°C for 1 h, and then centrifuged for 10 min at 4°C. Supernatants were discarded and pellets were washed with 70% ethanol, air-dried, and suspended in 10 µl TE buffer prior to loading on a 0.8% agarose/1× TAE gel. Gel electrophoresis and alkaline blotting onto positively charged nylon membranes were performed using standard protocols. Hybridization and detection were performed using the DIG Easy Hybridization, DIG Wash and Block Buffer Set, and DIG Luminescent Detection kit (Roche) according to manufacturer's instructions. Probes detecting restriction fragment length polymorphisms (RFLPs) were then hybridized to Southern blots of RIL DNAs digested with the appropriate enzyme.

Results and discussion

Fine genetic mapping of the *Lpa1* locus

Using an F_2 population derived from a cross between the mutant KB1-1 (*lpa1*/*lpa1*) and Zhe 733 (*Lpa1*/*Lpa1*), Larson et al. (2000) mapped the rice *Lpa1* locus to the long arm of chromosome 2 between the microsatellite markers RM207 and RM208. In the present study, RILs derived from the same cross were used to fine map the *Lpa1* locus. We initially analyzed the parental lines, KB1-1 and Zhe 733, using RM207 and RM208. Unexpectedly, RM208 was monomorphic and could not be used for analyzing this population. The RM207 was polymorphic, as previously reported, and was used to analyze 186 RILs (F_8 generation). Linkage analysis confirmed that the *Lpa1* locus is linked to RM207 (5.6 cM, 27 recombinants out of 186 RILs). To find markers flanking the *Lpa1* locus, we identified six microsatellite markers from the Gramene Database that are linked to RM207. Of these, only RM250 was polymorphic. The locus was mapped between RM207 and RM250 (30 cM, 61 recombinants out of 186 RILs). Thirty additional microsatellite markers in this region were identified from Gramene. The mapping of these markers using the 186 RILs indicated that the *Lpa1* locus is flanked by RM3542 (2.9 cM, seven recombinants out of 186 RILs) and RM482 (4.3 cM, 13 recombinants out of 186 RILs). An additional 155 RILs (F_7 generation) were analyzed using RM3542 and RM482, and one recombinant between RM3542 and the locus and four recombinants between RM482 and the locus were identified. Based on the total of 341 independently derived RILs, the *Lpa1* locus is approximately 2.4 cM from RM3542 and 3.5 cM from RM482.

Using the sequences of RM3542 and RM482, we performed a BLAST homology search. Two overlapping genomic clones, PAC AP004878 and BAC AP004048, were identified. Following alignment of the sequences of these two clones, the physical distance between the two markers was determined to be approximately 135 kb. Within this region, sequence analysis indicated that there are at least 25 putative ORFs, some of which appear to encode proteins with homology to kinases and one with homology to a phosphatase (Table 2).

Further delimitation of the *Lpa1* locus

In order to narrow down the physical region containing the *Lpa1* gene, we developed additional DNA markers. First, microsatellites were identified in this region using the SSRIT program, and primers were designed to amplify those with seven or more dinucleotide or trinucleotide repeats. Of the 20 primer pairs tested, only CA11 (Table 1) was polymorphic. CA11 maps between RM3542 and the *Lpa1* locus (two recombinants between CA11 and RM3452 and six recombinants between CA11 and *Lpa1*). Mapping with this marker reduced the region of interest to 100 kb, thereby eliminating a putative protein with homology to phosphoglycerate kinase from the list of interesting candidates (Table 2).

Given the low levels of polymorphism observed with the microsatellites examined in this region, primers were designed to amplify some of the putative ORFs within the region between CA11 and RM482. Four putative ORFs with homologies to known proteins [protein phosphatase homolog, (PPH); p-kinase2, (KN); histidine kinase, (HK); putative transferase, (PT); and ubiquitin ligase, (UL)] were selected, and primers were designed to amplify various regions of each ORF (Table 3). Amplification of the fragment KN2 resulted in the detection of a 500-bp product in KB 1-1 and a 600-bp product in Zhe 733 (data not shown). Other primer combinations were either monomorphic or did not consistently amplify products of the expected size (C. Andaya, data not shown). Some of the monomorphic markers were selected for use as RFLP probes based on DIG-dUTP labeling efficiency (Table 3). Of these, HK3 detected an RFLP between KB1-1 and Zhe 733 with the enzymes *Xho*I, *Eco*RI, and *Sal*I. The PPH2 and UL3 did not clearly detect polymorphism, while PT2 hybridized as a multicopy probe and could not be scored.

The KN2 and HK3 markers were used to analyze 18 recombinants that previously defined the locus. Based on this analysis, KN2 and HK3 represent the two closest flanking markers and delimit the locus to a physical region of 47 kb, which contains at least eight putative ORFs (Fig. 1). Three of these putative ORFs encode proteins with homologies to proteins of known function including a phosphatase, a ligase (zinc finger motif), and a transferase, while the five remaining are of unknown function.

Of the three putative ORFs with known function, the phosphatase homology is of particular interest as one proposed pathway of PA synthesis (Raboy et al. 2001; Pilu et al. 2003) may be impaired because of the failure of a phosphatase to produce a substrate needed to produce PA. The predicted zinc finger (also designated as ubiquitin ligase from our BLAST search) is another interesting candidate within the region. Zinc fingers are motifs common in many transcription factors that function in the recognition of specific DNA sequences (Laity et al. 2001). It is possible that this putative zinc finger controls the transcription of a gene involved in PA synthesis such as *MIPS*. Expression of the MIPS gene in the rice *lpa1* mutant has not been reported to date.

Implications and future directions

Despite the development of several low phytate crops, PA synthesis in plants is not well understood. Most of our current understanding of PA synthesis is based on mammalian systems and yeast studies. In these systems, glucose 6-phosphate is converted to inositol 3-phosphate

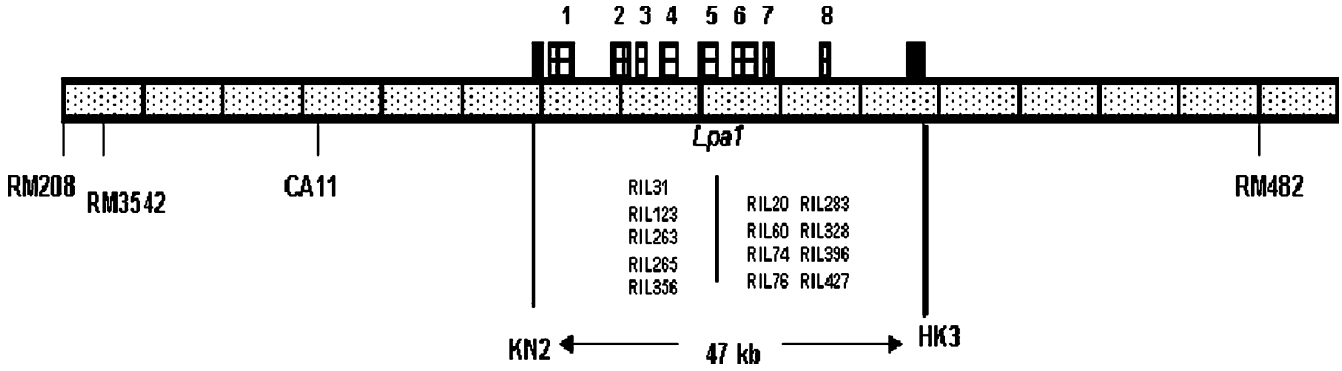

Fig. 1 Physical map of the *Lpa1* locus on rice chromosome 2. Putative ORFs in the region include: *1* protein phosphatase, *2* zinc finger/ubiquitin ligase, *3* kinesin light chain, *4* transferase, *5* DUF 260, unknown function, *6* unknown protein, *7* unknown protein, *8* DUF 296, unknown function. *Solid boxes* above KN2 and HK3 represent the p-kinase 2 and histidine kinase ORFs, respectively, used to delimit the locus

through the action of D- *myo*-inositol P_1 synthase (MIPS), the first enzyme in the PA biosynthetic pathway. Inositol 3-phosphate is converted to either inositol intermediates or phosphatidyl inositol intermediates and ultimately to PA through a series of phosphorylation events. This pathway is not well defined with respect to the sequential phosphorylation steps and the regulatory mechanisms involved (Pilu et al. 2003).

While the nature of the rice *lpa1* mutation remains to be determined, rice germplasm with the *lpa1* mutation has been released for use by breeders and other rice researchers (Rutger et al. 2004a, 2004b). Recently, Liu et al. (2004) reported the quantitative analysis of total P, phytic acid P, K, Mg, Ca, Fe, Mn, and Zn in whole grains, embryos, and rest-of-grain portions of wild-type and *lpa1* seed. The levels of total P, Ca, Mn, and phytic acid P in whole grains were lower in the *lpa1* seed than in the wild-type seed, while the levels of K, Mg, and Fe were similar, and that of Zn was higher. These results indicate that changes in PA affect the distribution of important micronutrients within the seed, which may have a significant impact on the nutritional value of *lpa1* rice.

Fine mapping of the *Lpa1* locus has resulted in the identification of a reasonably sized region that can be subcloned and used for functional complementation analysis via *Agrobacterium*-mediated stable transformation. Cloning of the *Lpa1* gene will contribute to a better understanding of PA metabolism in rice and other cereals and its role in development. The PCR-based markers reported here should also be useful for marker-aided introgression of the *lpa1* mutation by rice breeders interested in increasing P and micronutrient availability (Mendoza et al. 1998, 2001; Raboy et al. 2001; Adams et al. 2002).

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