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Characterization of a rice *dwarf and narrow leaf 2* mutant

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Abstract

The rice *dwarf and narrow leaf mutant 2* (*dnl2*) is dwarfed and forms narrow and brittle leaves. Its dwarfness was shown to be due to its shortened internodes resulting from a reduced size of the internode parenchyma cells. Its narrow and brittle leaves were attributed to a compromised ability to form vascular bundles but a reduced fiber content and thin cortical layer. However, response to the application of either gibberellin or brassinolide was not different between *dnl2* and its wild type. Transcription profiling indicates that a number of cell division/expansion-associated and crude fiber synthesis-related genes were down-regulated in the mutant. A genetic analysis revealed that the mutant phenotype was under monogenic control, and the gene responsible was mapped to a 50.1 kb genomic region on the long arm of chromosome 10. This region was shown to harbor 10 open reading frames. Although transcription profiling these genes indicates that three were differentially transcribed in the mutant, there was no sequence polymorphism in the coding sequence between the mutant and the wild type alleles.

Additional key words: fine-mapping, leaf and stem structure, mechanical strength, *Oryza sativa*, transcriptional analysis.

Introduction

The shoot is formed from a stem transporting water and nutrients and from leaves responsible for photosynthesis and transpiration. Both plant height and leaf area have been correlated with economic yield in a number of crops including rice (Cui *et al*. 2003, Tsukaya 2006, Micol 2009, Li *et al*. 2013). Leaf growth progresses by a combination of cell division and cell expansion, and a number of genes in rice responsible for these events have been identified and characterized (Weingartner *et al*. 2002, Chen et *al*. 2012). Growth is also mediated by interaction of various phytohormones, as was also shown by analysis of specific mutants unable to either synthesize or perceive a particular hormone (Nakamura *et al*. 2013, Wei *et al*. 2013). Gibberellins (GA) and brassinosteroids (BR) are particularly important in rice (Itoh *et al*. 2002, Gomi *et al*. 2004, Ueguchi-Tanaka *et al*. 2005, Jiang *et al*. 2013, Zhao *et al*. 2014, Zhou *et al*. 2014); the majority of GA- and BR-insensitive or deficient mutants exhibit dwarfism, and some form conspicuously narrow leaves. The rice genes *GA20ox2* (taking part in GA synthesis) and *GA2ox4* (in GA catabolism) have a major influence over vegetative growth (Ashikari *et al*. 2002, Mimura *et al*. 2012), whereas a number of genes involved in GA signaling pathways have been shown to be important during the whole development (Itoh *et al*. 2002, Gomi *et al*. 2004, Ueguchi-Tanaka *et al*. 2005, Wei *et al*. 2013).

Many dwarf phenotypes have been associated with the inability to synthesize or perceive BR (Hong *et al*. 2005, Tsuda *et al*. 2014), but the molecular basis is still not fully understood. At the same time, plant growth and development can also be controlled independently of GA or BR. An example is presented by mutation to certain cellulose synthase catalytic subunit-encoding genes, which induces lowering cellulose content and generating brittle stem and leaves (Ueguchi-Tanaka *et al*. 2003, Yang *et al*. 2014). A gene *CSLD4*, which encodes a cellulose synthase-like enzyme, is important for cell wall synthesis and rice growth (Li *et al*. 2009). Likewise, the loss of function of genes controlling lignin content can generate a brittle stem phenotype (Ueguchi-Tanaka *et al*. 2003, Yan *et al*. 2007, Zhang *et al*. 2009, 2010,

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Abbreviations: BR - brassinosteroids; *dnl2* - *dwarf and narrow leaf mutant 2*; EMS - ethyl methane sulfonate; GA - gibberellins; WT - wild type.

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Yoon *et al*. 2014).

Here, an ethyl methane sulfonate (EMS)-induced dwarf and narrow leaf mutant *dnl2* was used as the research object. The dwarf and narrow leaf characteristics were studied in detail using morphology and cytology methods. The mutant gene was fine mapped in a narrow chromosome region. These results might be useful for *DNL2* gene cloning and to improve our understanding of the molecular mechanism of regulation of plant height and leaf structure in rice.

Materials and methods

The rice *dwarf and narrow leaf mutant 2* (*dnl2*) is EMS-induced mutant from *Oryza sativa* L., a *japonica* type, cv. Zhonghua 11. The F_2 population bred from a cross *dnl2* × cv. Nanjing 11 (an *indica* type) was developed for the mutant gene mapping purpose. All materials were grown in the field at the China National Rice Research Institute, Hangzhou. Sowing and transplanting were made on 20 May and on 20 June, respectively. The *dnl2* and its wild type (WT) cv. Zhonghua 11 were transplanted into 3 plots, and each plot contained 48 plants (6 rows with 8 plants per row) with spacing of 15 × 20 cm. Standard cultivation practices were followed. Only 20 plants in the center of each plot were assessed for phenotyping. The breaking force of leaves and stems of the plants at physiological maturity was evaluated using a *HandPi HP-100* force gauge (www.handpi.com).

To evaluate responses to GA and BR, grains of the *dnl2* mutant and WT were surface-sterilized by immersion in distilled water containing a drop of *Tween 20* for 15 min, then thoroughly rinsed with water 3 times and laid on a 1.5 % (m/v) agar medium containing 0 - 0.1 mM gibberellic acid or 0 - 1 μM brassinolide. The seedlings were allowed to develop under a continuous irradiance of 1 000 μmol m^{-2} s^{-1} and a temperature of 30 °C for either 7 d (the GA plants) or 14 d (the BR plants). At the end of this period, the length of the second leaf (from the bottom) sheath of a set of 20 plants per treatment was measured.

The central section of the third stem internode and the flag leaf of both the *dnl2* plants and the WT plants were fixed in FAA (formaldehyde + glacial acetic acid + 50 % ethanol, 2:1:17, v/v/v) at 4 °C for 24 h following Yoshikawa *et al* (2013) with minor modifications. After a quick rinse in water, the material was dehydrated by passing through an ethanol series (concentrations of 30, 50, 70, 80, 90, 95, and 100 %; 5 min for each step), then steeped in 1-butanol, and finally embedded in *Paraplast Plus*. A series of 8 μm sections were prepared using a rotary microtome, and these were stained in Safranin containing Fast Green, and the samples were finally observed under a microscope (*Eclipse 80i*, *Nikon*, Japan).

At the heading stage, crude fiber content was determined using a slightly modified version of the *SN/T 0800.8-1999* protocol (www.cssn.net.cn). The flag leaf samples (0.5 - 3 g) of the *dnl2* and WT plants were properly weighed and defatted with petroleum ether. Then the samples were placed in a glass crucible and extracted by using a semi-automatic fiber analyzer (www.foss.dk), with a 1.25 % (m/v) sulfuric acid solution and then with a 1.25 % (m/v) sodium hydroxide solution. After drying at a temperature of 130 ± 2 ºC for 2 h, the crucible with the residue was weighed [m1]. Finally, the residue in the crucible was incinerated to a constant mass [m2] at a temperature of 550 ºC. The fiber in the sample was calculated as (m1 - m2) × 100/m1.

At the heading stage, RNA was isolated from flag leaves of the *dnl2* and WT plants using a *TRIzol* reagent, and it was reverse-transcribed by using the moloney murine leukemia virus reverse transcriptase (M-MLV) method. The first-strand cDNA was reverse transcribed from the DNase I-treated RNA with oligo(dT) as the primer. The cDNA was used as template for real time quantitative PCRs targeting 10 open reading frames, various cell division/expansion-related, cellulose encoding, and lignin synthesis genes (primer sequences given in Table 1 Suppl.). The rice *Ubiquitin* gene (GenBank accession AF184280) was used as a reference. Each 20 mm^3 of a real time quantitative PCR mixture contained 2 mm^3 of a template, 0.2 μM each primer, and a 1× *SYBR Green* PCR master mix (*TOYOBO Co.,* http://www.bio-toyobo.cn). The PCR was performed with a *Roche* light cycler 480 (http://www.roche-applied-science.com) using the following program: 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 30 s. Relative transcriptions were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

A mapping population was constructed from the F_2 generation of the cross *dnl2* × cv. Nanjing 11. The mapping was based on a set of 1 775 dwarfs, a narrow-leaved F_2 progeny, following the strategy of Zhang *et al*. (1994). Microsatellite and indel markers used for mapping are listed in Table 2 Suppl. The DNA was extracted from 30-d-old seedlings by using the cetyl trimethylammonium bromide (CTAB) method and subjected to PCRs, each of a 10-mm^3 mixture containing 25 ng of template DNA, 1 mm^3 of a 10× PCR buffer, 0.1 mM dNTP, 0.1 μM of each primer, and 0.1 U of Taq DNA polymerase. A cycling regime comprised an initial denaturation (95 °C for 3 min) followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and it was completed by a final extension (72 °C for 5 min). The PCR products were separated by electrophoresis through a 6 % (m/v) denaturing polyacrylamide gel and then visualized by silver staining.

Results

The height of the *dnl2* plants was distinctly less than that of the WT ones, a difference which was already noticeable at the late seedling stage became more marked as the plants developed (Fig. 1*A*,*B*). The mutant height at maturity was about 65 % of that of the WT plant (Fig. 1*B*,*F*). The mutant formed narrow leaves and a thin stem (Fig. 1*C*). Its panicle was reduced in size, comprising fewer primary branches (Fig. 1*D*,*J*). The spikelet number per panicle and grain size and mass were both less in the mutant than in the WT (Fig. 1*K*,*M*,*O*,*P*). Despite the mutant produced a higher number of panicles per plant than the WT (Fig. 1*G*,*H*), its grain productivity was lower (Fig. 1*Q*). Neither grain length nor panicle fertility were significantly affected by the mutation (Fig. 1*L*,*N*).

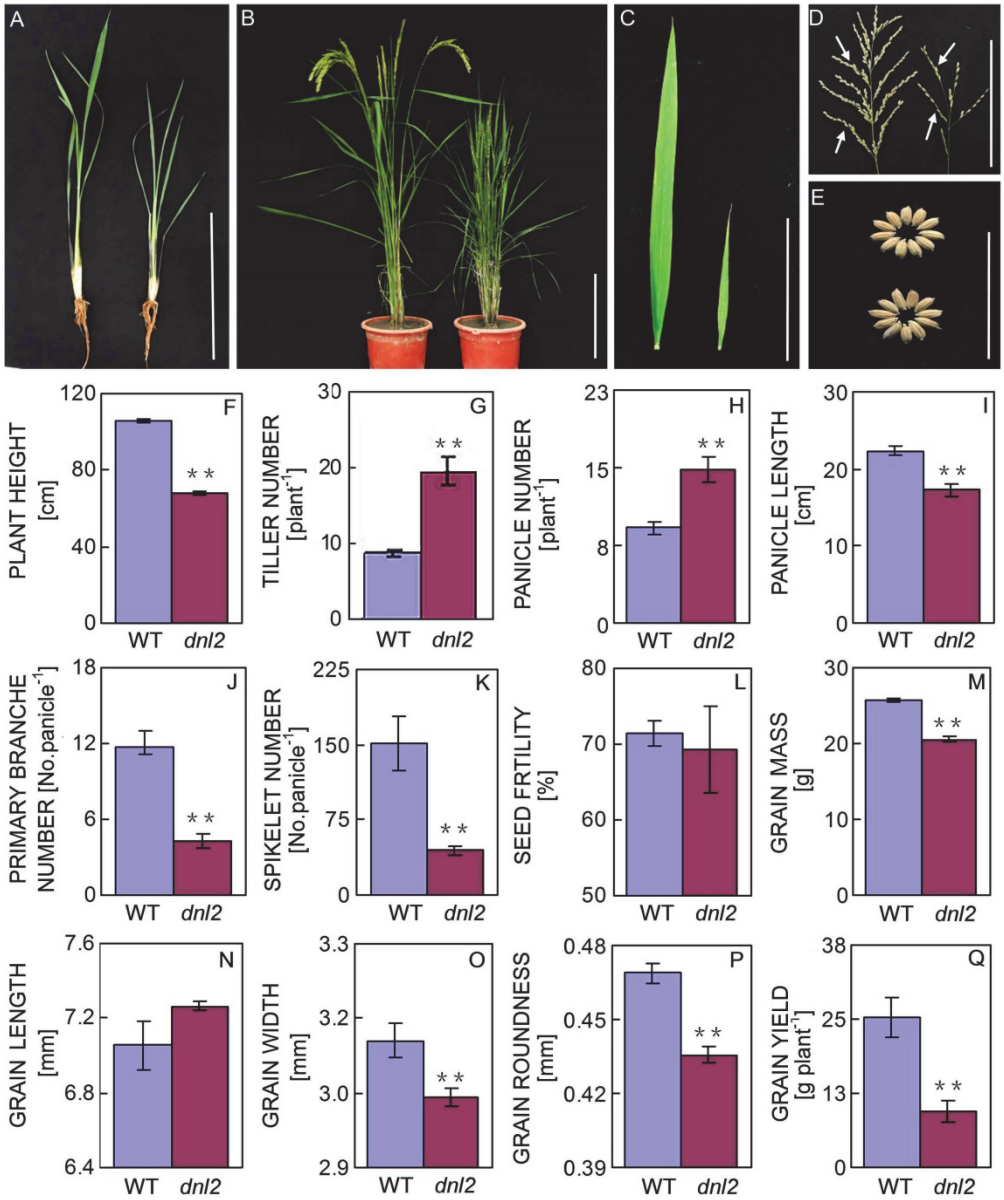

Fig. 1. The phenotype of the *dnl2* mutant. Wild type (WT) plants (*on the left*) and *dnl1* plants (*on the right*) 30 d after sowing (*A*) and at flowering (*B*); flag leaf (*C*), panicle (*D*), and grain (*E*) of main panicles of the wild-type plants (*above*) and *dnl2* plants (*below*). *Bar* = 10 cm (*A-D*) or 4 cm (*E*), the *white arrows* indicate primary branches (*D*). Plant height (*F*), the number of tillers per plant (*G*), the number of panicles per plant (*H*), panicle length (*I*), the number of primary branches per panicle (*J*), the number of spikelets per panicle (*K*), seed fertility (*L*), grain mass (*M*), grain length (*N*), grain width (*O*), grain roundness (*P*), and grain yield per plant (*Q*) of the wild-type plants and the *dnl2* plants. Means ± SDs, $n = 20$, * and ** indicate significant differences between the WT and the *dnl2* at $P \leq 0.05$ and $P \leq 0.01$, respectively.

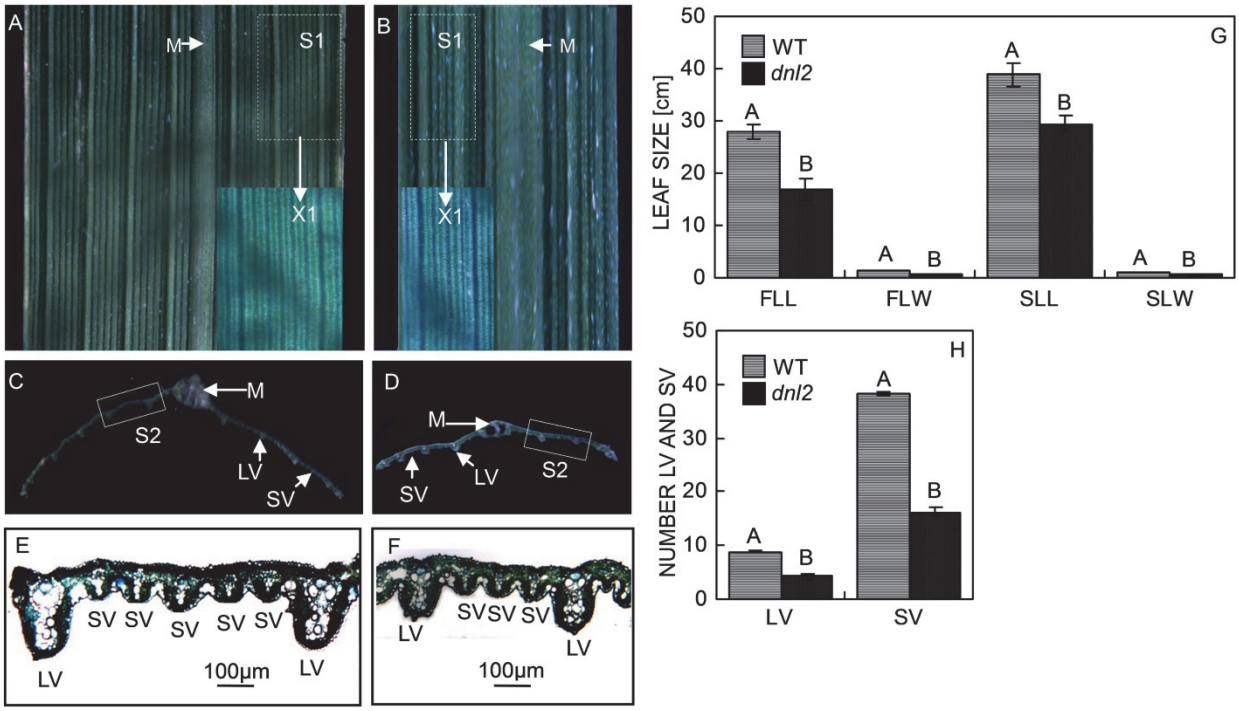

Fig. 2. The leaf surface view (*A*,*B*), leaf cross-section view (*C*,*D*), and paraffin section (*E*,*F*) of wild type (WT) and *dnl2* plants. X1 - the magnified view of the S1, S2 - the section of a leaf used for paraffin sectioning, M - midrib. Flag leaf length (FLL), flag leaf width (FLW), length of the second leaf from the top (SLL), and width of the second leaf (SLW) in the WT and *dnl2* plants (*G*). The number of large (LV) and small (SV) veins in vascular bundles of the WT and *dnl2* plants (*H*). Different letters in *G* and *H* indicate that the means differ at $P \leq 0.01$.

The mutant leaves were considerably reduced in size (Figs. 1*C*, 2*A*,*B*). The mean reductions in flag leaf length and width were 40 and 60 %, respectively, and in the second leaf from the top 25 and 47 %, respectively (Fig. 2*G*). The number of veins was greatly reduced in the mutant (by ~52 % for major veins and ~60 % for minor ones) (Fig. 2*E*,*F*,*H*). Consistent with this observation, the histological analysis shows a significant decrease in the number of vascular bundles carried by the mutant although vascular patterning remained unaffected (Fig. 2*E*,*F*). The mutant plants were not only shorter than the WT ones, but their stems were also thinner (Fig. 3*A*). Both the length and the diameter of the mutant stem internodes were smaller than those of the WT (Fig. 3*B*,*C*). When the longitudinal sections of typical internodes were inspected, it was noted that the parenchyma cells within the cortical layer of the mutant stem were smaller and more numerous (Fig. 3*D*,*E*). Although the number of the parenchyma cells in the internode of the *dnl2* was higher than in the WT, both the length and the width of the cells were significantly reduced (Fig. 3*F*-*H*).

The real time quantitative PCR-based assessment of transcript abundance of genes involved in cell division and expansion (cyclin *B1/2, cdc2/3, MCM3, RPA2A, RAN2,SPL16, H4, EXPA*, and *XTR*) shows that the mutant generated more transcripts of the cell division-associated genes *cdc2, CycB1, CycB2, MCM3, RPA2A, SPL16,* and *H4* and of the cell expansion gene *XTR* than did the WT, but less with respect to the cell division-associated gene *RAN2* and the cell expansion gene *EXPA* (Fig. 3*I*). The implication was that in the mutant, stem growth was inhibited due to abnormal expression of the whole suite of cell division- and cell expansion-associated genes. When the mutant seedlings were exposed to either gibberellic acid or brassinolide, the length of the second leaf sheath increased similarly as in the WT seedlings (Fig. 4*A*,*B*).

Leaves and stems of the mutant were more fragile than those of the WT as shown by a lower force required to disrupt them (Fig. 5*A*,*B*). Moreover, the crude fiber content of the vegetative tissue was lower in the mutant than in the WT plants (Fig. 5*C*). The real time quantitative PCR-derived transcript abundance of the brittle culm gene *OsBC1* in the mutant was significantly higher, whereas that of *OsBC12* and *OsBC14* was lower than in the WT (Fig. 5*D*). At the same time, the abundances of *OsCesA3, OsCesA4, OsCesA7, OsCesA9*, and *OsCesA11* (cellulose encoding genes) and of *OsCCR19* (lignin synthesis) transcripts were all lower than in the WT; the opposite was in the case of *OsCesA1, OsCesA2*, and *OsCesA5* (cellulose), and *OsCOA1* (lignin) encoding gene transcriptions (Fig. 5*E*,*F*).

The segregation between the WT and mutant

phenotype in the F_2 mapping population was consistent with a 3:1 ratio (data not shown), implying that dwarfness was inherited as a recessive trait under monogenic control. The mutated gene was coarsely mapped as lying between the microsatellite loci RM3834 and RM590 on the long arm of chromosome 10 (Fig. 6*A*). By increasing the progeny number screened and by adding further genotypic assays (Table 2 Suppl.), the mutated gene was eventually located within a 50.1 kb region bounded with two indel markers IM4 and IM10 (Fig. 6*B*,*C*).

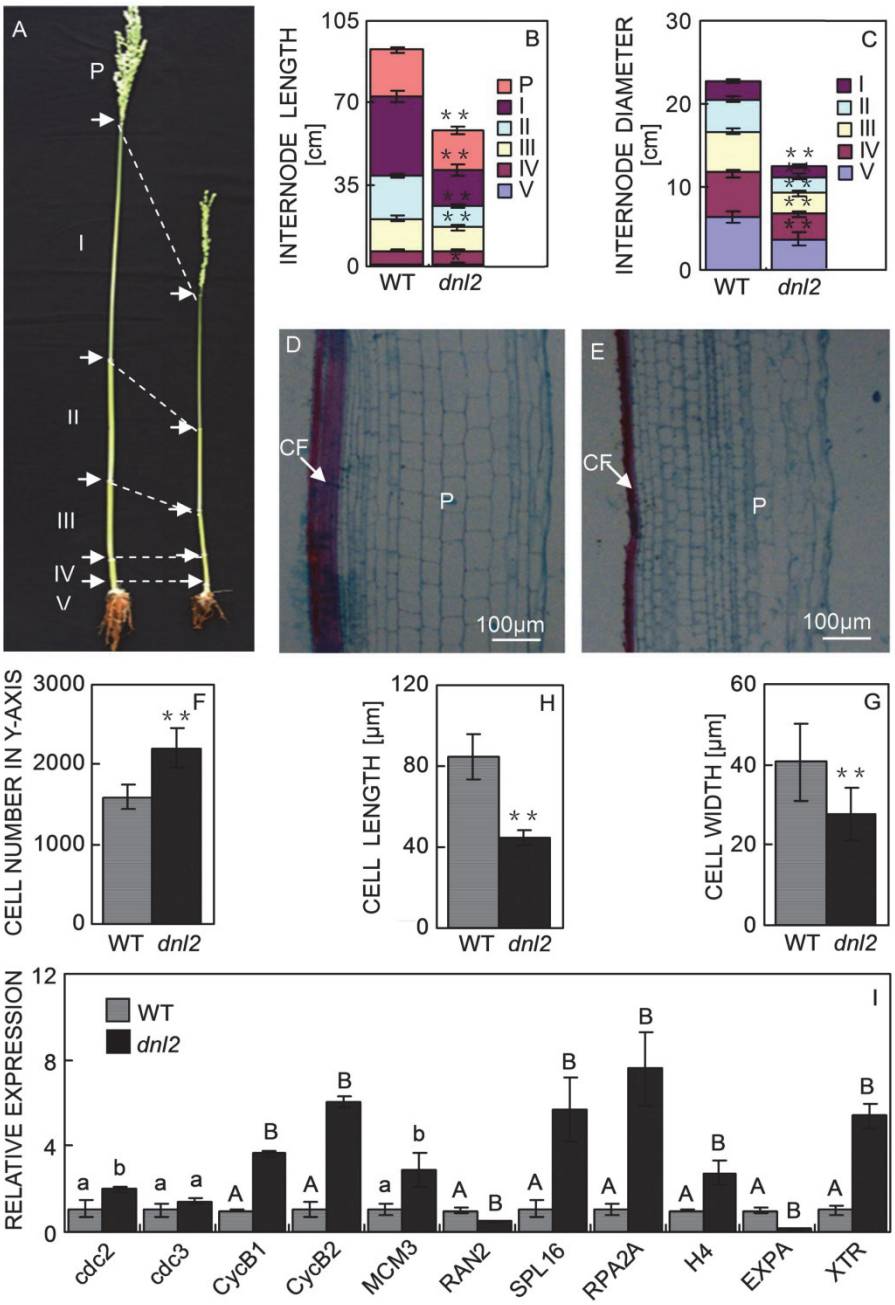

Fig. 3. *A* - Main culms of wild type (WT) plants (*on the left*) and *dnl2* plants (*on the right*) (the *arrows* indicate the positions of nodes I - V in a panicle P). *B*,*C* - The length and diameter of internodes and panicles. *D*,*E* - Parenchyma in the internode of the WT (*D*) and the *dnl2* mutant (*E*, CF - cortical fiber tissue, P - parenchyma). The total cell number in the y-axis of internode III (*F*), average cell length (*H*), and cell width (*G*) of the WT and the *dnl2* mutant. *I* - relative expression of cell division and expansion related genes. ** and different letters indicate that the means differ at $P \leq 0.05$ (a,b) and $P \leq 0.01$ (A,B), respectively.

A reference to the standard genome sequence of rice indicates that the IM4-IM10 region harbors 10 ORFs (as listed in Table 1). Transcription profiling with respect to all 10 candidate genes shows that the abundance of the

ORF3, *ORF7*, and *ORF8* transcripts was significantly lower in the *dnl2* than in the WT, whereas that of the other 7 did not differ between the mutant and the WT. However, re-sequencing all the 10 *ORF*s including *ORF3*, *ORF7*, and *ORF8* failed to identify any nucleotide polymorphisms between the *dnl2* and WT.

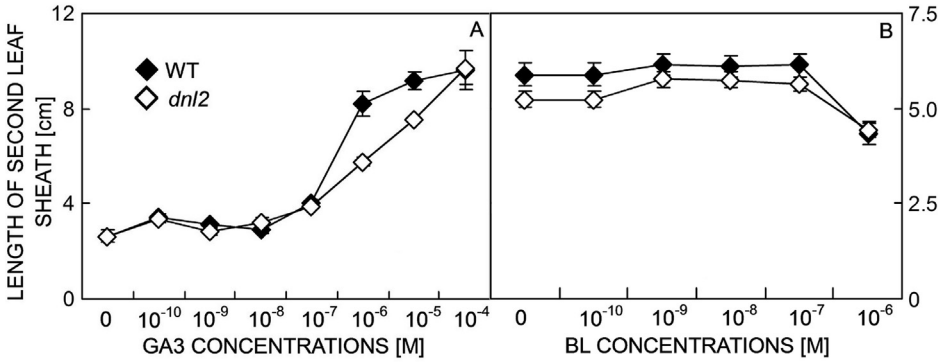

Fig. 4. The length of the second leaf sheath of wild type (WT) plants and *dnl2* plants in response to gibberellic acid (GA3; *A*) and brassinolide (BL; *B*) treatments. Means ± SDs; $n = 20$.

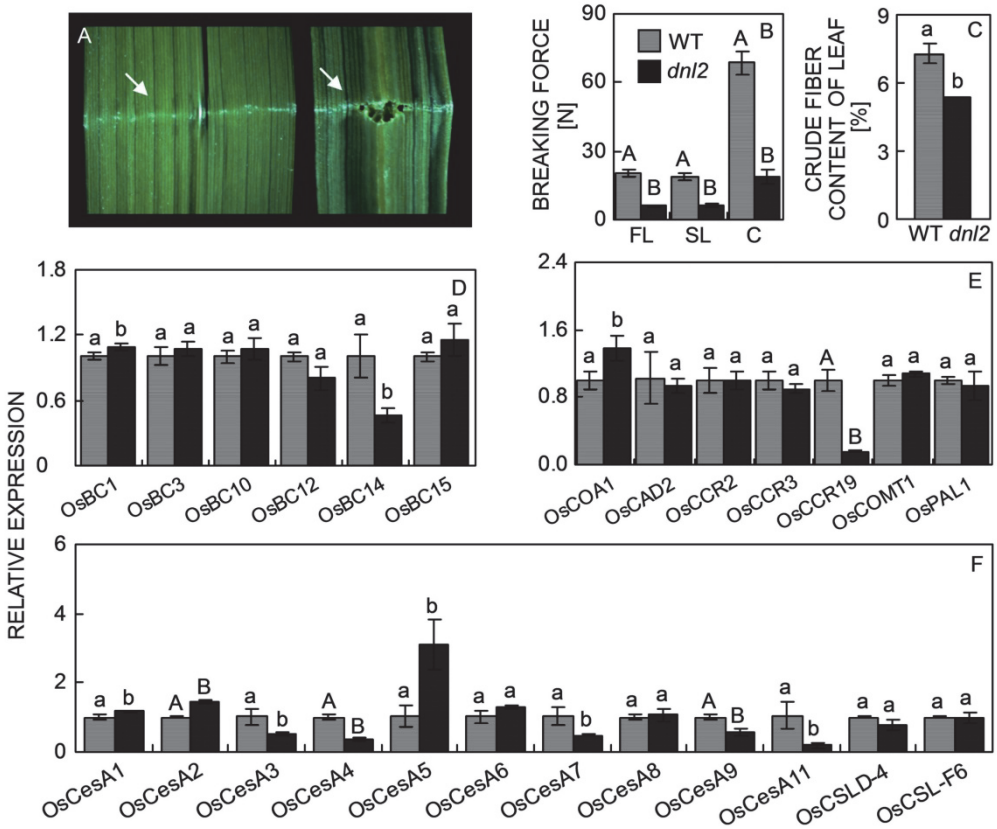

Fig. 5. Physical characteristics of leaves and stems of wild type (WT) and *dnl2* plants at maturity, and the transcription of cellulose synthesis- and lignin synthesis-associated genes. *A* - A normal leaf from the WT (*on the left*) and a brittle leaf from the *dnl2* mutant (*on the right*). The *arrow* indicates brittle aspect). *B* - The breaking force of the flag leaf (FL), second leaf (SL), and stem (C). *C* - Crude fiber content of the leaf. Transcript abundances of brittle culm genes (*D*), lignin synthesis genes (*E*), and cellulose synthesis genes (*F*). Means ± SDs; $n = 3$, different letters indicate that the means differ at $P \leq 0.05$ (a,b) and $P \leq 0.01$ (A,B).

Discussion

A large number of rice dwarf variants are known. Some, such as *d1* and *d11*, produce small grains, other, such as *d2, d6, tdd1, rl1*, *nal1, nal2, nal3*, *ndl*, *dtl1*, and *dnl1*, are malformed dwarfs and exhibit sometimes a narrow or

rolled leaf phenotype, whereas a further set (*htd1, htd2, d10*, *d27*, and *d53*) compensates for their shortened stature by tillering heavily (Tanabe *et al*. 2005, Qi *et al*. 2008, Li *et al*. 2009, Lin *et al*. 2009, Liu *et al*. 2009, Sazuka *et al*. 2009, Hu *et al*. 2010, Zhang *et al*. 2012, Ishiwata *et al*. 2013, Jiang *et al*. 2013, Wei *et al*. 2013, Zhou *et al*. 2014). Some of the dwarfs are phytohormone sensitive, whereas other are insensitive (Yamamuro *et al*. 2000, Ueguchi-Tanaka *et al*. 2005, Wei *et al*. 2013). Further, some dwarf-like *dnl2* affect plant growth and development independently of GA or BR (Ueguchi-Tanaka *et al*. 2003, Yang *et al*. 2014). The chromosome

Table 1. Predicted open reading frames (ORFs) and translation products for *DNL2*.

ORFs	Locus	Gene product name
ORF1	*LOC_Os10g42690*	*jmjC* domain containing protein, expressed
ORF2	*LOC_Os10g42700*	*CS* domain containing protein, putative, expressed
ORF3	*LOC_Os10g42710*	*RCD1*, putative, expressed
ORF4	*LOC_Os10g42720*	acyltransferase, putative, expressed
ORF5	*LOC_Os10g42724*	*VHS* and *GAT* domain containing protein, expressed
ORF6	*LOC_Os10g42730*	expressed protein
ORF7	*LOC_Os10g42750*	*CSLD1* - cellulose synthase-like family D, expressed
ORF8	*LOC_Os10g42754*	expressed protein
ORF9	*LOC_Os10g42760*	*PPR* repeat domain containing protein, putative, expressed
ORF10	*LOC_Os10g42770*	a_*IG002N01.7*, putative, expressed

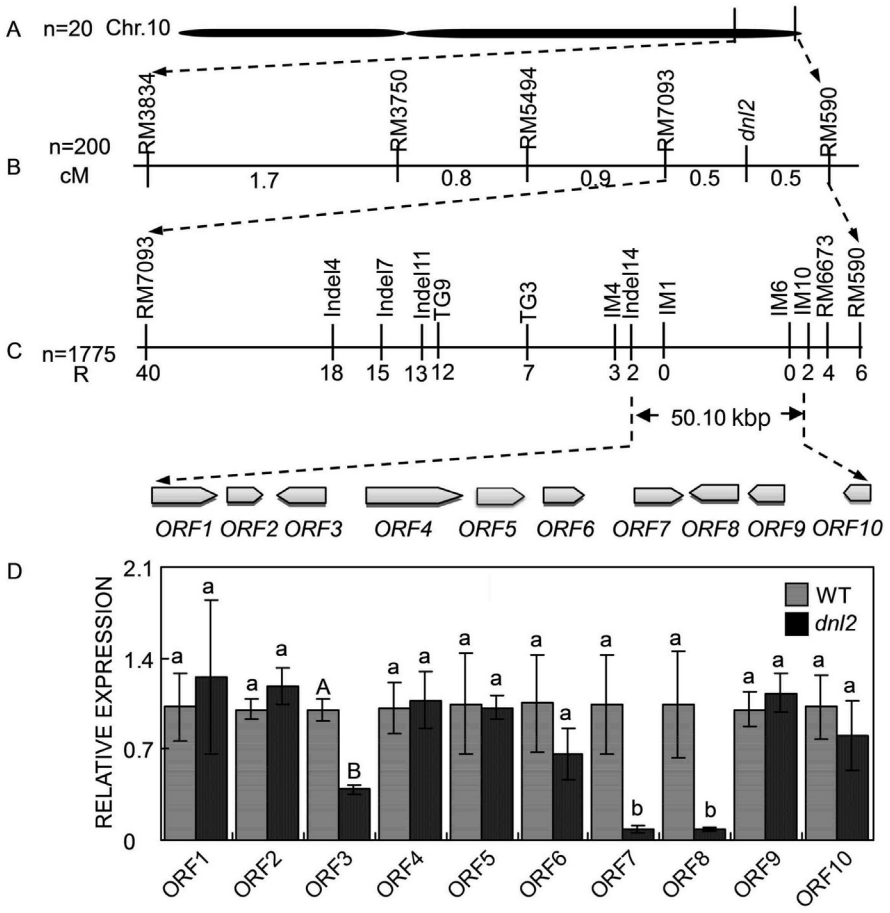

Fig. 6. Genetic mapping of a mutated gene in the *dnl2* mutant. *A* - The gene was initially coarsely located on rice chromosome 10 between RM384 and RM590. *B* - increasing the number of progeny screened to 200 located the gene between RM7093 and RM590. *C* - Fine-scale mapping based on 1 775 progeny placed the gene in a 50.1 kbp region, flanked by indels IM4 and IM10, which harbors 10 *ORF*s. *D* - The transcript abundance of each of the 10 *ORF*s in the wild type (WT) and the *dnl2* mutant. Means ± SDs; $n = 3$, different letters indicate significant differences between the WT and mutant at $P \leq 0.05$ (a,b) and $P \leq 0.01$ (A,B).

location of *dnl2* overlaps that of another dwarf mutant gene *dtl1* (Zhang *et al*. 2012); however, *dtl1* has not been cloned and only primary mapped. The phenotype of *dtl1* is different to *dnl2*, as it shows a twisty leaf and reduced tiller number, but *dnl2* does not. Besides, the leaves and stems of *dnl2* are brittle compared to its WT, but of *dtl1* are also not. So, we believe that *dnl2* is a new dwarf and narrow leaf mutant. The chromosomal region, into which *dnl2* maps, does not harbor any genes known to regulate plant growth. On the basis of transcription profiling, three genes *ORF3*, *ORF7*, and *ORF8* were considered as potential candidates for the mutation, but no sequence variation was detected in the alleles present in the mutant and the WT. The *ORF3* encodes a homolog of the RCD1 (RADICAL-INDUCED CELL DEATH) protein, which in *Arabidopsis thaliana* mediates various stress and developmental responses and interacts with certain transcription factors (Jaspers *et al*. 2009). The *ORF7* encodes a cellulose synthase-like protein (CSLD1), which has been associated with late pollen development in *A. thaliana* (Bernal *et al*. 2008). In rice, CSLD1 is also required for root hair morphogenesis (Chu *et al*. 2007), and *ORF8* encodes a protein of unknown function. Any of these three genes may be the candidate gene for the mutation since either some sequence variant(s) may be present outside the coding region or the variant may represent an epigenetic modification rather than a sequence mutation. The present data cannot exclude the possibility that one of other seven *ORF*s could also be a true candidate.

Growth of plant tissue involves a combination of cell division and cell expansion, processes which are controlled largely by cyclins and expansins (Weingartner *et al*. 2002). *Cyclin B* gene products interact with cyclin-dependent kinase 2 proteins to regulate cell division. The enhanced accumulation of cyclin *B1/2* and *cyclin-dependent like 2* transcripts displayed by the *dnl2* mutant and/or the lower abundance of the expansion gene *EXPA* transcript may explain the smaller size and greater number of parenchyma cells which formed in its stem. It appears that the mutant parenchyma cells divided freely but were unable to expand normally. The considerably increased accumulation of SQUAMOSA promoter binding protein-like 16 (*SPL16*) and X transposed region (*XTR*) transcripts may be a secondary consequence of the disrupted division/expansion ratio of the parenchyma cells. The product of *SPL16* is known to control grain size by promoting cell division and grain filling (Wang *et al*. 2012), whereas that of *XTR* genes participates in cell elongation and division (Bailey-Serres *et al*. 2008). We have noted that replication protein A isoform 2A (RPA2A) has an effect on cell division in rice (Xiao *et al*. 2006), and here it was observed that the abundance of the *RPA2A* transcript was delicately raised in *dnl2*.

Crude fiber, which consists of a mixture dominated by cellulose, hemicellulose, and lignin, has the role of providing structural support and plant mechanical protection. These compounds represent the end products of independent synthetic pathways (Ueguchi-Tanaka *et al*. 2003, Zhang *et al*. 2010, 2011, Yang *et al*. 2014, Yoon *et al*. 2014). The loss of function of a gene encoding a cellulose synthase catalytic subunit leads to development of brittle mature leaves in rice (Ueguchi-Tanaka *et al*. 2003). Any significant reduction in cellulose content was reported to result in stem brittleness along with dwarfism and withering the leaf tip (Zhang *et al*. 2011). Members of the cellulose synthase-like D family are important for plant growth and formation of cell walls (Li *et al*. 2009). Down-regulation of cellulose synthase (*CESA*) genes has the effect of lowering cellulose content, thereby weakening the mechanical strength of leaves (Yang *et al*. 2014). Taken together, reprogramming *CESA* genes and those encoding lignin could account for fragility of the *dnl2* vegetative tissue. On the other hand, suppression of lignin synthesis genes, such as *CCR19*, has promoted development of the grain abscission zone and thereby heightening the tendency of the grain to shatter (Yoon *et al*. 2014). Transcription of both the cellulose and lignin synthesis genes (*CESA3*, *CESA4*, *CESA7, CESA9*, *CESA11,* and *CCR19*) and the brittle related gene *BC12* was down-regulated in the *dnl2* mutant.

It is obvious that dysfunction of *dnl2* goes beyond dwarf and narrow leaf phenotypic expression since it also controls various developmental aspects in a plant. Considering the pleiotropic effect of this gene, a more comprehensive study is needed. In this regard, cloning *DNL2* would be a great contribution to the rice breeding program.

References

Ashikari, M., Sasaki, A., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Datta, S., Ishiyama, K., Saito, T., Kobayashi, M., Khush, G.S., Kitano, H., Matsuoka, M.: Loss-of-function of a rice gibberellins biosynthetic gene, *GA20* oxidase (*GA20ox-2*), led to the rice 'Green revolution. - Breed. Sci. **52**: 143-150, 2002.

Bailey-Serres, J., Voesenek, L.A.C.J.: Flooding stress: acclimations and genetic diversity. - Annu. Rev. Plant Biol. **59**: 313-39, 2008.

Bernal, A.J., Yoo, C.M., Mutwi, M., Jensen, J.K., Hou, G., Blaukop, F.C., Sørensen, I., Blancaflor, E.B., Scheller, H.V., Willats, W.G.: Functional analysis of the cellulose synthase-like genes CSLD1, CSLD2, and CSLD4 in tip-growing *Arabidopsis* cells. - Plant Physiol. **148**: 1238-1253, 2008.

Chen, M.L., Luo, J., Shao, G.N., Wei, X.J., Tang, S.Q., Sheng, Z.H., Song, J., Hu, P.S.: Fine mapping of a major QTL for flag leaf width in rice, *qFLW4*, which might be caused by alternative splicing of *NAL1*. - Plant Cell Rep. **31**: 863-872, 2012.

Cui, K.H., Peng, S.B., Xing, Y.Z., Yu, S.B., Xu, C.G., Zhang, Q.: Molecular dissection of the genetic relationships of source, sink and transport tissue with yield traits in rice. - Theor. appl. Genet. **106**: 649-658, 2003.

Gomi, K., Sasaki, A., Itoh, H., Ueguchi-Tanaka, M., Ashikari, M., Kitano, H., Matsuoka, M.: *GID2*, an F-box subunit of the *SCF E3* complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. - Plant J. **37**: 626-634, 2004.

Hong, Z., Ueguchi-Tanaka, M., Fujioka, S., Takatsuto, S., Yoshida, S., Hasegawa, Y., Ashikari, M., Kitano, H., Matsuoka, M.: The rice brassinosteroid-deficient dwarf2 mutant, defective in the rice homolog of *Arabidopsis DIMINUTO/DWARF1*, is rescued by the endogenously accumulated alternative bioactive brassinosteroid, dolichosterone. - Plant Cell **17**: 2243-2254, 2005.

Hu, J., Zhu, L., Zeng, D.L., Gao, Z.Y., Guo, L.B., Fang, Y.X., Zhang, G.H., Dong, G.J., Yan, M.X., Liu, J., Qian, Q.: Identification and characterization of *NARROW* and *ROLLED LEAF* 1, a novel gene regulating leaf morphology and plant architecture in rice. - Plant mol. Biol. **73**: 283-292, 2010.

Ishiwata, A., Misa, O., Hiroshi, N., Makio, K., Yusaku, N., Takahiro, Y., Misuzu, N., Sae, S.S., Akie, N., Masahiko, M., Hirano, H.Y., Yutaka, S.: Two *WUSCHEL*-related homeobox genes, *narrow leaf2* and *narrow leaf3*, control leaf width in rice. - Plant Cell Physiol. **5**: 779-792, 2013.

Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M., Matsuoka, M.: The gibberellin signaling pathway is regulated by the appearance and disappearance of *SLENDER RICE1* in nuclei. - Plant Cell **14**: 57-70, 2002.

Jaspers, P., Blomster, T., Brosché, M., Salojärvi, J., Ahlfors, R., Vainonen, J.P., Ramesha, A.R., Richard, I., Gerco, A., Franziska, T., Kirk, O., Jaakko, K.: Unequally redundant RCD1 and SRO1 mediate stress and developmental responses and interact with transcription factors. - Plant J. **60**: 268-279, 2009.

Jiang, L., Liu, X., Xiong, G., Liu, H., Chen, F., Wang, L., Meng, X., Liu, G., Yu, H., Yuan, Y., Yi, W., Zhao, L., Ma, H., He, Y., Wu, Z., Melcher, K., Qian, Q., Xu, H.E., Wang, Y., Li, J.: *DWARF 53* acts as a repressor of strigolactone signalling in rice. - Nat. Genet. **504**: 401-405, 2013.

Kim CM., Park, S.H., Je, B.II., Park, SH., Park, S.J., Piao, H. L., Eun, M.Y., Dolan, L., Han C.-D.: *OsCSLD*1, a cellulose synthase-like D1gene, is required for root hair morphogenesis in rice. - Plant Physiol. **143**:1220-1230, 2007.

Li, M., Xiong, G.Y., Li, R., Cui, J.J., Tang, D., Zhang, B.C., Pauly, M., Cheng, Z.K. and Zhou, Y.H.: Rice cellulose synthase-like D4 is essential for normal cell-wall biosynthesis and plant growth. - Plant J. **60**: 1055-1069, 2009.

Li, W., Chao, W., Hu, G.C., Li, X., Qian, W.J., Si, H.M., Sun, Z.X., Wang, X.C., Fu, Y.P., Liu, W.Z.: Characterization and fine mapping of a novel rice narrow leaf mutant *nal9*. - J. Integr. Plant Biol. **11**: 1016-1025, 2013.

Lin, H., Wang, R., Qian, Q., Yan, M., Meng, X., Fu, Z., Yan, C., Jiang, B., Su, Z., Li, J., Wang, Y.: *DWARF27*, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. - Plant Cell. **21**: 1512-1525, 2009.

Liu, W.Z., Chao W., Yaping, F., Guocheng, H., Huamin, S., Zhu L., Luan W.J., He Z.Q., Sun Z.X.: Identification and characterization of *HTD2*: a novel gene negatively regulating tiller bud outgrowth in rice. - Planta **230**: 649-658, 2009.

Livak, K.J., Schmittgen, T.D.: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{\Delta\Delta CT}$ method. - Methods. **25**: 402-408, 2001.

Micol, J.L.: Leaf development: time to turn over a new leaf? - Curr. Opin. Plant Biol. **12**: 9-16, 2009.

Mimura, M., Nagato, Y., Itoh, J.I.: Rice *PLASTOCHRON* genes regulate leaf maturation downstream of the gibberellin signal transduction pathway. - Planta **235**: 1081-1089, 2012.

Nakamura, H., Xue, Y.L., Miyakawa, T., Hou, F., Qin, H.M., Fukui, K., Shi, X., Ito, E., Ito, S., Park, S.H., Miyauchi, Y., Asano, A., Totsuka, N., Ueda, T., Tanokura, M., Asami, T. Molecular mechanism of strigolactone perception by *DWARF14*. - Nat. Commun. **4**: doi:10.1038/ncomms3613, 2013.

Qi, J., Qian, Q., Bu, Q.Y., Li, S.Y., Chen, Q., Sun, J.Q., Liang, W.X., Zhou, Y.H., Chu, C.C., Li, X.G., Ren, F.G., Klaus, P., Zhao, B., Chen, J.F., Chen, M.S., Li, C.Y.: Mutation of the rice *narrow leaf1* gene, which encodes a novel protein, affects vein patterning and polar auxin transport. - Plant Physiol. **147**: 1947-1959, 2008.

Sazuka, T., Kamiya, N., Nishimura, T., Ohmae, K., Sato, Y., Imamura, K., Nagato, Y., Koshiba, T., Nagamura, Y., Ashikari, M., Kitano, H., Matsuoka, M.: A rice tryptophan deficient dwarf mutant, *tdd1*, contains a reduced level of indole acetic acid and develops abnormal flowers and organless embryos. - Plant J. **60**: 227-241, 2009.

Tanabe, S., Ashikari, M., Fujioka, S., Takatsuto, S., Yoshida, S., Yano, M., Yoshimura, A., Kitano, H., Matsuoka, M., Fujisawa, Y., Kato, H., Iwasaki, Y.: A novel cytochrome P_{450} is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, *dwarf11*, with reduced seed length. - Plant Cell **17**: 776-790, 2005.

Tsuda, K., Nori, K., Hajime, O., Sarah, H.: Genome-wide study of *KNOX* regulatory network reveals brassinosteroid catabolic genes important for shoot meristem function in rice. - Plant Cell **26**: 3488-3500, 2014.

Tsukaya, H.: Mechanism of leaf-shape determination. - Annu. Rev. Plant Biol. **57**: 477-496, 2006.

Ueguchi-Tanaka, M., Murata, K., Yamazaki, M., Onosato, K., Miyao, A., Hirohiko, H.: Three distinct rice cellulose synthase catalytic subunit genes required for cellulose synthesis in the secondary wall. - Plant Physiol. **133**: 73-83, 2003.

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T.Y., Hsing, Y.I., Kitano, H., Yamaguchi, I., Matsuoka, M.: *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. - Nature **437**: 693-698, 2005.

Wang, S.K., Wu, K., Yuan, Q.G., Liu, X.Y., Liu, Z.B., Lin, X.Y., Zeng, R.Z., Zhu, H.T., Dong, G.J., Qian, Q., Zhang, G.Q., Fu, X.D.: Control of grain size, shape and quality by *OsSPL16* in rice. - Nat. Genet. **44**: 950-954, 2012.

Wei, X.J., Tang, S.Q., Shao, G.N., Chen, M.L., Hu, Y.C., Hu, P.S.: Fine mapping and characterization of a novel dwarf and narrow-leaf mutant *dnl1* in rice. - Genet. mol. Res. **12**: 3845-3855, 2013.

Weingartner, M., Helvia, R.P., Pavla, B., Karin, Z., Balázs, M., Consuelo, T., Erwin, H.B., László, B.: A plant cyclin B2 is degraded early in mitosis and its ectopic expression shortens

G2-phase and alleviates the DNA-damage checkpoint. - J. cell. Sci. **116**: 487-498, 2002.

Xiao, R., Wang, J.G., Liu, C.Y., Wang, Y., Wang, Y.Q., Zhai, J.X., Liu, J., Hong, X.H., Cao, X.F., Zhu, J.K., Gong, Z.Z.: *ROR1/RPA2A*, a putative replication protein A2, functions in epigenetic gene silencing and in regulation of meristem development in *Arabidopsis*. - Plant Cell **18**: 85-103, 2006.

Yamamuro, C., Ihara, Y., Wu, X., Noguchi, T., Fujioka, S., Takatsuto, S., Ashikari, M., Kitano, H., Matsuoka, M.: Loss of function of a rice *brassinosteroid insensitive1* homolog prevents internode elongation and bending of the lamina joint. - Plant Cell **12**: 1591-1606, 2000.

Yan, C., Yan, S., Zeng, X., Zhang, Z., Gu, M.: Fine mapping and isolation of *bc7*(t), allelic to *OsCesA4*. - J. Genet. Genomics **34**: 1019-1027, 2007.

Yang, C.H., Li, D.Y., Liu, X., Ji, C.J., Hao, L.L., Zhao, X.F., Li, X.B., Chen, C.Y., Cheng, Z.K., Zhu, L.H.: *OsMYB103*L, an *R2R3-MYB* transcription factor, influences leaf rolling and mechanical strength in rice (*Oryza sativa* L.). - BMC Plant Biol. **14**: 158-173, 2014.

Yoshikawa, T., Eiguchi, M., Hibara, K.I., Ito, J.I., Nagato, Y.: Rice *SLENDER LEAF 1* gene encodes cellulose synthase-like D4 and is specifically expressed in M-phase cells to regulate cell proliferation. - J. exp. Bot. **64**: 2049-2061, 2013.

Yoon, J., Cho, L.H., Kim, S.L., Choi, H., Koh, H.J., An, G.: The BEL1-type homeobox gene *SH5* induces seed shattering by enhancing abscission-zone development and inhibiting lignin biosynthesis. - Plant J. **79**: 717-728, 2014.

Zhang, B., Zhou, Y.: Rice brittleness mutants: a way to open the 'black box' of monocot cell wall biosynthesis. - J. Integr. Plant Biol. **532**: 136-142, 2011.

Zhang, B., Deng, L., Qian, Q., Xiong, G., Zeng, D., Li, R., Guo, L., Li, J., Zhou, Y.: A missense mutation in the trans-membrane domain of *CESA4* affects protein abundance in the plasma membrane and results in abnormal cell wall biosynthesis in rice. - Plant mol. Biol. **71**: 509-524, 2009.

Zhang, F.T., Fang, J., Sun, C.H., Li, R.B., Luo, X.D., Xie, J.K., Deng, X.J., Chu, C.C.: Characterization of a rice dwarf and twist leaf 1 (*dtl1*) mutant and fine mapping of *DTL1* gene. - Hereditas **34**: 79-86, 2012.

Zhang, M., Zhang, B., Qian, Q., Yu, Y., Li, R., Zhang, J., Liu, X., Zeng, D., Li, J., Zhou, Y.: Brittle *Culm12*, a dual-targeting kinesin-4 protein, controls cell-cycle progression and wall properties in rice. - Plant J. **63**: 312-328, 2010.

Zhang, Q.F., Shen, B.Z., Dai, X.K., Mei, M.H., Saghaia, M., Maroofo, Z., Li, B.: Using bulked extremes and recessive class to map genes for photoperiod-sensitive genic male sterility in rice. - Proc. nat. Acad. Sci. USA **91**: 8675-8679, 1994.

Zhao, J., Wang, T., Wang, M., Liu, Y., Yuan, S., Gao, Y., Yin, L., Sun, W., Peng, L., Zhang, W., Wan, J., Li, X.: *DWARF3* participates in an SCF complex and associates with *DWARF14* to suppress rice shoot branching. - Plant Cell Physiol. **55**: 1096-1109, 2014.

Zhou, F., Lin, Q.B., Zhu, L.H., Ren, Y.L., Zhou, K.N., Shabek, N.Z., Wu, F.Q., Mao, H.B., Dong, W., Gan, L., Ma, W.W., Gao, H., Chen, J., Yang, C., Wang, D., Tan, J.J., Zhang, X., Guo, X.P., Wang, J.L., Jiang, L., Liu, X., Chen, W.Q., Chu, J.F., Yan, C.Y., Ueno, K., Ito, S., Asami, T., Cheng, Z.J., Wang, J., Lei, C.L., Zhai, H.Q., Wu, C.Y., Wang, H.Y., Zheng, N., Wan, J.M.: *D14-SCFD3*-dependent degradation of *D53* regulates strigolactone signaling. - Nat. Genet. **504**: 406-410, 2014.