**FINE CULM1 (FC1) Works Downstream of Strigolactones to Inhibit the Outgrowth of Axillary Buds in Rice**

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(Received April 13, 2010; Accepted June 3, 2010)

Recent studies of highly branched mutants of pea, Arabidopsis and rice have demonstrated that strigolactones (SLs) act as hormones that inhibit shoot branching. The identification of genes that work downstream of SLs is required for a better understanding of how SLs control the growth of axillary buds. We found that the increased tillering phenotype of *fine culm1* (*fc1*) mutants of rice is not rescued by the application of 1 µM GR24, a synthetic SL analog. Treatment with a high concentration of GR24 (10 µM) causes suppression of tiller growth in wild-type plants, but is not effective on *fc1* mutants, implying that proper *FC1* functioning is required for SLs to inhibit bud growth. Overexpression of *FC1* partially rescued *d3-2* defects in the tiller growth and plant height. An in situ hybridization analysis showed that *FC1* mRNA accumulates in axillary buds, the shoot apical meristem, young leaves, vascular tissues and the tips of crown roots. *FC1* mRNA expression was not significantly affected by GR24, suggesting that transcriptional induction may not be the mechanism by which SLs affect *FC1* functioning. On the other hand, the expression level of *FC1* is negatively regulated by cytokinin treatment. We propose that *FC1* acts as an integrator of multiple signaling pathways and is essential to the fine-tuning of shoot branching in rice.

**Keywords:** *FINE CULM1 (FC1) • Rice • Shoot branching • Strigolactone • TCP transcription factor • Tiller.

**Abbreviations:** BAP, benzyl aminopurine; CaMV, cauliflower mosaic virus; CCD, carotenoid cleavage dioxygenase; epi-SDS, 2′-epi-5-deoxystrigol; GUS, β-glucuronidase; IPT, isopentenyltransferase; LC-MS/MS, liquid chromatography–tandem mass spectrometry; RT–PCR, reverse transcription–PCR; SAM, shoot apical meristem; SL, strigolactone

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**Introduction**

Plant architecture is determined by the pattern of shoot branching (McSteen and Leyser 2005). In most higher plants, shoot branches develop from axillary buds in the axils of leaves. Not all of the axillary buds develop, and each is subjected to a decision to continue growth or to become dormant, depending on a complex interplay between environmental and endogenous cues. Plant hormones are major players in the control of axillary bud growth. It has been known for a long time that two hormones in particular, auxin and cytokinin, are involved in this control. Auxin, which is supplied from the apical bud, indirectly suppresses axillary bud outgrowth, while cytokinins directly induce branching. During the past two decades, genetic and physiological analyses in pea and Arabidopsis have predicted the involvement of an additional, novel hormone in the control of shoot branching (for reviews, see Beveridge 2006, Ongaro and Leyser 2008). Recently it was demonstrated that the novel hormone, which inhibits bud outgrowth, is the group of compounds called strigolactones (SLs) or their downstream metabolites (Gomez-Roldan et al. 2008, Umehara et al. 2008).

Prior to the discovery of SLs as the branching hormone, *more axillary growth1* (*max1*) to *max6* in Arabidopsis and five *ramosus* (*rms*) mutants in garden pea (*Pisum sativum*) had been identified as components of a novel graft-transmissible branching signal pathway (Strinberg et al. 2002, Sorefan et al. 2003). Consistent with results obtained from grafting experiments, *max1*, *max3* and *max4* were shown to be SL deficient, and their defects were rescued by the external application of an SL (Gomez-Roldan et al. 2008, Umehara et al. 2008). On the other hand, a mutant of the *MAX2* gene, which encodes an F-box leucine-rich repeat (LRR)-containing protein, was not rescued by the SL (Strinberg et al. 2002). **MAX1** encodes...
CYP71A1, a class III cytochrome P450 (Booker et al. 2005). MAX3 and MAX4 encode carotenoid cleavage dioxygenases (CCDs) (Sorefan et al. 2003, Booker et al. 2004). The SL pathway seems to be well conserved across species (Beveridge and Kyozyuka 2010). Molecular cloning showed that pea RMS1, RMS4 and RMS5 are orthologs of MAX4, MAX2 and MAX3, respectively (Sorefan et al. 2003, Foo et al. 2005, Jhonson et al. 2006, Beveridge et al. 2009). Analyses of branching mutants in rice indicated that the pathway is also conserved in monocot species. We reported on five tillering dwarf mutants of rice, dwarf3 species. We reported on five tillering dwarf mutants of rice, dwarf3, d10, d14, d17 and d27 (Ishikawa et al. 2005). The high tillering dwarf1 (htd1) mutant, which resembles the five mutants, was also described (Zou et al. 2006). After the molecular cloning, it turned out that D3 and D10 are orthologs of MAX2/RMS4 and MAX4/RMS1, respectively (Ishikawa et al. 2005, Arite et al. 2007), while HTD1 encodes an ortholog of MAX3/RMS5, and is the same locus as D17 (Zou et al. 2006, Umehara et al. 2008). Meanwhile, D14 and D27 were shown to be novel genes that work in the SL pathway (Arite et al. 2009, Lin et al. 2009). D27 encodes an iron-containing protein and is likely to be involved in SL biosynthesis (Lin et al. 2009). The d14 mutant, also reported as d88 and htd2, is insensitive to exogenous SL application and contains elevated SL levels (Arite et al. 2009, Gao et al. 2009, Liu et al. 2009). Although its molecular function has not yet been determined, it is postulated that D14 also works in SL signaling (Arite et al. 2009). The mechanisms controlling cross-talk between the hormones are beginning to be elucidated. Recently it was revealed that one role of auxin is to suppress cytokinin biosynthesis in the stem (Tanaka et al. 2006, Shimizu-Sato et al. 2009). When the auxin supply from the apical bud is blocked, expression of isopentenytransferase (IPT) genes, which encode a rate-limiting enzyme of cytokinin biosynthesis, is rapidly up-regulated, and this results in the rapid synthesis of cytokinins in the stem. This cytokinin is transported to axillary buds and induces bud outgrowth. In addition, the auxin-dependent up-regulation of SL biosynthesis genes has been observed in all plant species analyzed so far (Arite et al. 2007, Heyward et al. 2009). Although actual changes in SL levels have not yet been observed, a likely scenario is that the apically derived auxin induces SL biosynthesis, and the SLs act as second messengers to inhibit axillary bud outgrowth. Furthermore, SL biosynthesis is controlled by feedback regulation (Arite et al. 2007, Heyward et al. 2009) and, at least in Arabidopsis, this feedback regulation is mostly dependent on auxin signaling (Heyward et al. 2009). Together, these observations suggest that the growth of axillary buds is controlled by multiple independent and interacting pathways (Dun et al. 2009). Despite the remarkable progress in our understanding of the frameworks that control axillary bud outgrowth, little is known so far about how SLs act to control shoot branching. As a first step towards understanding SL action at the molecular level, we report here that rice FINE CULM1 (FC1) partially works downstream of SLs to inhibit bud outgrowth. We propose that FC1 serves as a hub gene where multiple signals are integrated to fine-tune the development of axillary buds.

Results

Defects of fc1 mutants are not rescued by the application of SLs

Rice plants carrying recessive fc1 mutations show reduced plant height and increases in tiller numbers, thus resembling the tillering dwarf mutants d3, d10, d14, d17 and d27 (Fig. 1A; Takeda et al. 2003, Ishikawa et al. 2005). FC1 was previously reported as OsTBL1, an ortholog of maize teosinte branched (tb1) (Doebely et al. 1997, Takeda et al. 2003). FC1 encodes a protein of 388 amino acids that is a member of the TCP family of transcription factors (Fig. 1B). The TCP family proteins are divided into two subgroups, class I and class II. The class II proteins can be further divided into two clades, CIN and CYC/TBL1. FC1 belongs to the CYC/TBL1 subclade, along with the predicted products of two other rice genes, OsTCP15 and RETARDED PALEA DEVELOPMENT1 (REPI) (Fig. 1C; Yuan et al. 2008, Martin-Trillo and Cubas 2009). The previously described fc1-1 mutant allele contains a single base change resulting in a premature termination codon in the region of the gene that encodes the TCP domain (Fig. 1B; Takeda et al. 2003). We identified a new recessive allele, fc1-2, in a collection of plants that were regenerated from tissue culture (Fig. 1A, B). This mutant carries two deletions spanning a total of 27 bp within the region encoding the TCP domain. The TCP domain is crucial for dimerization and binding to target DNAs (Martin-Trillo and Cubas 2009), and thus it is likely that both fc1-1 and fc1-2 are null alleles of the FC1 locus. In comparison with other tillering dwarf mutants, fc1-1 and fc1-2 show weaker defects in both plant height and tiller number (Arite et al. 2007; Fig. 1A). In the five tillering dwarf mutants of rice the expression level of D10, which encodes a CCD8, is elevated due to feedback regulation, whereas no significant change in D10 expression was observed in the fc1 mutants (Fig. 1D; Arite et al. 2007). To explore the relationship between FC1 and the SL pathway, we first examined whether the defects of the fc1 mutants could be rescued by the exogenous application of an SL. As has been described previously, the application of 1 μM GR24 (a synthetic SL analog) was sufficient to inhibit the outgrowth of the first and second leaf buds of the SL-deficient mutant d10-2 (Fig. 2A, B). In contrast, growth of the first and second leaf buds was not suppressed by the same treatment in fc1-1 and fc1-2 (Fig. 2A, B). The endogenous levels of 2’-epi-SDS, a native SL of rice, were measured in roots (Fig. 2C) and root exudates (Fig. 2D) using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The levels of epi-SDS in roots and root exudates of fc1-2 were not significantly different from those of wild-type plants. These results suggested that FC1 works either independently from, or downstream of, the SL pathway.
Effects of high concentrations of an SL on tiller growth

We previously reported that the application of low concentrations (<1 µM) of an SL did not affect the outgrowth of tiller buds in wild-type plants, while treatment with higher SL levels suppressed the outgrowth (Umehara et al. 2008). In order to determine whether FC1 works independently from, or downstream of, the SL pathway, we tested whether FC1 function is required for the SL to suppress tiller bud outgrowth. Wild-type and mutant plants were grown in hydroponic culture for 6 weeks in the presence of 10 µM GR24 (Fig. 3). In wild-type plants this treatment completely inhibited the outgrowth of the third leaf tiller buds and significantly suppressed the growth of the fourth leaf tiller buds. In contrast, the third and fourth leaf tiller buds were not affected in fc1-1 and fc1-2 by the same treatment. Similar results were seen in d3-2, an SL-insensitive mutant. This indicated that FC1 function is required for SL to exert its effect of inhibiting bud outgrowth.
**Fig. 2.** *fc1* mutants are insensitive to GR24. (A) Responses of *d10-2* and *fc1-2* plants to GR24 application. Two-week-old wild-type (WT) and mutant plants were treated with 1 µM GR24. In the absence of GR24 treatment, the tillers in the axils of the first (blue arrowhead) and second (red arrowhead) leaves did not grow in the wild-type plants but did grow in the mutants. The outgrowth of tillers in *d10-2*, but not in *fc1-2*, was suppressed by the application of GR24. Scale bars = 1 cm. (B) Tiller numbers of *d10-2* and *fc1-2* plants with and without GR24 treatment. The numbers of tillers showing outgrowth (>2 mm) in six seedlings are shown for wild-type Nipponbare [WT (N)] and mutant plants. The blue and red bars indicate the first and second leaf buds, respectively. Error bars represent the SD (n = 6). (C) LC-MS/MS analysis of endogenous levels of epi-5DS in WT (N) and *fc1-2* mutants. Error bars represent the SD (n = 3). (D) LC-MS/MS analysis of epi-5DS levels in root exudates of WT (N) and *fc1-2* mutants. Error bars represent the SD (n = 3).

**Fig. 3.** Interaction of FC1 function and the SL pathway. (A) Responses of *fc1* mutant growth in a high concentration of GR24. Plants were grown in hydroponic culture with or without 10 µM GR24. The tiller lengths of the first to sixth leaves were measured 6 weeks after the initiation of the hydroponic culture. The sums of the average of tiller lengths of three plants are shown. The mutant *fc1-1* was derived from wild-type (WT) Taichung (T), and *fc1-2* and *d3-2* were derived from Nipponbare (N). (B) Partial rescue of the *d3-2* mutant phenotype by overexpression of FC1. Left, wild-type plant; centre, 35S-FC1/*d3-2* plant; right, *d3-2* plant. (C) Double mutant of *fc1-2* *d17-2*.
Genetic interaction between FC1 and the SL pathway

To understand the genetic interaction between FC1 and the SL pathway, FC1 was expressed under the cauliflower mosaic virus (CaMV) 35S promoter in d3-2 plants. Among 17 independent transgenic lines produced, seven lines showed partial rescue of defects observed in d3-2 with regard to the plant height, tiller number and stem thickness (Fig. 3b). Although Takeda et al. (2003) reported that overexpression of FC1 under the CaMV 35S promoter conferred a significant reduction of tiller number in wild-type plants, the effects of FC1 overexpression were not obvious in wild-type plants in our experiments. We crossed fc1-2 with d17-2, an SL-deficient mutant (Umehara et al. 2008). The fc1-2 d17-2 double mutant plant showed the d17-2-like phenotype (Fig. 3C). These analyses supported our notion that FC1 works, at least partially, downstream of the SL pathway.

Spatial distribution of FC1 mRNA

A broad spatial pattern of FC1 expression was described previously (Takeda et al. 2003); however, the details were not available. Because a precise determination of the site of FC1 action is a prerequisite to understanding its mode of action, we examined FC1 mRNA distribution in wild-type plants using RNA in situ hybridization. In serial sections of vegetative shoots, moderate levels of FC1 mRNA were detected in axillary buds (Fig. 4A–E). In rice, axillary meristem formation initiates when the subtending leaf reaches the P4 stage (Oikawa and Kyozuka 2009). Subsequently, the protrusion of a new axillary bud becomes evident at the P5 stage of leaf development. FC1 expression was observed in the axillary bud primordial at the P5 stage (Fig. 4A, C). FC1 mRNA continued to accumulate in the axillary buds through the P6 and P7 stages (Fig. 4A, D, E). FC1 mRNA was also detected in the shoot apical meristem, (SAM) the epidermis of leaves and vascular tissues (Fig. 4B, D, E). Particularly strong expression was observed in young leaves and the tips of crown roots (Fig. 4F–H). Despite its high expression in crown root tips, the role of FC1 in the root is unclear at this stage, since the fc1 mutants show no obvious root defects.

Hormonal control of FC1 mRNA expression

To understand the molecular basis of the relationship between FC1 and SL action, we then examined the control of FC1 expression by hormones. All hormones were supplied to 2-week-old seedlings via the roots. After treatment for 3h, RNA was extracted from the shoot apex containing the SAM, axillary buds, young leaves and nodes. Because our results suggested that FC1 probably functions downstream of SLs, we anticipated that FC1 may be positively regulated by SLs at the level of transcription. However, discordantly with this prediction, FC1 expression was not significantly affected by the application of GR24 (Fig. 5A, B). This was also the case for expression of OsTCP15 and REP1 (Supplementary Fig. S1).

Next, we examined whether expression of FC1 is influenced by auxin and cytokinin (Fig. 5). We previously reported that...
D10 expression was up-regulated by auxin, suggesting that SL production might be positively regulated by auxin (Arite et al. 2007). The up-regulation of D10 expression was confirmed by quantitative real-time reverse transcription–PCR (RT–PCR; data not shown). On the other hand, the expression level of D17/HTD1, which encodes CCD7, was not significantly affected by the same treatment with IAA (data not shown). The application of IAA caused no clear change in the expression level of FC1 (Fig. 5C, D). In contrast, benzyl aminopurine (BAP) treatment clearly suppressed FC1 expression in a concentration-dependent manner (Fig. 5E). We then asked whether the negative control of FC1 expression by BAP depends on the SL pathway. With or without BAP treatment, the levels of FC1 mRNA in d3 (an SL-insensitive mutant) and d10 (an SL-deficient mutant) were not significantly different from the levels in wild-type plants (Fig. 5E). These results implied that the negative control of FC1 expression by cytokinin is independent of the SL pathway. Furthermore, it is also indicated that the FC1 expression level is not controlled by the SL pathway. This is consistent with the fact that we did not observe a clear induction of FC1 expression after SL treatment (Fig. 5A, B). Expression of neither OsTCP15 nor REP1 was negatively regulated by cytokinin (Supplementary Fig. S2).

Finally, we tested the possibility that cytokinin biosynthesis is down-regulated by auxin through suppression of cytokinin biosynthesis genes, as is the case in pea (Tanaka et al. 2006). We chose four out of 10 IPT genes, OsIPT2, OsIPT4, OsIPT7 and OsIPT8 (Sakamoto et al. 2006), and their responses to auxin were examined (Fig. 5F, G). Expression of both OsIPT2 and OsIPT4 was clearly suppressed within 3 h after the auxin treatment. The same trend was observed for OsIPT7 and OsIPT8; however, due to their low expression level, it was difficult to draw an unequivocal conclusion (data not shown).

**Discussion**

**FC1 function is required for the action of SLs**

Several rice mutants that show reduced plant height and increased tiller numbers have been named 'tillering dwarf mutants'. Among the tillering dwarf loci, D3, D10, D14/D88/HTD2, D17/HTD1 and D27 are known to be involved in the SL pathway (Umehara et al. 2008, Arite et al. 2009, Liu et al., 2009). The fc1 mutants are also known as tillering dwarf mutants; however, the relationship with SLs remained to be determined (Takeda et al. 2003). Here we propose that FC1 functions as a regulator of tiller outgrowth downstream of the SL signal.
based on our results indicating that FC1 function is required for SLs to inhibit tiller outgrowth.

FC1 is an ortholog of TEOSINTE BRANCHED1 (TB1), which is one of the major loci responsible for domestication of maize (Doebley et al. 1997, Hubbard et al., 2002). Loss-of-function mutants of TB1 exhibit the excess branching phenotype which resembles the five d mutants. Because the amino acid sequence in the highly conserved TCP domain is disrupted in the fc1-1 and fc1-2 mutants, we assume that these are null alleles. Despite this, the fc1-1 and fc1-2 plants show weaker phenotypes than the other tillering dwarf mutants (Arite et al. 2007). This suggests that the function of FC1 as an inhibitor of bud outgrowth is likely to be shared with other proteins. Although strict orthologs of FC1/TB1 have not yet been found in eudicot genomes, the Arabidopsis genes BRANCHED1 (BRC1) and BRC2, encoding TCP proteins that are clustered in the CYC/TB1 clade, also act to inhibit bud outgrowth (Aguilar-Martinez et al. 2007, Martin-Trillo and Cubas 2009). Reductions in BRC1 mRNA levels in max mutants, and the induction of BRC1 expression by SLs, suggest that BRC1 (and possibly BRC2 too) functions downstream of the SL signal (Mashiguchi et al. 2009). This raises the possibility that other proteins in this clade may function downstream of SLs in the inhibition of bud outgrowth. In rice, REP1 and OsTCP15, which are closely related to BRC1 and BRC2 (Fig. 1C), are possible candidates for the co-operators of FC1. On the other hand, we showed that the high concentration of SL was not effective to suppress tiller growth in fc1 mutants, in spite of the presence of normal REP1 and OsTCP15 functions. This is not in favor of the prediction that REP1 and OsTCP15 work redundantly with FC1 as downstream components of the SL pathway. REP1 was identified as a controller of floral organ development, and no obvious defects in tiller growth have been reported for the rep1 mutants (Yuan et al. 2008). OsTCP15 function is currently unknown; however, considering its extremely low expression, we think it unlikely that OsTCP15 functions as the co-operator of FC1. A detailed analysis of rep1 and OsTCP15 loss-of-function mutants will aid our understanding of the mechanisms controlling tiller development in rice.

**Hormonal control of bud outgrowth**

It is well known that apically derived auxin indirectly suppresses bud outgrowth. One role of auxin is to regulate the level of cytokinin through negative control of IPT genes in the stem (Tanaka et al. 2006). When auxin levels are depleted, cytokinin biosynthesis is initiated and the synthesized cytokinin is transported to axillary buds where it induces bud outgrowth. At the same time, there is a link between auxin and SLs. Expression of the CCD7 and/or CCD8 genes is induced by auxin in pea, rice and Arabidopsis (Foo et al. 2005, Arite et al. 2007, Heyward et al. 2009). The induction of CCD mRNAs presumably leads to increases in SL levels, although this should be confirmed. Furthermore, it was recently shown that the direct application of SLs to axillary buds was sufficient to inhibit bud outgrowth in decapitated pea plants and in auxin signaling mutants of Arabidopsis (Brewer et al. 2009, Heyward et al. 2009). These results suggest that SL might act as a second messenger of auxin, and that the site of SL action is within the bud.

A likely scenario is that the apically derived auxin governs shoot branching through at least two pathways, one involving the maintenance of low cytokinin levels and the other involving the maintenance of high SL levels in axillary buds (Fig. 6; Beveridge and Kozuka 2010). Here, we showed that FC1 partially functions downstream of SLs, and that FC1 expression is negatively regulated by cytokinins. This led us to hypothesize that both pathways derived from auxin are finally integrated at FC1. To test this hypothesis further, it will be important to assess the biological effect of transcriptional regulation of the branching genes. In particular, it will be our first priority to

**Fig. 6** A model of the hormonal control of tiller bud outgrowth in rice. In this model, apically derived auxin negatively and positively regulates cytokinin (CK) and strigolactone (SL) biosynthesis, respectively. Subsequently, cytokinins negatively regulate expression of the TCP genes. The function of the TCP genes is positively controlled by SL at the level of mRNA accumulation in Arabidopsis, while the relationship between FC1 and SL remains unclear in rice. SL biosynthesis is under the control of feedback regulation. Solid lines show relationships that are confirmed in rice, while the dotted line represents a relationship that remains to be confirmed in rice.
determine whether the in planta SL levels are altered in response to changes in expression of D10, the putative SL biosynthesis gene. In addition, it will be essential to elucidate the molecular basis for the interaction between SLs and FC1 function. Our results showed that FC1 acts to suppress tiller growth as one of the downstream factors in the SL pathway. Identification of other components that control shoot branching under SL is obligatory for further understanding.

Recent molecular and genetic studies have demonstrated that molecular mechanisms controlling shoot branching are well conserved in distantly related species such as rice and Arabidopsis (Beveridge and Kyozuka 2010). At the same time, differences are also observed. As discussed earlier, Arabidopsis BRC1 expression seems to be dependent on SL levels while we found little, if any, dependence on SL in the expression of rice TCP genes in the CYC/TBI clade. On the other hand, the down-regulation of FC1 expression by cytokinin is evident in rice, whereas no significant change in BRC1 expression was observed in the amp1 mutants of Arabidopsis, in which cytokinin levels are increased (Aguilar-Martínez et al. 2007). Variations between species were also observed in auxin responsiveness and feedback regulation of the CCD genes (Heyward et al. 2009). These differences between species may reflect differences in their growth habits and branching patterns. Further studies of the components of the SL pathway will help us to understand how species-specific plant forms are achieved.

Materials and Methods

Plant materials

The fc1-1 mutant (cv. Taichung 65) was described previously (Takeda et al. 2003). The fc1-2 mutant (cv. Nipponbare) was isolated from a population of tissue culture-derived plants. Plants were grown in a growth chamber (14 h light at 28°C, 10 h dark at 24°C) or a glasshouse under natural light. FC1 promoter::GUS (β-glucuronidase) was described previously (Arite et al. 2007). d17-2 and d3-2 were described previously (Umehara et al. 2008).

SL analysis

SL treatments and SL analysis using LC-MS/MS were performed as described previously (Umehara et al. 2008, Arite et al. 2009). For the experiment involving treatment of plants with high concentrations of GR24, the plants were grown in 1 liter containers and the culture medium was changed every week. Tillers were scored 5 weeks after the start of the GR24 treatment.

Hormonal treatments

To assess the responsiveness to hormones and feedback regulation of gene expression, a segment of approximately 5 mm from the first node containing the SAM, a few axillary buds and the basal parts of the leaves was cut from 13-day-old seedlings treated with various concentration of IAA or BAP for 3 h.

In situ hybridizations

In situ hybridizations were performed as described by Kouchi et al. (1995). A partial cDNA of FC1 was PCR amplified by using the primer set 5′-TCTGAAATGCCCTTTGTCATCC-3′ and 5′-GATATCTCAGCAGTGTCGCCGAAT-3′. The PCR fragment was cloned into pGEM-T Easy vector (Promega) and linearized with an appropriate restriction enzyme. To make the antisense probe, in vitro transcription was performed using the linearized plasmid as a template, with the incorporation of digoxigenin (DIG)-UTP.

Real-time RT–PCR

Total RNA was extracted using a Plant RNA Isolation mini kit (Agilent). After DNase I treatment, first-strand cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen). The primer sets used to amplify the transcripts were as follows: forward and reverse primers for FC1, 5′-GGTATTGACEGATAGCTGAC-3′ and 5′-TCTGATGCATGCTGTGC-3′; for D10, 5′-CGTGGCCTATCTGGTGTG-3′ and 5′-CGACCTCCTTGAAGTCCTTG-3′; for Osipt1, 5′-CGATGGACGGATTGGAGTA-3′ and 5′-GTGGGCTCGAGTCTATT-3′; for Osipt2, 5′-AGTCA CCCCACAAAGTGCTTCTT-3′ and 5′-CTCTCCGTGAGC TTGTTTCGATG-3′; for Osipt4, 5′-AGTCTGACGTACGATCAAACTCC-3′ and 5′-TGTAAGCCCTGATGTC-3′; for Osipt7, 5′-GGGCGTCAAGTACGAGTCTGTGTTCC-3′ and 5′-GGCCTCAGTACA GGCTGAATC-3′; for Osipt8, 5′-GTCGACTACGAGATGTCTC GGCAAT-3′ and 5′-TGTTGGCCCTGATCGCTCTTCC-3′; and for Ubiquitin, 5′-AGAAGGACGTCGCACTACACAGTGTCC-3′ and 5′-GCCACGACGACGAAACAGC-3′. PCRs were performed with SYBR green I using a Light Cycler® 480 System II (Roche Applied Science).

Construction of CaMV 35S–FC1

A partial cDNA of FC1 containing the whole open reading frame was PCR amplified by using the primer set 5′-GATGTAGCTGACGCCTTACG-3′ and 5′-GCAATGACGCTGTTTCCG-3′. The PCR fragment was cloned into the SmaI site of pBS to make pBSFC1. Then, pBSFC1 was digested with XhoI and SpeI, and cloned into XhoI/SpeI sites of pMSh1, a binary vector containing the CaMV 35S promoter.

Supplementary data

Supplementary data are available at PCP online.

Funding

Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN) of Japan [to J.K. and S.Y.]; Ministry of Agriculture, Forestry and Fisheries [Genomics for Agricultural
Acknowledgments

We thank Kohki Akiyama for the internal standard of epi-SDS.

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