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ORIGINAL PAPER

Fine mapping and candidate gene analysis of *dense and erect panicle 3*, *DEP3*, which confers high grain yield in rice (*Oryza sativa* L.)

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Abstract Architecture of the rice inflorescence, which is determined mainly by the morphology, number and length of primary and secondary inflorescence branches, is an important agronomical trait. In the current study, we characterized a novel dense and erect panicle (EP) mutant, *dep3*, derived from the *Oryza sativa* ssp. *japonica* cultivar Hwacheong treated with *N*-methyl-*N*-nitrosourea. The panicle of the *dep3* mutant remained erect from flowering to full maturation, whereas the panicle of the wild type plant began to droop after flowering. The *dep3* mutation also regulated other panicle characteristics, including panicle length, grain shape and grain number per panicle. Anatomical observations revealed that the *dep3* mutant had more small vascular bundles and a thicker culm than wild type plants, explaining the EP phenotype. Genetic analysis indicated that the phenotype with the dense and EP was controlled by a single recessive gene, termed *dep3*. The *DEP3* gene was identified as the candidate via a map-based cloning approach and was predicted to encode a patatin-like phospholipase A2 (PLA2) superfamily domain-containing protein. The mutant allele gene carried a 408 bp genomic deletion within LOC_Os06g46350, which included the last 47 bp coding region of the third exon and the first 361 bp of the 3′-untranslated region. Taken together, our results indicated that the patatin-like PLA2 might play a significant role in the formation of vascular bundles, and that the *dep3* mutant may provide another EP resource for rice breeding programs.

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Introduction

Given the rapid growth in world population and the shrinking area of land available for agriculture, food shortage is a potentially serious global problem. As one of the most important cereal crops, rice is cultivated worldwide and serves as a carbohydrate source for more than half of the world's population. Accordingly, improving crop productivity by selecting for grain yield and optimal plant architecture has been the key focus of rice breeding programs (Sakamoto and Matsuoka 2004; Wang and Li 2008). The most striking examples arose in the 1960s during the "green revolution," when selection for a semi-dwarf stature in rice and wheat modified plant architecture and improved potential yield (Peng et al. 1999; Sasaki et al. 2002; Evenson and Gollin 2003).

Donald (1968) and Yoshida (1972) suggested that the erect panicle (EP) phenotype in wheat and barley is advantageous for not only reducing shade canopy area to increase photosynthesis efficiency, but also for improving physiological conditions in cultivated fields, including canopy illumination, temperature, humidity, and CO_2 aeration, which collectively increase population growth rate and grain yield. In rice, conventional cultivars usually

exhibit a drooping panicle after the grain fills out. The panicles of most old rice varieties are above the canopy of leaves. The panicles of new, improved rice varieties, however, are usually below the canopy of leaves. Because of the shading effect of panicles and their low photosynthetic activity, the spatial arrangement of panicles in improved rice varieties appears to be desirable (Yoshida 1972; Yang and Hwa 2008). Rice panicle architecture, associated with grain density, panicle length, grain number, and other features, is one of the target characteristics in rice breeding because of its strong association with grain yield.

Rice panicle architecture contributes not only to grain yield, but also to the ecological conditions of cultivated populations and the physicochemical properties of different varieties (Xu et al. 1996). Several genes affecting the panicle architecture of rice plants, such as *FZP*, *LAX*, *APO1*, *LOG*, *Gn1a*, and *SP1*, have been isolated (Komatsu et al. 2001, 2003; Ashikari et al. 2005; Ikeda et al. 2005; Kurakawa et al. 2007; Li et al. 2009). Most of these studies have focused on the development of spikelet or panicle size, however. Since the 1960s, many EP-type rice varieties have been developed and cultivated in the northern part of China. The EP-type varieties typically bear short, EPs and erect leaves, which enhance ventilation and light penetration and result in increased photosynthetic rates (Zhang et al. 2002a; Chen et al. 2007). The EP rice varieties tend to have greater lodging and fertilizer resistance as a consequence of decreased plant height (Xu et al. 1996).

Although the EP-type varieties have been propagated extensively throughout the northern part of China, the underlying genetic mechanisms have not been fully elucidated. To date, only a few genes responsible for the EP phenotype have been functionally characterized in rice. Initially, Zhu and Gu (1979) reported that EP traits are governed by a single recessive gene from the *japonica* cultivar, Xiaoli57. Analysis of major gene–polygene mixed inheritance models and joint analysis, however, revealed that the EP phenotype is controlled by a major gene with additive or dominant effects that are modified by polygenes (Zhang et al. 2002b). A major QTL responsible for the EP trait, *DEP1* (on chromosome 9 encoding a phosphatidylethanolamine-binding protein-like domain protein), was recently isolated in three independent studies (Huang et al. 2009; Wang et al. 2009; Zhou et al. 2009). Derived from a gain-of-function mutation caused by the truncation of *DEP1*, the *dep1* plant displays enhanced meristematic activity, resulting in reduced length of the inflorescence internode and an increased number of secondary branches and grains per panicle. In addition, four EP mutants controlled by the single recessive gene were identified. Zhou et al. (2008) reported an EP in introgression lines developed from *Oryza glaberrima*, an African cultivated rice, and mapped the gene between RM5879 and RM3332 on chromosome 4. Two allelic *ep2* mutants were derived from two *Oryza sativa* ssp. *indica* cultivars, Zhongxian 3037 and 93-11, by spontaneous mutation. The *dep2* mutant was isolated from the T-DNA insertion population. *DEP2* encodes a plant-specific protein without any known functional domain; however, it affects panicle outgrowth and elongation (Li et al. 2010). The *ep3* mutant was induced by treatment of *O. sativa* ssp. *japonica* cultivar Hwasunchal with *N*-methyl-*N*-nitrosourea (MNU); *EP3* encodes a putative F-box protein that plays a key role in protein interaction and signaling pathways (Piao et al. 2009).

In order to gain insight into the processes controlling panicle architecture, we identified a novel dense and EP mutant through chemical mutagenesis, and termed it *dep3*. In the current study, we characterized the phenotype of the *dep3* mutant, elucidated its effects on several agronomic traits, and identified the candidate *DEP3* using a map-based cloning strategy.

Materials and methods

Plant materials and phenotype data collection

The *dep3* mutant was induced by MNU treatment of rice *O. sativa* ssp. *japonica* cultivar Hwacheong (an elite Korean *japonica* cultivar) and has been maintained at the Rice Gene Bank of the Department of Plant Science, Seoul National University, Seoul, Korea. The parent plants and their F_1 and F_2 progenies were grown by conventional methods at the Experimental Farm of Seoul National University. All panicle and internode traits were measured during the maturation stage. Scored traits included the number of primary branches, number of secondary branches, number of panicles per plant, number of grains per panicle, grain yield per plant, culm length, panicle length, number of panicles per hill, number of spikelets per panicle, spikelet fertility, length of primary branches, length between nodes of secondary branches, grain length, grain width, grain thickness, and grain shape (length/width). The thickness and diameter of the first three internodes from the top were measured using light anatomical microscopy and the number of large/small vascular bundles of the uppermost internodes (NSVB/NLVB) was counted using paraffin sectioned slides. For all of these traits, we sampled 15 representative wild type plants and *dep3* mutant plants in the middle of each plot. The main culm panicle of each plant was chosen for trait measurement and further analysis.

Histological observations of the uppermost internodes

For paraffin sectioning, the uppermost internode tissues were prepared as described previously (Ji et al. 2006; Piao

et al. 2009) with slight modifications. Fresh uppermost internode tissues were collected in areas <1 cm from the panicle node at the ripening stage and fixed in FAA solution (50% ethanol, 5% acetic acid, 3.7% formaldehyde) at 4°C overnight. The next day, samples were dehydrated with 50–100% ethanol, cleaned with a series of washes with 25–100% xylene and then embedded in paraffin at 52–54°C (Sigma-Aldrich, St. Louis, USA). The samples were sectioned at a thickness of 10–15 μm using a rotary microtome (MICROM Lab, Walldorf, Germany). Cross and longitudinal sections were mounted on clean glass slides using Canada balsam and then the samples were stained with 1% (w/v) Safranin O and 0.5% (w/v) Fast green FCF (Sigma-Aldrich, St. Louis, USA). The prepared samples were observed under a light microscope at 100× magnification.

For scanning electron microscopy (SEM), samples were fixed overnight at 4°C in FAA. After dehydration in a graded ethanol series and substitution with 3-methyl-butyl-acetate, the samples were critical-point dried, sputter coated with platinum, and observed through a Stereoscan Leica Model 440 Scanning Electron Microscope (Leica, Cambridge, UK; http://www.leica-microsystems.com) at an accelerating voltage of 10–20 kV.

DNA extraction and PCR analysis

Total rice genomic DNA was extracted from the fresh leaf tissue of field-grown F_2 and parental plants as described previously (Causse et al. 1994). PCR conditions followed those of Qiao et al. (2010) with slight modifications of the annealing temperatures of primers.

Statistical and genetic analysis

For genetic analysis, two F_2 populations were developed from crosses between the *dep3* mutant and two rice cultivars: Hwacheong, which was the origin of the *dep3* mutant, and Milyang23, a Tongil-type rice derived from an *indica* × *japonica* cross with a genetic background resembling that of *indica*. The numbers of erect and curved panicle plants in the two F_2 populations were counted 30 days after heading. Chi-square tests were performed to determine whether the goodness-of-fit indicated conformity to a ratio of 3:1 in the F_2 populations.

Fine mapping of the *DEP3* gene

One F_2 population from the cross between *dep3* and Milyang23, which included a total of 917 F_2 plants, was used in locating and fine mapping of the *DEP3* locus. Equal amounts of DNA from ten mutant plants and ten wild type plants of the F_2 population were used to construct bulk pools for bulked segregant analysis (BSA; Michelmore et al. 1991). After BSA, we performed recessive class analysis (RCA) for the initial mapping of the *dep3* gene (Zhang et al. 1994) using STS markers designed by the Crop Molecular Breeding Lab, Seoul National University. Linkage analysis was conducted using MAPMAKER version 3.0 software (Lander et al. 1987). Map distances were estimated using the Kosambi equation (Kosambi 1944). For construction of a physical map of the *dep3* locus region, new STS markers were developed by designing primers based on differences in the DNA sequences between *indica* and *japonica* rice, which are available at http://www.ncbi.nlm.nih.gov/ (for *indica*) and at http://www.rgp.dna.affrc.go.jp/ (for *japonica*). Primer sequences and amplified lengths of the DNA markers used in this study are listed in Table 1.

Analysis of candidate genes

Based on the physical map of the *dep3* gene, corresponding bacterial artificial chromosome (BAC) or PAC sequences of the Nipponbare cv. in the *dep3* gene region were downloaded from the TIGR database (http://www.tigr.org). Open reading frames (ORFs) and potential exon/intron boundaries were predicted for the sequences described above using the Rice GAAS (Rice Genome Automated Annotation System; http://ricegaas.dna.affrc.go.jp/rgadb/). The ORF sequence of each predicted gene was used to search for its full length cDNA and EST (expressed sequence tag) in the KOME (http://cdna01.dna.affrc.go.jp/cDNA/) and Genbank databases. The translated amino acid sequences of candidate genes were subjected to BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) analysis for functional prediction. The full length genomic DNA sequence of each candidate gene was divided into several segments in order to design-specific PCR primers that were used to amplify genomic DNA from Hwacheong and the *dep3* mutant. PCR products were purified using a PCR purification kit for TA cloning (iNtRON Biotechnology, INC, Korea). Purified PCR product was introduced into the pGEM-T Easy Vector (Promega, USA) and then transformed into *Escherichia coli* strain DH5α. The recombinant plasmid was sequenced with an ABI Prism 3730 XL DNA Analyzer (PE Applied Biosystems, USA) and sequence alignment was performed with the BLAST network service (National Center for Biotechnology Information, NCBI).

The deletion of genomic fragments was determined by the specific primers for amplifying the deleted region and coding regions. The PCR amplification primer sets were 1F (5′-AGTGCAGGGCAACCAACTAC-3′), 1R (5′-TCATGGCGACTCGGACGAGG-3′), 2F (5′-AGTGCAGGGCAACCAACTAC-3′) and 2R (5′-ATTGCAAGCAACCC

Table 1 PCR-based molecular markers designed and utilized for fine mapping

Marker	Marker type	Size (bp)	Originated BAC	Forward primer sequence	Reverse primer sequence
S06100	STS	211	AP003568	5′-AGGAAGTGGCGGAGAGAAAC-3′	5′-ATAGCGCTACTGGCTTTTGC-3′
S06104	STS	164	AP005760	5′-CAAATTTACGGATAGGTTTAGTGGA-3′	5′-GGACCTATAAGGACTAAGGTGAGC-3′
RM30	SSR	140	AP005769	5′-GGTTAGGCATCGTCACGG-3′	5′-TCACCTCACCACACGACACG-3′
P01	STS	169	AP004744	5′-CGGAGAAGACACGGGAAGT-3′	5′-GAGCTGGGGAATGAACTGAG-3′
RM5509	SSR	255	AP005192	5′-GATGATCCATGCTTTGGCC-3′	5′-TTCCAGCAGAAAGAAGACGC-3′
P09	STS	214	AP005610	5′-CACGAATTGCACTCCATGAC-3′	5′-CGAATGCTGGAGCAGTTTTT-3′
P21	STS	227	AP004989	5′-TAAGCTTCCGCACAGTGTTG-3′	5′-AAATGTGCGGTTCTGTGTGA-3′
P22	STS	161	AP004989	5′-GGGCAGTCACAAATGGTGTA-3′	5′-ACCTGATCTCGGTGTCCTTG-3′
P23	STS	238	AP004989	5′-TGAACAGGACAAGGGATTCG-3′	5′-GGATCGATCGAGTTTTGCAT-3′
P12	STS	327	AP003728	5′-CTGTGAAATTGATGCGTGGT-3′	5′-ATGCACACAGCACAACACAA-3′
P14	STS	239	AP003728	5′-TGAACCGCTTCAAAACTCTAAG-3′	5′-GAGATTCGGAGGAGCACTTG-3′
P08	STS	214	AP003723	5′-TCTGTGCTCATTCTGCCAAG-3′	5′-GCTCCAACTGCCTAACAAGC-3′
S06110	STS	261	AP004797	5′-GGCTGGTCTAAACCCTGGTA-3′	5′-TTTGTTTTTGCATTGGTGGA-3′
S06113	STS	200	AP005395	5′-CGAGACCGAGACCGAATTTA-3′	5′-TGGCTAGCGTTTCGTGTCTA-3′
S06115	STS	189	AP005445	5′-TTGCATGGACACGATCAGTT-3′	5′-CGGAGATGAGGAAGGTGACT-3′

ACCTAC-3′). PCR amplicons were separated on horizontal 3% agarose gels.

Results

Phenotypic characteristics of the *dep3* mutant

To elucidate the molecular mechanisms underlying panicle morphology, we identified a new dense and EP rice mutant (*dep3*) from the *O. sativa* ssp. *japonica* cultivar Hwacheong population through mutagenesis with MNU. The *dep3* mutant did not exhibit a visibly abnormal phenotype during vegetative and early reproductive growth stages in comparison to wild type plants. After grain filling, however, the panicle of the *dep3* mutant remained erect from the flowering stage to the fully mature stage, whereas the panicle of the wild type plant began to droop after flowering (Fig. 1a, b). To better characterize the phenotype, we quantitatively compared panicle architecture and yield traits under field conditions. Differences between the wild type and *dep3* mutant were not detected in the heading date, length of the grain-filling period, culm length, or panicle number per plant (Table 2; Fig. 1f), but differences were evident in panicle architecture, inflorescence internode length, panicle length (Fig. 1c), and the number of both primary and secondary branches per panicle (Fig. 1d, e). Taken collectively, these traits conferred more grains per panicle in the *dep3* mutant (33.9%) (Fig. 1g). In addition to the differences in panicle architecture noted above, grain length and shape were significantly smaller and grain width and thickness were significantly greater in *dep3* plants (Table 3; Fig. 2a). Even though the 1,000-grain weight of the *dep3* mutant was slightly less than that of the wild type (24.4 g, wild type vs. 23.4 g, *dep3* mutant; not statistically significant), the *dep3* mutant did display a statistically significant increase in overall grain yield per plant (17.2%) in the field performance trials (Fig. 1h, i).

To explore whether the difference in grain size was caused by a decrease in cell number or cell size or both, epidermal cells outside of the rice glumes were observed using SEM. The *dep3* epidermal cells were shorter and wider than those of the wild type (Fig. 2b, c), causing changes in grain shape. These results suggested that the *DEP3* mutation is responsible for pleiotropic effects on the dense panicle, EP, and short and round grain shape.

The *dep3* mutant has more vascular bundles in the uppermost internodes

Erectness of the *dep3* mutant panicle indicated greater mechanical strength than in the wild type plant. For this reason, the thickness and diameter of the first three internodes from the top were examined using a light anatomical lens. Thickness and diameter of the *dep3* internodes were significantly greater than those of wild type plants. The difference in diameter increased gradually because both the value and the ratio increased from the uppermost internode to lower internodes, whereas the internode thickness ratio decreased (Table 3). To further evaluate alterations in cell wall structure, we compared the peripheral vascular tissues and sclerenchyma cells under the uppermost internode

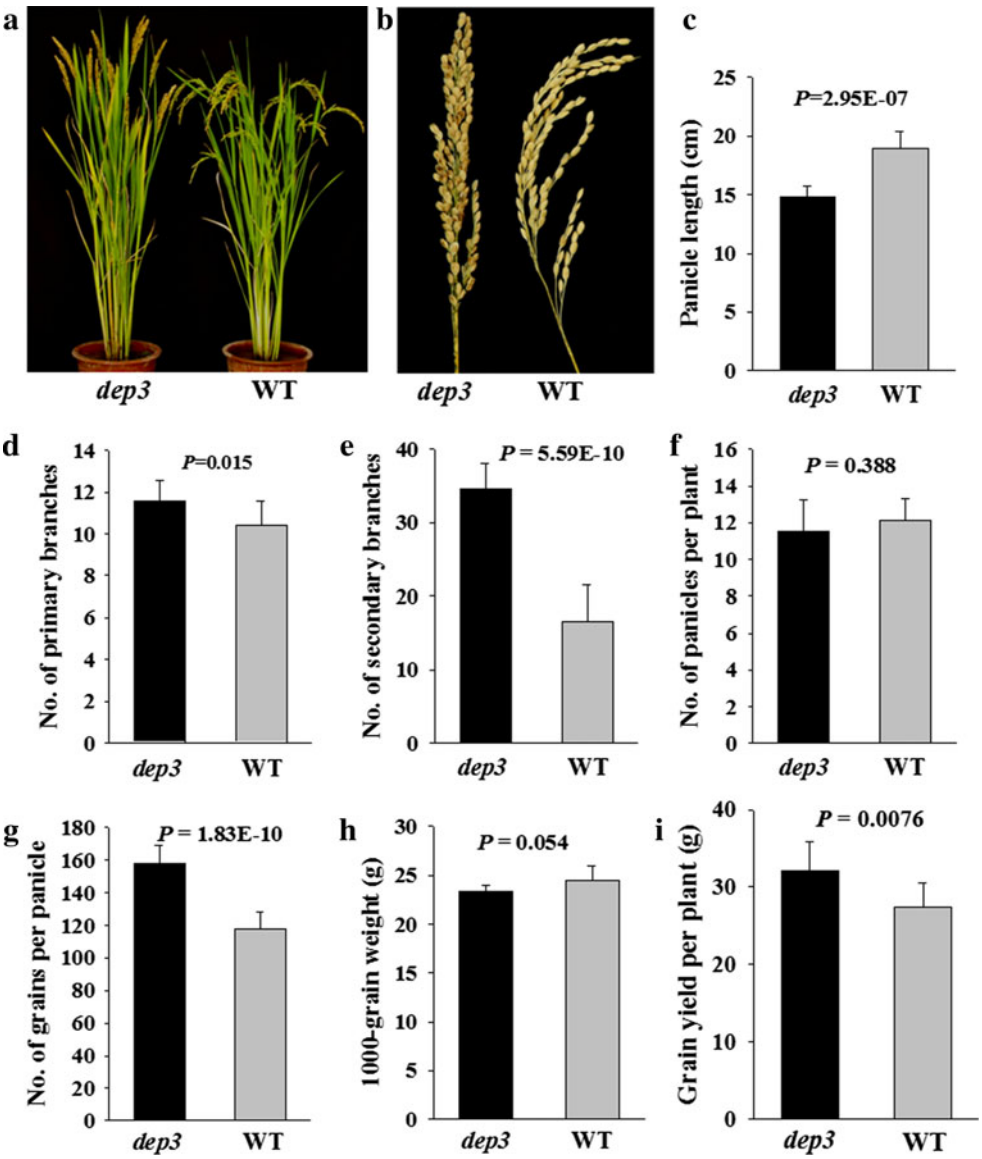

Fig. 1 Phenotype of the *dep3* mutant. **a** Plant phenotype after grain filling. **b** Phenotype with a dense and erect panicle at the mature grain stage. Comparison of panicle architecture and yield traits: **c** main panicle length. **d** Number of primary branches. **e** Number of secondary branches. **f** Number of panicles per plant. **g** Number of grains per main panicle. **h** 1,000-grain weight. **i** Grain yield per plant. *dep3* and wild type plants were grown in a standard paddy field at a planting distance of 30 × 15 cm. Fertilizer was applied at the rate of 10–80–80 kg ha^{-1} (nitrogen–phosphorus–potassium). All data are given as the mean ± SD (15–20). Student's *t* test was applied to test for significant differences

Table 2 Comparison of agricultural traits of *dep3* and the wild type cultivar, Hwacheong

Traits	HD	CL (cm)	SN	SF (%)	GL (mm)	GW (mm)	GT (mm)	GS	LPB (mm)	LBN (mm)
dep3	Aug. 20	79.7 ± 2.98	172.8 ± 11.9	91.4 ± 1.5	5.61 ± 0.23	3.61 ± 0.16	2.48 ± 0.09	1.56 ± 0.08	58.4 ± 0.49	5.1 ± 0.09
WT	Aug. 23	78.16 ± 2.39	120.6 ± 11.1	97.8 ± 1.3	6.87 ± 0.16	3.15 ± 0.11	2.19 ± 0.06	2.18 ± 0.08	78.2 ± 0.88	7.8 ± 0.14
Difference	NS	NS	**	**	**	**	**	**	**	**

HD heading date, *CL* culm length, *PN* number of panicles per hill, *SN* number of spikelets per panicle, *SF* spikelet fertility, *GL* grain length, *GW* grain width, *GT* grain thickness, *GS* grain shape (length/width), *LBP* length of primary branches, *LBN* length between nodes of the secondary branches, *WT* wild type, *NS* no significant difference

** $P < 0.01$. All data are given as the mean ± SD (15–20). Student's *t* test was applied to test for differences

epidermal layer. These tissues are presumed to provide mechanical strength to the plant body. The uppermost internodes of the *dep3* mutant had more small vascular bundles than in the wild type plants (Fig. 3a, b, d). The cells observed across longitudinal sections in the uppermost internodes of the *dep3* mutant were wider and shorter than those of wild type plants (Fig. 3c). These findings suggested that the thicker culm and greater number of vascular bundles increase the mechanical strength of the uppermost internodes, resulting in the EP. Moreover,

Table 3 Comparison of internode characteristics between *dep3* and wild type plants

Traits	Thickness of internode (mm)			Diameter of internode (mm)		
	First	Second	Third	First	Second	Third
dep3	0.39 ± 0.05	0.67 ± 0.08	0.88 ± 0.06	1.78 ± 0.11	4.11 ± 0.23	5.22 ± 0.25
WT	0.24 ± 0.03	0.43 ± 0.03	0.62 ± 0.06	1.33 ± 0.13	3.00 ± 0.22	3.53 ± 0.27
Difference	**	**	**	**	**	**

Thickness of internode [(outside diameter–inside diameter)/2], diameter of internode (outside diameter)

** $P < 0.01$. All data are given as the mean ± SD (15-20). Student's t test was applied to test for differences

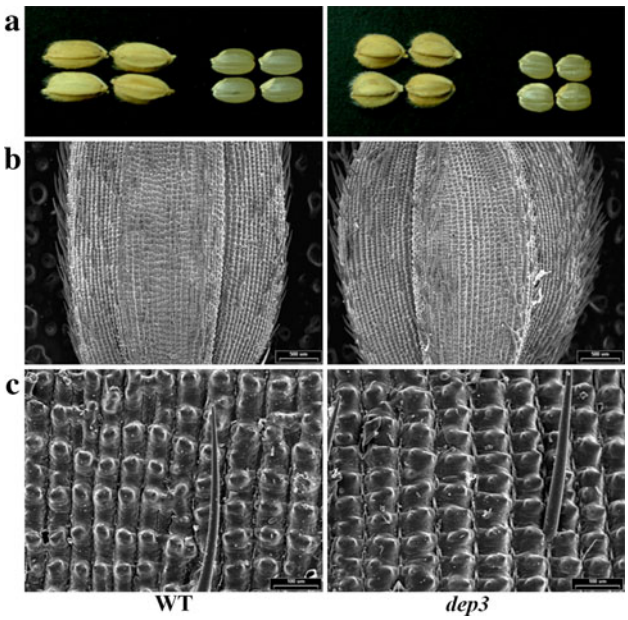

Fig. 2 Comparison of grain morphology between *dep3* and the wild type. **a** Mature paddy rice grain (*left*) and brown rice grains (*right*), showing differences in grain shape. **b** Scanning electron microscopy (SEM) analysis of the outer surface of rice glumes. **c** Magnified images of the region in **b**, showing differences in cell size. *Scale bars* 500 μm in **b**, and 100 μm in **c**

the secondary cell wall largely determines the mechanical characterization of the plant. It is composed of a rigid complex matrix of polymers and proteins, and coordinated deposition of these polymers is needed for proper assembly (Boudet et al. 2003). Accordingly, abnormal cell morphology and vascular tissues in the *dep3* might also affect secondary cell wall structure and associated mechanical traits.

Genetic analysis of the *dep3* mutant

The individual plants of F_1 and F_2 progenies from the crosses of *dep3* to Hwacheong and Milyang23 were investigated 30 days after heading. All the F_1 individuals from these crosses exhibited wild type panicle architecture, suggesting that the mutant trait was recessive. In the two F_2 populations, all of the segregation rates of the wild type and *dep3* plants fit a ratio of 3:1 ($\chi^2_C < \chi^2_{0.05} = 3.84$) (Table 4), demonstrating that the dense and EP trait was controlled by a single recessive nuclear gene, which we termed dense and EP 3 (*dep3*).

Fine mapping of the *DEP3* gene

To determine the chromosomal location of the *DEP3* gene, bulk-segregant analysis of F_2 plants from the *dep3*/Milyang23 cross was performed using 182 STS markers distributed over the 12 chromosomes (Michelmore et al. 1991) to detect flanking markers. One marker (S06110) on the long arm of chromosome 6 was linked to the mutant phenotype. By conducting RCA (Zhang et al. 1994) with 30 *dep3* mutant segregants, the *DEP3* gene was initially placed on the genome segment between the two flanking markers, RM30 and S06110 (Fig. 4a). To further define the position of the *DEP3* gene, nine PCR-based molecular markers within the flanking makers were designed based on sequences available in rice genomic databases (http://www.ncbi.nlm.nih.gov/ for *indica* and http://www.rgp.dna.affrc.go.jp/ for *japonica*) (Table 1) and genotyping was performed with the remaining plants from the F_2 mapping population. Ultimately, the *dep3* locus was delimited to a ~73 kb region on a Nipponbae BAC clone, AP004989 (Fig. 4b), and flanked by markers P21 and P23. No recombinant was identified for P22 in the F_2 mapping population (Fig. 4b), indicating that this marker was tightly linked to the *DEP3* gene.

The patatin-like phospholipase A is a candidate *DEP3* gene

Data obtained from fine mapping and available rice genome annotation databases (http://www.tigr.org, http://www.gramene.org) indicated that a total of six putative ORFs were located in this 73 kb genomic region. To determine which one carried the *dep3* mutation, the genomic DNA of the six candidate genes was sequenced. Among the mapping parents and the wild type, the sequence of LOC_Os06g46350 contained a polymorphism. No polymorphisms were detected in the five other candidate genes. Comparison of the LOC_Os06g46350

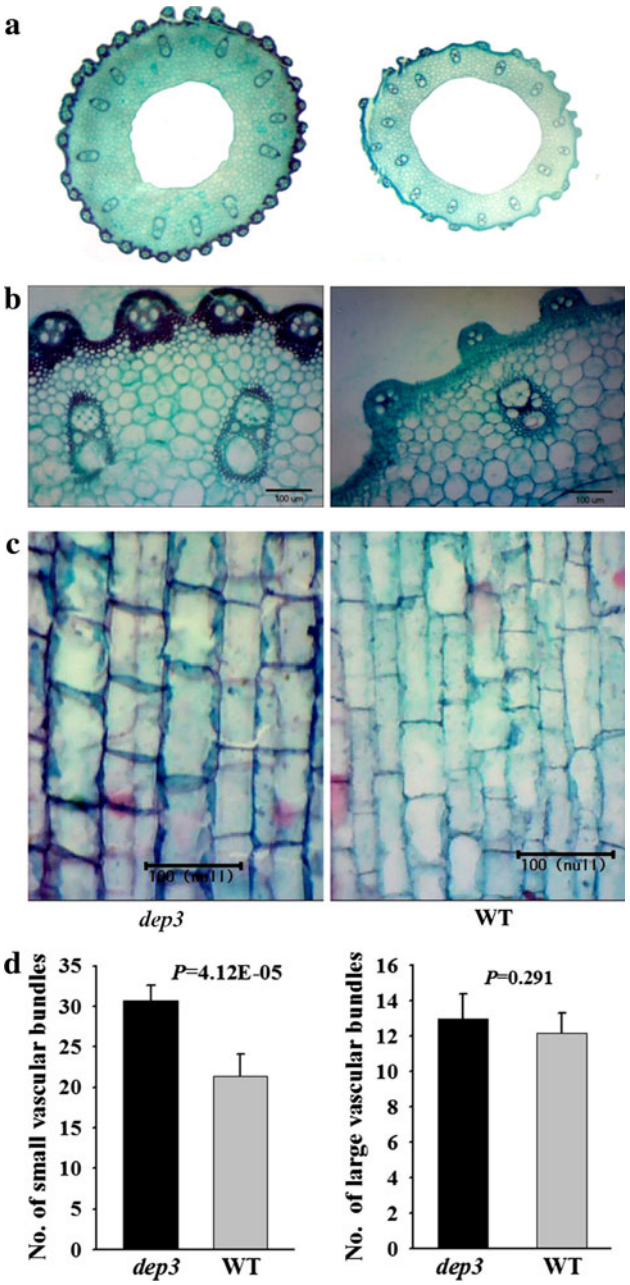

Fig. 3 Histological analyses of the uppermost internodes of the main culm at the mature stage of *dep3* and wild type plants. **a** Transverse section of the uppermost internodes, showing differences in morphology and vascular bundles. **b** Transverse section of the uppermost internodes at higher magnification. **c** Longitudinal section of the uppermost internodes, showing a slight reduction in cell size. **d** Comparison of the number of small and large vascular bundles. Data are given as the mean ± SD (15–20). Student's *t* test was applied to test for significant differences. *Scale bars* 100 μm

sequences of Hwacheongbyeo, wild type, and the *dep3* mutant revealed that the *dep3* allele carried a 408 bp genomic deletion, including the last 47 bp coding region of the third exon and the first 361 bp of a 3′-untranslated region (3′-UTR). The genomic fragment carrying the deletion was also confirmed through the use of two PCR primers located outside the potential deletion region (Fig. 4d). The LOC_Os06g46350 region consisted of three exons and two introns. The complementary DNA for this gene was 1,215 bp long and the deduced protein sequence consisted of 404 amino acids, including patatin and the phospholipase A2 (PLA2) superfamily domain, which are known to be key domains in lipid metabolism and plant signal transduction (Mansfeld 2009).

Table 4 Genetic analysis of the *dep3* mutant in rice

Cross combination	F_1 plants			F_2 plants			χ^2 (3:1)
	WT	dep	Total	WT	dep	Total	
dep3/Milyang23	19	0	19	711	206	917	3.14
dep3/Hwacheong	8	0	8	179	71	250	1.49

To determine whether the fragment deletion in the third exon of *DEP3* was present as a natural variant in other cultivars, ten typical *indica* and *japonica* rice cultivars were genotyped with specific primers that detect this deletion, which showed that all ten varieties carried the wild type allele (Fig. 4e). In addition, the genotypes associated with this specific marker were co-segregated with the matching phenotypes in the F_2 population from a cross between *dep3* and wild type plants (Fig. 4f). The difference in translated amino acids from the transcript of the *dep3* and the *dep3* genes was predicted using direct sequencing of the RT-PCR products (Fig. 5). Our results suggested that the *DEP3* gene mutation involved the substitution of four new amino acids and the deletion of 15 amino acids.

Discussion

Donald (1968) defined the "crop ideotype" as an idealized plant type with a specific combination of characteristics favorable for photosynthesis, growth, and grain production. Breeding programs aimed at developing ideotype lines have focused primarily on improving plant architecture. The most famous examples in breeding history are the introduction of the semi-dwarf gene and the erect leaf gene into cultivated rice and wheat (Evenson and Gollin 2003). Architecture of the rice inflorescence, particularly the panicle type, greatly influences agronomic value. For this reason, the panicle type has been a focus of breeding efforts for decades. Significant progress has been made in understanding the mechanistic control of the panicle type of rice, and the identification and functional analysis of genes conferring such characteristics is of value in rice breeding (Xu et al. 2005).

It has been reported that EP-type *japonica* rice cultivars possess increased lodging and fertilizer resistance as well

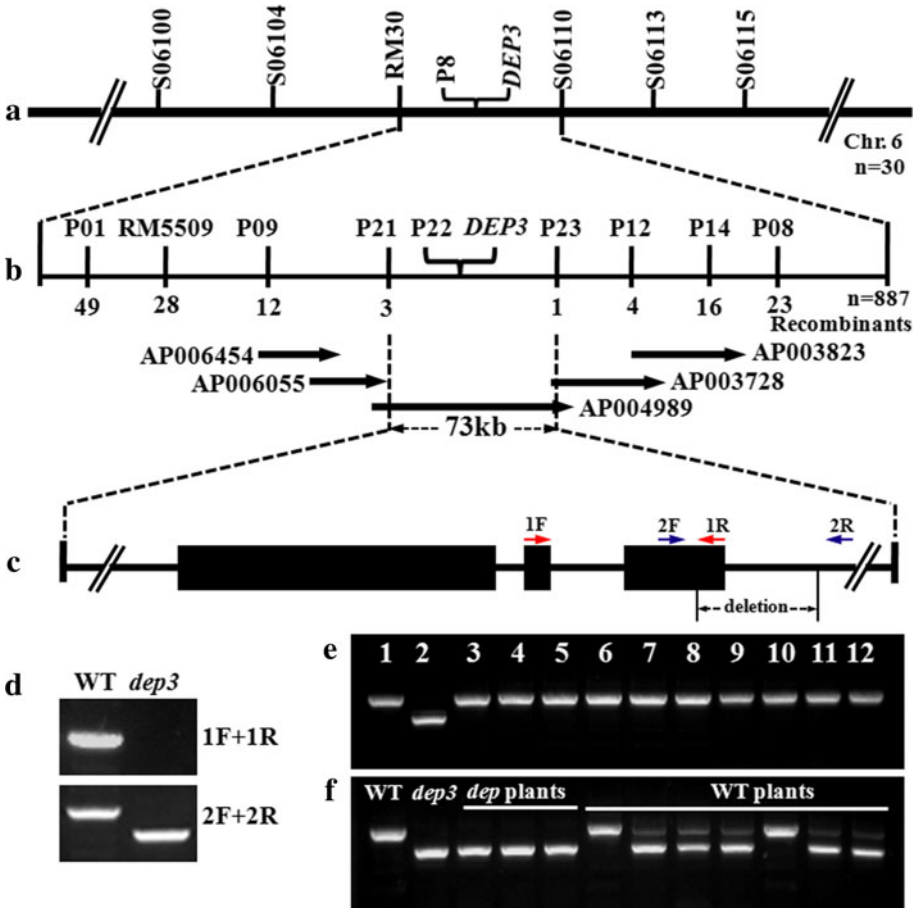

Fig. 4 Positional cloning of the *DEP3* gene. **a** The *DEP3* locus was mapped to chromosome 6 between markers RM30 and S06110 on the basis of genotyping of 30 *dep3* mutant segregants. **b** Fine mapping of the *DEP3* gene with markers based on the genome sequence. *Numerals* indicate the number of recombinants identified in 887 F_2 plants. The *DEP3* locus was narrowed to a 73-kb region of genomic DNA between markers P21 and P23. **c** The structure of *DEP3,* showing a 408-bp genomic deletion within the third exon and 3′-UTR of the LOC_Os06g46550 gene. *Closed boxes* indicate the coding sequence, and *lines between the boxes* represent introns. **d** PCR analyses with two pairs of specific primers confirmed the deletion of a genomic fragment. PCR primers were located within the deleted regions and/or coding regions; the amplified products were also confirmed by sequencing. **e** Confirmation of the genomic deletion in the *dep3* mutant by analysis of PCR amplicon size in the *dep3* mutant and 11 normal rice cultivars. *Lane 1* contains the wild type; *lane 2* contains the *dep3* mutant; *lanes 3–12* contain normal rice varieties (*japonica* cultivars by lane: *3* Nipponbare, *4* Koshihikari, *5* Dongjin, *6* Dianxi, *7* CP-SLO, and *indica* cultivars: *8* Milyang23, *9* IR64, *10* Habataki, *11* China1039, *12* Tadukan). **f** Co-segregation of the *dep3* mutant phenotype was analyzed by size comparison of PCR amplicons in the F_2 population from a cross between the *dep3* mutant and the wild type

as enhanced photosynthetic efficiency of the lower leaves, which increases grain yield primarily by increasing the number of secondary branches and the number of grains on each secondary branch (Chen et al. 2007; Huang et al. 2009). For these reasons, many high yielding varieties are currently being cultivated in China, and three EP genes have been isolated using a map-based cloning approach (Huang et al. 2009; Piao et al. 2009; Zhu et al. 2010; Li et al. 2010). However, inheritance of the EP phenotype has not been fully elucidated. In this study, we created a new *dense and erect panicle 3* (*dep3*) mutant by chemical mutagenesis. The panicle of the *dep3* mutant remained erect from flowering to grain maturity. The increased number of grains (or spikelets) per panicle in *dep3* was associated with an increased number of panicle branches, even with a reduction in rachis length. The *dep3* mutant had a rounder grain shape than the wild type and the 1,000-grain weight of *dep3* plants was slightly less than that of wild type plants. The overall grain yield per plant under field conditions was higher in the *dep3* mutant (Fig. 1). In addition, the overall shape of the *dep3* mutant plants was more compact than wild type plants. Moreover, our results also suggested that the phenotypic characteristics of the *dep3* closely resemble those of *dep1* except for culm length variance in comparison to their wild type (Huang et al. 2009).

In rice, the vascular bundle system of the culms, culm, and leaf veins provides mechanical support for the body of the rice plant (Ye 2002; Teale et al. 2006; Aohara et al.

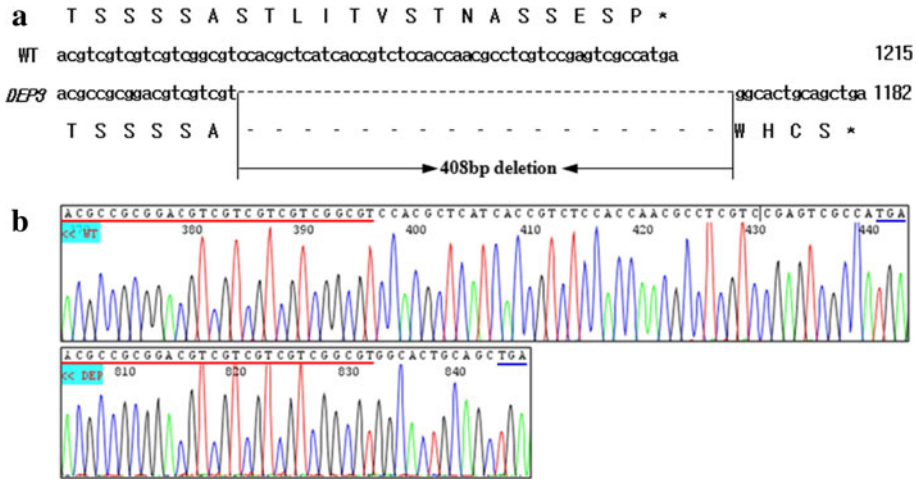

Fig. 5 Confirmation of the *dep3* mutant transcript by RT-PCR. **a** The complementary DNA and amino acid partial sequence alignment of *dep3* and WT. The *dotted line* indicates the deleted genomic region in the *dep3* mutant and the 408-bp deletion in LOC_Os06g46550 results in an amino acid deletion and substitution. **b** Sequencing chromatograms were constructed by sequencing of RT-PCR products of *dep3* and WT

2009). Panicle architecture is closely associated with the number of vascular bundles and EPs exhibit more small and/or large vascular bundles than the wild type (Huang et al. 2009; Piao et al. 2009; Zhu et al. 2010). In the current study, the uppermost internodes of the *dep3* mutant had thicker culms and smaller vascular bundles than the wild type, which might enhance both culm mechanical strength and water transport capacity. Moreover, hormones can affect patterns of the vascular system (Scarpella et al. 2003); auxin (by polar transport) is essential for vascular tissue formation and differentiation, brassinosteroids (BR) modulate vascular bundle numbers by promoting early procambial divisions (Ibanes et al. 2009), and gibberellin increases both large and small vascular bundles of the internodes (Shimizu and Takeoka 1966). Considering the similarities between the *dep3* mutant and gibberellin-treated plants, it seems likely that *DEP3* is involved in the gibberellin signaling required for normal development of panicles. The detailed molecular mechanism remains to be elucidated in future studies.

In the current study, the *DEP3* gene was mapped on chromosome 6 and was predicted to encode a patatin-like PLA2-containing protein similar to the animal iPLA2 type and highly conserved in all sequences found in protists, plants, and animals (Ryu 2004). In addition to functioning as a vacuolar enzyme, plant patatin-like phospholipase A (PLA) may have a role in auxin and elicitor signal transduction, where it hydrolyzes phosphatidylcholine and phosphatidylethanolamine within 1–5 min to generate free fatty acids and lysophospholipids as potential second messengers (Paul et al. 1998; Narvaez-Vasquez et al. 1999; Scherer et al. 2000). In *Arabidopsis thaliana*, ten patatin-related phospholipase A genes, also referred to as PLP, have been identified on the five chromosomes. All of the pPLA proteins tested so far possess phospholipase A2 activity. Compared to phospholipids, the catalytic rates of *At*PLAs are higher when galactolipids are used as substrates (Matos et al. 2001; La Camera et al. 2005; Yang et al. 2007). Although patatins are known as major storage proteins in potato tubers, *At*PLAs lacking an N-terminal signal peptide have been localized to the cytosol (Holk et al. 2002) and the PLA2 domain has also been proposed to participate in gravitropic responses of plants. *At*sPLA2-overexpressing or -silenced transgenic plants display altered gravitropism in their inflorescence culms and hypocotyls, suggesting that up- or down-regulation of *At*sPLA2 might disrupt the asymmetric cell growth of inflorescence culms and hypocotyls (Lee et al. 2003). To date, about 11 PLA genes in rice identified by Rice GAAS remain elusive. *DEP3* cloning provides an opportunity to investigate the molecular function of these genes in rice.

Panicle erectness has been emphasized in rice breeding programs in the northern part of China, resulting in enhanced grain yield. However, the EP sources, mainly derived from Balilla, cause a bottleneck effect in the genetic background when breeding for new varieties. Hence, the new *DEP* gene identified in this study not only diversifies rice mutation germplasm, but also provides a good starting point for elucidating the mechanisms underlying vascular bundle formation and inflorescence architecture. Further functional studies of the *DEP3* gene are underway, including analysis of gene expression patterns, enzyme activity assays, and gene identification with *Agrobacterium*-mediated transformation technology.

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