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Dwarf and short grain 1, encoding a putative U-box protein regulates cell division and elongation in rice

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ABSTRACT

Plant hormones coordinate a plant's responses to environmental stimuli and the endogenous developmental programs for cell division and elongation. Brassinosteroids are among the most important of these hormones in plant development. Recently, the ubiquitin-26S-proteasome system was identified to play a key role in hormone biology. In this study, we analyzed the function of a rice (*Oryza sativa*) gene, *DSG1*, which encodes a U-box E3 ubiquitin ligase. In the *dsg1* mutant (an allelic mutant of *tud1*), the lengths of the roots, internodes, panicles, and seeds were shorter than that in the wild-type, which was due to defects in cell division and elongation. In addition, the leaves of the *dsg1* mutant were wider and curled. The DSG1 protein is nuclear- and cytoplasm-localized and does not show tissue specificity in terms of its expression, which occurs in roots, culms, leaves, sheaths, and spikelets. The *dsg1* mutant is less sensitive to brassinosteroid treatment than the wild-type, and *DSG1* expression is negatively regulated by brassinosteroids, ethylene, auxin, and salicylic acid. These results demonstrate that *DSG1* positively regulates cell division and elongation and may be involved in multiple hormone pathways.

1. Introduction

The development of plant organs is controlled by a complex system that involves external and internal factors. In this system, plant hormones play a key role. Brassinosteroids (BRs) are among the most important plant hormones. Since the discovery of BRs, these polyhydroxylated steroid hormones have been shown to have important roles in regulating a myriad of physiological and developmental processes, including seed germination, skotomorphogenesis, flowering, and senescence (Clouse and Sasse, 1998). In the model plants *Arabidopsis* and rice, numerous genes involved in BR biosynthesis and gene regulation have been identified. Coupled with more recent biochemical approaches, these studies have provided fascinating insights into various aspects of plant steroid signaling, ranging from BR perception at the cell surface to the activation of transcription factors in the nucleus (Clouse and Sasse, 1998; Wang and He, 2004; Vert et al., 2005; Haubrick and Assmann, 2006; Kim and Wang, 2010; Gudesblat and Russinova, 2011; Zhu et al., 2013; Hu and Yu, 2014). According to the current understanding, BRs directly bind to the extracellular domain of the receptor-like kinase BRASSINOSTEROID INSENSITIVE1 (BRI1) (Li and Chory, 1997; Hothorn et al., 2011; She et al., 2011). When BR levels are high, BRs bind to and stimulate BRI1; the resulting downstream signal transduction leads to the inhibition of the GSK3-like kinase BRASSINOSTEROID INSENSITIVE2 (BIN2) and its homologs BIN2-LIKE1 (BIL1) and BIL2, which are pivotal suppressors of BR signaling (Li et al., 2001; Li et al., 2002; Li and Nam, 2002; Nam and Li, 2002; Tang et al., 2008; Kim et al., 2009; Yan et al., 2009; Kim and Wang, 2010; Guo et al., 2013; Hu and Yu, 2014). The inhibition of BIN2 leads to the dephosphorylation and nuclear accumulation of the BRASSINAZOLE RESISTANT1 (BZR1) family transcription factors, which regulate BR-responsive gene expression (He et al., 2002; Wang et al., 2002; Yin et al., 2002; Ryu et al., 2007; Li et al., 2001; Clouse, 2011; Hu and Yu, 2014). BR-related mutants display pleiotropic dwarf phenotypes caused by defects in cell elongation and differentiation, processes which determine organ length. The dwarf phenotypes include short leaf petioles and hypocotyls, dark-green and epinastic leaves, reduced apical dominance, and delayed flowering (Clouse et al., 1996; Li et al., 1996; Szekeres et al., 1996;

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Li and Chory, 1999; Krishna, 2003; Vleesschauwer et al., 2012; Hu and Yu, 2014).

Because plants are sessile organisms, plant hormones, such as BRs, abscisic acid (ABA), auxin (IAA), ethylene (ETH), gibberellin (GA), salicylic acid (SA) and jasmonic acid (JA), also play a key role in the selective removal of short-lived regulatory proteins, which allows plants to adapt rapidly to their environment and to redirect growth and development (Seo et al., 2014).

The ubiquitin-26S-proteasome system (UPS) is essential for this adaptation (Biedermann and Hellmann, 2011). Specific protein degradation is a key regulatory component of many fundamental cellular processes. This intracellular proteolysis is mediated predominantly by the ubiquitin-26S-proteasome system (Azevedo et al., 2001; Andersen et al., 2004). As in other eukaryotes, ubiquitin-dependent protein degradation in plants requires the sequential action of three enzymes: E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin ligase. The substrate specificity is conferred by the E3 ligase (Vierstra, 2009; Jung et al., 2015), which selects appropriate candidate proteins (Ciechanover, 1998). The E3 s are the most diverse components in the ubiquitination pathway, which, including proteins with RING, HECT, F-box, and U-box domains, annotated in *Arabidopsis thaliana* (Vierstra, 2009; Jung et al., 2015; Smalle and Vierstra, 2004; Zeng et al., 2004). The U-box is a relatively recently identified E3 ligase activity-related protein domain that was first shown in yeast to be involved in polyubiquitin chain assembly (Koegl et al., 1999). The U-box contains 70 amino acids and possesses a tertiary structure resembling that of the RING domain (Aravind and Koonin, 2000; Ohi et al., 2003; Zeng et al., 2004).

There are 77 PUBs (plant U-boxes) in rice, 55 of which have full-length cDNAs (Zeng et al., 2004; Yee and Goring, 2009). Their functions remain largely unknown. In this study, we provide detailed information on the defects resulting from a mutation (T-to-A transition) of *DWARF AND SHORT GRAIN 1*, an identical gene of *TUD1* (Hu et al., 2013; Ren et al., 2014); this evidence strongly suggests that *DSG1* is a multifunctional factor in rice. We also found that *DSG1* is probably involved not only in the BR pathway but also in some other hormone pathways to control cell elongation and differentiation. In addition, *DSG1* affects the development of vascular bundles in *dsg1* mutant plants and affects the expression of some leaf-development-related genes.

2. Materials and methods

2.1. Mutant materials and growth conditions

The stably inherited mutation *dsg1* was derived from the progeny of an indica restorer line, Jinhui10, treated with ethyl methyl sulfonate (EMS). Xinong1A was crossed with the *dsg1* mutant and the F_2 progeny were used for genetic analysis and mapping of the *DSG1* gene. Xinong1A is a cytoplasmic male sterile line bred by the Rice Research Institute of Southwest University, Chongqing, China. For expression analysis, various organs were collected, starting from the seedling and heading stage. For *DSG1* induction analysis, wild-type plants were hydroponically cultured with normal half-strength modified Hoagland medium after germination in a greenhouse for 10 days at 30 °C with a 14-h light (24 °C)/10-h dark cycle. Ten-day-old Jinhui10 plants were completely immersed in nutrition solutions containing different phytohormones, including brassinosteroid (50 μm), gibberellin (50 μm), auxin (50 μm), ethrel (100 mg/l), salicylic acid (100 μm), and abscisic acid (50 μm). Twenty plants were collected for RNA isolation. All experiments were repeated three times independently.

2.2. Characterization of mutant phenotypes

The phenotypes of the *dsg1* mutant and the wild-type Jinhui10 cultivar were compared throughout the growth period. Most of the agronomic traits were examined, including root length and seedling height, plant height, panicle and internode lengths, seed length and width, and seed setting rate.

An Olympus C-770 digital camera (Tokyo, Japan) was used to take photographs of plants at the seedling and mature stages and to compare the grain between the wild-type and the *dsg1* mutant. For SEM observations, the leaves and spikelets, outer and inner surfaces of sheaths, and outer and inner surfaces of lemma were observed with a Hitachi High-Tech scanning electron microscope, SU3500, under strong vacuum.

2.3. Histological observation

Fresh leaves from Jinhui 10 plants and mutants were obtained at the booting stage, fixed (FAA stationary liquid: 90 ml of 70% ethanol, 5 ml of 40% formaldehyde, 5 ml of glacial acetic acid), dehydrated, made transparent, and embedded in paraffin. Cross sections of 10 μm were cut, subjected to red and solid green staining, and photographed with a Zeiss fluorescence microscope.

2.4. Map-based cloning of the DSG1 gene

The *DSG1* gene was initially mapped to an interval between the SSR markers, RM3766 and SSR3-15, on chromosome 3. For fine mapping, 1394 dwarf individuals from the F_2 generation were analyzed with a set of linked primers (see Supplementary Table 2 online for sequences). *DSG1* was localized between two of these markers, SSR3-28 and RM14645, within a 190-kb region (Fig. 4A). The PCR products were separated on 10% polyacrylamide gels, and bands were visualized by a silver-staining method (Zhang et al., 2008).

2.5. Complementation of the dsg1 mutant

For functional complementation of the rice *dsg1* mutant, a genomic fragment of 5.1 kb containing the entire *DSG1* coding region, a 2650-bp upstream sequence, and a 1133-bp downstream sequence, was amplified from Jinghui10 and sub-cloned into the binary vector pCAMBIA1301 with a kanamycin resistance marker to generate the pCA1301-*DSG1* construct. Calli induced from homogeneous young *dsg1* panicles were used for transformation with *Agrobacterium tumefaciens* LBA4404 carrying the pCA1301-*DSG1* plasmid. Successfully transformed plants were identified by GUS staining.

2.6. Measure of E3 ubiquitin ligase enzyme activity

The procedures were carried out according to the instructions of the Plant E3/UBPL ELISA kit (Shanghai FanKe Biological Technology Co. Ltd.). 0.1 g grated fresh roots and leaves of the wild-type and *dsg1* mutant were collected at seeding stage and heading stage, and fixed with 400 ml PBS (PH7.2-7.4). Then froze the sample rapidly with liquid nitrogen, homogenized by hand, centrifuged 20 min at the speed of 2000–3000 rpm and removed the supernatant. Samples were detected under a microplate reader SYNERGY-H1 (BioTek).

2.7. Phylogenetic analysis

Homologous protein sequences from different organisms were acquired by blasting the DSG1 sequence against the phytozome using an expected threshold of 10^{-5} (http://phytozome.jgi.doe.gov/