the plant journal

SEB Society for Experimental Biology

The Plant Journal (2012) **72**, 829–842 doi: 10.1111/j.1365-313X.2012.05126.x

Dwarf and deformed flower 1, encoding an F-box protein, is critical for vegetative and floral development in rice (*Oryza sativa* L.)

Yuanlin Duan[1,2,†,*], Shengping Li[1,†], Zhiwei Chen[1,2], Leilei Zheng[1], Zhijuan Diao[1,2], Yuanchang Zhou[1,2], Tao Lan[1,2], Huazhong Guan[1,2], Runsen Pan[1,2], Yongbiao Xue[3] and Weiren Wu[1,2,*]

[1]*Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Fujian Agriculture & Forestry University, Fuzhou 350002, China,*

[2]*Fujian Provincial Key Laboratory of Molecular Marker-Assisted Breeding of Rice, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China, and*

[3]*State Key Laboratory of Molecular Developmental Biology, Institute of Genetics & Developmental Biology, Chinese Academy of Sciences and National Center for Plant Gene Research, Beijing 100101, China*

Received 5 May 2012; revised 7 August 2012; accepted 9 August 2012; published online 15 October 2012.
*For correspondence (e-mails: wuwr@fjau.edu.cn; ylduan863@fjau.edu.cn).
†These authors contributed equally to this work.

SUMMARY

Recent studies have shown that F-box proteins constitute a large family in eukaryotes, and play pivotal roles in regulating various developmental processes in plants. However, their functions in monocots are still obscure. In this study, we characterized a recessive mutant *dwarf and deformed flower 1-1* (*ddf1-1*) in *Oryza sativa* (rice). The mutant is abnormal in both vegetative and reproductive development, with significant size reduction in all organs except the spikelet. *DDF1* controls organ size by regulating both cell division and cell expansion. In the *ddf1-1* spikelet, the specification of floral organs in whorls 2 and 3 is altered, with most lodicules and stamens being transformed into glume-like organs and pistil-like organs, respectively, but the specification of lemma/palea and pistil in whorls 1 and 4 is not affected. *DDF1* encodes an F-box protein anchored in the nucleolus, and is expressed in almost all vegetative and reproductive tissues. Consistent with the mutant floral phenotype, *DDF1* positively regulates B-class genes *OsMADS4* and *OsMADS16*, and negatively regulates pistil specification gene *DL*. In addition, *DDF1* also negatively regulates the Arabidopsis *LFY* ortholog *APO2*, implying a functional connection between *DDF1* and *APO2*. Collectively, these results revealed that *DDF1*, as a newly identified F-box gene, is a crucial genetic factor with pleiotropic functions for both vegetative growth and floral organ specification in rice. These findings provide additional insights into the molecular mechanism controlling monocot vegetative and reproductive development.

Keywords: *Oryza sativa* L., *dwarf and deformed flower 1*, F-box, vegetative growth, floral organ development.

INTRODUCTION

F-box proteins are characterized by a conserved 'F-box' motif consisting of approximately 50 amino acids at the N terminus (Bai *et al.*, 1994; Kipreos and Pagano, 2000). Most F-box proteins reported so far have been identified as components of the Skp, Cullin, F-box-containing (SCF) complex that functions in defining substrate specificity in the ubiquitin–proteasome pathway. Several other F-box proteins with function in non-SCF complex have also been found (Clifford *et al.*, 2000; Galan *et al.*, 2001). F-box genes belong to a large family in plants, containing 42 groups, each of which has a distinct domain organization (Xu *et al.*, 2009). Studies have indicated that F-box genes play crucial roles in a number of biological processes in plants, including flower development, self-incompatibility, hormone response, circadian clock and photomorphogenesis, senescence, defense response, embryogenesis and seedling development (Moon *et al.*, 2004; Lechner *et al.*, 2006; Jain *et al.*, 2007; Schumann *et al.*, 2011). Until now, however, our knowledge about the functions of F-box genes in plants has remained very limited. Fewer than 5% in Arabidopsis F-box proteins (Schumann *et al.*, 2011) and only a few proteins in *Oryza sativa* (rice; Gomi *et al.*, 2004;

Ikeda *et al.*, 2007; Jain *et al.*, 2007) have been characterized for their functions.

Floral homeotic genes, most of which are MADS-box genes, have been known to play pivotal roles in specifying floral organs in eudicots. The molecular genetic mechanisms underpinning the development of floral organs by floral homeotic genes were first elucidated in Arabidopsis and *Antirrhinum* as the ABC model (Coen and Meyerowitz, 1991). The model is also applicable to rice, with modifications, although monocot flowers are quite different from eudicot in morphology (Ambrose *et al.*, 2000; Lee *et al.*, 2003; Nagasawa *et al.*, 2003; Whipple *et al.*, 2004; Kater *et al.*, 2006; Yamaguchi *et al.*, 2006; Dreni *et al.*, 2007; Ikeda *et al.*, 2007). In rice, the functions of B-class genes *OsMADS4* and *OsMADS16* were proven to be largely conserved (Nagasawa *et al.*, 2003), whereas the functions of C-class genes *OsMADS3* and *OsMADS58* were shown to have diverged from the Arabidopsis *AGAMOUS* gene, and the carpel specification appeared to be mainly determined by a distinct non-MADS-box homeotic gene *DROOPINGLEAF* (*DL*; Yamaguchi *et al.*, 2004, 2006). Nevertheless, a recent study on the *Osmads3 Osmads58* double mutant suggested that the two genes together exhibit a complete C-function in rice (Dreni *et al.*, 2011).

In addition to the ABC genes, several genes that regulate the expression of ABC genes in Arabidopsis have been identified, including *unusual floral organs* (*UFO*; Bowman *et al.*, 1992; Sakai *et al.*, 1995) and *Skp1-like 1* (*ASK1*; Zhao *et al.*, 1999). *UFO* encodes an F-box protein that mainly regulates B-class genes (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). The loss of *UFO* function results in homeotic transformation of floral organs in the second and third whorls, without greatly affecting the first and fourth whorls (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). *ASK1* shows somewhat similar effects to those of *UFO* on floral organ specification in terms of their mutant phenotypes. The proteins of these two genes interact to control floral organ identity in whorls 2 and 3. Interestingly, ASK1 is quite similar in sequence to the *Saccharomyces cerevisiae* (yeast) SKP1 protein, whereas UFO contains an F-box, a motif known to interact with SKP1 in yeast (Zhao *et al.*, 1999). Although these two genes act together in flower development, their functions in vegetative growth are quite different. A mutation in *ASK1* affects both vegetative growth and reproductive development, whereas a mutation in *UFO* does not influence vegetative growth (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). In the *ask1-1* mutant, rosette leaves are smaller and internodes in the floral stem are shorter than the wild type (Zhao *et al.*, 1999). Compared with Arabidopsis, much less is known about the regulation of floral organ identity genes in rice (Ikeda *et al.*, 2007; Horigome *et al.*, 2009; Xiao *et al.*, 2009; Duan *et al.*, 2010).

Vegetative growth and reproductive development are both important for high yield in cereal crops because plant architecture is the foundation of reproductive development, and floral development directly influences the quantity and quality of cereal grains. To date, only a few F-box genes with pleiotropic effects on vegetative growth and reproductive development have been identified. In rice, only *aberrant panicle organization 1* (*APO1*), the ortholog of Arabidopsis *UFO*, has been cloned and functionally characterized for its function in leaf emergence, panicle architecture and floral organ identity (Ikeda *et al.*, 2005, 2007; Ikeda-Kawakatsu *et al.*, 2009).

Here, we report a new pleiotropic F-box gene *DDF1* in rice. The *ddf1-1* plant is dramatically dwarfed, with much smaller vegetative organs and panicles, and its spikelet exhibits obvious defects in lodicule and stamen specification, resembling the defects found in Arabidopsis *ufo* mutants. *DDF1* is expressed throughout the whole plant, and can regulate the expression of floral homeotic genes involved in lodicule and stamen specification. This work demonstrates that *DDF1* is a critical gene for whole-plant development in rice.

RESULTS

ddf1-1 exhibits significant size reduction in the whole plant, except the spikelet

The mutant *ddf1-1* was discovered from a breeding population. The *ddf1-1* plant is much shorter and thinner than the wild type, and this difference is distinct at the early seedling stage and becomes more apparent as the plants develop. The final plant height of the mutant reaches only about half of that of the wild type (Figure 1a–e and Figure S1). All vegetative organs in *ddf1-1*, including stems, internodes, leaves and roots (both fibrous and lateral), are significantly shorter and thinner than in the wild type (Figure 1f–j and Figures S2 and S3). In addition, the *ddf1-1* panicle is also notably shorter and smaller, consisting of fewer primary and secondary branches and spikelets (Figure 1e,f; Figure S4). These phenotypes suggest that plant growth in *ddf1-1* is seriously stunted. However, the size of the spikelet, the numbers of various vegetative organs (e.g. internodes and tillers; Figure S5) and the time from sowing to heading (Figure 1e) remain unaffected in *ddf1-1*.

Cell division and cell expansion are inhibited in *ddf1-1*

The size of an organ depends on both cell number and cell size, which are related to cell division and cell expansion, respectively. A decrease in either cell number or cell size, or both, can result in a decrease of organ size. To find out the causes of organ size reduction in *ddf1-1*, we conducted microscopic observation on the internal structure of vegetative organs of the mutant by paraffin section. The results showed that in the *ddf1-1* leaf, parenchyma cells (Figure 2a,b) and epidermal cells (Figure 2c,d) are significantly reduced in size, mesophyll cells and abaxial sclerenchyma cells are notably reduced in number, and the abaxial

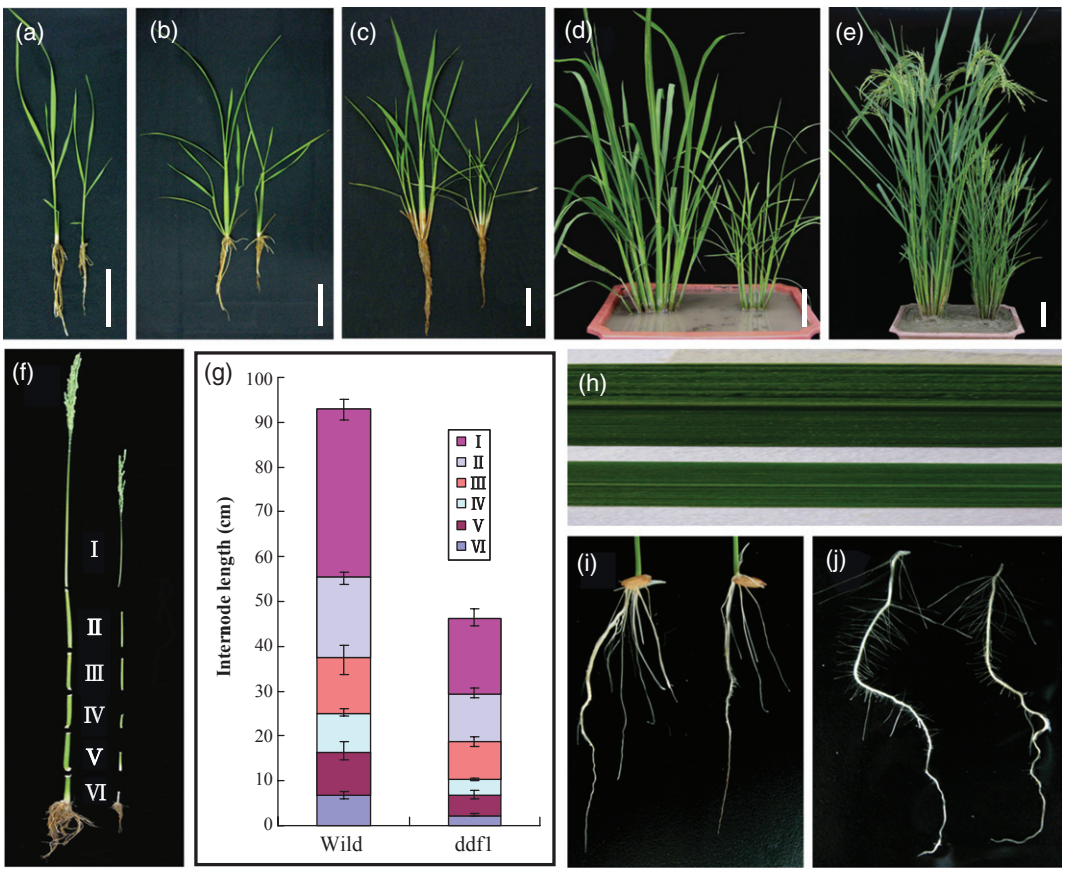

Figure 1. Comparison of morphological characters between *ddf1-1* and wild-type plants.
(a–e) Wild-type (left) and *ddf1-1* (right) plants at 15 (a), 30 (b), 45 (c), 60 (d) and 120 (e) days after sowing, respectively. Scale bars: 10 cm.
(f, g) Comparison of internode length of the main culm between wild type (left) and *ddf1-1* (right); I–VI, top-one to top-six internodes.
(h) Matured leaves of wild-type (upper) and *ddf1-1* (lower) plants.
(i, j) Comparison of fibrous roots (i) and lateral roots (j) in 7-day-old seedlings between wild-type (left) and *ddf1-1* (right) plants.

sclerenchyma cells are even completely absent in some cases (Figure 2a,b). In parallel with the reduction in cell size and cell number, the size of leaf veins in *ddf1-1* is reduced and the number of leaf veins is greatly decreased (to 36–44, with an average of approximately 40, compared with 60–76, with an average of approximately 68, in the wild type; Figure 2a,b).

Significant decreases in cell size and cell number are also observed in the *ddf1-1* root (Figure 2e–h) and stem (Figure 2i–n). Similar to the case of leaf veins, the number and size of vascular bundles in the *ddf1-1* stem are also reduced (to 24–28, with an average of approximately 26, compared with 30–36, with an average of approximately 33, in the wild type; Figure 2k,l). It is noticeable that both the root apical meristem (RAM) region (Figure 2e,g) and the shoot apical meristem (SAM) region (Figure 2i,j) in *ddf1-1* are significantly smaller than in the wild type. As all underground organs (roots) and above-ground organs (stems, leaves and flowers) are established from RAM and SAM by cell division, respectively, the decrease of RAM and SAM regions might be the direct cause of the dwarfism and thin stature of *ddf1-1*.

Based on the above observations, we conclude that the overall dwarfism and thin stature of *ddf1-1* is caused by the reduction of both cell number and cell size, suggesting that both cell division and cell expansion are suppressed in *ddf1-1*.

The expression of cell division/expansion-related genes is downregulated in *ddf1-1*

The above results of histological analysis suggest that *DDF1* probably plays essential roles in plant growth by affecting both cell division and cell expansion in rice. Many genes related to cell division or cell expansion have been known, such as xyloglucan endotransglycosylase-related genes (*XTR*), α-*Expansin* (*EXPA*), *Ras*-like nuclear GTPase (*RAN*) and *Histone* (Tobina *et al.*, 2003; Chen *et al.*, 2011). To better understand the results of histological observation and the role of *DDF1* in plant growth, we examined the expression of two genes that are mainly related to cell division (*Histone H4* and *OsRAN2*) and two genes that are mainly related to cell elongation (*OsXTR2* and *OsEXPA2*; Table S1) in *ddf1-1* young leaves by quantitative RT-PCR (qRT-PCR). The results

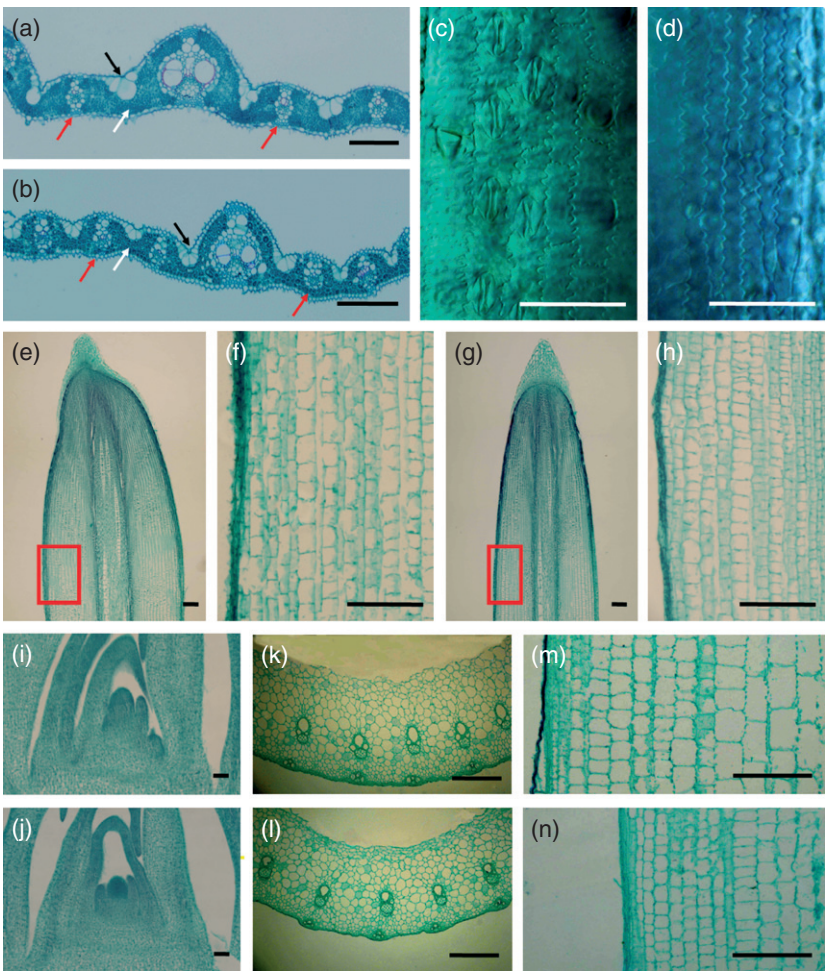

Figure 2. Histological characterization of the leaf, root and stem of *ddf1-1* plants.
(a, b) Transverse sections of wild-type (a) and *ddf1-1* (b) leaves. Black arrow, parenchyma cells; white arrow, mesophyll cells; red arrow, abaxial sclerenchyma cells.
(c, d) Leaf epidermal cells in wild-type (c) and *ddf1-1* (d) plants.
(e–h) Longitudinal section of root tip of wild-type (e, f) and *ddf1-1* (g, h) plants. (f) and (h) are enlarged pictures of the regions indicated by red rectangles in (e) and (g), respectively.
(i, j) Stem primordia of wild-type (i) and *ddf1-1* (j) plants.
(k, l) Transverse sections of the top-second internode of wild-type (k) and *ddf1-1* (l) plants.
(m, n) Longitudinal sections of the top-second internode of wild-type (m) and *ddf1-1* (n) plants.
Scale bars: c, d, f, h, k–n, 50 μm; a, b, e, g, 100 μm; i, j, 200 μm.

showed that the expression levels of all the genes tested are dramatically decreased in *ddf1-1* (Figure 3). This is compatible with the morphological phenotype of *ddf1-1* and the results of histological analysis, suggesting that *DDF1* probably affects cell size and cell number by regulating the expression of cell division/expansion-related genes, so as to direct vegetative organs developing into their normal sizes.

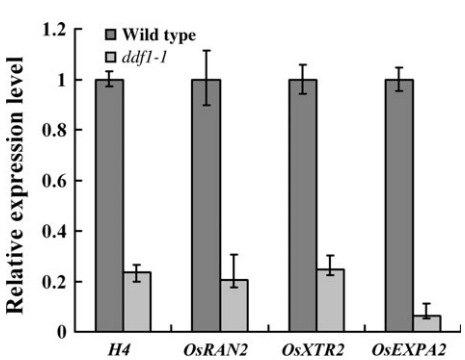

Figure 3. Expression analysis of several genes related to cell elongation and division in young leaves of wild-type and *ddf1-1* plants.

ddf1-1 also shows significant structural abnormalities in florets

The *ddf1-1* floret size is normal, but its floral morphology is abnormal (Figure 4a). A wild-type floret comprises four whorls of floral organs: a lemma and a palea (whorl 1), two lodicules (whorl 2), six stamens (whorl 3) and a carpel (whorl 4; Figure 4b,i). The *ddf1-1* floret also has four whorls, but its floral organs all exhibit morphological abnormalities to different degrees. The *ddf1-1* lemma and palea are both somewhat distorted in shape and cannot close together tightly (Figure 4a). Inside them, the two lodicules in whorl 2 are thoroughly transformed into glume-like organs (GLOs; Figure 4c–f). In some cases there are one or two extra GLOs generated in whorl 2 (Figure 4j,k; Table 1). In whorl 3, the six stamens are transformed into a variable number (usually 4–6) of pistil-like organs (PLOs; Figure 4c–f; Table 1), with occasionally one or two stamens remaining at the site (Figure 4f,k; Table 1). The PLOs are obviously aberrant, and do not have a uniform appearance (Figure 4d,f,j,k). Rarely, a chimera of stamen and pistil tissues can be found in whorl 3 (Figure 4f). Neither the remaining stamens nor the

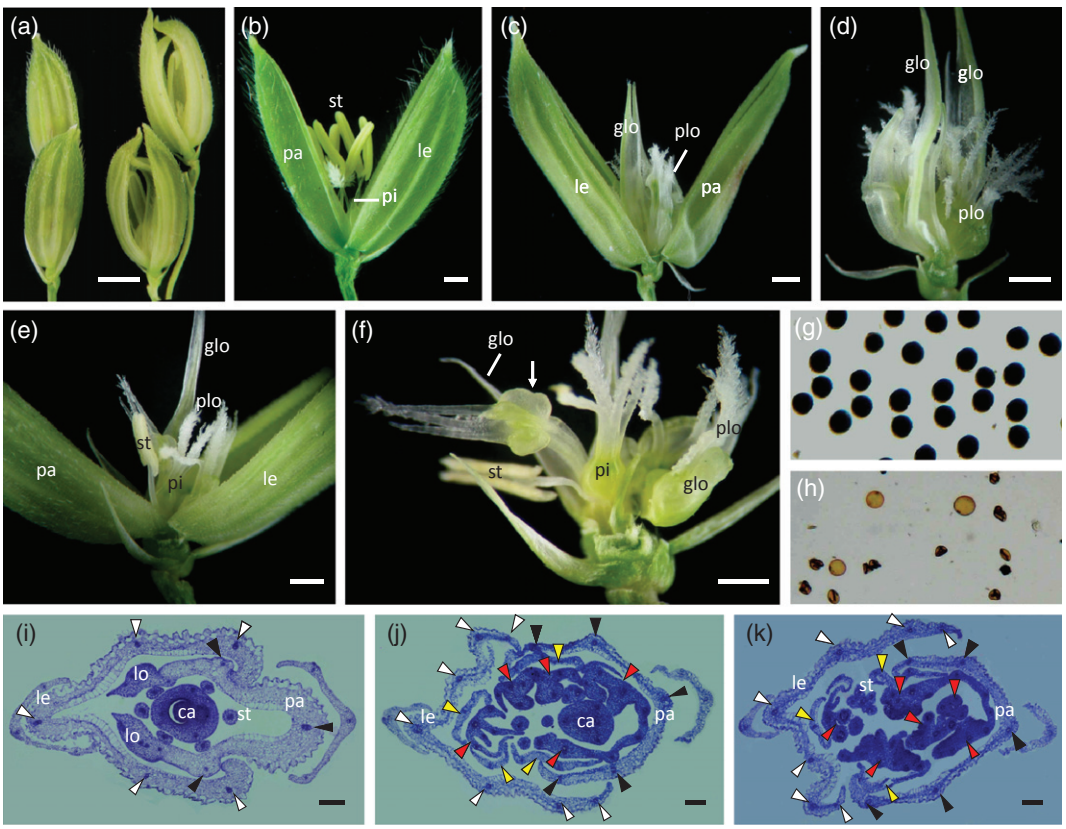

Figure 4. Phenotypes of *ddf1-1* florets.
(a) Spikelets of wild-type (left) and *ddf1-1* (right) plants.
(b) An open wild-type floret.
(c–f) Two typical *ddf1-1* florets: the lemma and palea were removed in (d) and (f), and the white arrow in (f) indicates a chimera of stamen and pistil tissues.
(g, h) Pollen grains in wild-type stamen (g), and the remaining stamen or chimeric organ of *ddf1-1* (h).
(i–k) Transverse sections of wild-type (i) and *ddf1-1* (j, k) spikelets; white and blank arrowheads indicate vascular bundles in lemma and palea, respectively; yellow and red arrowheads indicate glume-like organs (glo) and pistil-like organs (plo); le, lemma; lo, lodicule; pa, palea; pi, pistil; st, stamen. Scale bars: a, 5 mm; b–f, 1 mm; i–k, 100 μm.

Table 1 Numbers of floral organs in whorls 2 and 3 in *ddf1-1* florets[a]

	Whorl 2			Whorl 3							
	Glume-like organs			Stamen			Pistil-like organs				Chimeric organs
Whorl	1	2	>2	0	1	2	3	4	5	6	1
Numbers	14	157	65	215	17	4	7	83	94	52	9
Percentage	5.9	66.5	25.4	91.1	7.2	1.7	3.0	35.2	39.8	22.0	3.8

[a]A total of 236 *ddf1-1* florets were observed.

stamen–pistil chimera can produce fertile pollen grains (Figure 4g,h). Therefore, nearly all *ddf1-1* florets are male sterile. Nevertheless, the pistil in whorl 4 still has normal function in most *ddf1-1* florets, which can produce seeds after being pollinated by wild-type pollens, although the pistil may contain a few (usually one or two) additional styles in some cases (Figure 4f,j).

Transverse section analysis of *ddf1-1* florets confirmed the results of morphological observation (Figure 4i–k). In particular, it clearly revealed the structural abnormalities in *ddf1-1* lemma and palea. The *ddf1-1* lemma and palea are thinner than the wild-type ones, but they possess more vascular bundles (there are five and three vascular bundles in the wild-type lemma and palea, respectively, whereas

there are seven and five vascular bundles in the *ddf1-1* lemma and palea, respectively; Figure 4i–k). These structural abnormalities are likely to cause the distorted shapes of *ddf1-1* lemma and palea (Figure 4j,k), which in turn result in an imperfect match between the lemma and palea, leading to a partly opened floret in *ddf1-1* (Figure 4a).

To further clarify the morphogenesis of *ddf1-1* florets, we examined young spikelets of both the mutant and the wild type at early developmental stages by scanning electron microscopy (Figure 5). In a wild-type floret, floral organ primordia emerge from the floral primordium, and develop into the correct organs from the outermost whorl (lemma and palea) to the innermost whorl (carpel; Figures 5a–d). In the *ddf1-1* floret, the primordia of the four-whorl floral organs can also emerge in the correct sequence at the right positions. However, although the primordia in whorls 1 and 4 can correctly develop into lemma/palea (Figure 5e–i) and pistil (Figure 5g–i), respectively, the primordia in whorls 2 and 3 have changed identities, developing into variable numbers of GLOs (Figure 5f–i) and PLOs (Figure 5g–i), respectively.

The above observations indicate that in typical *ddf1-1* florets, the identities of lodicule and stamen are seriously altered, and these organs have been transformed into variable numbers of GLOs and PLOs, respectively, whereas the identities of lemma/palea and pistil are hardly affected, suggesting that the *DDF1* gene is critical for the specification of lodicule and stamen in rice.

The *ddf1-1* phenotype is caused by a single nucleotide substitution

We had previously mapped the *DDF1* gene to a 165-kb region on the long arm of chromosome 6 (Li *et al.*, 2011). In this study, using a larger F_2 population, and several new SSR and InDel markers (Table S2) that we have developed ourselves, we further narrowed down the position of *DDF1* to a 45-kb interval, in which there were eight annotated open reading frames (Figure 6a). Sequence analysis revealed that one of the eight genes had a single base transversion

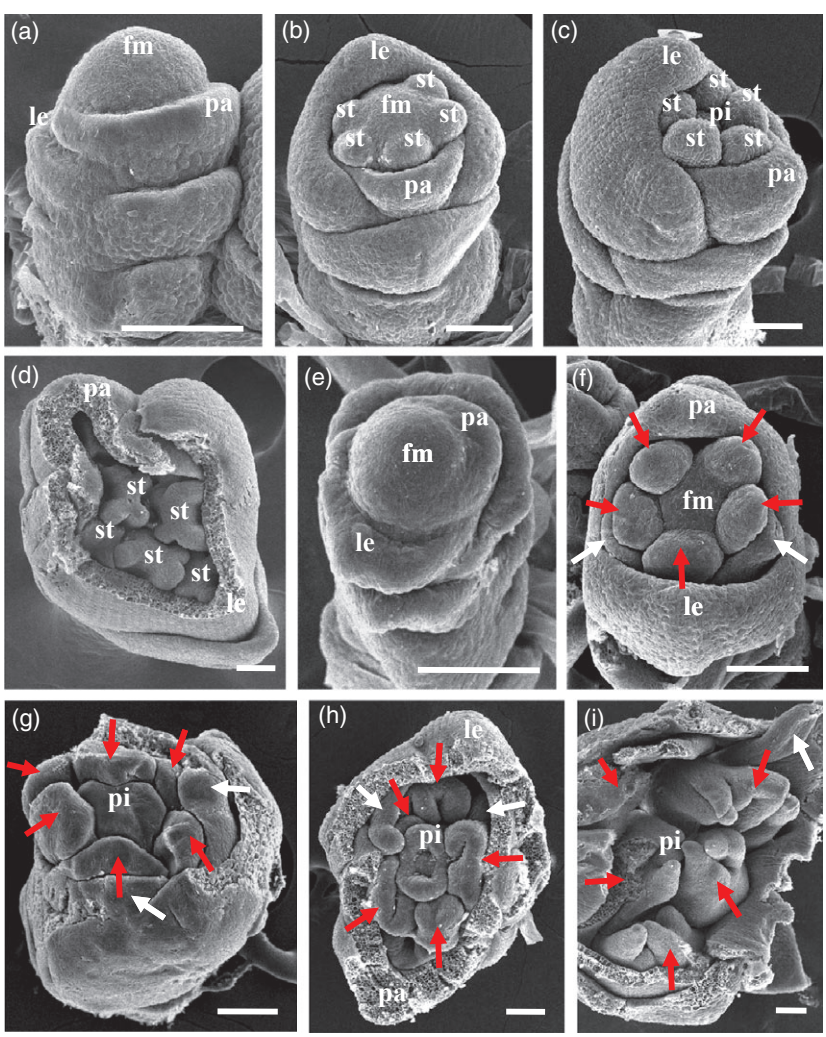

Figure 5. Scanning electron microscopy of wild-type and *ddf1-1* spikelets at early developmental stages.
(a–d) Wild-type spikelets. (e–i) *ddf1-1* spikelets. White arrows indicate two glume-like organs transformed from lodicule primordia; red arrows indicate pistil-like organ primordia or pistil-like organs transformed from stamens. Abbreviations: fm, floral meristem; glo, glume-like organs; le, lemma; lo, lodicule; pa, palea; pi, pistil; plo, pistil-like organs; st, stamen. Scale bars: 50 μm.

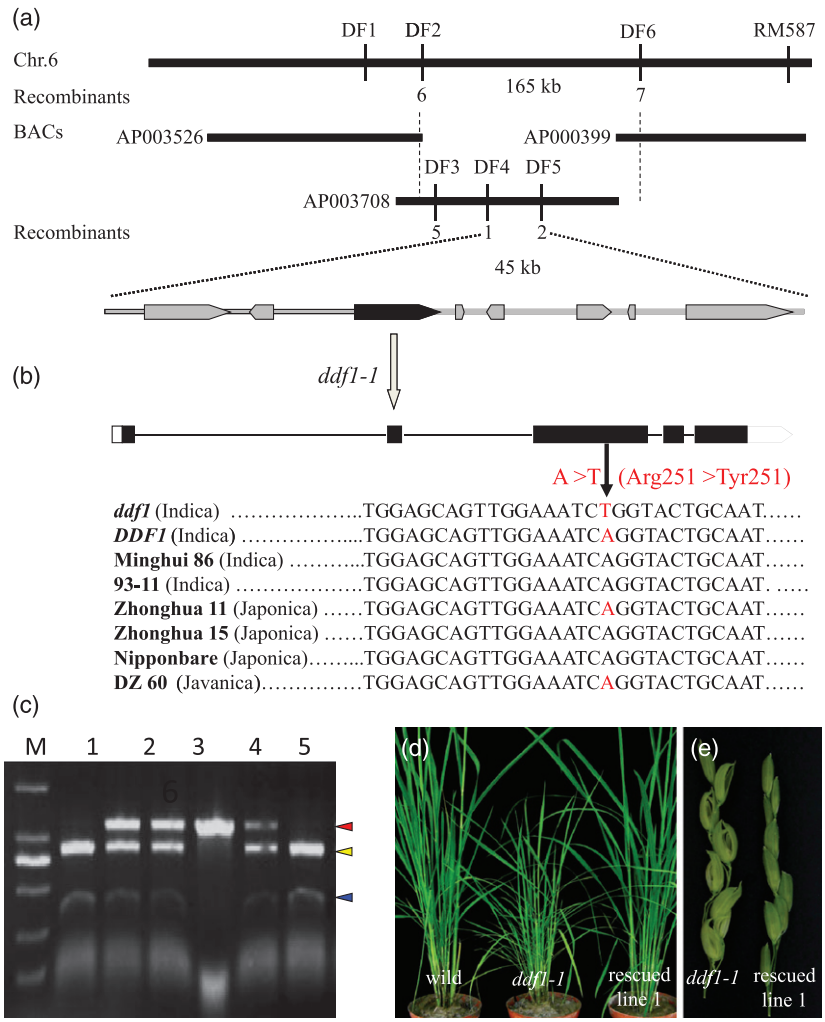

Figure 6. Map-based cloning of *DDF1*.
(a) Physical mapping of *DDF1*. The number under each marker indicates recombination events that occurred between the marker and *DDF1*.
(b) Mutation position of *ddf1-1*. A single base transversion from A to T was found inside the third exon of a gene in *ddf1-1*. Six other varieties all have the wild-type allele.
(c) Co-segregation analysis of the mutation site. Only the mutant showed a 1174-bp band (indicated by the red arrowhead), the wild type showed two digested bands, at 867 bp (yellow arrowhead) and 317 bp (blue arrowhead), respectively, and the heterozygote showed all the three bands. Lane M, DL2000 molecular weight marker; lanes 1–6, *ddf1-1* heterozygote selfing progeny plants.
(d, e) Genetic complementation of the *ddf1-1* mutant. In the rescued lines, all the mutant phenotypes (including dwarf stature, small stem, leaves, root and panicle, and deformed floral organs) were recovered; (d) and (e) indicate the phenotype of plant and spikelet.

(A → T) inside the third exon, resulting in a conversion of Arg to Tyr at the 251st amino acid in the encoded polypeptide (Figure 6b); no base alteration was found in the other seven genes. By examining this base in six other rice varieties, we found that they all belonged to the wild-type genotype (Figure 6b). In addition, we found that this base happened to be located in a restriction site of enzyme *Alw*NI, and that its transversion mutation removed the restriction in *ddf1-1*. Hence, we could conveniently use *Alw*NI to detect the single nucleotide polymorphism (SNP) at the mutated base (A versus T) in the F_2 population. The result showed that this SNP site completely co-segregated with the mutant trait (Figure 6c). The above results strongly suggest that this single base transversion mutation was likely to be the molecular cause of *ddf1-1* mutant phenotypes. Therefore, we took this gene as the candidate of *DDF1*.

To validate the candidate gene, we constructed a plasmid *pCAMBIA1300-DDF1* carrying an 8869-bp wild-type genomic DNA, which covered the entire *DDF1* candidate gene and its upstream promoter region. By introducing the plasmid into *ddf1-1*, all the mutant phenotypes (including dwarf stature, smaller stems, leaves, roots and panicles, and aberrant spikelet) were rescued (Figure 6d,e). Thus, the *DDF1* gene was confirmed.

DDF1 encodes an LRR-type F-box protein, anchored in nucleolus

DDF1 covers approximately 4.5 kb of the genome, comprising five exons and four introns (Figure 6b). A BLAST search and protein function analysis (http://smart.embl-heidelberg.de) revealed that *DDF1* encodes a putative leucine-rich repeat (LRR)-type F-box protein consisting of 519 amino acids (Figure S6). Further analysis identified five other LRR-type F-box proteins with surprisingly high similarities to DDF1 in rice. Among them, Os06g04690 and Os06g04980 have an identity of approximately 96 and 90% to DDF1, respectively, and their genes are both located closely to *DDF1* in the genome, implying that there might be tandem or segmental duplication events occurring in that region (Xu *et al.*, 2009). In addition, DDF1 also showed high similarity to

HSLF2-S1, an LRR-type F-box protein of *Hordeum bulbosum*, which is possibly related to self-incompatibility (Kakeda, 2009; Figure S6).

To understand the evolution of DDF1, we constructed a phylogenetic tree based on the full amino acid sequences of DDF1, HSLF2-S1 and another 16 F-box proteins involved in the regulation of flower development, self-incompatibility, hormone response, circadian clock and photomorphogenesis. The results indicated that *DDF1* constituted a clade with *HSLF2-S1*, whereas the other sixteen genes, including *APO*, *UFO* and *FIM* (Fimbriata), which act on flower development, were separate from this clade (Figure 7a), suggesting that DDF1 is possibly an ortholog of *Hordeum HSLF2-S1*.

Moreover, we constructed a DDF1-GFP fusion protein expression vector to transform young onion epidermal cells with *Agrobacterium tumefaciens*. The result indicated that the DDF1-GFP fusion protein is expressed specifically in nucleus (Figure 7a), suggesting that DDF1 is a nuclear protein.

The expression pattern of *DDF1* is consistent with the phenotypes of *ddf1-1*

To reveal the expression pattern of *DDF1* we constructed a fusion of *DDF1* promoter and GUS (β-glucuronidase) reporter gene, and transferred it into rice via *A. tumefaciens*. A histochemical examination of GUS activity in transgenic plants showed that *DDF1* is expressed almost in all tissues throughout the lifetime of the plant (Figure 8a), which is in agreement with the overall plant mutant phenotype of *ddf1-1*. During early vegetative growth, *DDF1* expression is strong in all vegetative organs, including root (Figure 8aA), leaf (Figure 8aB), sheath (Figure 8aC) and young stem (Figure 8aD), but is weak in the stem apical meristem (Figure 8aD). At the jointing stage, *DDF1* expression is strong in node regions and medium in elongating internodes, but is very weak or even null in developed internodes (Figures 8aE–G). This is compatible with the inference obtained above that *DDF1* plays important roles in both cell proliferation and cell expansion.

The spatial and temporal expression pattern of *DDF1* in reproductive organs also appears to be consistent with the phenotypes of *ddf1-1*. In young panicles, *DDF1* expression is strong in both spikelets (especially in lemma and palea) and branches (Figures 8aG,H). As the development of the spikelet proceeds, *DDF1* expression in lemma and palea gradually declines, whereas that in stamens gradually increases (Figures 8aI,J). In mature flowers, *DDF1* expression is mainly concentrated in stamens, and is also found in lodicules, to a considerable degree, but is weak or even absent in lemma, palea, filaments and pistil (Figures 8aI–L). We also employed the *in situ* hybridization to analyze the expression pattern of *DDF1* in young spikelets at early developmental stages (Figure 8b). *DDF1* expression was detected in floral meristems and the primordia of all floral organs, including lemma, palea, lodicule, stamen and carpel, and was especially strong in stamen primordia and developing stamens (Figure 8bA–D). These results were largely in agreement with those of the GUS analysis.

DDF1 regulates the expression of several floral organ specification genes

The floral phenotype of *ddf1-1* and the expression pattern of *DDF1* in the floret strongly suggest that *DDF1* possibly regulates the expression of floral organ identity genes during floral organ differentiation. To address this question, we analyzed the transcription levels of nine floral organ identity genes by qRT-PCR, including three *AP1*-like genes (*OsMADS14*, *OsMADS15* and *OsMADS18*), three B-class genes (*OsMADS2*, *OsMADS4* and *OsMADS16*), two C-class genes (*OsMADS3* and *OsMADS58*) and one carpel specification gene (*DL*), in young panicles of 2–20 mm in length (i.e. within stage 7 of inflorescence, the period of floral organ differentiation; Ikeda *et al*., 2004) from *ddf1-1* and wild-type plants. The results showed that the two B-class genes *OsMADS4* and *OsMADS16* are significantly downregulated in

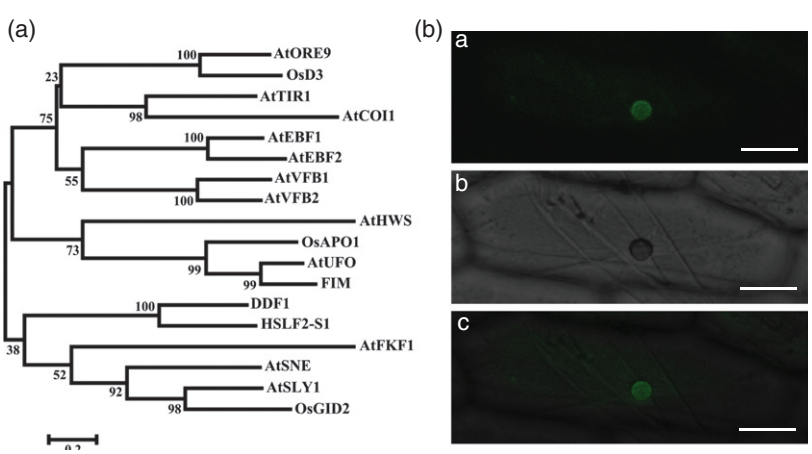

Figure 7. Sequence analysis and subcellular location of DDF1.
(a) A phyllogenetic tree generated from the full deduced amino acid sequences of DDF1 and some other F-box proteins from rice, Arabidopsis, *Antirrhinum* and *Hordeum* using the neighbor-joining method. Bootstrap values generated with 100 replicates are indicated beside the nodes. The bar on the bottom indicates the branch length equivalent to 0.2 amino acid changes per residue.
(b) Subcellular location of DDF1 with GFP: (A) GFP filter; (B) bright field; (C) merged image of GFP filter and bright field. Scale bars: 50 μm.

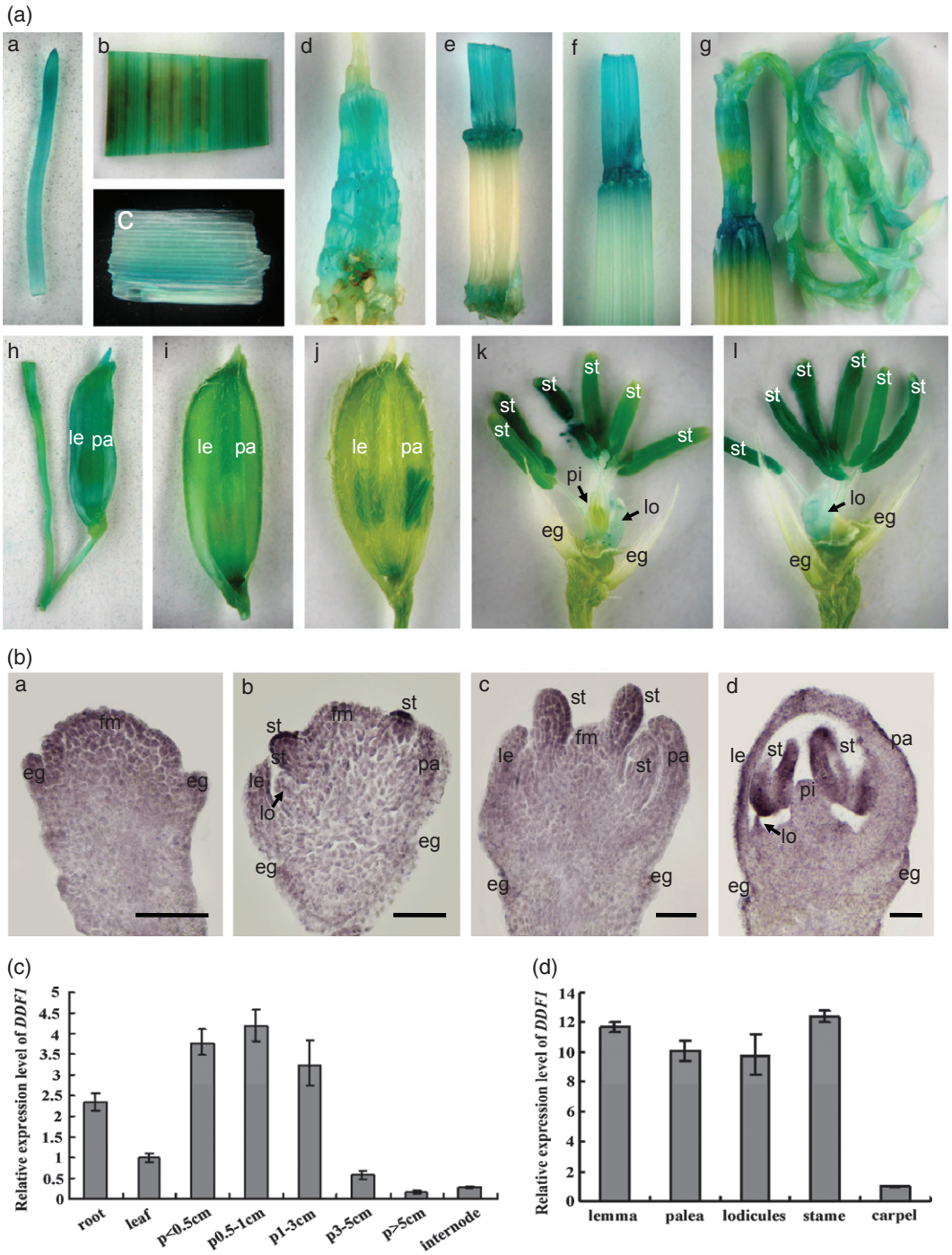

Figure 8. Expression pattern of the *DDF1* gene during vegetative growth and floral organ development in rice.
(a) GUS analysis of *DDF1* expression: (A) root; (B) leaf; (C) sheath; (D) stem at tillering stage; (E, F) culm at bolting stage; (G) young panicle; (H, I) young and developing spikelet; (J–L) maturing spikelet; lemma and palea were removed in (K), and lemma, palea and pistil were removed in (L). Abbreviations: e.g, extra glum; le, lemma; pa, palea; pi, pistil; st, stamen.
(b) Localization of *DDF1* transctipts in young wild-type spikelets. Abbreviations: e.g, empty glume; fm, floral meristem; le, lemma; lo, lodicule; pa, palea; pi, pistil; st, stamen. Scale bars: 50 μm.

ddf1-1, whereas *DL* is significantly upregulated (Figure 9a). Among the *AP1*-like genes, *OsMADS14* and *OsMADS15* appeared to be slightly upregulated and downregulated, respectively, whereas *OsMADS18* remained unchanged.

To validate the qRT-PCR results and further discern the relationships between *DDF1* and floral organ specification genes, we examined the temporal and spatial expression patterns of two downregulated genes (*OsMADS4* and

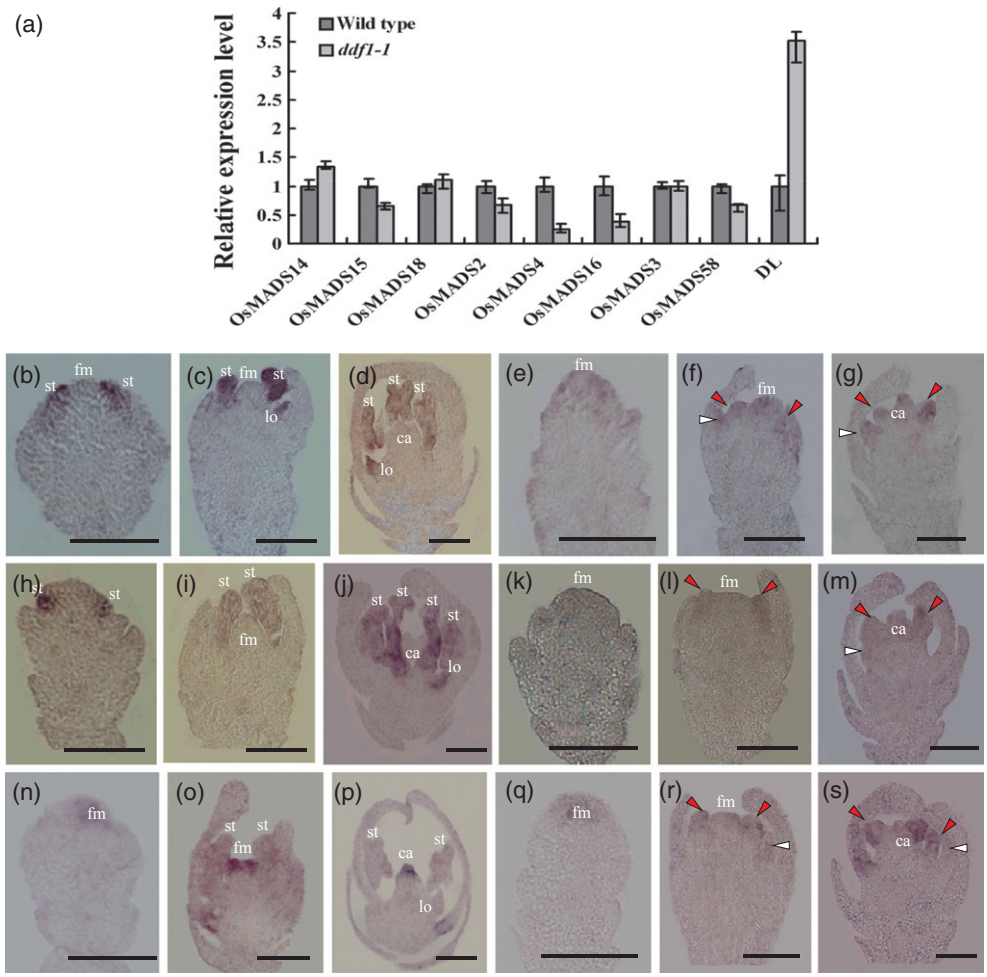

Figure 9. Expression analyses of floral identity genes in *ddf1-1* and wild-type young spikelets.
(a) qRT-PCR analyses of the expression of floral identity genes.
(B–S) RNA *in situ* hybridization analysis of *OsMADS4* and *OsMADS16* and *DL* expression.
(b–g) Expression of *OsMADS4* in wild-type (b–d) and *ddf1-1* (e–g) spikelets.
(h–m) Expression of *OsMADS16* in wild-type (h–j) and *ddf1-1* (k–m) spikelets.
(n–s) Expression of *DL* in wild-type (n–p) and *ddf1-1* (q–s) spikelets. Red arrowheads indicate pistil-like organ primordia or pistil-like organs; white arrowheads indicate glume-like organ primordia or glume-like organs. Abbreviations: ca, carpel; fm, floral meristem; lo, lodicule; st, stamen. Scale bars: 100 μm.

OsMADS16) and one upregulated gene (*DL*) by *in situ* RNA hybridization in wild-type and *ddf1-1* plants (Figures 9b–s).

The two B-class genes *OsMADS4* and *OsMADS16* showed similar expression patterns in the wild-type spikelet. They were both expressed in the primordia of lodicule and stamen, as expected, and the expression was detectable throughout the development of the two floral organs (Figures 9b–d,h–j; Nagasawa *et al.*, 2003). In the *ddf1-1* spikelet, the two genes were also expressed in the whorls in which they were expected, but their expression levels were significantly reduced. Counter to expectation, no expression was detected in the initiating regions of whorls 2 and 3 where GLOs and PLOs should develop, respectively (Figure 9e,k), and only very weak expression was observed during the development of GLOs and PLOs (Figure 9f,g,l,m).

DL plays a specific role in carpel development (Yamaguchi *et al.*, 2004). In the wild-type floret, *DL* expression was first detected in the region where carpel primordia should initiate (carpel anlagen). Afterwards, the expression domain of *DL* was restricted to the carpel anlagen and carpel primordia (Figures 9n–p; Yamaguchi *et al.*, 2004). In the *ddf1-1* floret, the *DL* expression domain was expanded to the primordia in whorl 3, where the ectopic PLOs should develop, and the expression of *DL* continued during the development of both PLOs and the original carpel (Figure 9s). The expansion of the expression domain might explain the higher *DL* expression level in *ddf1-1* young panicles than in the wild type.

Taken together, the results of qRT-PCR analysis and *in situ* RNA hybridization analysis were consistent, suggesting that *DDF1* positively regulates the expression of

OsMADS4 and *OsMADS16*, and negatively regulates the expression of *DL* during lodicule and stamen specification.

DDF1 negatively controls *APO2* expression

Aberrant panicle organization 2 (*APO2*) is the ortholog of Arabidopsis *LEAFY* (*LFY*) in rice. *APO2* shows a similar expression pattern to that of *DDF1* in developing spikelets; its loss-of-function mutant also exhibits somewhat similar phenotypes to those of *ddf1-1* (Ikeda-Kawakatsu *et al.*, 2012). These similarities between *APO2* and *DDF1* imply that the two genes might be closely related in function. To understand the relationship between them, we examined *APO2* expression in young *ddf1-1* panicles by qRT-PCR. The result showed that *APO2* expression is enhanced in *ddf1-1* compared with that in the wild type (Figure S7), suggesting that *DDF1* negatively controls *APO2* transcription.

DISCUSSION

DDF1 is generally required for normal organ size in rice

Cell division and cell expansion are the foundations of plant growth and development, and enable the plant and various organs to develop into the correct sizes. Recent studies have uncovered some key regulatory genes (growth-promoting and growth-restricting factors) and pathways that affect plant organ size by altering either cell number or cell size, or both (Krizek, 2009; Guo *et al.*, 2010; Moyroud *et al.*, 2010). However, the intrinsic mechanisms responsible for organ size variation are yet to be well understood (Guo *et al.*, 2010).

In this study, we have identified an F-box gene *DDF1* that appears to play a general role in the control of organ size in rice. In the mutant *ddf1-1*, all vegetative organs exhibit a significant reduction in size (Figure 1). Inside the organs, both cell number and cell size are apparently reduced (Figure 2), suggesting that both cell division and cell expansion are inhibited in the mutant. Consistent with this, the expression levels of four genes, *Histone H4*, *OsRAN2*, *OsXTR2* and *OsEXPA2*, which are essential for cell division and cell expansion in rice (Cho and Kende, 1997; Huang *et al.*, 2000; Uozu *et al.*, 2000; Jan *et al.*, 2004; Yokoyama *et al.*, 2004; Mu *et al.*, 2005; Zang *et al.*, 2010; Chen *et al.*, 2011), are all dramatically downregulated in *ddf1-1* (Figure 3). In addition, the expression of *DDF1* in culms, as revealed by GUS detection, is concentrated in the cell division and elongation zone of internodes, but is very weak or even undetectable in the mature zone of internodes (Figure 8aD–F). These results strongly suggest that *DDF1* positively controls both cell division and cell expansion, and controls organ size by affecting cell division and cell expansion concurrently.

In addition to vegetative organs, *DDF1* also affects the size of the inflorescence. Similar to the situation of vegetative organs, the size of panicle in *ddf1-1* is also greatly reduced, with significantly fewer primary branches and spikelets (Figure 1e,f and Figure S4). Studies have shown that the number of primary branches in rice is determined by the initial size of the reproductive shoot apex, and that the number of florets per primary branch is determined by cell division activity in the subsequent growth of the apex (Mu *et al.*, 2005). In *ddf1-1*, the initial size of reproductive shoot apex is obviously smaller than that in the wild type (Figure 2). This is consistent with the phenotype of smaller panicles in *ddf1-1*.

DDF1 is also required for normal flower development in rice

Floral ABC genes are specifically expressed in the floral meristem and in the primordia of floral organs generated from the floral meristem to elaborate floral organ structures. Proper regulation of floral homeotic gene expression ensures the development of floral organs in the correct number, type and spatial arrangement (Liu and Mara, 2010). To date, several genes that control the development of floral organs via regulating the expression of ABC genes in specific domains have been identified (Bowman *et al.*, 1992; Sakai *et al.*, 1995; Yamaguchi *et al.*, 2004; Liu and Mara, 2010). However, knowledge on the regulation of floral organ identity genes in rice remains very limited.

In this study, we have identified a new F-box gene *DDF1* that plays a crucial role in the specification of floral organs in whorls 2 and 3. In most *ddf1-1* spikelets, lodicules and stamens in whorls 2 and 3 are transformed into GLOs and PLOs, respectively, whereas the identity specification of lemma/palea in whorl 1 and pistil in whorl 4 is hardly affected (Figure 4). According to the ABC model, the floral phenotype of *ddf1-1* implies a reduction of B function and an increase of C function in the mutant florets, suggesting that *DDF1* positively regulates B-function genes but negatively regulates C-function genes. This was verified by the results of qRT-PCR and *in situ* hybridization analyses, in which the expression of B-class genes *OsMADS4* and *OsMADS16* was significantly reduced, whereas the expression of *DL*, a specific gene with strong C function, predominantly determining carpel identity in rice (Yamaguchi *et al.*, 2004), was significantly increased, and its expression domain was also expanded from the carpel primordium in whorl 4 to the stamen primordia in whorl 3, where the PLOs subsequently emerged (Figure 9).

Taken together, we have reason to infer that *DDF1* affects the specification of floral organs in whorls 2 and 3 by regulating B-function and C-function genes (i.e. enhancing the former, but suppressing the latter). Therefore, *DDF1* is also crucial for flower development in rice.

The possible molecular mechanism of *DDF1* function

The F-box gene *UFO* plays a critical role in flower development in Arabidopsis, and controls the specification of floral

organs in whorls 2 and 3 by positively regulating the expression of B-class genes (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). This is quite similar to the function of *DDF1* in rice. Studies indicated that *UFO* activates B-class genes by cooperating with *LFY*, which encodes a plant-specific transcriptional factor (Weigel *et al.*, 1992; Levin and Meyerowitz, 1995; Honma and Goto, 2000). More recently, it was revealed that UFO functions as a co-factor for LFY-induced transcription of B-class gene *AP3*, and regulates the activity of LFY in a proteasome-dependent manner (Chae *et al.*, 2008). It has been proposed that the molecular interaction between UFO and LFY in Arabidopsis might represent a general mechanism in plants, as homologs of UFO and LFY have been identified in a wide array of plant species (Chae *et al.*, 2008), and some studies have shown that UFO or its homologs are activators of LFY proteins (Lippman *et al.*, 2008; Souer *et al.*, 2008).

APO1 and *APO2* are the orthologs of *UFO* and *LFY* in rice, respectively. The mechanism of UFO–LFY interaction has proven to be conserved in rice, although the functions of *APO1* and *APO2* have partially diverged from those of *UFO* and *LFY* (Ikeda *et al.*, 2007; Ikeda-Kawakatsu *et al.*, 2012). *APO1* and *APO2* both affect vegetative and reproductive development simultaneously. Their loss-of-function mutants display similar phenotypes in plant growth and inflorescence differentiation, namely, shortened plastochron, more leaves and a smaller inflorescence, with fewer spikelets (Ikeda *et al.*, 2005, 2007; Ikeda-Kawakatsu *et al.*, 2012). These similarities between *apo1* and *apo2* suggest a close relationship between *APO1* and *APO2*. It has been demonstrated that *APO2* negatively controls *APO1* transcription, and that APO1 and APO2 act cooperatively to control panicle development (Ikeda-Kawakatsu *et al.*, 2012).

Nevertheless, the phenotypes of *apo1* and *apo2* spikelets are quite different. In *apo1*, stamens are transformed into lodicules, whereas in *apo2*, lodicules are transformed into glume-like organs and stamens are transformed into carples and lodicules (Ikeda *et al.*, 2005, 2007; Ikeda-Kawakatsu *et al.*, 2012). In contrast, the *apo2* spikelet appears quite similar to that of *ddf1-1*. Moreover, the *APO2* expression pattern in floral meristems and developing floral organ primordia is similar to that of *DDF1* as well. These similarities imply that there might be a close relationship between *APO2* and *DDF1*. The functional similarities between *UFO* and *DDF1* mentioned above also provide a clue for the possible connection between *APO2* and *DDF1*. This is preliminarily supported by the result of qRT-PCR analysis (Figure S7), in which *DDF1* appears to negatively control *APO2* transcription.

In addition, studies have suggested that Arabidopsis LFY and its homologs in other plants have reserved an ancestral role in regulating cell division and arrangement (Moyroud *et al.*, 2010). This has proven true for *APO2* in rice, which decides leaf size by regulating the cell number (Ikeda-Kawakatsu *et al.*, 2012). *APO1* has also been found to affect cell division, which promotes cell proliferation in the meristem (Ikeda-Kawakatsu *et al.*, 2009). This might result from the interaction between *APO1* and *APO2*, explaining how APO1 and APO2 cooperatively control panicle development in rice (Ikeda-Kawakatsu *et al.*, 2012). Likewise, *DDF1* controls organ size via affecting cell division and expansion, and the phenotype of reduced leaf and panicle sizes in *apo2* partially resembles that in *ddf1-1*. The analogue of the relationship between *DDF1* and *APO2* with that between *APO1* and *APO2* on the genetic control of cell division and plant development further suggests that *DDF1* might also function in connection with *APO2* as *APO1*. In other words, both APO1 and DDF1 might be required to activate APO2 at different sites and times during plant development. Thus, *ddf1-1* and *apo1* might each reveal a part of the *APO2* function.

In conclusion, as a newly identified F-box gene in rice, *DDF1* is crucial for vegetative growth and reproductive development. Although its precise mechanism is still unclear, we still have reasons to speculate that DDF1 might act as a co-factor for APO2-induced transcription of floral homeotic genes (e.g. *OsMADS4*, *OsMADS16* and *DL*), and regulate the activity of APO2 in a proteasome-dependent manner. It will be interesting to further investigate whether and how DDF1 acts as a co-factor to activate APO2 at different sites and times during plant development in rice.

EXPERIMENTAL PROCEDURES

Plant materials

The *ddf1-1* mutant was discovered in rice breeding materials (*Oryza sativa* sub. *indica* cultivar). As the mutant almost couldn't set seed, the mutant *ddf1-1* was maintained through heterozygotes, which were identified using their progeny lines. The homozygous normal individuals in the progeny lines of *ddf1-1* heterozygotes were used as the wild-type control.

Microscopic observation

Young spikelet, young root, the top-second internode and leaf were observed by paraffin section. The materials were fixed with a formalin: acetic acid: alcohol solution at 4°C overnight, dehydrated in an ethanol series, completely stained in phenodin, rinsed to transparency in xylene, embedded in paraffin, sliced into 8–10-μm sections and attached to microscope slides, and finally observed and photographed using a light microscope (VANOX AH3; Olympus, http://www.olympus-global.com).

Scanning electron microscopy observation

Young panicles (1–10 mm) were observed by scanning electron microscopy (SEM; Duan *et al.*, 2010). Panicles were fixed in 2.5% glutaric dialdehyde and washed with a sodium phosphate buffer (0.1 M, pH 7.0), further fixed in 1% osmic acid for 1–2 h, and again washed with the sodium phosphate buffer (0.1 M, pH 7.0), dehydrated with an ethanol series, incubated in ethanol-isoamyl acetate and then in isoamyl acetate, dried, mounted and coated with gold,

and finally observed with an XL30 ESEM scanning electronic microscope (Phillips, http://www.philips.com).

Positional cloning of DDF1

The *ddf1* locus was previously mapped onto a 165-kb region between two simple sequence repeats markers, DF2 and DF5 (Li *et al.*, 2011). An F_2 population of 2525 mutants was further used to narrow down the *ddf1* locus onto a 45-kb region. The candidate gene was identified by sequencing analysis. An 8869-bp DDF1 genomic DNA fragment covering a 3730-bp promoter and a 609-bp 3′ region was subcloned into a binary vector *pCAMBIA1300* and introduced into *ddf1-1* mutant embryonic calli by *Agrobacterium tumefaciens*-mediated transformation (Hiei *et al.*, 1994).

Promoter activity analysis

A 3724-bp promoter located upstream of the coding region was fused to the GUS reporter gene with the nopaline synthase terminator and cloned into pCAMBIA1391Z (*DDF1-GUS*). The *DDF1-GUS* plasmid was introduced into EHA105 and further into japonica cultivar Zhonghua-15 to generate 46 independent transgenic lines. A histochemical assay for GUS activity in transgenic plants was performed as described by Jefferson *et al.* (1987).

Subcellular localization

The DDF1-GFP fusion was made by in-frame fusion of the 1536-bp full-length *DDF1* coding sequence with GFP. The fusion gene was inserted into vector pCAMBIA1302T and further into EHA105. Transient expression of the DDF1-GFP fusion and GFP alone (as a control) in onion epidermal cells was performed as described by Eady *et al.* (2000). The samples were observed with an OLYMPUS IX71 microscope.

Real-time quantitative PCR

The expression levels of genes for cell division and expansion in leaves of a young seedling, the expression traits of *DDF1* in roots, leaves, stems, young panices and spikelet, the expression levels of floral identity genes in young panicles (5–10 mm in length) and the expression levels of *APO2* in young panicles (5–10 and 10–20 mm in length) were analyzed using real-time quantitative RT-PCR. Isolation of total RNAs was performed using Trizol Reagent kit (Invitrogen, http://www.invitrogen.com). Reverse transcription of total RNA was performed using the Primescript™ RT reagent kit (TaKaRa, http://www.takara-bio.com). The cDNA samples were diluted to 8 and 2 ng μl^{-1}. Triplicate quantitative assays were performed using SYBR Premix Ex Taq II (TaKaRa) with a Mastercycler ep realplex^4 sequence detection system (Eppendorf, http://www.eppendorf.com). Amplification of *Actin* was used as an internal control to normalize all data. The primers used for qRT-PCR analysis are listed in Table S1.

mRNA in situ hybridization

mRNA *in situ* hybridization was essentially performed as previously described by Lai *et al.* (2002). Young panicles (2–20 mm in length) were fixed with formalin : acetic acid : alcohol solution and embedded in paraffin. The tissues were sliced into 8-mm sections with a rotary microtome and attached to microscope slides. The primers used for mRNA *in situ* hybridization are listed in Table S3.

ACKNOWLEDGEMENTS

We thank Dr Baocai Tan (Chinese University of Hong Kong, Hong Kong) for a critical reading of the article. We are grateful to the anonymous reviewers for offering valuable suggestions to improve the article. We also thank Zhiqin Guan, Rong Zheng, Weiqi Tang, Lihui Lin, Xuzhang Zhang, Damei Mao, Fan Yang, Yichang Liu and Hanjun Wu (Fujian Agriculture & Forestry University) for their help with the experiment. This work was supported by the National Natural Science Foundation of China (30970175 and 30671122), Research Foundation for the Doctoral Program of Higher Education of China (20093515110003), National High Technology Research and Development Program of China (2006AA10Z128), and Natural Science Foundation of Fujian Province of China (B0620001).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Plant height of the wild type and *ddf1-1* on different days after sowing.

Figure S2. Internode diameter of wild-type and *ddf1-1* plants.

Figure S3. Leaf size of wild-type and *ddf1-1* plants.

Figure S4. Primary branch number, secondary branch number and spikelet number of wild-type and *ddf1-1* plants.

Figure S5. Tiller number of wild-type and *ddf1-1* plants at different days after sowing.

Figure S6. Alignment of deduced amino acid sequences of DDF1 and some other LRR-type F-box proteins from rice and *Hordeum*.

Figure S7. qRT-PCR analyses of *APO2* expression levels in wild-type and *ddf1-1* young panicles.

Table S1. Primers used for quantitative RT-PCR analysis.

Table S2. Primers used for fine-mapping *DDF1*.

Table S3. Primers used for the RNA *in situ* hybridization assay.

Please note: As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

REFERENCES

Ambrose, B.A., Lerner, D.R., Ciceri, P., Padilla, C.M., Yanofsky, M.F. and Schmidt, R.J. (2000) Molecular and genetic analyses of the *silky1* gene reveal conservation in floral organ specification between eudicots and monocots. *Mol. Cell*, **5**, 569–579.

Bai, C., Richman, R. and Elledge, S.J. (1994) Human cyclin F. *EMBO J.* **13**, 6087–6098.

Bowman, J.L., Sakai, H., Jack, T., Weigel, D., Mayer, U. and Meyerowttz, E. (1992) *SUPERMAN,* a regulator of floral homeotic genes in *Arabidopsis*. *Development*, **114**, 599–615.

Chae, E., Tan, Q.K., Hill, T.A. and Irish, V.F. (2008) An Arabidopsis F-box protein acts as a transcriptional co-factor to regulate floral development. *Development*, **135**, 1235–1245.

Chen, N., Xu, Y., Wang, X., Du, C., Du, J., Yuan, M., Xu, Z. and Chong, K. (2011) *OsRAN2*, essential for mitosis, enhances cold tolerance in rice by promoting export of intranuclear tubulin and maintaining cell division under cold stress. *Plant Cell Environ.* **34**, 52–64.

Cho, H.T. and Kende, H. (1997) Expression of expansin genes is correlated with growth in deepwater rice. *Plant Cell*, **9**, 1661–1671.

Clifford, R., Lee, M.H., Nayak, S., Ohmachi, M., Giorgini, F. and Schedl, T. (2000) FOG-2, a novel F-box containing protein, associates with the GLD-1 RNA binding protein and directs male sex determination in the C. elegans hermaphrodite germline. *Development*, **127**, 5265–5276.

Coen, E.S. and Meyerowitz, E.M. (1991) The war of the whorls: genetic interactions controlling flower development. *Nature*, **353**, 31–37.

Dreni, L., Jacchia, S., Fornara, F., Fornari, M., Ouwerkerk, P.B.F., An, G., Colombo, L. and Kater, M.M. (2007) The D-lineage MADS-box gene Os *MADS13* controls ovule identity in rice. *Plant J.* **52**, 690–699.

Dreni, L., Pilatone, A., Yun, D., Erreni, S., Pajoro, A., Caporali, E., Zhang, D. and Katera, M.M. (2011) Functional analysis of all *AGAMOUS* subfamily members in rice reveals their roles in reproductive organ identity determination and meristem determinacy. *Plant Cell*, **23**, 2850–2863.

Duan, Y.L., Diao, Z.J., Liu, H.Q., Cai, M.S., Wang, F. and Wu, W.R. (2010) Molecular cloning and functional characterization of *OsJAG* gene based on a complete-deletion mutant in rice (*Oryza sativa* L.). *Plant Mol. Biol.* **74**, 605–615.

Eady, C.C., Weld, R.J. and Lister, C.E. (2000) *Agrobacterium tumefaciens* mediated transformation and transgenic-plant regeneration of onion (*Allium cepa* L.). *Plant Cell Rep.* **19**, 376–381.

Galan, J.M., Wiederkehr, A., Seol, J.H., Haguenauer-Tsapis, R., . Deshaies, R.J., Riezman, H. and Peter, M. (2001) Skp1p and the F-box protein Rcy1p form a non-SCF complex involved in recycling of the SNARE Snc1p in yeast. *Mol. Cell. Biol.* **21**, 3105–3117.

Gomi, K., Sasaki, A., Itoh, H., Ueguchi-Tanaka, M., Ashikari, M., Kitano, H. and Matsuoka, M. (2004) 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.

Guo, M., Rupe, M.A., Dieter, J.A., Zou, J., Spielbauer, D., Duncan, K.E., Howar, R.J., Hou, Z. and Simmons, C.R. (2010) Cell number regulator1 affects plant and organ size in maize: implications for crop yield enhancement and heterosis. *Plant Cell*, **22**, 1057–1073.

Hiei, Y., Ohta, S., Komari, T. and Kumashiro, T. (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. *Plant J.* **6**, 271–282.

Honma, T. and Goto, K. (2000) The Arabidopsis floral homeotic gene PISTILLATA is regulated by discrete cis-elements responsive to induction and maintenance signals. *Development*, **127**, 2021–2030.

Horigome, A., Nagasawa, N., Ikeda, K., Ito, M., Itoh, J. and Nagato, Y. (2009) Rice OPEN BEAK is a negative regulator of class 1 knox genes and a positive regulator of class B floral homeotic gene. *Plant J.* **58**, 724–736.

Huang, J., Takano, T. and Akita, S. (2000) Expression of α-expansin genes in young seedlings of rice (*Oryza sativa* L.). *Planta*, **211**, 467–473.

Ikeda, K., Sunohara, H. and Nagato, Y. (2004) Developmental course of inflorescence and spikelet in rice. *Breed. Sci.* **54**, 147–156.

Ikeda, K., Nagasawa, N. and Nagato, Y. (2005) ABERRANT PANICLE ORGANIZATION 1 temporally regulates meristem identity in rice. *Dev. Biol.* **282**, 349–360.

Ikeda, K., Ito, M., Nagasawa, N., Kyozuka, J. and Nagato, Y. (2007) Rice *ABERRANT PANICLE ORGANIZATION 1*, encoding an F-box protein, regulates meristem fate. *Plant J.* **51**, 1030–1040.

Ikeda-Kawakatsu, K., Yasuno, N., Oikawa, T., Iida, S., Nagato, Y., Maekawa, M. and Kyozuka, J. (2009) Expression level of ABERRANT PANICLE ORGANIZATION1 determines rice inflorescence form through control of cell proliferation in the meristem. *Plant Physiol.* **150**, 736–747.

Ikeda-Kawakatsu, K., Maekawa, M., Izawa, T., Itoh, J.I. and Nagato, Y. (2012) ABERRANT PANICLE ORGANIZATION 2/RFL, the rice ortholog of Arabidopsis LEAFY, suppresses the transition from inflorescence meristem to floral meristem through interaction with APO1. *Plant J.* **69**, 168–180.

Jain, M., Nijhawan, A., Arora, R., Agarwal, P., Ray, S., Sharma, P., Kapoor, S., Tyagi, A.K. and Khurana, J.P. (2007) F-Box proteins in rice. Genome-Wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. *Plant Physiol.* **143**, 1467–1483.

Jan, A., Yang, G., Nakamura, H., Ichikawa, H., Kitano, H., Matsuoka, M., Matsumoto, H. and Komatsu, S. (2004) Characterization of a Xyloglucan endotransglucosylase gene that is up-regulated by gibberellin in rice. *Plant Physiol.* **136**, 3670–3681.

Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) Gus fusion: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901–3907.

Kakeda, K. (2009) S locus-linked F-box genes expressed in anthers of Hordeum bulbosum. *Plant Cell Rep.* **28**, 1453–1460.

Kater, M.M., Dreni, L. and Colombo, L. (2006) Functional conservation of MADS-box factors controlling floral organ identity in rice and Arabidopsis. *J. Exp. Bot.* **57**, 3433–3444.

Kipreos, E.T. and Pagano, M. (2000) The F-box protein family. *Genome Biol.* **1**, REVIEWS 3002.1–3002.7.

Krizek, B.A. (2009) Making bigger plants: key regulators of final organ size. *Curr. Opin. Plant Biol.* **12**, 17–22.

Lai, Z., Ma, W., Han, B., Liang, L., Zhang, Y., Hong, G. and Xue, Y. (2002) An F-box gene linked to the self-incompatibility (S) locus of Antirrhinum is expressed specifically in pollen and tapetum. *Plant Mol. Biol.* **50**, 29–41.

Lechner, E., Achard, P., Vansiri, A., Potuschak, T. and Genschik, P. (2006) F-box proteins everywhere. *Curr. Opin. Plant Biol.* **9**, 631–638.

Lee, S., Jeon, J.S., An, K., Moon, Y.H., Lee, S., Chung, Y.Y. and An, G. (2003) Alteration of floral organ identity in rice through ectopic expression of *OsMADS16*. *Planta*, **217**, 904–911.

Levin, J. and Meyerowitz, E.M. (1995) *UFO*: an Arabidopsis gene involved in both floral meristem and floral organ development. *Plant Cell*, **7**, 529–548.

Li, S.P., Duan, Y.L., Chen, Z.W., Guan, H.Z., Wang, C.L., Zheng, L.L., Zhou, Y.C. and Wu, W.R. (2011) Genetic analysis and gene mapping of *DDF1*, a pleiotropic gene involving in both vegetable and reproductive growth in rice. *Hereditas*, **33**, 1374–1379.

Lippman, Z.B., Cohen, O., Alvarez, J.P., Abu-Abied, M., Pekker, I., Paran, I., Eshed, Y. and Zamir, D. (2008) The making of a compound inflorescence in tomato and related nightshades. *PLoS Biol.* **6**, 2424–2435.

Liu, Z. and Mara, C. (2010) Regulatory mechanisms for floral homeotic gene expression. *Semin. Cell Dev. Biol.* **21**, 80–86.

Moon, J., Parry, G. and Estelle, M. (2004) The ubiquitin-proteasome pathway and plant development. *Plant Cell*, **16**, 3181–3195.

Moyroud, E., Kusters, E., Monniaux, M., Koes, R. and Parcy, F. (2010) LEAFY blossoms. *Trends Plant Sci.* **15**, 346–352.

Mu, C., Nemoto, K., You, Z. and Yamagishi, J. (2005) Size and activity of shoot apical meristems as determinants of floret number in rice panicles. *Plant Prod. Sci.* **8**, 51–58.

Nagasawa, N., Miyoshi, M., Sano, Y., Satoh, H., Hirano, H., Sakai, H. and Nagato, Y. (2003) *SUPERWOMAN1* and *DROOPING LEAF* genes control floral organ identity in rice. *Development*, **130**, 705–718.

Sakai, H., Medrano, L.J. and Meferowitz, E.M. (1995) Role of SUPERMAN in maintaining Arabidopsis floral whorl boundaries. *Nature*, **378**, 199–203.

Schumann, N., Navarro-Quezada, A., Ullrich, K., Kuhl, C. and Quint, M. (2011) Molecular evolution and selection patterns of olant F-box proteins with C-terminal kelch repeats. *Plant Physiol.* **155**, 835–850.

Souer, E., Rebocho, A.B., Bliek, M., Kusters, E., de Bruin, R.A.M. and Koes, R. (2008) Patterning of inflorescences and flowers by the F-Box protein DOUBLE TOP and the LEAFY Homolog ABERRANT LEAF AND FLOWER of Petunia. *Plant Cell*, **20**, 2033–2048.

Tobina, H., Uozu, S., Matsuoka, M., Kitano, H. and Hattori, K. (2003) Gene expression pattern of cell division/elongation factors in rice dwarf mutants. In *Advances in Rice Genetics* (Khush, G.S., Brar, D.S. and Hardy, B., eds). Manila: IRRI, pp. 470–472.

Uozu, S., Tanaka-Ueguchi, M., Kitano, H., Hattori, K. and Matsuoka, M. (2000) Characterization of XET-related genes of rice. *Plant Physiol.* **122**, 853–859.

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F. and Meyerowitz, E.M. (1992) LEAFY controls floral meristem identity in Arabidopsis. *Cell*, **69**, 843–859.

Whipple, C.J., Ciceri, P., Padilla, C.M., Ambrose, B.A., Bandong, S.L. and Schmidt, R.J. (2004) Conservation of B-class floral homeotic gene function between *maize* and *Arabidopsis*. *Development*, **131**, 6083–6091.

Wilkinson, M.D. and Haughn, G.W. (1995) *UNUSUAL FLORAL ORGANS* controls meristem identity and floral organ primordia fate in *Arabidopsis*. *Plant Cell*, **7**, 1485–1499.

Xiao, H., Tang, J., Li, Y. *et al.* (2009) *STAMENLESS 1*, encoding a single C2H2 zinc finger protein, regulates floral organ identity in rice. *Plant J.* **59**, 789–801.

Xu, G., Ma, H., Nei, M. and Kong, H. (2009) Evolution of F-box genes in plants: different modes of sequence divergence and their relationships with functional diversification. *PNAS*, **106**, 835–840.

Yamaguchi, T., Nagasawa, N., Kawasaki, S., Matsuoka, M., Nagato, Y. and Hirano, H.Y. (2004) The YABBY gene DROOPING LEAF regulates carpel specification and midrib development in Oryza sativa. *Plant Cell*, **16**, 500–509.

Yamaguchi, T., Lee, D.Y., Miyao, A., Hirochika, H., An, G. and Hirano, H.Y. (2006) Functional diversification of the two C-class genes *OsMADS3* and *OsMADS58* in *Oryza sativa*. *Plant Cell*, **18**, 15–28.

Yokoyama, R., Rose, J.K.C. and Nishitani, K. (2004) A surprising diversity and abundance of Xyloglucan Endotransglucosylase/Hydrolases in rice: classification and expression analysis. *Plant Physiol.* **134**, 1088–1099.

Zang, A., Xu, X., Neill, S. and Cai, W. (2010) Overexpression of *OsRAN2* in rice and Arabidopsis renders transgenic plants hypersensitive to salinity and osmotic stress. *J. Exp. Bot.*, **61**, 777–789.

Zhao, D., Yang, M., Solava, J. and Ma, H. (1999) The *ASK1* gene regulates development and interacts with the *UFO* gene to control floral organ identity in *Arabidopsis*. *Dev. Genet.*, **25**, 209–223.