

Rice monoculm mutation *moc2*, which inhibits outgrowth of the second tillers, is ascribed to lack of a fructose-1,6-bisphosphatase

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Abstract We characterized a rice monoculm mutant *moc2*, which showed significantly reduced tiller numbers, pale-green leaves, a reduced growth rate, and a consequent dwarf phenotype. The monoculm feature was attributed to a deficiency in the efficient outgrowth of tiller buds, although the *moc2* mutant produced tiller buds. Inconsistent change was observed in the expression of genes involved in tiller bud outgrowth, suggesting that the *moc2* mutant has a defective function necessary for the tiller bud outgrowth. The gene responsible for the *moc2* mutant was mapped to a locus encoding cytosolic fructose-1,6-bisphosphatase 1 (FBP1), in which a Tos17 retrotransposon was inserted in exon 4. Reverse-transcription PCR for the *FBP1* gene amplified a shorter transcript from the *moc2* mutant than from the wild-type plant. The sequence of the shorter transcript revealed a deletion of exon 4 by abnormal splicing, and the resulting frameshift generated a new translation termination signal. The *moc2* mutant showed a very low level of FBPase activity, suggesting that it involves a loss-of-function mutation of *FBP1*. Cytosolic FBPase is considered a key enzyme in the sucrose biosynthesis pathway. Defective FBPase activity is anticipated to lead a shortage of sucrose supply, which probably causes the inhibition of tiller bud outgrowth in the *moc2* mutant. The monoculm phenotype of the *moc2* mutant supports the idea that sucrose supply may be an important cue to outgrow tiller buds.

Key words: Defective outgrowth of tillers, loss-of-function mutant, map-based cloning, rice (*Oryza sativa*), sucrose starvation.

Monocot plant species usually bear many culms, formed by tillering, which increase their productivity. Rice, a monocot plant, is one of the most important cereal crops in the world. A rice culm branches from a tiller bud at the basal node. A rice plant usually produces more than 10 branches during the stage of vegetative growth. Tiller numbers are generally regarded as the factor determining grain yield because tillers are specialized branches bearing panicles. Therefore, tillering is an important agronomic trait for grain productivity in rice (Wang and Li 2011).

Rice tillering occurs in two steps: the formation of an axillary bud at the leaf axil and its subsequent outgrowth. The axillary meristems are formed as lateral organs from the shoot apical meristems on every leaf axil and differentiate into axillary buds. The outgrowth activity of the axillary buds is controlled by multiple genetic, developmental, and environmental signals (Beveridge

et al. 2003; Leyser 2003; Shimizu-Sato and Mori 2001). Rice branching is very different from that of dicot plants, such as *Arabidopsis*, but these two species may share conserved mechanisms for the regulation of branching (Wang and Li 2011).

Many mutants with reduced culm numbers have been investigated. The rice *moc1* mutant plant has only one main culm, without tillers, because of a defect in the formation of tiller buds. The gene responsible for this mutation, *MONOCULM1* (*MOC1*), encodes a transcription factor that transcribes to a putative GRAS family nuclear protein that is mainly expressed in the axillary buds and functions to initiate axillary buds and promote their outgrowth (Li et al. 2003). It was recently reported that rice tillering is regulated by degradation of *MOC1* by APC/C, and that TAD1 functions as a coactivator of APC/C to target *MOC1* for degradation in a cell-cycle-dependent manner (Xu et al. 2012).

Abbreviations: FBPase, fructose-1,6-bisphosphatase; RT-PCR, reverse-transcriptase mediated PCR.

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Strigolactones have been shown to act as endogenous inhibitors of axillary bud outgrowth in rice, and their levels are elevated under phosphate starvation (Umehara et al. 2008, 2010). Mutants of the strigolactone biosynthetic pathway, such as those involving *HTD1*, *HTD2*, *D10*, *D27*, and *D3*, have been identified as having reduced culm numbers or bushy phenotypes (Arite et al. 2007; Ishikawa et al. 2005; Lin et al. 2009; Liu et al. 2009; Zou et al. 2006). *FINE CULM1* (*FC1*) acts downstream from the strigolactones as an integrator of multiple signaling pathways by which the shoot branching in rice is fine-tuned (Minakuchi et al. 2010).

It has also been suggested that other factors may be involved in tillering. A series of rice mutants with reduced culm numbers, *rcn1* to *rcn9*, has been reported. *RCN1*, to which is attributed the *rcn1* mutation, encodes an ATP-binding cassette subfamily G protein that acts independently of *D3* (Yasuno et al. 2007, 2009). Maize *RA3* encodes a trehalose-6-phosphatase that functions through the predicted transcription factor *RA1* to regulate inflorescence branching by modifying of a sugar signal that moves into the axillary meristems (Sato-Nagasawa et al. 2006). The wheat *tin* mutant showing reduced tillering is attributed to the early cessation of tiller bud outgrowth during the transition of the shoot apex from the vegetative to the reproductive stage. This mutant displays the upregulation expression of a gene induced by sucrose starvation, the downregulation of a sucrose-inducible gene, and a reduced sucrose content in the dormant *tin* buds. This suggests the importance of sucrose in shoot branching and the optimization of tillering because it affects the timing of internode elongation (Kebrom et al. 2012).

We isolated a spontaneous rice mutant, *moc2*, that showed a monocolm phenotype similar to that produced by the *moc1* mutation. In this paper, we report the characteristics of the mutant and the isolation of the gene responsible for this mutant with a map-based cloning method.

Materials and methods

Plant materials

The *moc2* mutant was isolated from a pool of mutant lines that were established by the regeneration of cultured rice (*Oriza sativa* cv. Nipponbare) cells. The rice mutant lines, NC0041, NE4831, and NG1467, were provided by the Rice Genome Resource Center, National Institute of Agrobiological Sciences, Japan. The seeds were germinated at 30°C in a dark chamber and grown in pots in the summer in our growth area under natural open-air conditions. *FBP1* gene-expressed transformants and the control plants were grown in a growth chamber under the 14 h-light (28°C)/10 h-dark (24°C) conditions.

PCR, RT-PCR and Realtime Quantitative RT-PCR

Genomic DNA was extracted according to the method of Murray and Thompson (1980). PCR was performed using Blend-Taq[®] thermostable DNA polymerase (Toyobo, Japan). Total RNA was extracted from each tissue as described previously (Imamura et al. 2007). The first-strand cDNA was synthesized from 1 µg of total RNA using a ReverTra Ace cDNA synthesis kit (Toyobo) with an oligo-dT₂₀ primer. The following primers were used for PCR: primer 1, CGT CAA GGCTCT CGT CAG; primer 2, ACG CCA CAG TCG ATG TTG; and primer 3; ATG GACTGG ACATCCGATGG. To detect *Actin1* mRNA (accession no. AK100267), the primer set used was CCT CAT GAA GAT CCT GAC GG and GTA CTC AGC CTT GGCAAT CC. Realtime quantitative PCR was performed using the ABI PRISM 7000 sequence detection system (Applied Biosystems) with an SYBR Green Real-time PCR mix (Toyobo). The value of *Actin1* mRNA was used for data normalization as a positive control.

Genome mapping of the *moc2* locus and DNA sequence analysis

For genome mapping, we used approximately 6000 F3 seeds that were obtained from the F2 plant derived from a cross between the *moc2* mutant (a Japonica cultivar in a Nipponbare background) and Kasalath (an Indica cultivar). From among these seedlings, those showing the monocolm phenotype were selected and used for genome mapping. The *moc2* locus was fine mapped by PCR of sequence tag sites using DNA markers (Shen et al. 2004), with previously and newly established primers based on the nucleotide polymorphisms in the corresponding regions of Nipponbare and Kasalath, according to She et al. (2010). The previously established, public available markers can be found at the Rice Genome Research Program website (<http://rgp.dna.affrc.go.jp/whoga/index.html>). The newly established DNA markers are listed in Supplementary Table 1.

Microscopic analysis

Rice tissues from 20-day-old plants were excised transversely with a knife and fixed for 16 h at 4°C with a mixture of 0.25% paraformaldehyde and 0.25% acetic acid in 50% ethanol, dehydrated with ethanol and *tert*-butanol, and then embedded in Paraplast Plus (Oxford Labware, St. Louis, MO, USA). Microtome sections (10 µm thickness) were mounted on glass slides and stained with Safranin-Fast Green FCF (Sigma-Aldrich, St. Louis, MO, USA). The samples were examined with an Olympus BX51-FL microscope (Tokyo, Japan).

Analysis of the cytosolic FBpase activity

Proteins were extracted from the powdered leaves of 4-week-old plants, as described previously (Asano et al. 2002). Cytosolic FBpase activity was measured spectrophotometrically by coupling fructose-6-phosphate formation to the reduction of NADP in the presence of phosphoglucose isomerase and glucose-6-phosphate dehydrogenase, according to Jang et al.

(2003).

Determination of soluble sugars and starch

Leaf sheaths were harvested at the daytime. Sucrose, glucose, fructose and soluble starch were measured according to Stitt *et al.* (1989) using Food Analysis F-kit system (R-Biopharm AG, Darmstadt, Germany).

Results

Phenotype of the *moc2* mutant

The *moc2* mutant was isolated for its monocolm phenotype from a pool of mutant lines produced by regeneration of cultured rice cells likely as the Tos17 rice mutants (Miyao *et al.* 2003). The *moc2* mutant phenotype includes reduced tiller numbers, and most plants are composed of a single culm (Figure 1A). Thus, this phenotype represents a kind of monocolm mutant. When the mutant plants were grown under high-temperature conditions in the summer, they sometimes produced second tillers (Figure 1A). However, the plants usually grew with a single culm until the reproductive stage. The average number of tillers in this mutant was 1.1, whereas it is 8.4 in the wild-type plant (Figure 1B). Compared with the wild-type plant, the *moc2* mutant showed reduced plant height and shoot length. The decelerated growth rate of the *moc2* mutant was evident 10 days after germination and thereafter, resulting in a dwarf phenotype (Figure 1C). Significant differences were detected in the color and size of all the leaves of the *moc2* mutant compared with those of the wild-type plant. The *moc2* plant produced pale-green leaves, whose lengths and widths were less than two thirds of the wild-type ones (Figure 1D).

The timing of heading and the date of flowering were similar to those of the wild-type plants. The *moc2* mutant was fertile, and set adequate normal seeds by self-pollination. However, the *moc2* mutant produced significantly small panicles, and the grain number in each ear was clearly reduced. A high level of fertility and a high germination rate were observed in the *moc2* mutant seeds, as for the wild-type plants.

To determine whether the monocolm phenotype is attributable to the inhibition of the tiller bud formation or of their subsequent outgrowth, we microscopically examined sections in the region of the basal node in the *moc2* mutant. As shown in Figure 2, the formation of tiller buds was detected in the *moc2* mutant, as in the wild type. However, no extension or outgrowth of branches was observed in the *moc2* mutant, suggesting that the growth of the tiller buds was inhibited or that the tiller buds failed to enlarge to generate individual organs.

Fine mapping of the gene responsible for the *moc2* mutant

We then undertook to identify the gene responsible for the *moc2* mutant by map-based cloning using the F3 progeny of a cross between the *moc2* mutant (Japonica background) and the Indica cultivar, Kasalath. Approximately 6000 F3 lines were developed and analyzed. The *moc2* phenotype segregated in a ratio of 3:1 in the F2 progeny, suggesting that the gene responsible for the *moc2* mutant was a single locus in the genome. Using 1289 lines of individual F3 progeny displaying the *moc2* phenotype, a fine map of the locus was created. The candidate gene was initially mapped to a region of 82 kb between two newly established molecular markers, P0505D12-58k and P0505D12-3, in a 146.4 cM region of chromosome 1 (Figure 3A).

To narrow the region containing the gene responsible for the *moc2* mutant, we further analyzed this region using other molecular markers. However, this was not successful because the structures of the Nipponbare and Kasalath genomes are highly diverged, where a transversion/translocation is suggested to have occurred.

Therefore, we examined the differences in the transcripts that were expected to originate in this region, where 11 putative genes are predicted. We detected the transcripts from these genes in Nipponbare leaves. Among them, we found an obvious divergence between the *moc2* mutant and the wild-type plants in the size of the transcripts of the Os01g0866400 gene, which encodes cytosolic fructose-1,6-bisphosphatase 1 (FBP1) (Figure 3B).

Reduced amounts of the transcripts from Os01g0866800, which encodes a putative tubby-like F-box protein 10, were also detected in the *moc2* mutant (Figure 3B). However, the nucleotide sequence of the corresponding gene, including the surrounding region in the *moc2* mutant, was identical to that of the wild-type gene, indicating that this gene was not responsible for the *moc2* mutant. However, the reduction in the expression of the transcripts of this gene could not be explained.

Determination of the gene responsible for the *moc2* mutant

The nucleotide sequence of the Os01g0866400 gene was determined. A Tos17 retrotransposon was found to be inserted in the *moc2* mutant gene. The full-length cDNA corresponding to the Os01g0866400 gene is registered in GenBank under accession number AK119536. The wild-type gene is predicted to contain 12 exons. In the *moc2* mutant, Tos17 is inserted in exon 4 (Figure 4A).

To verify that this locus is responsible for the *moc2* mutant, we analyzed the genomic status of the Os01g0866400 gene in the F3 plants. All the F3 plants showing the *moc2* phenotype were homozygous for the corresponding insertion, whereas the F3 plants showing

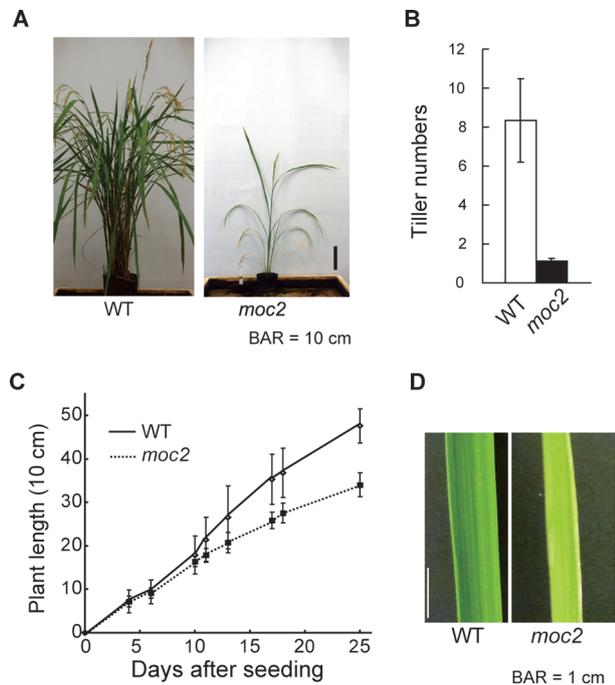


Figure 1. Phenotype analysis of the *moc2* mutant. (A) Features of 4-month-old wild-type (left) and *moc2* (right) plants. The bar indicates 10 cm. (B) Average tiller numbers of the mature plants. (C) Time course of shoot growth. Shoot length is shown. Solid and broken lines indicate the growth curves of the wild-type and *moc2* plants, respectively. (D) A representative mature leaf from each of the 4-week-old wild-type and *moc2* plants.

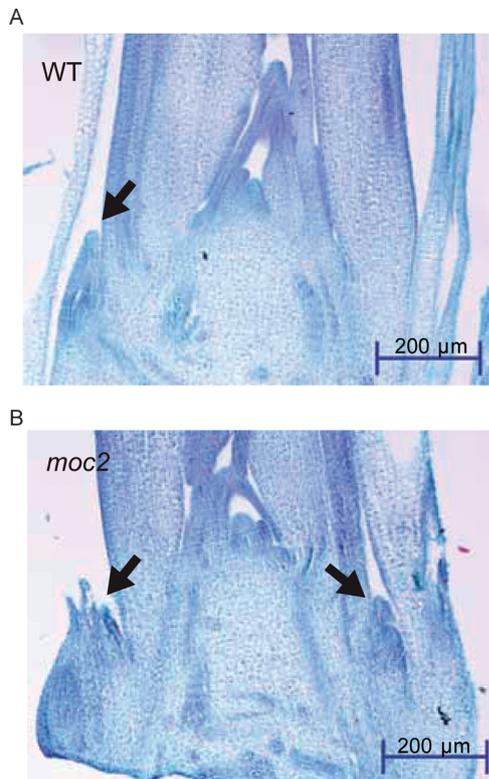


Figure 2. Morphological observation of tiller buds at the basal node in the wild-type (A) and the *moc2* mutant (B) plants. Arrows indicate tiller buds. Bar=200 μ m.

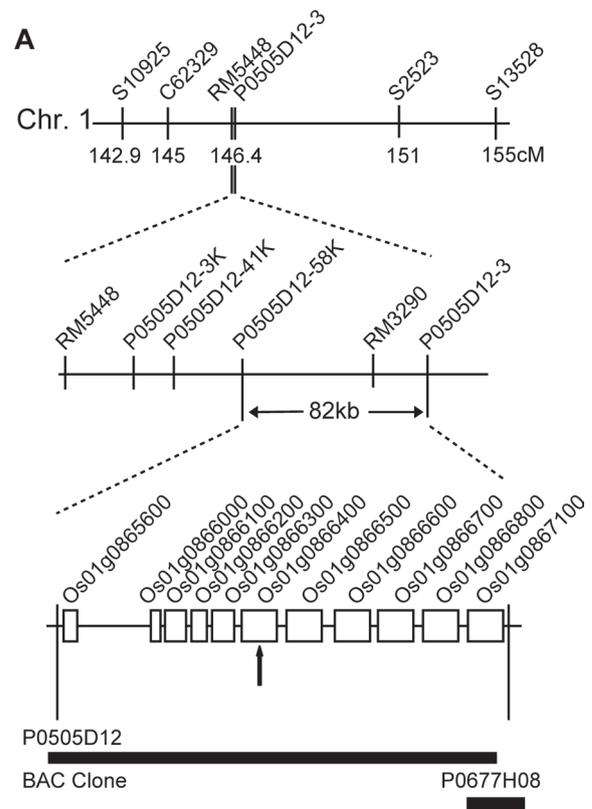


Figure 3. Determination of the gene responsible for the *moc2* mutant. (A) Fine-mapping of the *moc2* locus. The region of the genome containing the gene responsible for the *moc2* mutant was mapped to a 146.4-cM region of chromosome 1, and narrowed to an 82-kb region with newly created markers (P0505D12-58k and P0505D12-3). This region contains 11 predicted genes, shown as open boxes. The names of the genes are shown above the boxes. The BAC clones of the corresponding region are shown as the bars below the boxes. The bold arrow indicates the position of the candidate gene responsible for the *moc2* mutation. (B) Detection of the transcripts of the genes in the 82-kb region of the wild type plant (left) and the *moc2* mutant (right). RNA was prepared from leaves of 4-week-old plants. The bold arrow highlights the position of the specific transcript detected in the *moc2* mutant. The transcripts were detected by RT-PCR with primers designed based on the corresponding nucleotide sequences, which are shown in Supplementary Table 2.

the wild-type phenotype only contained the wild-type allele or were heterozygotes (Figure 4B). These results indicate that the mutant gene and the mutant

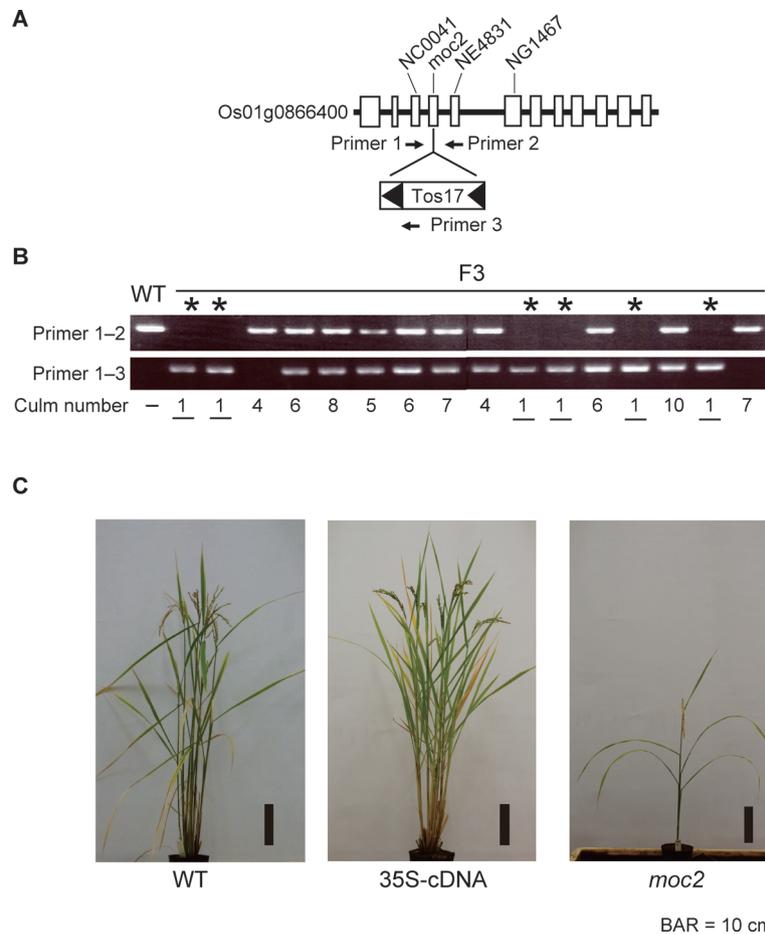


Figure 4. Structure of the Os01g0866400 gene, and its linkage to the *moc2* phenotype. (A) Exon/intron structure of the Os01g0866400 gene. Exons are indicated by open boxes. The sites of insertion of Tos17 in the mutants (NC0041, *moc2*, NE4831, and NG1467) are indicated by vertical lines on the boxes. The inserted Tos17 in the *moc2* mutant is indicated by the box with arrowheads. The PCR primers used are shown as arrows, with their names. (B) Segregation of the Os01g0866400 gene in F3 progeny obtained by crossing the *moc2* mutant with the wild-type plant. The presence of the mutant gene was determined by PCR. Primers 1–2 and 1–3 indicate the results of PCR amplification using primers 1 and 2, primers 1 and 3, respectively. The culm numbers of the mature plants are shown below the line. Asterisks indicate the F3 lines showing the *moc2* phenotype. (C) Photographs of the wild-type (WT) plant, a mature transformant plant containing the *FBPI* cDNA driven by the 35S promoter (35S-cDNA), and the mature *moc2* mutant (*moc2*). These plants were grown in a growth chamber. Bar=10 cm.

phenotype cosegregated. The tight linkage between the mutant allele and the *moc2* phenotype suggests that this gene is responsible for the *moc2* mutant. We introduced the *FBPI* cDNA under the control of the CaMV 35S promoter into the *moc2* mutant. This transformant showed the same phenotype as the wild-type plants, producing multiple tillers (Figure 4C), suggesting that the normal tillering phenotype was restored by the *FBPI* gene.

Expression of the mutant gene

A reverse-transcription PCR (RT-PCR) analysis of the Os01g0866400 gene detected a shorter transcript in the *moc2* mutant (Figure 3B). A nucleotide sequence analysis of this transcript identified a 62-nt deletion in the coding region. Abnormal splicing was considered to have occurred between exon 3 and exon 5, causing the skipping of exon 4 (Figure 5). The resulting frameshift generated a new translation termination signal in the

region corresponding to exon 5 (Figure 5), presumably disrupting the gene function.

Analysis of the mutants of the *FBPI* gene

FBPI mutants have already been characterized and shown severe growth retardation (Lee *et al.* 2008), which differs from the phenotype of the *moc2* mutant. We analyzed the characteristics of the other cytosolic *FBPI* mutants, NC0041, NE4831, and NG1467, which have been registered as mutants of the Os01g0866400 gene. Each of them contains an insertion of Tos17, in exon 3, exon 5, and exon 6, respectively (Figure 4A). These mutants are maintained as heterozygous plant lines because they are sterile. Plants homozygous for these mutations have been generated but exhibit severe growth retardation, ultimately causing plant death in the early stage of vegetative growth, as reported previously (Lee *et al.* 2008). A few of the homozygous NC0041 lines survived for several months, but these plants displayed a

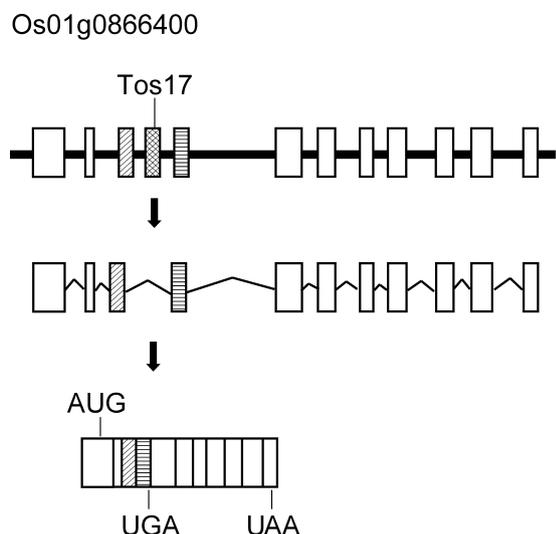


Figure 5. Schematic representation of the structure of the Os01g0866400 transcript in the *moc2* mutant. Exons are indicated by boxes. Exons 3, 4, and 5 are shown as shaded boxes. The regions spliced out are shown with inverted-V-shaped lines. The sites of the initiation codon (AUG), the newly generated termination codon (UGA), and the original termination codon (UAA) are shown in the schematic figure of the mRNA detected in the *moc2* mutant.

severe dwarf phenotype with a delayed growth rate, and did not reach to the reproductive stage (Figure 6A).

To explore the causes of the differences in the *moc2* and NC0041 phenotypes, we examined the expression of the *FBP1* gene in these mutants. RT-PCR showed that the transcripts of the mutant gene of the *moc2* mutant were shorter than those of the wild-type plants, which may be attributable to the deletion of exon 4. In contrast, no transcripts from the mutant gene were detected in NC0041 (Figure 6B). There are three FBPase isozymes in rice, and FBP1 functions as the major enzyme in the leaves (Lee et al. 2008). We analyzed the FBPase activity in the leaves of the *moc2* and NC0041. Both *moc2* and NC0041 showed very low levels of FBPase activity, which were 2% lower than that of the wild-type plant, although the FBPase activity in the *moc2* mutant was greater than that in the NC0041 mutant (Inset in Figure 6C).

Sucrose content and gene expression involved in sucrose starvation

In the wheat *tin* mutant, which has reduced numbers of tillers, the sucrose-starvation-inducible and sucrose-inducible genes are upregulated and downregulated, respectively, because the sucrose content of the mutant tiller buds is reduced (Kebrom et al. 2012). We determined the expression of the homologous rice genes, which encode an asparagine synthase (Os06g0265000) and a pyrophosphate-dependent phosphofructokinase (Os06g0326400), respectively. As shown in Figure 7A, the sucrose-inducible gene was strongly underexpressed in the *moc2* mutant compared with the wild-type. The

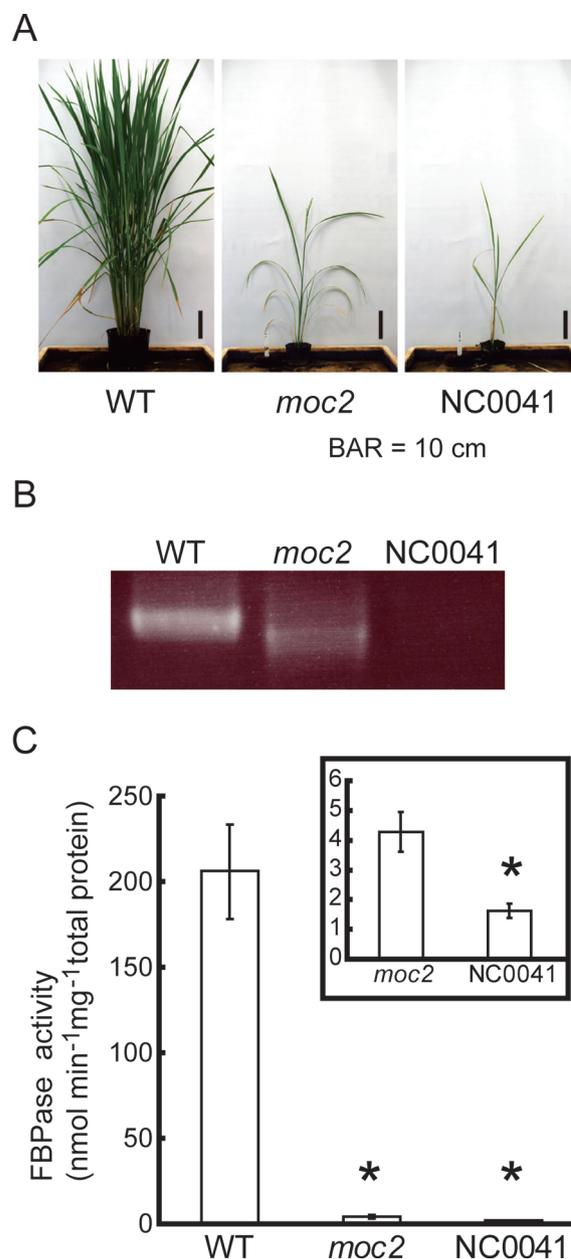


Figure 6. Analysis of the mutant alleles of the *moc2* mutant. (A) Features of 3-month-old plants of the *moc2* (center) and NC0041 (right) mutants, and the wild type (left). (B) Expression analysis of the Os01g0866400 gene in the *moc2* and NC0041 mutants and the wild-type plant by RT-PCR. (C) The cytosolic FBPase activity in the *moc2* and NC0041 mutants, and the wild-type plant. Each data point represents the mean (\pm SE) of three separate experiments. Asterisks indicate significance at $p < 0.05$.

sucrose-starvation-inducible gene did not show a statistically significant increase in the expression. Next, we measured the amount of sugars and soluble starch in leaf sheaths. As shown in Figure 7B, sucrose content was significantly reduced in the *moc2* mutant, whereas amount of glucose and soluble starch was similar with the wild-type plant. These results indicate that a shortage of sucrose occurs in the *moc2* mutants.

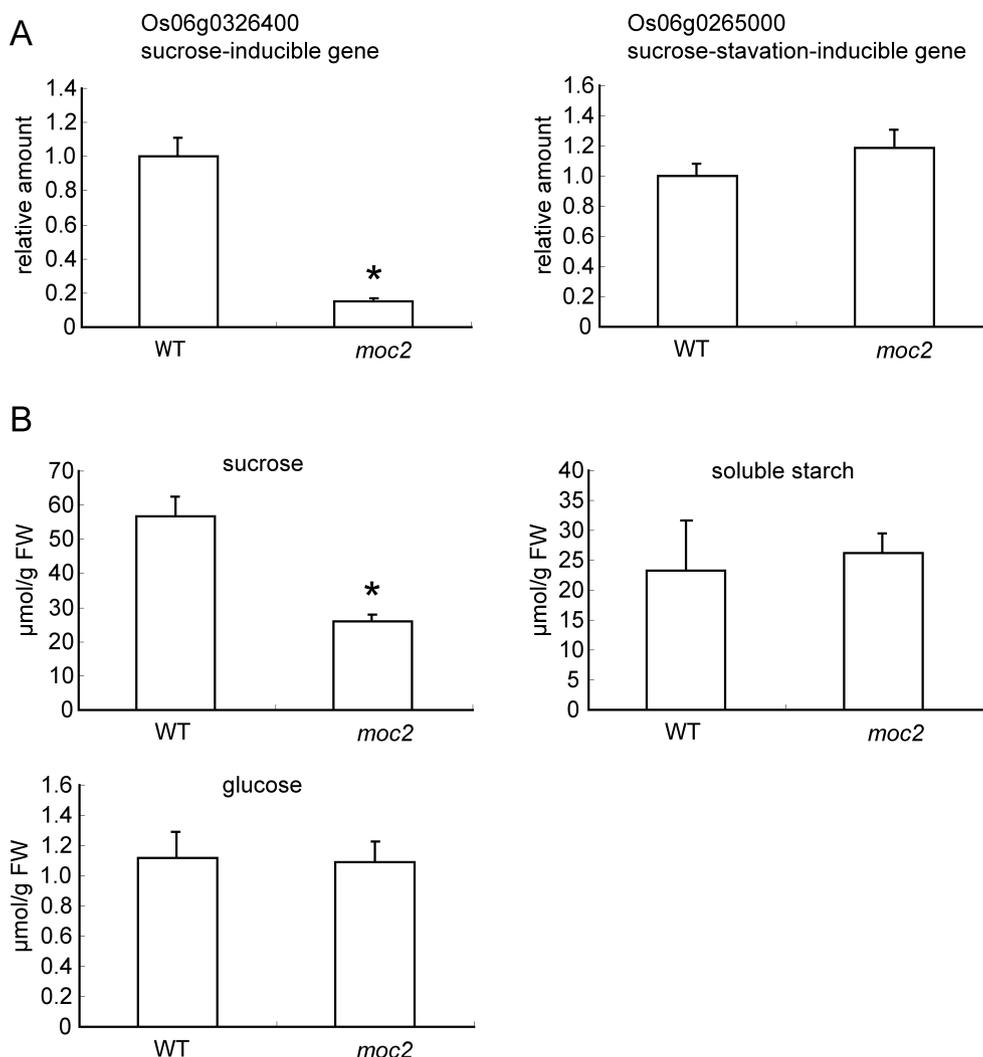


Figure 7. Expression of the genes that are sucrose-starvation-inducible and sucrose-inducible, and amount of soluble sugars and starch. Panel (A) indicates amount of the transcripts, which were detected by realtime quantitative RT-PCR. RNA was prepared from leaves of 4-week-old plants. Relative values to those of the wild type plant are indicated. The amount of the transcript in the wild-type plant was set at 1.0. The primers used are listed in Supplementary Table 2. Each data point represents the mean (\pm SE) of three separate experiments. (B) The amounts of sucrose, glucose, and soluble starch in the mature leaf sheaths at the 4-weeks-old plants are shown. Error bars indicate the SE ($n=3$). Asterisks indicate significance at $p<0.05$.

Gene expression involved in tillering

To examine the gene expression involved in tiller formation, a *MOC1* gene expression was analyzed. The *MOC1* gene was expressed in the *moc2* mutant, and the expression was rather higher than that of the wild-type plant (Figure 8A). This result is consistent with the morphological observation.

HTD1, *D10*, and *D3* are involved in the inhibition of the tiller bud outgrowth mediated by strigolactones (Umehara et al. 2010). We analyzed the expression of these orthologous genes (Os04g0650600, Os01g076400, and Os06g0154200, respectively) in the *moc2* mutant. Among these genes, the *HTD1* expression was higher in the *moc2* mutant (Figure 8B), but the other genes showed no significant differences in these expression between *moc2* and wild-type plants (Figure 8C,D). Therefore, strigolactones do not appear to be involved in the

monoculm phenotype of *moc2*. We also determined the expression of the *RCN1* and *TB1* genes (Os03g0281900 and Os03g0706500, respectively). *RCN1* controls shoot branching by promoting the outgrowth of lateral shoots, and the *TB1* gene negatively regulates lateral branching (Yasuno et al. 2009; Takeda et al. 2003). They showed no significant difference in these expression between *moc2* and wild-type plants (Figure 8E,F). These results suggest that these genes are expressed independently of the *moc2* mutation.

Discussion

We characterized a rice monoculm mutant, *moc2*, which showed a significant reduction in tiller numbers, pale-green leaves, a reduced growth rate, and consequent dwarf features (Figure 1). These characteristics are

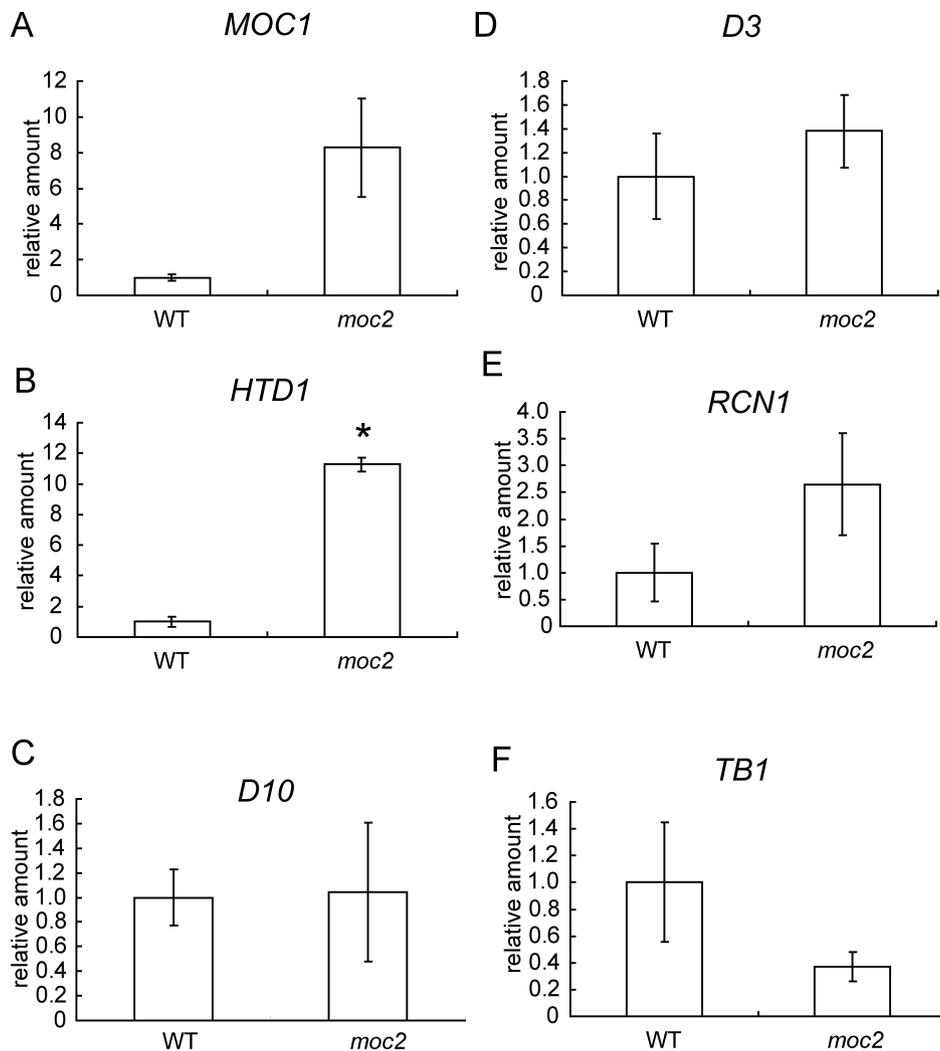


Figure 8. Expression of the *MOC1*, *HTD1*, *D10*, *D3*, *RCN1*, and *TB1* genes in the *moc2* mutant. Amount of the transcripts were detected by realtime quantitative RT-PCR. Relative values to those of the wild type plant are indicated. RNA was prepared from shoots of 6-week-old plants. The amount of the transcript in the wild-type plant was set at 1.0. The primers used are listed in Supplementary Table 2. Each data point represents the mean (\pm SE) of three separate experiments. Asterisks indicate significance at $p < 0.05$.

similar to those of the *moc1* mutant, which lacks a transcription factor involved in the regulation of the cell cycle during axillary bud formation and outgrowth (Li et al. 2003). However, the *moc2* mutant produced tiller buds, unlike the *moc1* mutant (Figure 2). The *MOC1* gene expression was also detected in the *moc2* mutant (Figure 8A). These facts suggest that the monocult phenotype of the *moc2* mutant was attributed to a deficiency in the outgrowth of the axillary buds.

We determined the gene responsible for the *moc2* mutant with map-based cloning, and identified it as the cytosolic *FBP1* gene (Figure 3). The *FBP1* gene in the *moc2* mutant contains an insertion of Tos17 in exon 4 (Figure 4). This insertion induces a cryptic splicing event that deletes exon 4 from the mRNA. In this way, a frameshift occurs that generates a new termination codon in the region corresponding to exon 5, causing a loss of function in the *FBP1* gene.

We found three other mutants of the *FBP1* gene, all of which contain insertions in exonic regions (Figure 4A). These mutants were sterile and show more severe growth inhibition than the *moc2* mutant (Figure 6A). FBPase activities are greatly reduced in both the *moc2* mutant and the NC0041 mutant (Figure 6C). Three isoforms of FBPase have been reported in rice, and the FBP1 functions as the major enzyme in leaves (Lee et al. 2008). The remaining FBPase activities in all these mutants were attributed to the other FBPase isoforms.

The *moc2* mutant has a moderate phenotype compared with those of the other mutants, in so far as it shows viable growth until the reproductive stages and is fertile. In the *moc2* mutant, the mutant gene is transcribed and spliced to form a shorter mRNA, lacking exon 4, in which Tos17 is inserted, whereas no transcripts from the mutant gene were detected in the NC0041 mutant (Figures 5 and 6B). A short protein could be translated

from the *moc2* mutant transcript, but it is assumed to have no FBPase activity because the mutation generates a new translation termination signal in the middle of the gene (Figure 5). These results suggest that both mutants involve the loss of FBP1 function, although the FBPase activity of the *moc2* mutant is greater than that of the NC0041 mutant (Figure 6C). The reason for the difference in the FBPase activity remains to be solved.

Tillers are the grain-bearing branches of monocot plants. Normally, a tiller bud arises from the axil of each leaf on the main stem of a rice plant, but only those on the non-elongated basal internodes potentially develop into tillers. The final tiller number is determined not only by how many tiller buds form, but also by how many tiller buds are capable of outgrowth (Wang and Li 2011).

The plant hormones (auxins, cytokinins, and strigolactones) are central to the control of bud activation, and move throughout the plant forming networks of systemic signals (Domagalska and Leyser 2011; Zou *et al.* 2006). Strigolactone, in particular, contribute greatly to the regulation of tiller formation and shoot branching, and the genes that participate in strigolactone biosynthesis greatly affect the developmental and environmental control of tillering (Arite *et al.* 2007; Ishikawa *et al.* 2005; Lin *et al.* 2009). We examined the expression of the *HTD1*, *D10*, *D3*, *RCN1*, and *TB1* genes, which function in the regulation of axillary bud outgrowth. *HTD1* and *D10* act in the strigolactone biosynthesis pathway, and *D3* acts in a step downstream from strigolactone synthesis (Umehara *et al.* 2010). *RCN1* and *TB1* are involved in controlling lateral branching or tiller outgrowth (Takeda *et al.* 2003; Yasuno *et al.* 2009). In the *moc2* mutant, although high-level expression of the *HTD1* gene was detected, no significant changes were detected in the expression of the other genes compared with the wild type (Figure 8). These results suggest that the monocolm phenotype of the *moc2* mutant is derived from some mechanism other than those mediated by these genes.

Sugars are the primary products of photosynthesis and perform multiple roles in plants, as energy and carbon transport molecules, hormone-like signaling factors, osmotica and the source of materials from which plants make proteins, polysaccharides, oils, and woody materials (Halford *et al.* 2011). Cytosolic FBPase catalyzes the first irreversible reaction in the conversion of triose phosphates to sucrose, and is a key enzyme in the sucrose biosynthesis pathway. It has been reported that high levels of FBPase in a transgenic tobacco plant led to a significant increase in the growth rate under elevated CO₂ conditions, and that the change in carbon partitioning affected the photosynthetic capacity and morphogenesis of the plant at elevated CO₂ levels (Tamoi *et al.* 2011). In contrast, a reduction in cytosolic FBPase activity results in the inhibition of sucrose synthesis,

the accumulation of phosphorylated intermediates, the *Pi*-limitation of photosynthesis, and the stimulation of starch synthesis (Serrato *et al.* 2009; Strand *et al.* 2000). In the *moc2* mutant, the expression level of the sucrose-inducible gene was significantly reduced (Figure 7A), and significant reduction of sucrose content was detected (Figure 7B). These observations show that defective FBPase activity should lead to a shortage of sucrose.

The wheat *tin* mutant shows reduced tillering, which is attributed to the early cessation of tiller bud outgrowth during the transition of the shoot apex from the vegetative to the reproductive stage. *tin* represents a novel type of reduced-tillering mutant associated with precocious internode elongation, which diverts sucrose away from tiller development (Kebrom *et al.* 2012). The monocolm feature of the *moc2* mutant is caused by a mutation in FBPase, which is the key enzyme for sucrose supply in the source organs. Therefore, the *tin1* mutation suggests that the defective FBPase activity in *moc2* may suppress the outgrowth of the tiller buds. In the *moc2* mutant, the sucrose supply may be insufficient for the outgrowth of the tillers, but enough to allow the growth of the monocolm to maturity, in some way. The nature of this mutant may provide a good system in which to explore the role of sucrose in the outgrowth of tiller buds, because it is normally fertile.

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Supplementary Table 1. List of newly established DNA markers for genome mapping.

Name of marker	Forward sequence (5'→3')	Reverse sequence (5'→3')	*RE
S10925	TGCCTCTGTTATGGCGA	TACAACAAGTAGTTACAAATCACA	<i>EcoRI</i>
C6232	TGTGGTGAAACGGGCATAGC	AAGGACCGGTGAACACGATG	
RM5448	GGGGCCCTTAGAGTTTTTAC	CTGCTACAGGACAAAACCAG	
P0505D12-3k	ACGATCCAACCAATACTGGG	AGCGTGTAATACAGATGCACC	<i>Alw441</i>
P0505D12-41k	AAGCGAATTCCCAAAGTGAA	CGGCCTCGCCTAGTATATGT	<i>PvuI</i>
P0505D12-58k	CCTCCTCTTTCATCTTCTC	TCCCAACCGATCGATCTC	
RM3290	GGATCCTCTCGTCGCTGTC	GTTAGCATCGGCAGATAGGC	
P0505D12-3	GGGTTAGGGTGGTTATGCCT	GCCACCGTATCTAACCGAGA	<i>HaeII</i>
S2523	AGGAGAAGAGGAGAGCAAGA	CAAACATCAAGCCCACAGAG	<i>SalI</i>
S13528	AGCACATTCGGCAGTTTCTC	GCACGCCATACAACAGCCTC	

* RE: Name of restriction enzyme by which the amplified fragment was digested when used as the CAPS marker.

Supplementary Table 2. List of PCR primers.

Name of the gene	Forward primer (5'→3')	Reverse primer (5'→3')
Os01g0865600	GCCGAAGAAGAGTTCACGTT	CCTAAAGGCTGTATCTGTGCTG
Os01g0866000	ATCTGCCTCTCCGAGTTCG	CCACAGTATCTCGGATGACAA
Os01g0866100	GCGGAGCTCTTAGAAGGA	CCAAAACACTACAAGTCGTTCA
Os01g0866200	GCATTCGAAGCTTGTAGAAGA	CAAACACCAACCAAGGAACC
Os01g0866300	AAGCCAAATCCACGCATC	GTTCAATGAATGTGTTAGTTGC
Os01g0866400	AGAGCGCGAGGCAGAGTAA	GTAAGGCCAGGGAGTCCATT
Os01g0866500	ATCCGGCGTTTCTTTGAAG	AGAAAGTGACCTGCGGTGC
Os01g0866600	ATCCCAAGTCGTCTTCCTCC	GAGCTGACGCACATCTCAA
Os01g0866700	GGGTAAGCTAGGGTTTAGGT	GTAGCACTTCAGCACATAACA
Os01g0866800	AACTCCTTCGCAACCTGGT	CTCTATCATGCATGAGGGCA
Os01g0867100	TGGAGCGGAGACAATGATA	TTCTCTGCGACCGAAGTATG
Os06g0326400	ATGTCATCAGCCACGCCTTC	CTCCCACTGAAGACAACACCAAC
Os06g0265000	GTGATGCTATTGGCATTGTCC	GGAAGGATATGAATCGTCCG
<i>MOC1</i>	GCGTCATGTTCTTGACAACC	CGAGTAGTGATCCATGGCCAC
<i>HTD1</i>	GCTGCACATGTCAAGCTGCTC	TTTATGAACGACGGGTCCAG
<i>D10</i>	GCTGTACAAGTTCGAGTGGCACC	ATGAAGTGGAACGTCACGAACG
<i>D3</i>	GGATGGTGCCATTGCTCCAC	CGATAATGACATGCAAACTGGC
<i>TB1</i>	TCCCATCAGTAAAGCATGCTTCC	GATGGTGGTGGTGGTGGT
<i>Rcn1</i>	GCAAGAGCACGCTCATCGAC	GAGATGACCTTGAGCAGTTGC