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ORIGINAL PAPER

High-resolution genetic mapping and candidate gene identification of the *SLP1* locus that controls glume development in rice

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Abstract *Stunted lemma palea 1* (*slp1*) is a rice mutant that displays dwarfism, shortened inflorescence lengths, severely degenerated lemmas/paleas, and sterility. The *SLP1* locus was mapped between markers RM447 and D275 in the distal region of the long arm of chromosome 8, using the F2 progeny derived from the cross between the *Slp1/slp1* mutant (*Oryza sativa* subsp. *japonica*) and the variety Taichung Native 1 (TN1, *O. sativa* subsp. *indica*). The *SLP1* locus was further delimited to a 46.4-kb genomic region containing three putative genes: *OsSPL16*, *OsMADS45*, and *OsMADS37*. Comparisons of the sequence variations and expression levels of the three candidate genes between wild-type plants and homozygous *slp1* mutants suggested that a missense mutation in the sixth amino acid of the OsSPL16 protein was likely responsible for the *slp1* mutant phenotypes.

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Introduction

Studies on the molecular mechanisms of flower development usually adapt the ABCE model, which was originally established from floral mutant analyses using two eudicot species, *Arabidopsis* and *Antirrhinum.* The flowers of both species bear four types of floral organs that are arranged in concentric whorls: sepals, petals, stamens, and carpels. The ABCE model proposed that the floral organ identity genes were grouped into four classes (A, B, C, and E). Genes within the same class and/or between two different classes coordinately regulate the development of a floral organ. Class A genes specify sepals and co-regulate the differentiation of petals with class B genes. Class B and C genes specify stamens, whereas class C genes specify carpels. Finally, class E is essential for the specification of all whorls of the floral organs (reviewed in Krizek and Fletcher 2005). Nevertheless, not all flowers in angiosperms have distinct sepals and petals, especially those of monocots. For example, rice bears a lemma and palea at the outermost whorl and lacks petals (Ikeda et al. 2004). Several lines of evidence have shown that genes homologous to the class A genes in monocots did not fulfill the A function, which suggested that the genes that control the development of the outermost floral organ might not be conserved (reviewed in Litt and Kramer 2010).

Recent studies on several glume mutants in rice have shed light on the molecular mechanism of lemma and palea development. Three rice mutants that affect the development of the lemma and palea have been reported: the *leafy hull sterile1* (*lhs1*) mutant exhibited overdevelopment of leafy lemmas/paleas (Jeon et al. 2000); the *supernumerary bract* (*snb*) mutant had multiple rudimentary glumes in an alternative phyllotaxy with lemma/palea-like organs that were transformed from lodicules

(Lee et al. 2006); and the *retarded palea* (*rep1*) mutant displayed significantly retarded palea development (Yuan et al. 2009). The *lhs1* and *snb* genes were homologous to class E and class A floral organ identity genes, respectively, whereas *rep1* belongs to the TCP gene family (Cubas et al. 1999), which are not floral organ identity genes. In addition to these three mutants, two additional glume mutants have been characterized. The *palealess* (*pal*) mutant had an abnormal palea that was replaced by two leaf-like organs and was mapped to the short arm of chromosome 6 (Luo et al. 2005). The *abnormal hull* (*ah*) mutant showed severe degeneration of the lemma and palea. Its stamens and lodicules were homeotically transformed into pistils and palea/lemma-like structures, respectively. The phenotype of *ah* is similar to that of the B loss-of-function *SPW1* gene, which was reported previously in rice (Zhang et al. 2007).

In the current study, a new rice glume mutant *stunted lemma palea 1* (*slp1*), which shows extreme degeneration of the lemma/palea, was characterized. Genetic evidence confirmed that the *slp1* mutant phenotype was controlled by a single gene. The *SLP1* locus was delimited to a 46.4-kb genomic region at the distal part of the long arm of chromosome 8. Three genes, two MADS-box-like genes and one SBP-like gene, were annotated in this genomic region. Analyses on DNA sequence variations and gene expression level differences between the wild-type and mutant plants suggested that a missense mutation in the sixth amino acid of the SBP-like protein was likely responsible for the *slp1* mutant phenotype.

Materials and methods

Mutants and mapping population

The *slp1* mutant was originally identified from F5 progeny derived from the cross between Taikeng2 (TK2) and a M6 individual of the sodium azide-treated Tainung67 (TNG67) (Wang et al. 2002), which are both japonica rice. The *slp1* mutant was maintained by self-pollination of the heterozygous mutants due to complete sterility of the homozygous mutants.

For genetically mapping the *SLP1* locus, a heterozygous mutant plant was used as the female parent and crossed with the indica rice Taichung Native 1 (TN1) to produce F1 seeds. Five F1 plants that showed the heterozygous mutant phenotype were self-pollinated to produce bulk F2 seeds for genetic mapping. The high-resolution genetic mapping population was grown in the experimental farm of National Taiwan University in the fall of 2008. All other rice plants were grown in the phytotron of National Taiwan University with day/night temperatures of 30/25°C.

Phenotypic characterization

Progenies derived from the self-pollinated heterozygous mutant plant were classified into three genotypic groups based on their spikelet phenotypes: normal spikelets (designated as the *Slp1/Slp1* genotype), intermediate degenerated lemmas/paleas (designated as the *Slp1/slp1* genotype), and severely degenerated lemmas/paleas and complete sterility (designated as the *slp1/slp1* genotype). Individuals among different genotypic groups were considered to be nearly isogenic lines because the heterozygous *slp1* mutant used in the current study was the self-pollinated progeny of the original *slp1* mutant for more than ten generations. Seven individuals from each group were randomly chosen for measurements of the plant height, inflorescence length, spikelet length, and spikelet number per inflorescence. The inflorescence from the primary tiller of each plant was used to measure inflorescence length and spikelet number per inflorescence. From the same inflorescence, the first two spikelets from the tip of the primary branch above the rachis were used to measure the spikelet length.

Plant height was measured as the height from the soil surface to the tip of the tallest inflorescence. Inflorescence length was the length between the tip of the primary branch above the rachis and the rachis base at the lowest primary branch (Ikeda et al. 2004). Spikelet length was measured from the tip of the lemma to the base of the empty glumes. The data for each trait were subjected to analysis of variance using the ANOVA procedure of SAS version 9.2 (SAS Institute 2009). Comparisons of the mean and least significant differences between genotypes were assessed with Tukey's studentized range test at $\alpha = 0.05$.

The Chi-square goodness-of-fit test was used to test the hypothesis that the wild-type allele of the TN1 variety was incompletely dominant to the *slp1* mutant allele. Phenotypic data from two consecutive generations were used. In the F1 hybrids, the expected ratio of the wild-type phenotype (*Slp1/Slp1*) to the mutant phenotype (*Slp1/slp1*) was 1:1. In the F2 mapping population, the expected 1:2:1 ratio of the three phenotypic groups (*Slp1/Slp1*:*Slp1/slp1*:*slp1/slp1*) was tested. A statistically insignificant Chi-square test indicated that the observed ratio between the phenotypic groups did not deviate from the expected ratio.

Selection and development of DNA markers

One hundred ninety-nine simple sequence repeats markers (SSR markers) were selected to identify possible DNA polymorphisms between the homozygous *slp1* mutant and the Taichung Native 1 variety and were based on the information provided in two databases: Gramene (International Rice Genome Sequencing Project 2005; http://www.gramene.org/markers/microsat/) and ASPGC (Academia

Sinica Plant Genome Center) (Lin et al. 2008). A total of 82 polymorphic SSR markers were identified and employed in the coarse genetic mapping experiment. These SSR markers were spaced approximately 5 Mb from each other and were expected to be linked to all genes in the genome.

For high-resolution genetic mapping, InDel markers and single nucleotide polymorphism (SNP) markers were developed based on the information from the MSU Rice Genome Annotation Project Database (Ouyang et al. 2007; http://rice.plantbiology.msu.edu/), in which the DNA polymorphisms between two rice genomes, Nipponbare (japonica rice) and 93-11 (indica rice), are annotated. Primers were designed using Primer3 (Rozen and Skaletsky 2000; http://frodo.wi.mit.edu/). The primer sequences of the polymorphic markers and the corresponding annealing temperatures are listed in the Supplementary Materials.

Marker analysis and genetic mapping

DNA was extracted from leaves individually collected from 45- to 50-day-old plants using the protocol described by Murray and Thompson (1980). Polymerase chain reaction (PCR) was carried out based on the procedures described by Sambrook and Russell (2001). The PCR products were separated on 2% Agarose SFR™ gels (Amresco, Solon, OH, USA) using the TBE buffer system and stained with ethidium bromide. For the analysis of a SNP marker, PCR products were digested with the CelI nuclease using the protocol described by Till et al. (2003). The CelI enzyme was purified according to the same protocol. To genotype a SNP marker for an individual, two CelI enzyme reactions were performed: one using the PCR product from an F2 individual only, and another using a mixture of PCR products from the F2 individual and the maternal plant (Supplementary Materials).

Genetic linkage between the *SLP1* locus and each of the 82 SSR markers was tested using the MAPMAKER/EXP version 3.0 software, and data were collected from 42 F2 rice plants. The genotype of the *SLP1* locus in each plant was deduced from the plant phenotype. The order of the DNA markers in the same linkage group was based on the rice physical map. Genetic distances of the linkage map were estimated using MAPMAKER/EXP version 3.0 with the Kosambi mapping function (Lander et al. 1987; Lincoln et al. 1992).

DNA sequencing

The rice PAC clone P0702E04 (GI: 42761368), which contains the delimited genomic region of the *SLP1* locus, was used as the reference for DNA sequencing. The annotated coding sequences in the three candidate genes *OsSPL16*, *OsMADS45*, and *OsMADS37* were targeted for DNA sequencing. The primer pairs for DNA sequencing can be found in the Supplementary Materials. Genomic DNA extracted from wild-type plants and the homozygous *slp1* mutants were used as the DNA templates for PCR. To avoid false nucleotide polymorphisms introduced from PCR amplification, three independent PCR amplicons in each combination of primer pairs and DNA templates were mixed. The mixed PCR amplicons were then treated with the ExoSAP-IT® enzyme (USB, Cleveland, OH, USA) and submitted for DNA sequencing. The DNA sequences were assembled using the SeqMan Pro applications in Lasergene Suite v. 8.1 (DNASTAR Inc, Madison, WI, USA). The putative coding sequence of *OsSPL16* from the *slp1*/*slp1* genotype was deposited in GenBank (GI:310756532).

RT–PCR analysis

Inflorescences from the *Slp1*/*Slp1* genotype and the *slp1*/*slp1* genotype at two inflorescence developmental stages were harvested for RNA extraction. For the *Slp1*/*Slp1* genotype, inflorescences with 1.5–10 mm and 40–220 mm lengths were defined as the inflorescence developmental stages In7 and In8, respectively (Ikeda et al. 2004). For the *slp1*/*slp1* genotype, inflorescence lengths of 1.5–10 mm and 20–50 mm were defined arbitrarily as the developmental stages In7 and In8, respectively, in the current study. Three immature inflorescences from a single plant for each developmental stage were collected as replicates for each genotype. Total RNA extraction and first-strand cDNA synthesis were conducted using the TRIzol® reagent, RNase-free DNase I, and the SuperScript™ III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

Transcript levels of each candidate gene were detected by RT–PCR analysis. Briefly, 3 μL of 10× diluted first-strand cDNA solution was used as the template for subsequent PCR amplification. The following primers were used for the RT–PCR analyses: *OsSPL16*_F, AGCCAGA TCCCATGAACTC; *OsSPL16*_R, ATTTGGTGGTGCGT GTAGTAT; *OsMADS45*_F, CTGGAGGAAAGCAAC CATGT; *OsMADS45*_R, ATGGGGGCATGTAGGTGTT; *OsMADS37*_F, GAGCGGGCTGTTCAAGAAG; *OsMAD S37*_R, ATTGCAATCAACCCGAGATTC; *eEF-1α*_F, TT TCACTCTTGGTGTGAAGCAGAT; *eEF-1α*_R, GACT TCCTTCACGATTTCATCGTAA. To avoid overestimation of gene expression due to genomic DNA contamination, all primer pairs were designed to span an intron. The PCR reactions were performed with a cycle of 94°C for 30 s and 27–30 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 15 s. The optimal number of cycles of RT–PCR for each gene was determined empirically (Supplementary

Materials). The *eEF-1α* gene was chosen as the internal control (Jain et al. 2006).

Results

Genetic analysis of the *slp1* mutant

The *stunted lemma palea 1* (*slp1*) mutant phenotypes were hypothesized to be controlled by a single locus because the progeny derived from a self-pollinated fertile *slp1* mutant showed three distinct phenotype groups that fit the 1:2:1 ratio (normal spikelets:intermediate degenerated lemmas/paleas:severely degenerated lemmas/paleas and completely sterile) (Liu 2003). To confirm the inheritance pattern of the *slp1* mutant, the segregation ratios of distinct phenotype groups were examined. The segregation ratio of the F1 progeny derived from the cross between a fertile *slp1* mutant and the indica rice variety TN1 fit the expected ratio of 1:1 ($\chi^2_{1,0.05} = 0.11 < 3.84$). The segregation ratio of the F2 progeny also fit the expected ratio of 1:2:1 ($\chi^2_{2,0.05} = 2.75 < 5.99$). These results confirmed that the *slp1* mutant phenotype was controlled by a single locus. In addition, the wild-type allele from TN1 displayed a similar spikelet phenotype as the wild-type allele from TK2. The *SLP1* genotype for each phenotypic group was assigned as follows: *Slp1*/*Slp1* for normal spikelets; *Slp1*/*slp1* for intermediate degenerated lemmas/paleas, which was the fertile *slp1* mutant; and *slp1*/*slp1* for severely degenerated lemmas/paleas, which was the sterile *slp1* mutant.

Phenotypic characterization of the *slp1* mutant

To understand the function of the *SLP1* gene, agronomic traits in the vegetative and reproductive stages of individuals with different *SLP1* genotypes were compared (Table 1). There were no significant phenotypic differences among the three genotypes before the tilling stage. The differences in plant height became obvious at the heading stage (Fig. 1a). The mature leaves of the homozygous *slp1* mutants were shorter and more fragile than those of wild-type plants. There were also no significant differences in the flowering time, number of floral organs, and the structure of the primary/secondary branches among the different genotypes. However, the homozygous *slp1* mutants showed remarkably abnormal inflorescence lengths and glume architectures (Fig. 1b–d, Table 1). In wild-type plants, the inflorescence length was 18.6–22.0 cm, and one inflorescence had 95–131 spikelets. In the homozygous *slp1* mutants, inflorescences were 3.5–6.0 cm long, and each had 14–46 spikelets. Moreover, the homozygous *slp1* mutants showed severely degenerated lemmas and paleas, which resulted in empty glumes, complete sterility, and unclosed lemmas/paleas (Fig. 1c, d). The average spikelet length was 2.3 mm in the homozygous *slp1* mutants, which is statistically significantly different from 5.1 mm in the heterozygous *slp1* mutants and 8.1 mm in the wild-type plants (Table 1).

Genetic mapping of the *SLP1* locus

Genetic linkages between the *SLP1* locus and SSR markers were examined using 42 F2 rice plants derived from a cross between the *slp1* mutant (a japonica rice variety) and the indica rice variety TN1. Among the 82 tested SSR markers, only the markers RM23477 and RM23652 showed linkage with the *SLP1* locus. This result suggested that the *SLP1* locus was located close to these markers at the distal region of the long arm of chromosome 8 (Fig. 2a). To confirm the mapped location of the *SLP1* locus, 134 additional F2 individuals were used to construct a linkage map around the *SLP1* locus by determining their *SLP1* phenotypes and the genotypes of four polymorphic markers between RM23477 and RM23652. The *SLP1* locus was mapped on the chromosomal region between markers RM447 and D275 (Fig. 2b).

Eight F2 individuals with a single crossover event in the D275–RM447 interval were screened from 982 F2 progenies and used to build a high-resolution map of the *SLP1* locus. These recombinant individuals were further genotyped with eight polymorphic markers between D275 and RM447 (Fig. 2c, d). The *SLP1* locus was eventually delimited in the SNP471–SNP386 interval, which corresponded to 15,137–61,541 bp of the rice PAC P0702E04. According to the MSU Rice Genome Annotation Project Database (http://rice.plantbiology.msu.edu/; Ouyang et al.

Table 1 Major phenotypic differences among three genotypes of the *SLP1* locus

Genotypes	Phenotypes[†]			
	Plant height (cm)	Inflorescence length (cm)	Spikelet length (mm)	Spikelet number per inflorescence
Slp1/*Slp1*	95.0[a]	20.3[a]	8.1[a]	111.3[a]
Slp1/*slp1*	97.1[a]	17.7[a]	5.1[b]	90.1[b]
slp1/*slp1*	58.1[b]	4.6[b]	2.3[c]	27.3[c]

[†] Means within a column not followed by the same letter are significantly different ($P < 0.05$) based on Tukey's studentized range test

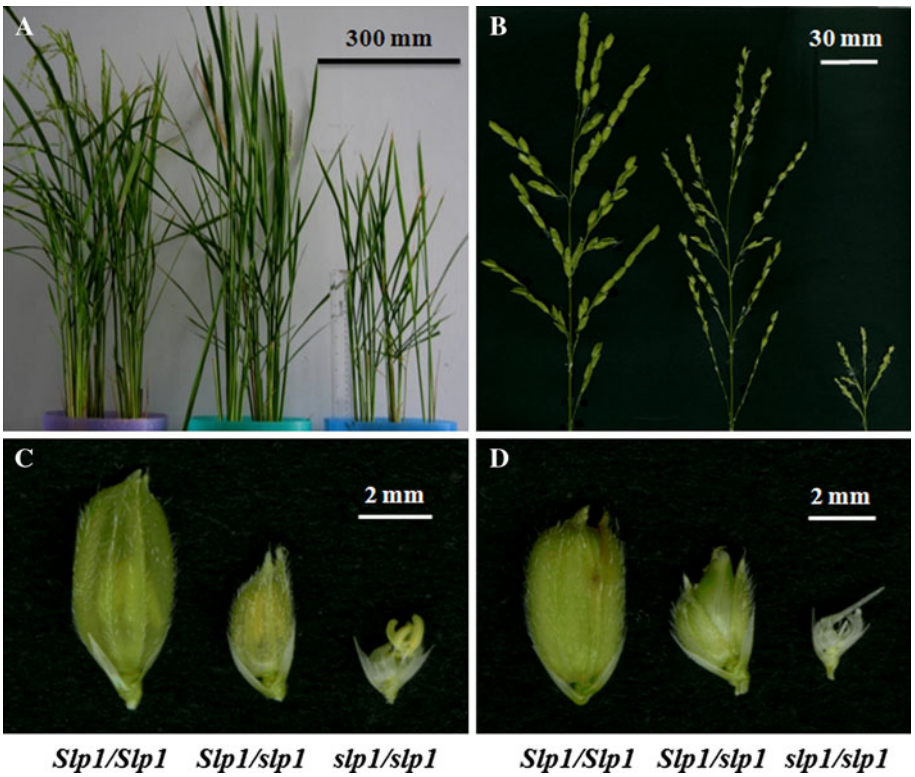

Fig. 1 Phenotypic comparisons between three genotypes of the *SLP1* locus. **a** Plant height. The *slp1/slp1* genotype showed a dwarf phenotype that began during the heading stage. **b** Inflorescence. The structures of the primary and secondary branches were not different among the three genotypes of the *SLP1* locus. Inflorescence length and grain number were significantly reduced in the *slp1/slp1* genotype. **c** Spikelet. The numbers of floral organs were not different among the three genotypes of the *SLP1* locus, but lemmas and paleas were severely degenerated in the *slp1/slp1* genotype. **d** Grain. The *slp1/slp1* genotype was complete sterility and had unclosed lemmas/paleas. The *Slp1/slp1* genotype was semi-sterile (less than 30%) and also had unclosed lemmas/paleas

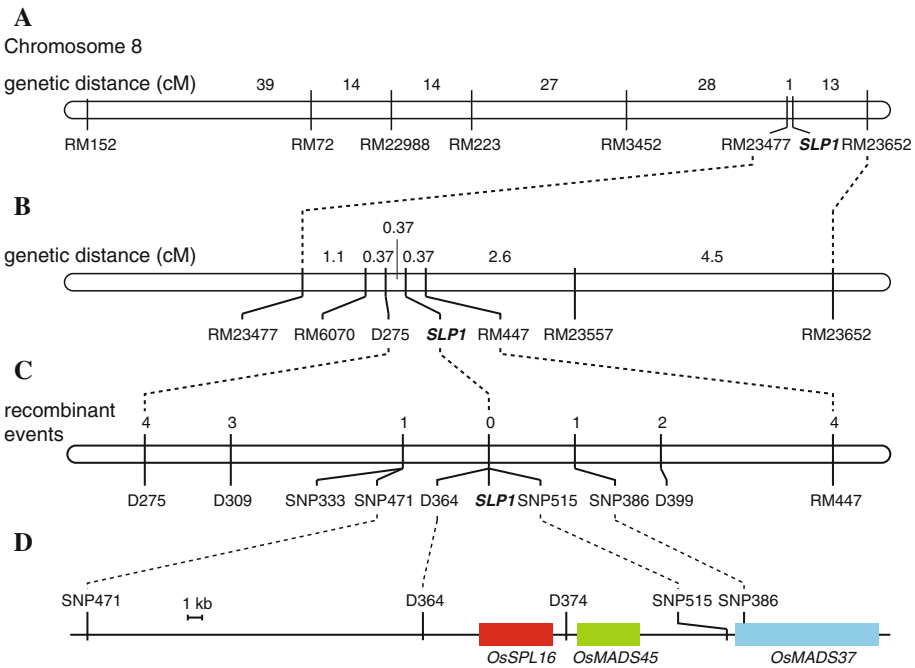

Fig. 2 Genetic mapping of the *SLP1* locus. **a** The *SLP1* locus was mapped to the distal region of the long arm of chromosome 8 using 42 F2 individuals. **b** Genetic map. *SLP1* was delimited between the markers D275 and RM447 using 134 F2 individuals. **c** High-resolution genetic map. This map was constructed using eight F2 individuals with a single recombination that occurred between the markers RM447 and D275. The eight recombinant F2 individuals were screened from 982 F2 plants. The *SLP1* locus was delimited between the markers SNP471 and SNP386 and co-segregated with the markers D364, D374, and SNP515. **d** The physical map around the *SLP1* locus. Three putative genes, *OsSPL16*, *OsMADS45*, and *OsMADS37,* were annotated within the *SLP1*-delimited genomic region

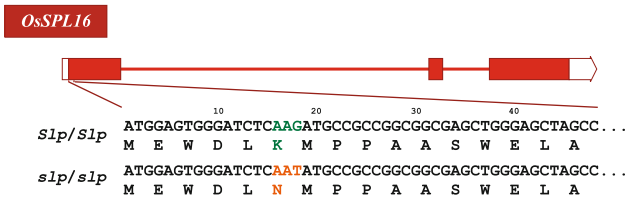

Fig. 3 Sequence comparison of the *OsSPL16* gene between the *Slp1/Slp1* and *slp1/slp1* genotypes. The SNP in the *OsSPL16* gene resulted in a missense mutation in the sixth amino acid of the OsSPL16 protein, which resulted in the substitution of lysine (K) by asparagine (N)

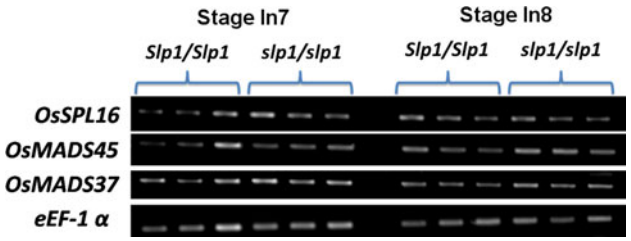

Fig. 4 Comparisons of the expression levels for three candidate genes, *OsSPL16*, *OsMADS45*, and *OsMADS37*, during two inflorescences developmental stages. All three candidate genes showed similar expression levels between the *Slp1/Slp1* and *slp1/slp1* genotypes. Stage In7 was defined as the differentiation of floral organ, and stage In8 was defined as the rapid elongation of rachis and branches (Ikeda et al. 2004). The expression level of the *eEF-1α* gene was used as an internal control. All primer pairs for RT–PCR analysis were designed so that they spanned an intron to avoid the problem of genomic DNA contamination in the RNA solutions

2007), the *SLP1*-delimited genomic region contained two full-length putative genes, *OsSPL16* and *OsMADS45* (also known as *OsMADS7*), and 611 bp of the *OsMADS37* genomic sequence downstream from its start codon (Fig. 2d).

Analyses of candidate genes

Coding sequence variations between the *Slp1/Slp1* and *slp1/slp1* genotypes for three candidate genes were examined by PCR-sequencing, and only one SNP was identified. The SNP was located in the first exon of *OsSPL16*, which corresponded to position 42865 of the PAC P0702E04, and resulted in a missense mutation in the sixth amino acid of the OsSPL16 protein, producing the substitution of lysine with an asparagine (Fig. 3).

To determine whether the expression levels of the candidate genes were correlated with the mutant phenotypes, transcript levels were compared between the *Slp1/Slp1* and *slp1/slp1* genotypes for the three candidate genes during the inflorescence developmental stages In7 and In8. It was hypothesized that the *slp1* mutant phenotypes, including degenerated lemmas/paleas and shortened inflorescence lengths, might be correlated with a defect in gene expression during floral organ differentiation (stage In7) and rapid elongation of rachis and branches (stage In8), respectively (Ikeda et al. 2004). Investigation of the expression levels for all candidate genes, however, showed no significant differences between the wild-type and the mutant genotypes during the two selected developmental stages (Fig. 4).

Discussion

The MADS-box candidate genes are probably not the *SLP1* gene

OsMADS45 (also known as *OsMADS7* in Arora et al. 2007) and *OsMADS37* belong to the MADS-box family of genes, which are known to be transcription factors that control flower development. *OsMADS37* is one of five MADS-box genes that belong to the MIKC* subgroup, but its function is still unknown (Arora et al. 2007). In *Arabidopsis*, five of the six MIKC* gene transcripts accumulate at high levels in pollen and have been suggested to regulate the development and maturation of pollen (Verelst et al. 2007; Adamczyk and Fernandez 2009). In the current study, DNA variations were not found at the 686 bp sequences of *OsMADS37* in the *Slp1/Slp1* and *slp1/slp1* genotypes. Moreover, the expression levels of *OsMADS37* in the *Slp1/Slp1* and *slp1/slp1* genotypes were not significantly different during both inflorescence developmental stages. These results suggested that *OsMADS37* probably is not the *SLP1* gene.

The biological function of *OsMADS45* is currently unknown. *OsMADS45* is homologous to the *Arabidopsis AtSEP3* gene, an E-class floral organ identity gene (Malcomber and Kellogg 2005; Honma and Goto 2001). Five E-class floral organ identity genes, *OsMADS1*, *OsMADS5*, *OsMADS24* (also known as *OsMADS8*), *OsMADS34* (also known as *OsMADS19*) and *OsMADS45* (also known as *OsMADS7*), have been identified in rice (reviewed in Kater et al., 2006). Two of the five E-class genes, *OsMADS1* and *OsMADS5,* have been functionally analyzed. The loss-of-function mutant of *OsMADS1*, *lhs1*, exhibited leafy paleas and lemmas, altered numbers of stamens, and transformed lodicules into palea/lemma-like organs (Jeon et al. 2000). The loss-of-function mutant *osmads5* showed only slight defects in the development of lodicules (Agrawal et al. 2005). To summarize, none of rice mutants that are homologous to *OsMADS45* showed similar phenotypes to the *SLP1* mutant. Moreover, expression of *OsMADS45* was detected in immature seeds, spikelet primordia, developing lodicules, stamens, and pistils (Pelucchi et al. 2002; Arora et al. 2007; Bai et al. 2008) but not in glumes. These data implied that *OsMADS45* is probably not involved the processes of glume development. In the current study, no

DNA variations were found in the coding sequence of *OsMADS45* between wild-type plants and *slp1* mutants. In addition, the expression levels of *OsMADS45* in the *Slp1*/*Slp1* and *slp1*/*slp1* genotypes were not significantly different during both inflorescence development stages. These results suggest that *OsMADS45* probably is not the *SLP1* gene.

OsSPL16 is likely the candidate gene for *SLP1*

OsSPL16 belongs to the *SPL* (*SQUAMOSA promoter-binding-like*) gene family, which comprises 16 and 19 genes in *Arabidopsis* and rice, respectively (Xie et al. 2006). Studies in *Arabidopsis* suggested that the *SPL* genes are involved in the regulation of flowering time, pollen sac development, and shoot maturation (Cardon et al. 1997; Unte et al. 2003; Schwarz et al. 2008). The function of the *SPL* gene family in rice has been primarily investigated using *Osmi156*-overexpressing rice plants (Xie et al. 2006). Over-expression of *Osmi156* in rice resulted in decreases in the transcript levels of three *OsSPL* genes (*OsSPL14*, *OsSPL16*, and *OsSPL18*) in the inflorescence and four *OsSPL* genes (*OsSPL2*, *OsSPL12*, *OsSPL13*, and *OsSPL14*) in the flag leaves and produced dramatic morphological changes, including dwarfism, late flowering, and reduced inflorescence length, spikelet number, and spikelet length. Most of the *OsSPL* genes share sequence similarity only in the highly conserved *SQUA* promoter-binding protein (SBP) domain. Based on a phylogenic analysis, the closest paralogous gene to *OsSPL16* was *OsSPL18*, probably because it is located in a putative duplicated genomic region (Xie et al. 2006). Two additional rice *SPL* genes, *OsSPL1* and *OsSPL2,* clustered with *OsSPL16* in the same branch. Among 19 *OsSPL* genes, only *OsSPL14* has been individually characterized. *OsSPL14* regulated the tiller number and the secondary branching number of inflorescences (Jiao et al. 2010; Miura et al. 2010). *OsSPL14* was clustered with *OsSPL7* and *OsSPL17* and showed very low sequence similarity to *OsSPL16* (Xie et al. 2006). A monogenic mutant of the *OsSPL16* gene has not been previously reported.

The following observations suggest that *OsSPL16* is likely the *SLP1* gene. First, the organs in which *OsSPL16* is expressed correspond to the organs in which the *slp1* mutant phenotypes are observed; specifically, expression of *OsSPL16* was detected in young stems, young leafs, and all developmental stages of panicles (Xie et al. 2006), and the *slp1* mutant phenotypes showed dwarfism, shorter leaves, shorter inflorescence lengths, and degenerated lemmas/paleas. Moreover, a missense mutation in the sixth amino acid of the OsSPL16 protein was found when the *Slp1*/*Slp1* and *slp1*/*slp1* genotypes were compared, while the expression level of the *OsSPL16* gene was not significantly different between the wild-type plants and the *slp1* mutant plants.

The missense mutation in the sixth amino acid of the *OsSPL16* gene and the homologous *Teosinte Glume Architecture1* (*TGA1*) gene in maize revealed a parallel developmental change on glumes. *TGA1* was identified as the major quantitative trait locus responsible for the change of glume architecture between maize and teosinte (Doebley et al. 1990). A previous study suggested that the substitution of lysine (K) in teosinte to asparagine (N) in maize at amino acid six of the TGA1 protein is the causative mutation for the differences in glume size between teosinte and maize (Wang et al. 2005). Teosinte with the introgressed maize *TGA1* allele had degenerated glumes, while normal teosinte had large and hard glumes. In contrast, maize with the introgressed teosinte *TGA1* allele had enlarged glumes, while normal maize had smaller glumes (Wang et al. 2005). In the current study, the amino acid substitution of lysine (K) in the wild-type allele to asparagine (N) in the mutant allele at position six of the SPL protein was detected, and larger glumes were found in the wild-type plant. While additional experimental evidence is required to conclude that *OsSPL16* is the *SLP1* gene, the association between the changes in glume size and the same missense mutation in the homologous genes between maize and rice reinforces our proposition that *OsSPL16* is likely the *SLP1* gene.

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