Characterization and gene cloning of the rice (*Oryza sativa* L.) dwarf and narrow-leaf mutant *dnl3*

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ABSTRACT. The dwarf and narrow-leaf rice (*Oryza sativa* L.) mutant *dnl3* was isolated from the *Japonica* cultivar Zhonghua 11 (wild-type). *dnl3* exhibited pleiotropic developmental defects. The narrow-leaf phenotype resulted from a marked reduction in the number of vascular bundles, while the dwarf stature was caused by the formation of foreshortened internodes and a reduced number of parenchyma cells. The suggestion that cell division is impaired in the mutant was consistent with the transcriptional behavior of various genes associated with cell division. The mutant was less responsive to exogenously supplied gibberellic acid than the wild-type, and profiling the transcription of genes involved in gibberellin synthesis and response revealed that a

lesion in the mutant affected gibberellin signal transduction. The *dnl3* phenotype was inherited as a single-dominant gene, mapping within a 19.1-kb region of chromosome 12, which was found to harbor three open reading frames. Resequencing the open reading frames revealed that the mutant carried an allele at one of the three genes that differed from the wild-type sequence by 2-bp deletions; this gene encoded a cellulose synthase-like D4 (CSLD4) protein. Therefore, *OsCSLD4* is a candidate gene for *DNL3*. *DNL3* was expressed in all of the rice organs tested at the heading stage, particularly in the leaves, roots, and culms. These results suggest that *DNL3* plays important roles in rice leaf morphogenesis and vegetative development.

Key words: Cellulose synthase-like D4; Map-based cloning; Rice; Gibberellin signaling; Dwarf and narrow-leaf mutant

INTRODUCTION

The yield potential of rice (*Oryza sativa* L.) is strongly influenced by the plant's morphology, as demonstrated to such great effect by the positive impact on productivity of the Green Revolution semi-dwarf varieties. The genetic basis of plant morphology in rice has been intensively studied (Arite et al., 2007). The mechanistic basis of leaf shape and structure is important in this context, because the leaf is the site of energy capture through photosynthesis. Many leaf development mutants have been discovered that produce a variety of unusual shapes and structures, including narrow leaves, rolling leaves, needle-like leaves, and thread-like or radical leaf lamina (Yan et al., 2008).

The control of plant height in rice is closely tied to the synthesis and responses of a range of phytohormones (Itoh et al., 2002; Gomi et al., 2004). The majority of mutants deficient for the synthesis of, or insensitive to, gibberellic acid (GA3) or brassinosteroid is dwarfed, and exhibit other agronomic defects. The dwarf mutants *sd1*, *d18*, and *d35* are all associated with defective gibberellin synthesis; in *sd1*, this arises from a compromised gene that encodes *GA2ox20* (Sasaki et al., 2002), while *d18* and *d35* carry mutations to the genes that encode *GA3ox2* and *KO2*, respectively (Itoh et al., 2001, 2004). All three of these mutants produce low levels of active gibberellin, and the phenotype can be rescued by supplying GA3. In contrast, the dwarf mutants *d1*, *slr1*, *gid1*, and *gid2* are all insensitive to exogenously supplied GA3, and their endogenous gibberellin content is higher than in wild-type (WT) plants; these mutants are gibberellin non-responsive (Sasaki et al., 2003). Plant leaves and all other lateral organs are derived from founder cells of the peripheral zone in the shoot apical meristem. The founder cells differentiate into leaf primordia, which result in a flat-leaf formation after differentiation along the adaxial-abaxial, apical-basal, and radial axes (Scanlon et al., 2000; Bowman et al., 2002); the establishment of these axes is essential for leaf morphogenesis.

Cellulose provides mechanical strength to the plant cell wall (McNeil et al., 1984). The most important genes underlying cellulose synthesis are those that encode cellulose synthase active subunits (*CESA*) and *CESA-like* (*CSL*) proteins, which lack the N terminal zinc finger domains characteristic of *CESA*s (Cutler and Somerville, 1997), and which harbor sequence motifs characteristic of β-glycosyltransferases. CSLs tend to accumulate in the Golgi apparatus, which is the site of hemicellulose synthesis. The loss-of-function of various

*CSLD*s results in reduced lignin content, and consequently plants develop a brittle culm (Yan et al., 2007; Yoon et al., 2014). The rice gene *OsCSLD4* has been shown to encode a cellulose synthase-like enzyme that is important for cell wall synthesis and plant growth (Li et al., 2009), as well as being required for cell division and proliferation during the M phase of the cell cycle (Yoshikawa et al., 2013). However, *OsCSLD4* has not been investigated in phytohormone-mediated plant developmental processes.

In the present study, we investigated the rice dwarf narrow-leaf mutant *dnl3* and demonstrate that it is non-responsive to GA3. Positional cloning revealed that the gene compromised in the mutant is *OsCSLD4*. Therefore, the results of this study clarify the molecular mechanisms of *OsCSLD4* in rice growth and development.

MATERIAL AND METHODS

Field- and laboratory-based phenotyping

dnl3 is a rice ethyl methanesulfonate-induced dwarf and narrow-leaf mutant obtained from the *Japonica*-type cultivar Zhonghua 11. The F_2 population derived from a *dnl3* x Nanjing 11 (an Indica-type cultivar) cross was developed for gene mapping. Ten plants of each of the *Japonica*-type cultivar Zhonghua 11 and its derived dwarf and narrow-leaf mutant *dnl3* were grown in the field at the China National Rice Research Institute, Hangzhou, China, and their agronomic traits were evaluated. The central sections of five samples from both the second culm internode and the flag leaf of *dnl3* and WT plants were fixed in two parts formaldehyde to one part glacial acetic acid to 17 parts 50% (v/v) ethanol for 24 h at 4°C, rinsed in water, dehydrated by passing through an ethanol series (5 min per step), steeped in 1-butanol, and finally embedded in Paraplast Plus® (Structure Probe Inc., West Chester, PA, USA). After preparing sections with a rotary microtome, each 8-µm section was stained in Safranin containing Fast Green.

Phytohormone-induced shoot elongation

The phytohormones GA3 and brassinolide (BL) stimulate culm elongation in rice. To investigate the hormonally mediated development of the gene compromised in the *dnl3* mutant, mutant and WT seedlings were exposed to exogenous GA3 and BL. Surface-sterilized grains were plated on 1.5% agar containing a range of either GA3 (0-0.1 mM) or BL (0-1 µM) concentrations, and the germinated seedlings were grown under continuous fluorescent lighting at 30°C for either 7 (GA3 test) or 14 days (BL test). At the end of the treatment, the lengths of the second leaf sheath of 20 plants per treatment were measured.

Evaluation of endogenous phytohormone content

At the heading stage, the abscisic acid, indole acetic acid, GA3, salicylic acid, and jasmonic acid contents of the *dnl3* and WT leaves were quantified. Three plants per genotype were sampled, and each assay was performed in triplicate. High-performance liquid chromatography-mass spectrometry was used for extraction and subsequent quantification (Almeida Trapp et al., 2014).

Fine mapping of the gene compromised in the *dnl3* mutant

Two F_2 populations were analyzed in order to establish the mode of inheritance of the *dnl3* mutant phenotype: one was bred from the cross Zhonghua 11 x *dnl3* (201 individuals) and the other from Nanjing11 x *dnl3* (3383 individuals). The set of dwarf, narrow-leaf *dnl3* x Nanjing 11 F_2 segregants was genotyped using a panel of 300 microsatellite (simple sequence repeat) markers distributed over the full set of 12 rice chromosomes (http://www.gramene.org/), in order to obtain a map location for the gene compromised in the *dnl3* mutant. The microsatellite and indel markers used for the mapping are listed in Table 1. The parental and F_2 segregants' DNA was extracted from 30-day-old seedlings using a cetyltrimethylammonium bromide-based method (Wang et al., 2011). Each 10 μL polymerase chain reaction (PCR) contained 25 ng template DNA, 1.0 μL 10X PCR buffer, 0.1 mM dNTP, 0.1 μM of each primer, and 0.1 U *Taq* DNA polymerase. The amplification protocol comprised an initial denaturation step (95°C/3 min) followed by 35 cycles of 95°C/30 s, 55°C/30 s, 72°C/40 s, and was completed by an extension step (72°C/8 min). The amplicons were electrophoretically separated through a 6% non-denaturing polyacrylamide gel and visualized by silver staining (Xu et al., 2011).

Table 1. Primers used for fine mapping *DNL3*.

Primers designations	Forward primers (5'-3')	Reverse primers (5'-3')
RM28438	GTTCGTGAGCCACAACAA	GTTAAATGCTCCACCAAA
Indel1	GCATGGTATAACAAGGCA	GGGTGTCCAACAGCTAAT
Indel2	CTTAACCCTGGTAGGTCC	CCAATCAATTTTATACTGCA
Indel3	ACATCAAGAAGTTATTTG	AAACACCTTTTAAGTACA
Indel4	TGTTATTACCACATCGTTT	GCATTACTGCCATCCAAG
RM28448	CATCTTCAGCTTTACTCCG	GAGATGATCCGCTTCCTTC

Quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted using an RNAprep Pure Plant Kit (Beijing Dingguo Biotechnology Co. Ltd., China) following the manufacturer protocol. The first-strand cDNA was synthesized from DNase I-treated RNA with an oligo $(dT)_{18}$ primer in a 20-μL reaction using a SuperScript® III Reverse Transcriptase Kit (Toyobo Co. Ltd., Japan) following the manufacturer protocol. Each 20-μL qRT-PCR was conducted using TaKaRa SYBR® Premix *Ex Taq* II, and the reactions were run using a LightCycler® 480 device (Roche). The primer sequences are presented in Table 2; the rice ubiquitin gene *Os03g0234200* was chosen as the reference. The $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) was used to calculate relative transcript abundances.

RESULTS

Phenotypic effects of the *dnl3* mutation

The *dnl3* mutant seedling was somewhat shorter than the WT, a difference that became more apparent as the plants developed (Figure 1A and B). At maturity, the height of the mutant was about 60%, and the width of its flag leaf about 50%, of those of the WT (Figure 1C, D, G, and I). The mutant's panicle was markedly reduced in size, and consisted of fewer primary branches (Figure 1E and K), while grain size (in terms of length, width, thickness, and weight)

was substantially reduced and seed set was lowered (Figure 1F, J, and L-O). However, the plant tillered more profusely than the WT (Figure 1B, C, and H). The overall yield of grain per plant was significantly lower than that achieved by the WT (Figure 1P).

Table 2. Primers used for the quantitative reverse transcription-polymerase chain reaction analysis of the transcription of related genes.

Genes names	Forward primers (5'-3')	Reverse primers (5'-3')
Cdc2	CCGCCTCGCTATCAACG	TCGACCTCGCCATCACC
Cdc3	ACCTCCTCTTCTCCGACTGC	TCCGTGAACCCGCTTGG
CycB1	ATCTCACCGTTCCTACAGCATA	CGTACTGCATCAGTGCCAATT
MCM3	GCCAAGTCCCAGCTCCTAA	GCAGCGGTCAAACCAACAC
RAN2	AGCCAAGCAGGTCACATTCC	AGGTTCGGGTCACCAGCAAG
SPL16	ACTACACGCACCACCAAATC	CCTGGAGGAAAGGGAAGC
RPA2A	GCAGCAATCTGGCATCACC	CAATCCCACAAGCCTAACG
H4	GCATCTCGGGGCTCATCTAC	CATGGCGGTGACGGTCTT
EXPA	AAGAGCCAGCCGATGCG	CGGAAGGAGGCGACGAA
D1	AATGCGGTAATCTTTTGTGCTG	AGAGTTCCTTGGTCTCCATCATTC
SLR1	GGTGCGGCCAAGGATCGTCA	GAGGAGCGTGCTCGCCTGTTT
GLD1	AGGACAGGGACTGGTACTGGAAG	TCGTGGTAGTGGACGGTGTTGG
GLD2	GTGCGGGAGTGGGCGAACCT	GGCGGATGTAGACAGCGTGGAG
EUI1	CGGGCTTGCTTTGGGAGTGAT	GCGAAGGGATGCTGAAGATGACG
GA20ox2	CGCTGTCGAACGGGAGGTATAAG	CATGAGGTCGGCCCAGGTGAA
GA20ox3	CGAGTTCACGCAGAGGCACTACC	TTGCTTGATCCAGGCGACGAAGG
GA3ox2	CCGACGAGTTGCTGAGGTTGT	GCACGAAGGTGAAGAAGCCCGAG
GA2ox1	CGAGGGCAAGGAGATGTGGG	GTGGGCTGCTGGCTGTGATT
GA2ox3	GCGGGTGATGGAGGCGATGT	TTGAGCGGAGCACGGAGACG
OsCesA1	GGCATTCAGGGTCCAGT	CCAAATCAGCCTCAGTTAGT
OsCesA2	ATCTATTTGCGGTCTTCCA	AAATCGCCTTCTTCATCAG
OsCesA3	GGGCAGATGGTTGATGAC	TTGAACAGGTAGATTGGGAT
OsCesA4	GGTCGTGGAGGAGGAAGT	CCTGGTGATCGCTGAGTT
OsCesA5	TTCCAACGCAATCAATAAC	TTCCAACCAAACCCTTCA
OsCesA6	GGTATGGGTATGGTGGTGG	GCAGGCAAGGTACAGTAAGC
OsCesA7	CCACGAGCCCGTCAACT	CAGATCCTTCCCAGCCAC
OsCesA8	CCTTGAGATGAGGTGGAGT	CTTGAGGAGACCCTGGAA
OsCesA9	TCGGACGGAAGGTTTG	GTTGCGGTTGGCGTAT
OsCSLD1	CTCTGGTCGGTGGTCTTCG	TCGGTTGCTCCGTCGTG
OsCSLD2	TGCTGTGCCACCTCTACCCG	CCCGACCAGACGAAGACGATG
OsCSLD3	TTCTTCAACTTCTGGGTCCTCTT	ACCACCACCGGCGTCTT
OsCSLD4	TGCTGTGCCACCTCTACCCG	CCCGACCAGACGAAGACGATG
OsCSLD5	TGCTGCTCATCACCATCACCC	AACTGCTCGTTCCGCCACC

Inspection of the leaf venation showed that fewer veins had formed in the mutant than in the WT (Figure 2A-F), and a histological analysis revealed that the vascular patterning was unaltered, although the number of vascular bundles was reduced (Figure 2E and F).

The *dnl3* culm was shorter and thinner than that formed in the WT plant (Figure 3A, B, and D-G). Cell length in the internode was similar in the mutant and the WT, while cell number was drastically reduced in the former (Figure 3C and H-I).

The *dnl3* mutant was relatively unresponsive to exogenously applied GA3. GA3 treatment induced less elongation in the mutant's second leaf sheath than was induced in the WT (Figure 4A), but there was no difference between the mutant's and the WT's responses to BL treatment (Figure 4B). Considerably more GA3 had accumulated in *dnl3* than in the WT (Figure 4C). The qRT-PCR analysis indicated that the transcription of *GID1*, *EUI1*, and *GA20ox2* (GA3-related genes) was similar in the WT and the mutant, whereas that of *D1*, *SLR1*, and *GID2* (GA3-response genes) and *GA20ox3*, *GA3ox2*, *GA2oX1*, and *GA2ox3* (GA3

synthesis genes) differed (Figure 4D). This suggests that the product of the gene mutated in *dnl3* probably interacted with a gibberellin metabolic pathway.

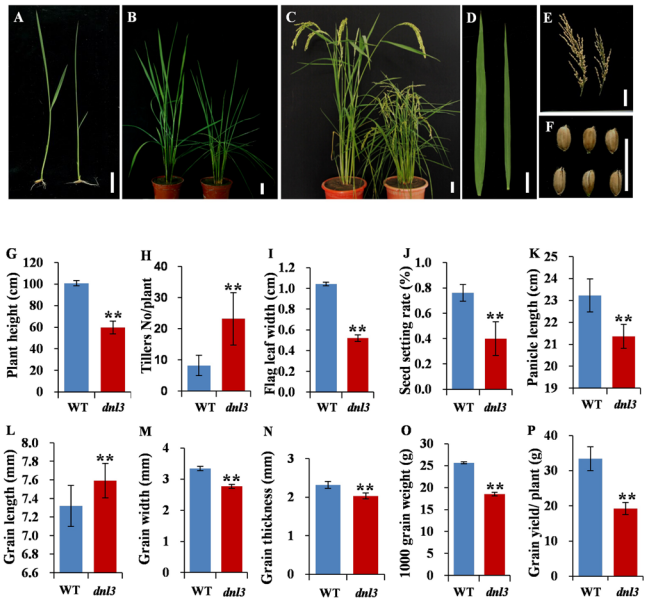

Figure 1. Phenotypic effect of the *dnl3* mutation: **A.** 30-day-old plants, **B.** plants at the tillering stage, **C.** plants at maturity, **D.** the flag leaf, **E.** the panicle, and **F.** the grain. In (A-E), the wild-type (WT) plant is shown on the left, and in (F) the WT grain is shown above the mutant grain. Bars in (A-E), 5 cm, and in (F), 2 cm. Comparative performance of the WT and mutant: **G.** plant height, **H.** tiller number, **I.** flag leaf width, **J.** seed set, **K.** panicle length, **L.** grain length, **M.** grain weight, **N.** grain thickness, **O.** 1000-grain weight, and **P.** grain yield per plant.

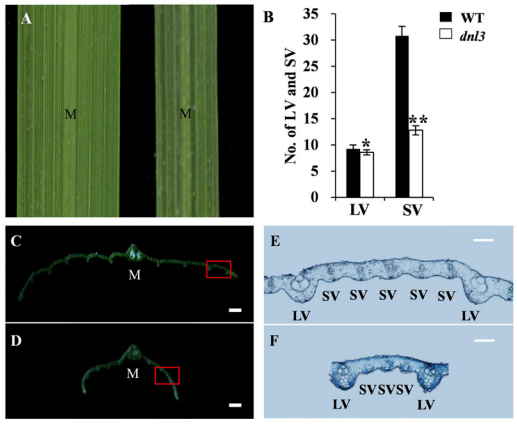

Figure 2. Effect of the *dnl3* mutation on leaf structure. **A.** Leaf surface of a wild-type (WT) plant (left) and the *dnl3* mutant (right). **B.** Number of large (LV) and small (SV) vascular bundles (means ± SE; N = 10). *,**Means differed significantly at $P \leq 0.05$ and $P \leq 0.01$, respectively. **C.** and **D.** Cross-section of a WT (C) and a *dnl3* mutant (D) leaf. Bar, 50 µm. **E.** and **F.** Magnified images of the boxed areas shown in (C) and (D). Bar, 100 µm. M = middle of the left in (A), (C), and (D).

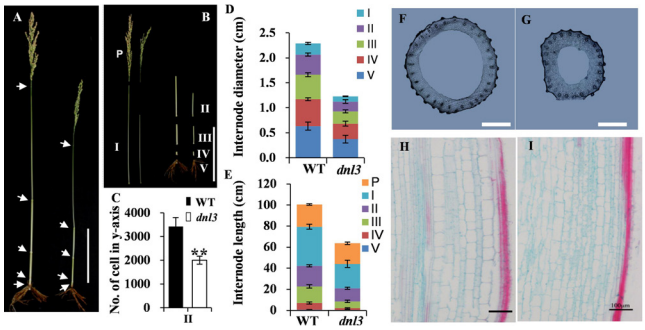

Figure 3. Effect of the *dnl3* mutation on culm structure. **A.** Leading culms of a wild-type (WT) plant (left) and the *dnl3* mutant (right). Arrows indicate the position of nodes. Bar, 20 cm. **B.** and **E.** Panicles and internodes on the leading culm of the WT plant (left) and the *dnl3* mutant (right). Internodes are labeled I-V, counting from the top of the culm. Bar, 20 cm. **C.** Cell numbers in internode II. **D.** Internode diameter. **F.** and **G.** Cross-section of internode II of the WT plant (left) and the *dnl3* mutant (right) in mature plants. Longitudinal sections: **H.** WT plant and **I.** *dnl3* mutant internode II. Bar, 100 µm.

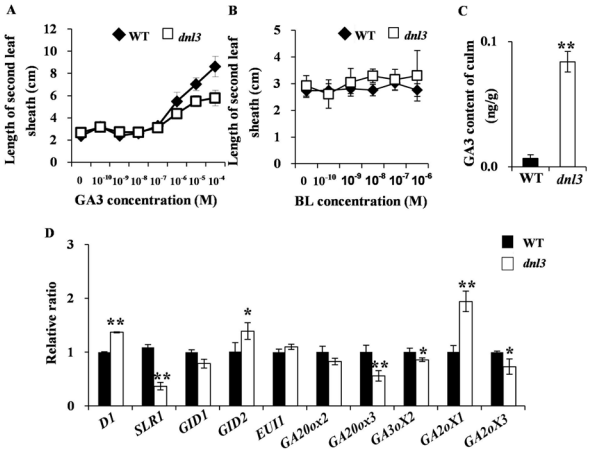

Figure 4. Elongation of the second leaf sheath in response to treatment with **A.** gibberellic acid (GA3) and **B.** brassinolide (BL) in wild-type (WT) (diamonds) and *dnl3* mutant (squares) plants (means ± SE; N = 20). **C.** High-performance liquid chromatography-mass spectrometry quantification of endogenous GA3 in the culm (means ± SE; N = 3). **Significantly different to WT ($P \leq 0.01$). **D.** Transcription profiling of genes controlling gibberellin synthesis and response (means ± SE; N = 3). *,**Significantly different to WT at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Positional cloning of the gene compromised in the *dnl3* mutant

The genetic analysis indicated that the segregation pattern of WT to *dnl3* types in the two F_2 populations was consistent with a monogenic 3:1 ratio (Table 3). The simple sequence repeat-based linkage and fine mapping analyses identified the location of *DNL3* to a 19.1-kb interval on rice chromosome 12, lying between the indel markers Ind2 and Ind3 (Figure 5A and B). This region harbors three open reading frames (ORFs) (rice.plantbiology.msu.edu/index.shtml). Resequencing the ORFs revealed that the mutant differed from the WT by a 2-nt deletion (amino acid) in the second ORF, resulting in the formation of a premature stop codon (Figure 5C); the ORF is predicted to encode a cellulose synthase-like D4 (*CSLD4*).

Table 3. Segregation data for the *dnl3* mutant phenotype.

Hybridized combination	No. of normal plants	No. of mutant plants	Chi-square value (3:1)	P value
Zh11/*dnl3*	148	53	0.201	0.65
Nj11/*dnl3*	2543	840	0.052	0.82

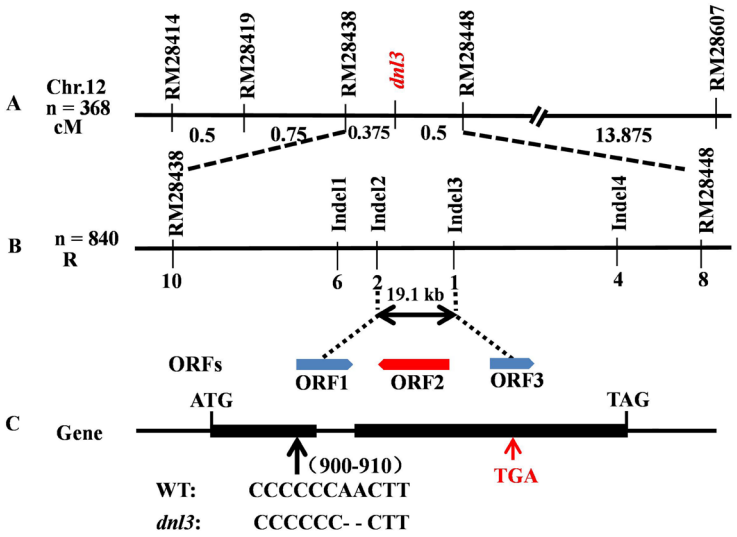

Figure 5. Positional cloning of the gene compromised in the *dnl3* mutant. **A.** Mapping based on 368 F_2 progeny; the gene was located on chromosome 12 between the simple sequence repeat loci RM28438 and RM28448. **B.** Fine-scale mapping based on 840 progeny placed the gene in a 19.1-kbp region, flanked by Ind2 and Ind3, which harbored three open reading frames (ORFs). **C.** Structure of ORF2. ATG and TAG, start and stop codons, respectively; heavy lines denote exons, and the intervening thin line an intron. The black arrow indicates the site of the 2-nt amino acid deletion in the *dnl3* mutant. The red arrow indicates the premature stop codon formed in the *dnl3* mutant allele.

Transcription profiling of *CSLD4* and genes related to cellulose synthesis and cell elongation/division

A qRT-PCR was conducted in order to examine the transcription pattern of *dnl3*. *DNL3* was expressed in all of the tissues and organs tested at the heading stage, including flag leaves, flag leaf sheaths, the culms of the second internodes, panicles, and roots. The *CSLD4* (*DNL3*) transcript was abundant in the WT leaves, roots, and culms, but not in these organs in the mutant (Figure 6A). When the transcriptions of other *CSLD* genes and members of the *CESA* gene family were assayed, transcription differences between WT and the *dnl3* mutant were apparent for all of the *CSLD* genes, and for *CESA2*, *CESA3*, *CESA4*, *CESA5*, and *CESA7*, but not for *CESA1*, *CESA6*, *CESA8*, or *CESA9* (Figure 6B). The abundances of transcripts generated from the cell division/expansion-related genes *CDC2*, *CDC3*, *MCM3*, *RPA2A*, *SPL16*, and *H4* were lower in *dnl3* than in the WT, while those of *RAN2* were substantially increased; the transcript abundances of both *EXPA* and *CYCB1* were unaffected by the mutation (Figure 6C).

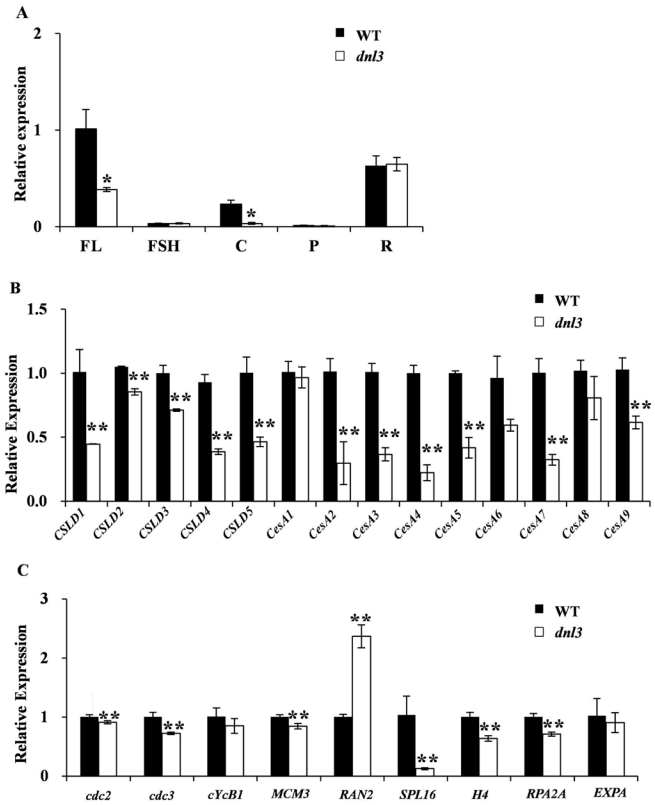

Figure 6. Effect of the *dnl3* mutation on the transcription of related genes. **A.** Quantitative reverse transcription-polymerase chain reaction analysis of CSLD4 transcription throughout the wild-type (WT) and *dnl3* mutant plants; FL, flag leaf; FSH, flag leaf sheath; C, culm; P, panicle; R, root (means ± SE; N = 3). *Significantly different to WT ($P \leq 0.05$). **B.** Transcription of genes encoding cellulose synthase active subunits (CESA) and CESA-like genes (CSL) (means ± SE; N = 3). *,**Significantly different to WT at $P \leq 0.05$ and $P \leq 0.01$, respectively. **C.** Transcription profiling of genes controlling cell elongation/division in the young leaf (means ± SE; N = 3). **Significantly different to WT ($P \leq 0.01$).

DISCUSSION

Mutagenesis experiments in rice have produced a large number of narrow-leaf mutants, and the genes underlying some of these (*dnl1*, *dnl2*, *nal1*, *nal2*, *nal3*, *nal4*, *nal5*, *nal6*, and *nal7*) have been identified (Fujino et al., 2008; Qi et al., 2008; Wei et al., 2013; Adedze et al., 2016). An even larger number of dwarf mutants are known, including some that produce small grains (*d11*, *d1*, and *d18*), some that form narrow or rolled leaves (*d2*, *d6*, *tdd1*, *rl1*, *nal2*, *nal3*, *dtl1*, and *dnl1*), and some that tiller profusely (*htd1*, *htd2*, *d10*, *d27*, and *d53*) (Qi et al., 2008; Liu et al., 2009; Sazuka et al., 2009; Wei et al., 2013; Zhao et al., 2014). Like *dnl3*, both *dnl1* and *dnl2* combine a dwarfed stature with a narrow-leaf phenotype, while the dwarfism character has not been reported in *nal* mutants. The *dnl3* mutant also exhibited a reduction in culm diameter, panicle size, grain set, and grain weight, but formed more tillers than the WT. Leaf morphology can have a profound impact on photosynthetic efficiency, and consequently

on the productivity of the plant, while plant height is also potentially a key determinant of rice yield (Sakamoto and Matsuoka, 2006).

Plant height in rice is strongly determined by the synthesis of, and responsiveness to, various phytohormones (Itoh et al., 2004; Arite et al., 2007; Li et al., 2009). The pathways mediated by the gibberellins are currently the best understood. Dwarf mutants in which gibberellin synthesis is compromised lack sufficient gibberellin, and tend to respond positively to an exogenous supply of GA3, whereas mutants in which the responsiveness to gibberellin has been abolished are unaffected by GA3 treatment (Ikeda et al., 2001; Sasaki et al., 2003). The limited shoot elongation response of *dnl3* to exogenous GA3 indicates that the mutant falls into the class of less-responsive types. Consistent with this conclusion, in the *dnl3* mutant, the abundance of transcripts produced by the gibberellin synthesis genes *GA20ox3*, *GA3ox2*, *GA2oX1*, and *GA2ox3* and the gibberellin response genes *D1*, *SLR1*, and *GID2* differed from that in the WT, presumably reflecting the plant's ability to sense its endogenous gibberellin content.

The *dnl3* mutant exhibited shortened culm internodes. Internode elongation is driven by cell division in the intercalary meristem, followed by cell elongation in the elongation zone (Hoshikawa, 1989). A comparison of longitudinal sections taken from the *dnl3* and WT internode II failed to show any significant difference in cell size, but there was a marked reduction in cell number in the mutant. This contrasts with the basis of the narrow-leaf phenotype of the *Arabidopsis thaliana ANGUSTIFOLIA* mutant, which is caused by a reduced cell size without any decrease in cell number (Tsuge et al., 1996). In the rice *nal2* and *nal3* mutants, the number of large leaf veins is marginally lower than in the WT, while the number of small ones is reduced by almost 50% (Cho et al., 2013). The *dnl3* mutant exhibited a significant decrease in the number of small vascular bundles. The growth of plant tissue involves a combination of cell division and cell expansion, processes that are largely controlled by the products of genes that encode cyclin expansions (Kawamoto et al., 1997; Weingartner et al., 2003). The enhanced accumulation of *CYCB1/2* and *CDL2* transcripts in the mutant and/or the reduced presence of *EXPA* transcripts may explain the smaller size and greater number of parenchyma cells that formed in its culm (Xia et al., 2006).

The gene underlying the *dnl3* mutation was revealed via positional cloning to encode a CSLD4 protein. The *CSLD* family of genes is ubiquitous in land plants; it shares more sequence homology with the genes encoding cellulose synthase than any of the members of eight other *CSL* sub-families. The rice genome harbors five *CSLD* genes. The phenotype of the *CSLD1* loss-of-function mutant shows that the gene is required for root hair development; in the mutant, root hairs are initiated, but elongate poorly and develop kinks and swellings along their lengths (Kim et al., 2007). The phenotype of the loss-of-function of *CSLD4* has shown that this gene is important for normal cell wall formation and plant growth (Li et al., 2009). Our results show that it also affects other plant traits. *nd1*, *nrl1*, and *sle1* mutants also have *CSLD4* as a candidate gene, and *dnl3* may be an allelic mutant gene of *nd1/nrl1/sle1*. The four mutants, including *dnl3*, exhibit common traits, such as a narrow-leaf phenotype. However, *nd1* is dwarfed and narrow-leaved, while *nrl1* and *sle1* only have narrow leaves. In addition, anther dehiscence is compromised in the *sle1* mutant, which results in the production of few mature pollen grains (Li et al., 2009; Hu et al., 2010; Wu et al., 2010; Yoshikawa et al., 2013). Besides the dwarfism and narrow-leaf traits, *dnl3* exhibits other pleiotropic developmental defects that are characterized by its low responses to phytohormones, which suggests that *CSLD4* may be involved in phytohormone regulation. The most probable explanation for this apparent inconsistency is that *CSLD4* may be the gene that is compromised in all of these mutants,

and their different phenotypes are caused by different mutations. These results suggest that *DNL3* plays an important role in plant growth and development, and is essential for gibberellin signaling in rice. Further research is required to better understand this mechanism in rice.

Conflicts of interest

The authors declare no conflict of interest.

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