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).
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; 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 *RA1* 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 (Satoh-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 monoculm 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 (*Oryza 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-dT<sub>20</sub> primer. The following primers were used for PCR: primer 1, CGT CAA GGC TCT CGT CAG; primer 2, ACG CCA CAG TCG ATG TTT; and primer 3, ATG GACTTGG ACA TTCG GAT GG. To detect *Actin1* mRNA (accession no. AK100267), the primer set used was CCT CAT GAA GAT CCT GACGG and GTA CTC AGC CTT GGC AAT 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 monoculm 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 phosphoglucone isomerase and glucose-6-phosphate dehydrogenase, according to Jang et al.
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 monoculm 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 monoculm 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 plant produced pale-green leaves, whose lengths and widths were less than two thirds of the wild-type ones (Figure 1D).

To determine whether the monoculm 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 homoyzgous for the corresponding insertion, whereas the F3 plants showing
Rice FBPase mutation affects tillering

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 μm.

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
The 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 FBP1 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 FBP1 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 FBP1 gene**
FBP1 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 FBP1 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 heterozygotic 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...
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 (Inlet 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 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.
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 RCNI and TB1 genes (Os03g0281900 and Os03g0706500, respectively). RCNI 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
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 monoculm 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 isozymes 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 isozymes.

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 monoculm 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 monoculm 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 monoculm 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.

Acknowledgments

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References


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

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<th>Name of marker</th>
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*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.**

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