Theor Appl Genet (2014) 127:241–250
DOI 10.1007/s00122-013-2213-7

ORIGINAL PAPER

Characterization and genetic mapping of a *Photoperiod-sensitive dwarf 1* locus in rice (*Oryza sativa* L.)

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Received: 30 April 2013 / Accepted: 4 October 2013 / Published online: 25 October 2013

Abstract Plant height is an important agronomic trait for crop architecture and yield. Most known factors determining plant height function in gibberellin or brassinosteroid biosynthesis or signal transduction. Here, we report a *japonica* rice (*Oryza sativa* ssp. *japonica*) dominant dwarf mutant, *Photoperiod-sensitive dwarf 1* (*Psd1*). The *Psd1* mutant showed impaired cell division and elongation, and a severe dwarf phenotype under long-day conditions, but nearly normal growth in short-day. The plant height of *Psd1* mutant could not be rescued by gibberellin or brassinosteroid treatment. Genetic analysis with R_1 and F_2 populations determined that *Psd1* phenotype was controlled by a single dominant locus. Linkage analysis with 101 tall F_2 plants grown in a long-day season, which were derived from a cross between *Psd1* and an *indica* cultivar, located *Psd1* locus on chromosome 1. Further fine-mapping with 1017 tall F_2 plants determined this locus on an 11.5-kb region. Sequencing analysis of this region detected a mutation site in a gene encoding a putative lipid transfer protein; the mutation produces a truncated C-terminus of the protein. This study establishes the genetic foundation for understanding the molecular mechanisms regulating plant cell division and elongation mediated by interaction between genetic and environmental factors.

Communicated by M. Wissuwa.

R. Li and J. Xia contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-013-2213-7) contains supplementary material, which is available to authorized users.

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Introduction

Plant height is one of the most important traits in crop breeding. The introgression of semi-dwarf traits into cereal crop cultivars, a breakthrough in the 'Green Revolution', produced substantial increases in crop yields (Hedden 2003; Jiang et al. 2007). In rice (*Oryza sativa* L.), about 70 dwarf and semi-dwarf mutants have been reported (Matsuo et al. 1997; http://www.shigen.nig.ac.jp/rice/oryzabaseV4).

Plant height is determined by complex genetic networks. Dwarfism arises from various types of defects, and gibberellin (GA) and brassinosteroid (BR) are two major factors, which have been the most intensely studied. Most dwarf mutants are deficient in the biosynthesis or perception of these phytohormones (Sun 2011; Yang et al. 2011). Genes that encode the GA receptor GA-INSENSITIVE DWARF1 (GID1), DELLA proteins, and the F-box protein GA-INSENSITIVE DWARF2 (GID2) have been identified (Gomi et al. 2004; Ikeda et al. 2001; Peng et al. 1997; Sasaki et al. 2003; Silverstone et al. 1998; Ueguchi-Tanaka et al. 2005), and an integrated picture of the GA signal transduction pathway has started to emerge (Sun 2011). In Arabidopsis (*Arabidopsis thaliana*), several factors for perception and transduction of the BR signal, such as BRI1, a membrane-localized LRR receptor-like kinase (RLK) (Peng and Li 2003; Wang and He 2004), BAK1, which interacts with BRI1 (Li et al. 2002; Nam and Li

2002), and the downstream factors BRI1 kinase inhibitor 1 (BKI1), BIN2, BES1, and BZR1 (He et al. 2002; Wang and Chory 2006; Wang et al. 2002; Yin et al. 2002; Zhao et al. 2002) also have been identified by screening for BR signaling mutants. Other than these two major pathways, however, little is known about other mechanisms controlling cell division, cell elongation and plant height. Also, plant growth is affected by environmental conditions, but how environmental factors interact with the genetic factors that determine plant height remains unclear.

GA- and BR-related mutants exhibit their typical phenotypes (Komorisono et al. 2005). For example, rice GA-related mutants are typical dwarfs with deep green and rough leaves (Sakamoto et al. 2004). By contrast, rice BR-deficient mutants have typical dwarf phenotypes, but also have abnormal morphologies including malformed leaves with twisted and stiff blades (Hong et al. 2004). Therefore, characterization of dwarf mutants with phenotypes that differ from the typical GA- or BR-related phenotypes can reveal novel defects related to cell division and elongation. For example, analysis of the rice dwarf mutant *d3*, which exhibits dwarfism and increased tiller numbers, reveals that *D3* encodes an F-box Leu-rich-repeat protein orthologous to the *Arabidopsis* MAX2/ORE9 (Ishikawa et al. 2005). Similarly, study of the rice dwarf mutant *dgl1*, which shows defects in root and flower elongation, and has leaves with a unique rounded tip, reveals that *dgl1* encodes a microtubule-severing katanin-like protein, which is important in cell division and elongation in plants (Komorisono et al. 2005).

In this study, we identified and characterized a novel dwarf mutant, *Photoperiod-sensitive dwarf 1* (*Psd1*), which arose from tissue culture of a *japonica* rice (*O. sativa* ssp. *japonica*) cultivar. *Psd1* showed a severe dwarf phenotype with dark green leaves, similar to GA-related mutants, but it could not be rescued by GA and BR treatments. *Psd1* is photoperiod-sensitive; the dwarf phenotype exhibited in long-day (LD) growth conditions is suppressed under short-day (SD), suggesting that *Psd1* represents a different class of mutant from those reported previously. The *Psd1* phenotype is controlled by a dominant locus on chromosome 1. By fine-mapping, the *Psd1* locus was determined in an 11.5-kb region, and sequencing analysis detected a mutation site in a gene encoding a lipid transfer protein, thus this gene is considered as the candidate of *Psd1*.

Materials and methods

Plant materials

The *Psd1* mutant was obtained from callus-regenerated plants of a *japonica* cultivar Zhonghua 11 (ZH11) (See Supplementary materials for details). R_1 population ($N = 128$) of *Psd1* was used for genetic analysis. An *indica* cultivar Dular (with wild-type *SD1*, plant height is ~160 cm) was crossed with *Psd1*, and then 101 tall plants selected from 390 F_2 individuals were used for linkage analysis and primary mapping with molecular markers. Then additional 1017 tall plants from larger F_2 populations ($N = 4{,}105$) of this cross were used for fine-mapping of *Psd1*. These materials for genetic mapping were grown from May to July, the long-day season, at the experimental farm of South China Agricultural University (Guangzhou, longitude 113°17′, north latitude 23°8′).

Phenotyping and genotyping

Plant height (from ground to the top of panicles) was measured to phenotype R_1 and F_2 segregating populations. In the R_1 population and F_2 population of the *Psd1* × ZH11 cross grown in LD condition, individuals with height <30 cm were classified as dwarf plants (wild-type ZH11 grown in LD condition had an average plant height of 85.3 cm); In the F_2 population of the *Psd1* × Dular cross grown in LD condition, individuals with height <55 cm were classified as dwarf plants (the tall plants had an average plant height of 129.2 cm). Offspring (F_3 plants) of some key F_2 recombinant plants for fine-mapping were planted and their segregation was observed so as to confirm the phenotype of these recombinant plants. Leaf tissues of the materials were collected for DNA extraction and genotyping.

Microscopy

Elongating regions of the culm internodes of *Psd1* and ZH11 were sliced into 8–10 μm sections using a Leica RM2135 microtome (Germany) and were observed under bright field using a Carl ZEISS Axio Observer Z1 microscope (Germany).

Photoperiod sensitivity tests

Psd1 and ZH11 plants were planted in Guangzhou on May 20th, and they headed in late July. Natural day-length in this season ranges 13.2–14.0 h, a LD condition. The SD-treatment (ca. 10 h of day-length) of the plants grown in the same season was performed by moving the plants grown in pots into a dark box every day from 16:00 (at ~20:00 the plants were moved out from the box). Plant height was measured and number of tillers was recorded 10 days after heading.

GA and BR treatments

GA and BR treatments were performed as previously reported (Komorisono et al. 2005) with minor

modifications. *Psd1* and ZH11 seeds were sterilized with 1.5 % NaClO for 30 min twice and washed five times in sterile distilled water. The seeds were then placed on 0.75 % agar plates, grown under fluorescent light at 30 °C for 4 days, and then transferred to 0.75 % agar plates containing MS medium and various concentrations of GA3 or BR (Fig. 2a, b). The length of the third-leaf sheath was measured at 4 days after application of GA3 or 2 weeks after application of BR.

Molecular markers development and assays

More than 600 SSR markers (RM-series) distributed over the whole rice genome were screened for polymorphisms between ZH11 and Dular. A number of new insertion/deletion (InDels) markers were also identified based on rice genome sequences (http://www.ncbi.nlm.nih.gov/) by blasting the sequences of Nipponbare (*japonica*) and 93-11 (*indica*). PCR reactions were performed using a TaKaRa PCR Thermal Cycler TP650 (Japan) under conditions as follows: 94 °C for 3 min; 33 cycles of 95 °C for 15 s, 56 °C for 30 s and 72 °C for 20 s. Finally, 270 polymorphic markers between ZH11 and Dular were identified.

Because SSR or InDel markers were not enough for fine-mapping in the primarily mapped *Psd1* locus region (approximately 300 kb in length), genomic DNA fragments of ZH11 and Dular, about 1 kb in length, were amplified by PCR and sequenced to find single nucleotide polymorphisms (SNPs) between the parents. Identified SNP markers (Table S2) were analyzed based on semi-nested PCR, in which forward and reverse primers were used for first round PCR (33 cycles of 95 °C for 15 s, 56 °C for 30 s and 72 °C for 30 s), then forward primers together with the allele-specific primers were used for second round PCR, with 100-fold diluted primary PCR products and 12 cycles of 95 °C for 15 s, 48.5 °C for 30 s and 72 °C for 10 s. The second round PCR products were examined on 2 % agarose gels. Marker ORF2-3′ (Table S2) was based the mutation site in an ORF (ORF2) within the mapped region, and the genotyping was performed with high-resolution melting (HRM) analysis as described by Li et al. (2010).

Linkage analysis and gene mapping

Using 270 polymorphic markers, 22 tall plants (with wild-type, homozygous recessive allele *psd1*+) selected from a small F_2 population (*Psd1* × Dular, $N = 390$, containing 101 tall plants) were used to find out the chromosomal region linked to the target gene. Then all the 101 tall plants were used to construct the primary map of *Psd1*. Linkage analysis and the genetic distance (cM) calculation between the markers (loci) were conducted by MAPMAKER3.0 (Lander et al. 1987). The newly developed InDel and SNP markers in the anchored region and 1017 tall plants selected from larger F_2 population ($N = 4{,}105$) were used for fine-mapping of *Psd1*. The target chromosomal region was determined according to the numbers of chromosomal recombinant events between the markers and the *Psd1* locus.

RNA isolation and quantitative RT-PCR analysis

Elongating stems and leaf blades of *Psd1* and ZH11 plants (45 days after planted, under LD condition) were harvested at 12:00 a.m. and 0:00. RNA extraction followed the Trizol reagent protocol provided by the manufacturer (Invitrogen, USA) with subsequent DNase I (Invitrogen, USA) treatment. The RNAs (1.5 μg each sample) were used to synthesize first-strand cDNA with an oligo (dT) primer in 20 μl reaction volume using SuperScript II (Invitrogen, USA). PCR was performed in a total volume of 20 μl with 1 μl of the reverse transcription (RT) products, 0.3 μM gene-specific primers (Table S3), and 1 unit of ExTaq (TaKaRa, Japan). Quantitative RT-PCR (qRT-PCR) was performed using Bio-Rad CFX Connect Real-Time PCR Detection System (USA) and the Bio-Rad SYBR Green PCR kit, with the following profile: 95 °C for 30 s; 40 cycles of 95 °C for 5 s, 58 °C for 20 s, 72 °C for 10 s. Rice *Actin1* was used as the internal control. The relative expression levels were analyzed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Results

Identification and characterization of the *Psd1* mutant

Somatic mutation is a common phenomenon in tissue culture of plants. In our test of the regeneration efficiency of calli from the *japonica* cultivar ZH11, we obtained a dwarf mutant, as a somatic mutation, from the regenerated lines (R_0 generation). We named this mutant *photoperiod-sensitive dwarf 1* (*Psd1*) due to its photoperiod-sensitive nature (see below).

The *Psd1* mutant showed a severe dwarf phenotype, with plant height of about 25 cm, when grown under natural LD conditions (Fig. 1a; Table 1; Table S1). The heading time of major tillers of *Psd1* plants was similar to that of ZH11 (Fig. 1a, b). However, most tillers of *Psd1* failed to head under LD (Fig. 1a). The total number of the headed culm internodes of *Psd1* was the same as ZH11, but the length of each elongated internode was substantially shorter (Fig. 1c). The panicles of *Psd1* were small (<8 cm in length), and the tillers that did not head developed only stunted inflorescences (Fig. 1c). Transverse sections of elongated internodes of *Psd1* and ZH11 showed that the structure of parenchyma cells and vascular bundles of *Psd1*

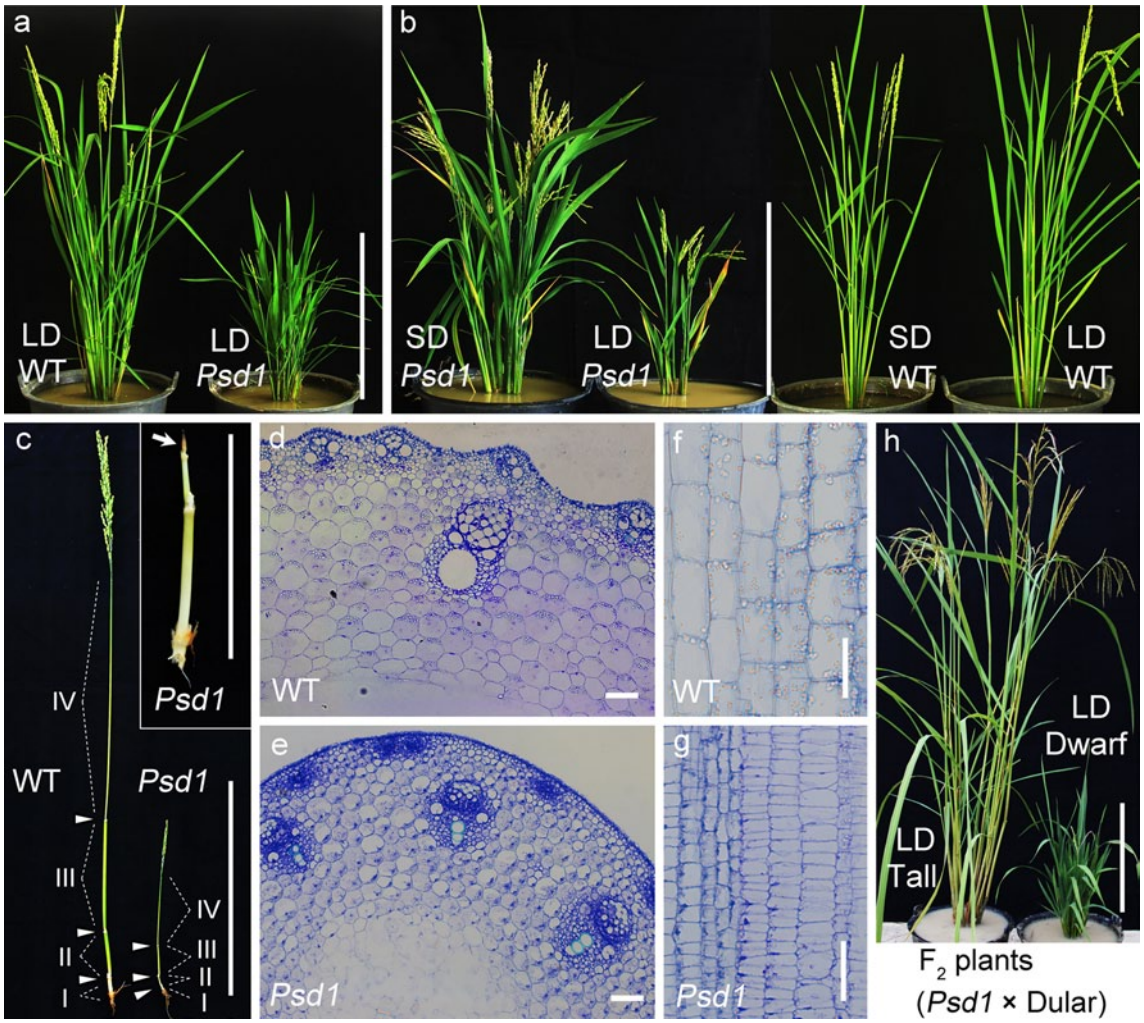

Fig. 1 Phenotypic characterization of the *Psd1* mutant. **a** Wild-type ZH11 (*left*) and *Psd1* (*right*) plants 10-day after heading, grown in a long-day (LD) season. *Bar* 30 cm. **b** *Psd1* plants grew sturdier and taller in short-day (SD) conditions than in LD conditions. ZH11 plants (WT) grown in LD were slightly taller than those grown in SD conditions. The plants were planted in Guangzhou on May 20th and they headed in late July, with the average natural day-length of 13.2–14 h (LD); SD-treatment (~10 h day-length) in the same season was performed by moving the plants to a *dark box* every day from 16:00 to ~20:00. *Bars* 30 cm. **c** Culms of wild-type (*left*) and *Psd1* (*right*) plants. *Arrowheads* indicate nodes, and I to IV indicate internodes. *Bar* 30 cm. *Top right corner* shows the culm of a *Psd1* tiller that failed to head. *Arrow* indicates the stunted inflorescence. *Bar* 6 cm. Transverse sections of the elongated regions of internodes of wild-type (**d**) and *Psd1* (**e**) plants. *Bars* 50 μm. Longitudinal sections of the elongated regions of internodes of wild-type (**f**) and *Psd1* (**g**) plants. *Bars* 50 μm. **h** Tall (*left*) and dwarf (*right*) plants from F_2 population (*Psd1* × Dular), 10-day after heading, grown in a long-day (LD) season. *Bar* 30 cm

was different from ZH11 (Fig. 1d, e); the average number of cells and the cell size of the internodes in *Psd1* were 38.6 and 78.2 % of those in ZH11, respectively (Table 2). Longitudinal sections also showed that the cell length of the internodes in *Psd1* was much shorter, only 21.2 % of that in ZH11 (Fig. 1f, g; Table 2). These observations indicate that cell division and elongation were impaired in the *Psd1* mutant.

We observed that the plant height of *Psd1* mutant was increased to about 58 cm when the plants were grown from August to October, the SD season with the day-lengths of 12.9–11.6 h (Table S1). This suggested that the *Psd1* phenotype might be affected by day-length. To test this hypothesis, we grew *Psd1* plants under natural LD (13.2–14.0 h) season (May to July) and artificial SD (ca. 10 h) conditions in the same season (May to July), respectively. Indeed, under SD *Psd1* plants grew sturdier and taller (55.7 cm) than those under LD (25.2 cm); by contrast, ZH11 plants grown under LD were slightly taller than those in SD (Fig. 1b; Table 1). These results indicated that the dwarf phenotype of *Psd1* is suppressed under SD. Therefore, *Psd1* is a novel photoperiod-sensitive dwarf mutant.

Table 1 Tiller numbers and plant height of ZH11 and *Psd1* plants in natural long-day (LD) and artificial short-day (SD) conditions

Population	SD/LD	No. tiller	Plant height (cm)
Psd1	SD	11.2 ± 2.1	55.7 ± 3.2
	LD	6.5 ± 1.3	25.2 ± 3.1
ZH11	SD	10.7 ± 2.5	76.4 ± 3.4
	LD	7.2 ± 1.9	85.3 ± 3.7

The plants were grown in pots in Guangzhou on 20th May, 2001 and the major tillers headed in late July, with the average natural day-length of 13.2–14.0 h (LD); SD-treatment (~10 h day-length) of the plants in the same season was performed by moving the plants to a dark box every day from 16:00 to ~20:00. Values are mean ± SD, $N = 15$

Table 2 Average length and numbers of cells in elongating regions of culm internodes of *Psd1* and ZH11

Lines	Cell length (μm)[a]	Cell diameter (μm)[a]	Cell no. of transverse section[b]
Psd1	11.0 ± 1.2	28.7 ± 6.6	2,883.6 ± 179.0
ZH11	51.9 ± 6.9	36.7 ± 8.0	7,468.4 ± 556.4

Values are mean ± SD

[a] $N = 30$ cells

[b] $N = 5$ transverse sections

Responses of *Psd1* mutant to GA and BR

Psd1 mutant responded to exogenous bioactive GA to a similar degree as wild-type rice, but exogenous GA could not rescue the *Psd1* dwarf phenotype (Fig. 2a). Also, the dwarf phenotype could not be rescued by exogenous BR (Fig. 2b). Moreover, the sheaths of *Psd1* seedlings could elongate to the similar length as ZH11 under complete darkness (Fig. 2c, d; Table 3), a phenotype different from that of BR-related mutants, in which sheaths could not elongate in the dark (Hong et al. 2003). These characterizations suggest that *Psd1* may not be deficient in the biosynthesis and perception of GA or BR.

Psd1 dwarfism is caused by a dominant allele

Since *Psd1* was generated from somatic mutation during callus regeneration, and the dwarf phenotype appeared in the R_0 generation, it is likely that the mutant is dominant. Indeed, crossing the original *Psd1* R_0 mutant with wild-type ZH11 pollen produced both dwarf and wild-type plants (Table 4). Further segregation analysis of R_1 progenies of *Psd1* showed that 93 of 128 plants were dwarf, which fitted the expected ratio of 3:1 (dwarf:tall, $\chi_{3:1} = 0.26$) (Table 4). These results indicated that *Psd1* is controlled

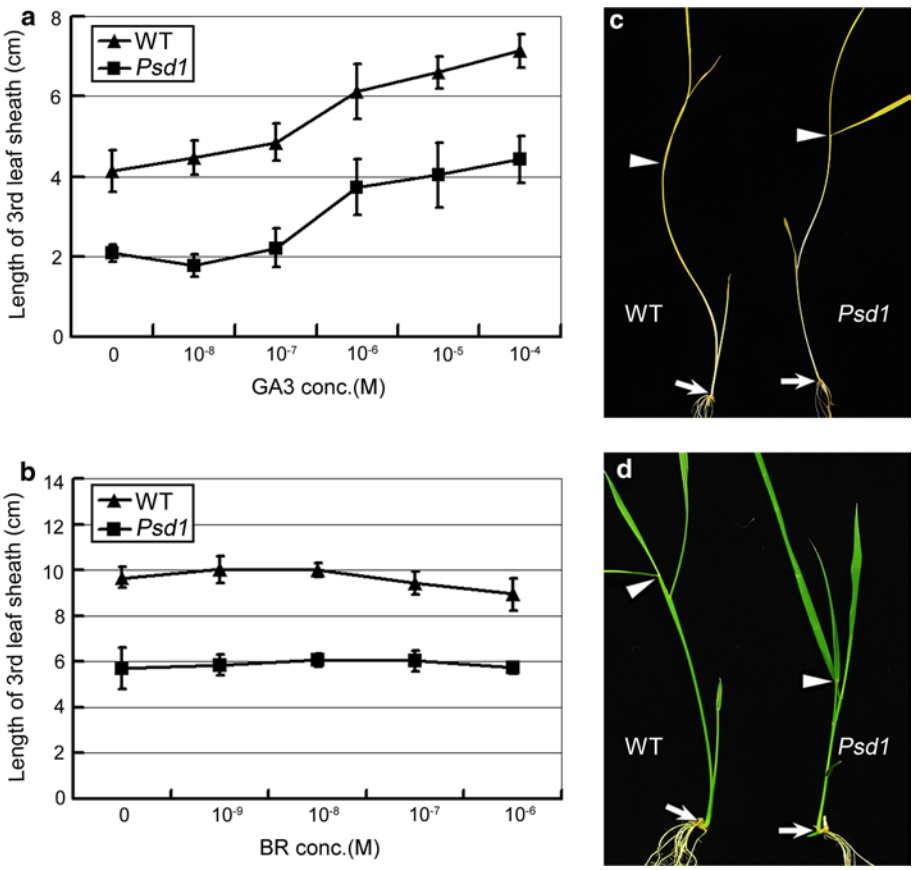

Fig. 2 Elongation of the third-leaf sheath in response to GA3, BR or dark treatments. Elongation of the third-leaf sheath in response to GA3 (**a**) or BR (**b**). The sheath length was measured at 4 days after application of GA3 or 2 weeks after application of BR. Wild-type ZH11 plants were used as controls. *Error bars* the SD from the mean ($N = 15$). Photomorphogenic phenotypes of the mutant (*right*) and the wild-type (*left*) grown in the dark (**c**) or in condition of 12 h light per day (**d**). Plants were germinated and grown on MS medium for 3 weeks under complete dark or fluorescent light (12 h) per day. *Arrows* indicate the position of nodes, and *arrowheads* indicate the leaf collars

Table 3 Length of the third-leaf sheath of *Psd1* and ZH11 plants grown in dark and under light

Line	Day length (h)	Length of the third-leaf sheath (cm)	Increasing ratio (0/12 h)
Psd1	0	12.6 ± 1.3	2.1
	12	5.9 ± 0.5	
ZH11	0	13.7 ± 0.9	1.4
	12	9.6 ± 0.4	

Values are mean ± SD, $N = 20$. Plants were germinated and grown on MS medium for 3 weeks under complete dark or fluorescent light 12 h per day

Table 4 Segregation of populations of R_1 and F_2 from crosses between *Psd1* and ZH11 or Dular

Cross (population)	Generation	Phenotype	No. plants	χ^2 (dwarf:tall)
Psd1 × ZH11	F_1	Dwarf	4	NT[a]
		Tall	1	
Psd1	R_1	Dwarf	93	0.26 (3:1; $P > 0.05$)
		Tall	35	
Psd1 × Dular	F_2	Dwarf	289	0.12 (3:1; $P > 0.05$)
		Tall	101	

[a] χ^2 analysis was not tested due to that only five F_1 plants were obtained

by a dominant locus in the genetic background of ZH11. In addition, segregation analysis with an F_2 population from crossing *Psd1* with an *indica* cultivar Dular (wild-type with tall plant height) (Fig. 1h; Table 4) also showed that the *Psd1* dwarfism was controlled by a single dominant locus. Here the recessive allele of the *Psd1* locus from wild-type (tall) ZH11 and Dular is designated as *psd1*+.

Molecular mapping of *Psd1*

Since the *Psd1* dwarf phenotype is a quality trait controlled by a mutant dominant allele, the F_2 plants from the *Psd1* × Dular cross could be clearly classified into the dwarf and tall groups. The tall plants were expected to contain the homozygous wild-type allele *psd1*+ from Dular. Therefore, we selected 22 tall F_2 plants for the linkage analysis. By screening 270 polymorphic markers distributed throughout the rice 12 chromosomes in these tall F_2 individuals, a marker 135250 located on chromosome 1 showed the homozygous *Dular* genotype in all of the 22 tall F_2 individuals, suggesting a linkage of this marker to the *Psd1* locus. Indeed, analysis of more F_2 plants with *Dular*/*Dular* genotype for the marker 135250 showed an average plant height of 129.2 cm, greatly taller than those (46.7 and 36.4 cm) with *ZH11*/*Dular* and *ZH11*/*ZH11*

Table 5 Plant height of F_2 plants with different genotype for the marker 135250 linked to the *Psd1* locus

Population	Genotype	Plant height (cm)[a]
F_2 plants (*Psd1* × Dular)	*Dular*/*Dular*	129.2 ± 13.1 (tall)
	ZH11/*Dular*	46.7 ± 4.3 (dwarf)
	ZH11/*ZH11*	36.4 ± 3.2 (dwarf)

[a] The plants were grown in the LD season. Plant height values are mean ± SD, $N = 50$

genotypes (Table 5), supporting the linkage between 135250 and *Psd1* locus. Four other polymorphic markers RM302, RM486, 135680 and 136730 (Table S2) linked to 135250 were further employed to construct a primary map for *Psd1* using 101 tall F_2 individuals. As a result, *Psd1* was bounded between RM486 and 135250, an about 300-kb interval (Fig. 3a).

For further fine-mapping of *Psd1*, four new SNP markers 35.087, 35.112, 35.124 and 35.191 and one InDel marker 135136 (Table S2) were developed in the mapped RM486-135250 region. These new markers were used to analyze 1017 tall plants obtained from a total of 4105 F_2 individuals. Finally, the location of the *Psd1* locus was narrowed down to an 11.5-kb region defined by two markers 35.112 and 35.124, which was located on a BAC clone sequence P0485B12 (Fig. 3b, c).

Sequencing analysis detected a mutated candidate gene

The sequence annotation database (http://ricegaas.dna.affrc.go.jp/rgadb/) indicates that two predicted genes (ORF1: LOC_Os01g60730; ORF2: LOC_Os01g60740) are present in the mapped 11.5-kb region (Fig. 3c). ORF1 and ORF2 encode, respectively, a putative RING-H2 finger protein and a putative non-specific lipid transfer protein (LTP), both supported by the presence of cDNA sequences in the database.

In order to find out the mutant *Psd1* gene, genomic DNA fragments of the two genes (including promoter regions) were amplified by PCR from ZH11 and homozygous *Psd1* plants, respectively, and sequenced. The results indicated that a mutation site, with a single-nucleotide deletion and a single-nucleotide substitution, was present in ORF2 of *Psd1* plants (Fig. 3d), which caused a frame-shift from the 101th codon, thus producing a varied and truncated C-terminal sequence of the protein (Fig. 3e). No sequence variation was detected in the ORF1 region. A new marker (ORF2-3′) was developed based on the mutation site in ORF2, and this marker showed completely co-segregation with the target locus (Fig. 3b). These results suggest that ORF2 is the candidate gene of the *Psd1* locus.

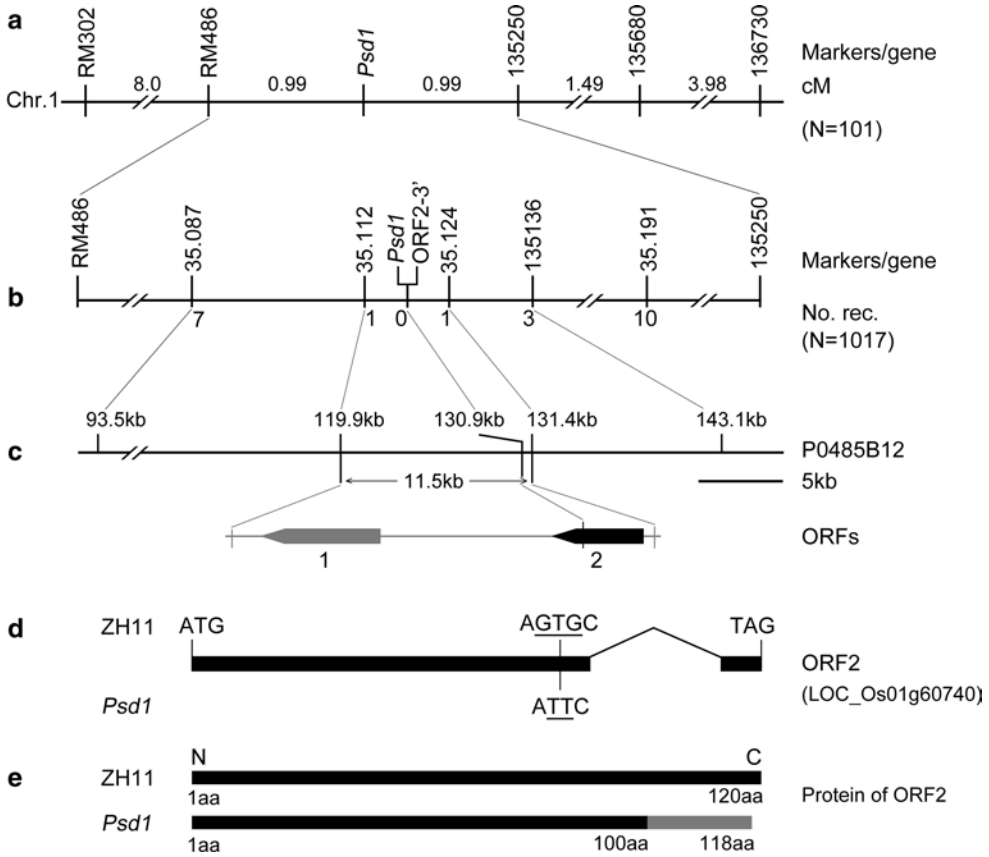

Fig. 3 Genetic and physical mapping of the *Psd1* locus and candidate gene analysis. **a** Linkage map on chromosome 1 constructed using 101 tall plants selected from 390 F_2 individuals. The *Psd1* locus was mapped to a region (~300 kb) between markers RM486 and 135250. The *long horizontal lines* show the physical map around the *Psd1* locus. *Numbers* show genetic distance (centiMorgan, cM) between adjacent markers. **b** The *Psd1* locus was restricted to a 11.5-kb region between markers 35.112 and 35.124 using 1017 tall plants selected from 4,105 F_2 individuals. Number of chromosomal recombinant event (No. Rec.) between the markers and the *Psd1* locus is indicated below the markers. **c** Annotated ORFs in the mapped region corresponding to a BAC clone P0485B12. **d** Sequence comparison of ORF2 between ZH11 and *Psd1*. The structure of ORF2 is based on the RiceGAAS system (http://ricegaas.dna.affrc.go.jp/rgadb/). **e** The LTP protein structures encoded by ORF2 between ZH11 and *Psd1*. The shaded C-terminal region in *Psd1* mutant shows the frame-shift and truncated sequence

Expression analysis of genes in the mapped region

Based on the Rice Expression Profile Microarray Database (http://ricexpro.dna.affrc.go.jp/), ORF1 is expressed mainly in leaf blade, with different levels in the daytime and at the night (Fig. S1a), while ORF2 is expressed in elongating stem, vegetative leaf sheath and young inflorescence (Fig. S1b). Because plants regenerated from tissue culture may produce stably inheritable epigenetic changes (genomic imprinting) that affect gene expression (Stroud et al. 2013), we examined the expression of the two ORFs in the wild-type and mutant plants under LD condition, but detected no obvious expression change (Fig. 4a, b), suggesting that genomic imprinting was unlikely involved in these genes. Therefore, ORF1 that does not vary in the DNA sequence is unlikely to be the candidate gene for *Psd1*.

Expression analysis of flowering-related genes in the *Psd1* mutant

To investigate whether the *Psd1* mutant affects the rice flowering pathways, we examined the expression of several heading-related genes, *Hd3a*, *RFT1, Ehd1, Ghd7,* and *DTH8* in the *Psd1* and ZH11 plants grown under LD condition. No obvious expression change of these genes was detected in the mutant and wild-type plants (Fig. 4c).

Discussion

Plant height regulated by photoperiod

Here a novel photoperiod-sensitive dominant dwarf mutant, *Psd1*, is identified. *Psd1* has a severe dwarf phenotype

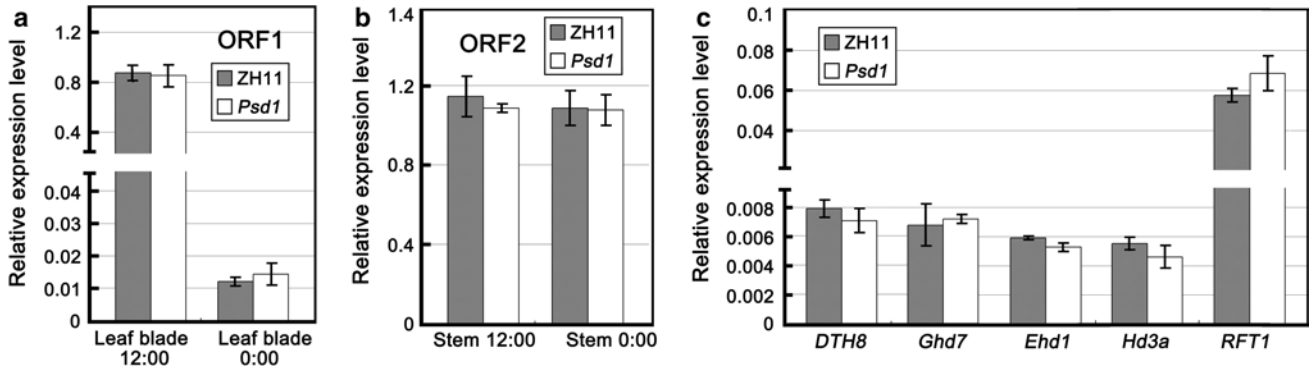

Fig. 4 Expression analysis of ORF1, ORF2 and flowering-related genes in the *Psd1* mutant by qRT-PCR. **a**, **b** Expression of ORF1 and ORF2 in leaf blades and elongating stems, respectively, of *Psd1* and ZH11 plants (45 days after planted, under LD condition). The samples were harvested at 12:00 and 0:00. Relative expression levels represent the ratios of the examined genes to *Actin1*. **c** Expression of five flowering-related genes in leaf blades of the *Psd1* and ZH11 plants (45 days after planted) harvested at 12:00

under LD conditions with reduced cell number, cell size, and elongation of culm internodes, but *Psd1* plant height is largely restored under SD conditions (Fig. 1). Although fewer tillers of *Psd1* headed under LD (Fig. 1a, b), most tillers were able to develop into stunted inflorescences, but failed to head due to severely impaired elongation of the internodes. Some heading-date genes such as *Ghd7* and *DTH8* have pleiotropic effect to affect the plant height in rice (Xue et al. 2008; Wei et al. 2010), however, the severe dwarf phenotype of *Psd1* is completely different to the plant height variation slightly affected by the *Ghd7* and *DTH8* pathways. In addition, the expression levels of the florigen genes *Hd3a* and *RFT1* and its upstream regulators *Ehd1, Ghd7* and *DTH8* were similar between the *Psd1* and ZH11 plants (Fig. 4c). Therefore, the *Psd1* mutation involves the pathway(s) for the inflorescence development and stem elongation, but not those for the vegetative-to-inflorescence phase transition.

PIF4 and phytochromes (mainly phyB) play crucial roles in regulation of photoperiodic elongation of hypocotyls in *Arabidopsis thaliana* (Nomoto et al. 2012). However, photoperiod-mediated regulation of plant height remains unknown, and no photoperiod-dependent dwarf mutant has been reported. Plant height of wild-type ZH11 is less affected by photoperiod (Fig. 1b), whereas the *Psd1* dwarf phenotype is dependent on LD conditions, suggesting that the *Psd1*-related genetic network is regulated by day-length dependent signals.

The *Psd1*-related genetic network may not involve the GA and BR signaling pathways

It is interesting about the relationship between the *Psd1*-related genetic network and those involving biosynthesis and signal transduction of GA and BR, which determine plant height (Wang and Li 2008). The *Psd1* mutant exhibits no similarity of plant morphologies to BR-related mutants (Fig. 1); BR-related mutants show abnormal morphologies, including malformed leaves with twisted, stiff blades (Hong et al. 2004). Also, the seedling leaf sheaths of *Psd1* could elongate in dark conditions (Fig. 2c, d; Table 3), a phenotype different from BR-related dwarf mutants, which could not elongate in darkness (Hong et al. 2003). Moreover, the *Psd1* dwarf phenotype could not be rescued by exogenous BR (Fig. 2b). Based on these characterizations, we consider that *Psd1* is not a mutant involved in BR-related processes.

Although *Psd1* seedlings showed slightly increased plant height in response to exogenous GA3, similar to the GA-response of wild-type seedlings, the dwarf phenotype could not be rescued by this phytohormone (Fig. 2a). In fact, ZH11 and Dular contain wild-type *SD1* gene (data not shown), which encodes a GA 20-oxidase (GA20ox) involved in GA biosynthesis (Monna et al. 2002; Sasaki et al. 2002; Spielmeyer et al. 2002). However, the presence of *SD1* did not affect the plant height segregation of the populations from the crosses between *Psd1* and ZH11 or Dular (Fig. 1h). Therefore, we propose that the *Psd1* mutant may not be deficient in the biosynthesis and signal transduction of GA or BR, but rather represents a novel genetic network independent of the GA and BR signaling pathways for plant height control.

The LTP gene may involve in plant growth control

We have mapped the *Psd1* locus to an 11.5-kb region containing two ORFs (Fig. 3). Based on the sequencing and expression analyses, we expect that ORF2 is the candidate gene for *Psd1,* since this gene has a mutation that causes a frame-shift and produces amino acid variation in the C-terminal of the encoded putative LTP (Fig. 3d, e). LTPs are small soluble proteins that facilitate the transfer

of fatty acids, phospholipids, glycolipids, and steroids in plants (Wang et al. 2012). LTPs isolated from tobacco (*Nicotiana tabacum* L.) are able to enhance the extension of different wall specimens (Nieuwland et al. 2005). In addition, it is found that a small secreted peptide LTP5 of *Arabidopsis thaliana* plays a role in growth of pollen tube tip, and pollen tube tip growth is disturbed in *ltp5-1* mutant plants (Chae et al. 2009). These researches suggest that LTPs may participate in regulation of plant cell division and elongation. The putative LTP gene identified in this study may involve in rice growth control. However, the mechanism by which *Psd1* and photoperiod interact to control the growth of *Psd1* plants needs further studies to reveal.

In summary, *Psd1* is a novel photoperiod-sensitive dwarf mutant in plants. Although this mutant may not be a suitable material for rice breeding, our findings here provide a genetic foundation for understanding new molecular mechanisms of plant cell division and elongation mediated by environmental factor(s).

Acknowledgments This work was supported by grants from the National Natural Science Foundation of China (31271301) and the Ministry of Science and Technology of China (2012AA10A303).

Conflict of interest The authors declare no conflict of interest.

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