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Control of tillering in rice

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Tillering in rice (*Oryza sativa* L.) is an important agronomic trait for grain production, and also a model system for the study of branching in monocotyledonous plants. Rice tiller is a specialized grain-bearing branch that is formed on the unelongated basal internode and grows independently of the mother stem (culm) by means of its own adventitious roots[1]. Rice tillering occurs in a two-stage process: the formation of an axillary bud at each leaf axil and its subsequent outgrowth[2]. Although the morphology and histology[2,3] and some mutants of rice tillering[4] have been well described, the molecular mechanism of rice tillering remains to be elucidated. Here we report the isolation and characterization of *MONOCULM 1* (*MOC1*), a gene that is important in the control of rice tillering. The *moc1* mutant plants have only a main culm without any tillers owing to a defect in the formation of tiller buds. *MOC1* encodes a putative GRAS family nuclear protein that is expressed mainly in the axillary buds and functions to initiate axillary buds and to promote their outgrowth.

To identify genes involved in the control of rice tillering, we have screened for mutants with altered tiller numbers from collections derived from spontaneous mutations or γ-ray radiation and ethyl methanesulphonate (EMS) mutagenesis. A spontaneous *monoculm 1* (*moc1*) mutant is of particular interest, because *moc1* plants nearly completely lose their tillering ability, producing only one main culm, in contrast to the multiple tillers in wild-type plants (Fig. 1). Genetic analysis with reciprocal crosses between *moc1* and wild-type plants revealed that *moc1* possesses a recessive mutation in a single nuclear locus. Allelic tests between the *moc1* mutant and five recessive tillering mutants with reduced culm number (*rcn1* to *rcn5*)[4] indicated that *MOC1* is a previously unknown locus that is involved in the control of rice tillering.

In the seedling stage, no obvious morphological difference could be observed between *moc1* and wild-type plants. However, during the tillering stage, beginning from the fourth complete leaf formation, tillers emerged from sheaths of the subtending leaves in wild-type plants, but no tillers arose from leaf axils of *moc1* plants (Fig. 1a–d). Up to the heading stage, wild-type rice plants produced not only primary tillers on the main culm, but also secondary ones on the primary tiller culms. In *moc1* mutants, however, no primary tillers other than a main culm could be observed, and therefore no secondary tillers were seen either (Fig. 1e, f). Similarly, *moc1* panicles also produced much fewer rachis-branches and spikelets than did wild-type plants (Fig. 1g, h). In contrast to phenotypic alterations observed in aerial organs, roots appear to be unaffected in *moc1* plants (Fig. 1a–f).

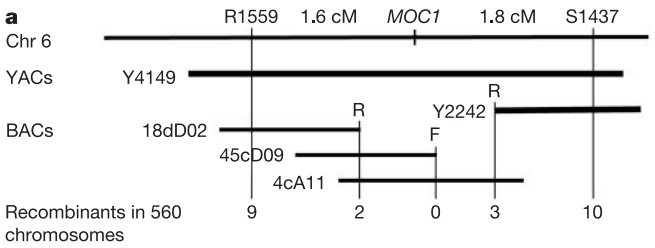
The *MOC1* locus was mapped primarily to the long arm of chromosome 6 of *O. sativa* between markers R1559 and S1437 (Fig. 2a), and was subsequently fine-mapped to a 20-kilobase (kb) region using newly developed molecular markers (Fig. 2a, b; Supplementary Table 1). Annotation of the 20-kb sequence identified an open reading frame (ORF) that encodes a protein highly homologous (44% identity) to the tomato LATERAL SUPPRESSOR (LS)[5]. In tomato, loss-of-function mutation in *LS* causes a branchless phenotype owing to a failure in axillary meristem initiation. This result suggests that the rice ORF with homology to the tomato *LS* is very probably the *MOC1* gene.

We therefore amplified the corresponding ORF from *moc1* and wild-type plants with polymerase chain reaction (PCR) and sequenced it. DNA sequence comparison revealed a 1.9-kb retrotransposon inserted in this ORF in the *moc1* mutant. Confirmation of the retrotransposon-interrupted ORF as *MOC1* was achieved by functional complementation. A binary plasmid carrying a 3.2-kb wild-type genomic fragment containing the entire ORF plus a 1.5-kb upstream region (pC8247), but not the one carrying a C-terminal truncation (pC8247S) (Fig. 2b), was able to rescue the monoculm phenotype of the *moc1* mutant (Fig. 1i, j). In DNA blot analysis of T_2 progeny from a self-pollinated transgenic line, the co-segregation of the transgene and the tiller phenotype is further evidence that the ORF homologous to *LS* is indeed the *MOC1* gene (data not shown). DNA blot analysis also indicated that *MOC1* is a single-copy gene in the rice genome.

The *MOC1* complementary DNA was cloned by reverse transcription (RT)–PCR using total RNA prepared from rice seedlings. Alignment of the cDNA and genomic DNA sequences revealed no introns in the *MOC1* gene, as is the case in the tomato *LS*[5]. The first in-frame ATG of the transcript (position 1) initiates an ORF encoding a protein of 441 amino-acid residues (Fig. 2c; Supplementary Fig. 1a). In *moc1*, the 1.9-kb retrotransposon is inserted at position 948, causing a premature translation stop that results in a truncated fusion protein of 338 amino-acid residues with the last 22 residues encoded by the retrotransposon sequence (Fig. 2c; Supplementary Fig. 1b).

Figure 1 Phenotype and complementation of the *moc1* mutant. **a**–**f**, Comparison of tillering abilities between wild-type and *moc1* plants at the onset of tillering stage (**a**, **b**), at the peak of tillering stage (**c**, **d**), and at the heading stage (**e**, **f**). Arrows show emerging tillers in wild-type plants or empty leaf sheaths in the *moc1* mutant. **g**, **h**, Panicles of wild-type and *moc1* plants. Arrows show bract nodes missing rachis-branches in the *moc1* plant. **i**, **j**, The *moc1* plants harbouring one and three *MOC1* transgene copies.

Homology analysis demonstrated that MOC1 is a member of the plant-specific GRAS family proteins[6], which contain several subfamilies including MOC1 and LS for axillary branching[5], SCR and SHR for root radial patterning[7,8], PAT1 for light signalling[9], and GAI, RGA, SLR1, RHT1 and D8 for gibberellin-acids signalling and plant height[10–13]. The GRAS family proteins are characterized by a conserved VHIID motif flanked by two leucine heptad repeats, a conserved C-terminal region, and an N-terminal region varying in length and sequence (Fig. 2c) that may confer specificity[9]. In addition, the MOC1 C terminus also contains an SH2-like domain[14] followed by a conserved tyrosine (Fig. 2c), a potential site for phosphorylation.



c

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MOC1 MLRSLHSSSSSDTDNNSGGCKNNGGGGGEAAAVEGGGDQRAVAAAAPSTR  51
  LS MLGSPGSSSSQSHPHHDEESSDHHQQRRFTATTIT-TTTITTSPAIQIR  50

MOC1 DLLLACADLLQRGDLPAARRAAEIVLAAAASPRGDAADRLAYHFARALALR 102
  LS QLLISCAELISQSDFSAAKR-LLTILSTNSSPFGDSTERLVHQFIRALSLR 100

MOC1 VD----AKAGHGHVVVGGGAARPASSG--------AYLAPNQIAPFLRFAHL 142
  LS LNRYISSTTNHFMTPVETTPTDSSSSSLALIQSSYLSLNQVTPFIRFTQL 151

MOC1 TANQAILEAVDG-ARRVHILDLDAVHGVQWPPLLQAIAERADPALGPPEVR 192
  LS TANQAILEAINGNHQAIHIVDFDINHGVQWPPLMQALADRYP----APTLR 198

MOC1 VTGAGADRDTLLRTGNRLRAFARSIHLPFHFTPLLLSCATTAPHHVAGTST 243
  LS ITGIGGNDLDTLRRTGDRLAKFAHSLGLRFQFHPLYIANNNHDHD------- 242

MOC1 GAAAAASTAAAATGLEFHPDETLAVNCVMFLHNLAG-HDRLAAFLKWVKAM 293
  LS ------EDPSIISSIVLLPDETLAINCVFYLHRLLKDREKLRIFLHRVKSM 287

MOC1 SPAVVTIAERFAGGGGGGGDHIDDLPRRVGVAMDHYSAVFPALEATVPPGS 344
  LS NPKIVTIAEKFAN------HNHPLFLQRFIFALDYYIAVFDSLEATLPPGS 332

MOC1 RERLAVEQEVLGREIEAAVGPSGGRWWRGIER---WGGAARAAGFAARPLS 392
  LS RERMTVEQVWFGREIVDIVAMEGDKRKERHERFRSWEVMLRSCGFSNVALS 383

MOC1 AFAVSQARLLLRLHYPSEGYLVQEARGACFLGWQTRPLLSVSAWQPSS    442
  LS PFALSQAKLLLRLHYPSEGYQLGVSSNSFFLGWQNQPLFSISSWR      428
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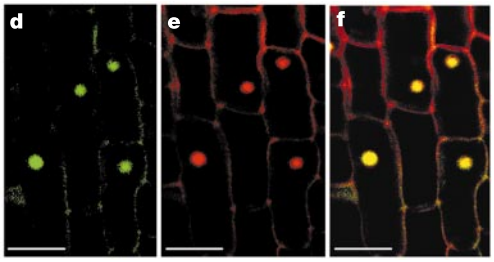

Figure 2 Molecular identification of *MOC1*. **a**, *MOC1* was mapped within the BAC clone 4cA11. BAC, bacteria artificial chromosome; cM, centimorgans; YAC, yeast artificial chromosome. **b**, *MOC1* was further localized within a 20-kb region and covered by plasmids P4123 and P4124 from the 4cA11 shotgun library. Plasmids containing the entire (pC8247) or truncated (pC8247S) *MOC1* were constructed for complementation. **c**, Alignment of rice MOC1 and tomato LS. The serine homopolymeric stretch is in blue, the conserved leucine in leucine heptad repeats in red, the VHIID motif in green, the invariant arginine in SH2-like domain in purple, and the conserved tyrosine in yellow. Red triangle indicates retrotransposon insertion site. **d**–**f**, The MOC1–GFP fusion protein is nuclear-localized. The figure shows MOC1–GFP green fluorescence in the nucleus (**d**), the same cells stained with PI (**e**), and their merged image (**f**). Scale bars, 10 μm.

On the basis of findings that some GRAS members such as RGA[11] and SLR1[12] possess nuclear localization signals (NLS), the GRAS family proteins have been proposed to function as transcription factors[6,14]. Surprisingly, no conventional NLS domains thus far characterized[15] could be predicted in MOC1 (Fig. 2c). To test whether or not MOC1 is localized in the nucleus, a *GFP* reporter gene encoding green fluorescent protein was fused in-frame to the last codon of *MOC1* to produce a MOC1–GFP fusion protein in the transgenic rice plants. The MOC1–GFP green fluorescent signal was detected mainly in the nuclei of stable transgenic rice plants (Fig. 2d–f), indicating that MOC1 is a nuclear-localized protein and presumably functions as a transcription regulator. There may be an unidentified NLS in MOC1 or MOC1 may be imported into the nucleus through an NLS-independent mechanism.

To find out what role *MOC1* plays in rice tillering, we characterized the *moc1* mutant at both anatomical and histological levels. In wild-type rice seedlings, tiller bud formation was easily recognizable along the unelongated basal internodes (Fig. 3a). In contrast, no tiller buds could be observed in the *moc1* mutant (Fig. 3b), indicating that the monoculm phenotype of *moc1* is probably caused by a failure in the formation of tiller buds. In the longitudinal sections of the tip part of wild-type seedlings, tiller buds at different developmental stages could be observed (Fig. 3c, e, g, i), but not in the *moc1* mutant (Fig. 3d, f, h, j). The three tiller buds that emerged at the axils of the 6th, 5th and 4th leaves in the wild-type plants correspond approximately to the three stages of axillary bud formation proposed in *Arabidopsis*[16]; that is, axillary cell division

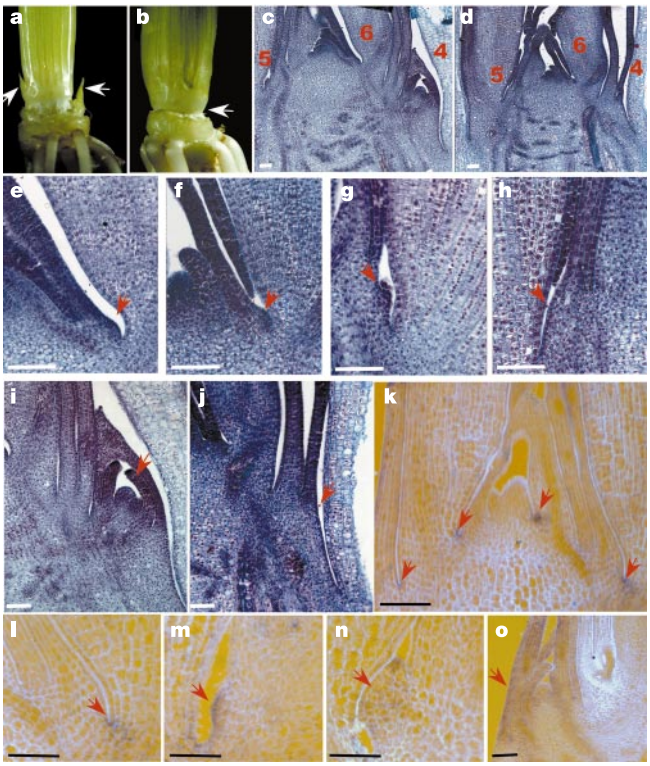

Figure 3 Microscopic studies on rice tiller bud formation. **a**, **b**, Tiller buds of four-leaf stage seedlings. Arrows indicate tiller buds in the wild-type and barren tillering nodes in *moc1*. **c**–**j**, Longitudinal sections of four-leaf stage shoot apexes. **c**, **d**, Overall structure of wild-type and *moc1* shoot apexes. Number indicates the 4th, 5th or 6th leaf. **e**, **g**, **i**, The small protuberance, axillary meristem and tiller bud formed in the 6th, 5th and 4th leaf axils in wild type. **f**, **h**, **j**, The void 6th, 5th and 4th leaf axils in *moc1*. **k**–**o**, RNA *in situ* hybridization of *MOC1*, showing specific expression in the leaf axils (**k**), in a few epidermal cells prior to any visible morphological changes (**l**), in a small protuberance before axillary meristem appearance (**m**), in axillary meristem (**n**), and in a matured tiller bud (**o**). Arrows show the *MOC1* expression sites. Scale bars, 100 μm.

(stage 1, Fig. 3e), appearance of the axillary meristems (stage 2, Fig. 3g) and differentiation of the first several axillary leaf primordia (stage 3, Fig. 3i). The defect in the formation of tiller buds in *moc1* plants could be observed as early as stage 1 (Fig. 3e, f), and no axillary meristems (Fig. 3g, h) and tiller buds (Fig. 3i, j) were formed in the *moc1* mutants. Therefore, the monoculm phenotype of *moc1* can be specifically attributed to a failure in the initiation of axillary meristems.

The requirement of *MOC1* for axillary meristem initiation and tiller bud formation was confirmed by investigating its spatial and temporal expression patterns (Fig. 3k–o). RNA *in situ* hybridization showed that *MOC1* expression could be detected in a small number of epidermal or subepidermal cells at the leaf axils, before any visible morphological changes (Fig. 3l). Subsequently, *MOC1* expression was observed in the small protuberance (Fig. 3m) and axillary meristem (Fig. 3n), and extended to the entire tiller buds including the axillary leaf primordia and young leaves (Fig. 3o). In contrast to high-level expression in the axillary meristems, *MOC1* expression was undetectable in the shoot apical meristem (SAM) (Fig. 3k). These results strongly suggest that *MOC1* has a critical role in the initiation of axillary meristems and formation of tiller buds.

In wild-type rice plants, a tiller bud is normally formed at each leaf axil, but only those formed on the unelongated basal internodes can grow out into tillers and those formed on the elongated upper internodes become arrested (Fig. 4a)[1]. Secondary tillers are usually formed in wild-type plants, but higher-order tillers such as tertiary, quaternary and quinternary ones are seldom developed[2,17] (Fig. 4c).

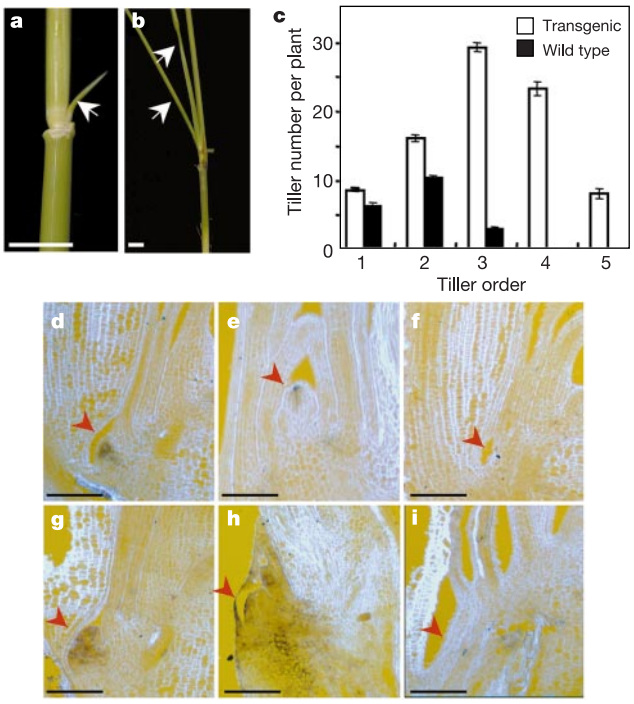

Figure 4 Promotion of *MOC1* on tiller bud outgrowth and *OSH1* and *OsTB1* expression in wild-type and *moc1* plants. **a**–**c**, Tiller bud outgrowth. **a**, The single arrested tiller bud (arrow) formed on the elongated upper internode of wild-type plants. **b**, Two tillers (arrows) formed on one single elongated upper internode in *MOC1* transformants. Scale bars, 1 cm. **c**, Outgrowth of higher-order tillers in *MOC1* T_2 transformants. Tiller numbers were counted at maturity stage and shown as mean ± s.e. ($n = 10$). **d**–**i**, *OSH1* and *OsTB1* expression in wild-type and *moc1* plants. **d**–**f**, *OSH1* RNA *in situ* hybridization, showing expression in axillary meristem (**d**) and in the SAM of tiller bud (**e**) in wild type, but no expression in the *moc1* leaf axil (**f**). **g**–**i**, *OsTB1* RNA *in situ* hybridization, showing expression in axillary meristem (**g**) and in the entire tiller bud (**h**) in wild type, but no expression in the *moc1* leaf axil (**i**). Arrows indicate the expression sites. Scale bars, 100 μm.

However, this tillering pattern was altered in all 15 independent *MOC1* transgenic lines obtained in the complementation tests. In the *MOC1* transformants, most tiller buds formed on the elongated upper internodes (Fig. 4b), higher-order tiller buds (Fig. 4c) were able to develop into tillers and more than one tiller was formed at some leaf axils (Fig. 4b). The altered tiller phenotype was inheritable in the T_2 progeny (Supplementary Table 2).

These observations suggested that in addition to its role in the formation of tiller buds, *MOC1* also functions in promoting tiller bud outgrowth in the rice genetic background. The enhanced tillering ability of *MOC1* transformants resulted in 2–3-fold more tillers than in wild-type rice plants (Fig. 1j; Supplementary Table 2). Of the five lines we examined, the two lines harbouring three transgene copies produced many more tillers than the three other lines harbouring one copy (Supplementary Table 2), suggesting that the enhanced tillering ability was probably due to slight over-expression of the *MOC1* gene.

We have also found that the plant height of the high-tillering *MOC1* transformants was significantly reduced (Fig 1j; Supplementary Table 2). This negative correlation between tiller number and plant height has also been observed in high-tillering dwarf mutants and cultivated rice varieties[4,18]. The finding that *MOC1* affects both rice tillering and plant height will prompt us to investigate their molecular mechanisms further.

Tillering is a complex process in which expression of many genes must be fine-tuned. Given the importance of MOC1 in both tiller bud formation and outgrowth, and its nuclear localization, *MOC1* is probably a key regulator in this complicated process. To identify possible downstream genes regulated by *MOC1*, we examined the expression of several important genes involved in shoot meristem or branch development. Among them, the *OSH1* gene is a key regulator of meristem initiation and maintenance[19], and the *OsTB1* gene is a rice orthologue of the maize *TB1* that is expressed in axillary buds and regulates axillary bud outgrowth[20–22]. RT–PCR analysis revealed that expression of both *OSH1* and *OsTB1* was significantly reduced in the *moc1* mutant (Supplementary Fig. 2).

This result was confirmed by *in situ* examination of *OSH1* and *OsTB1* expression patterns during tiller bud formation. In wild-type plants, besides the expression in SAM (data not shown), *OSH1* was also expressed in the axillary meristem (Fig. 4d) and the apical meristem of tiller buds (Fig. 4e). In the *moc1* mutant, however, *OSH1* expression could not be detected in the leaf axils (Fig. 4f), although the SAM expression was unaffected (data not shown). A similar observation was also made for the *OsTB1* expression in wild-type and *moc1* plants (Fig. 4g–i). These findings indicate that *OSH1* and *OsTB1* might be regulated by *MOC1* in the tillering process, and *MOC1* may act as a master regulator in the control of rice tillering.

MOC1 is the first functionally defined gene, to our knowledge, that controls tillering in rice, although several genes involved in dicotyledonous plant branching have been isolated[5,23,24]. Given the mutant's phenotype and similarity of sequences, *MOC1* is probably the rice orthologue of tomato *LS*. However, *MOC1* has distinct functions, such as promotion of tiller bud outgrowth and a negative effect on plant height, which may reflect the fundamental difference between monocotyledonous tillering and dicotyledonous branching.

Tillering is one of the most important agronomic traits because tiller number per plant determines panicle number, a key component of grain yield[18]. As a key regulator of tillering, *MOC1* (and its homologues in other cereals) could make a significant contribution to future improvement of these crops. □

Methods

Plant materials

The rice *moc1* mutant was obtained from a natural occurrence in a *japonica* cultivar, H89025. A mapping population of 2,010 F_2 mutant plants was generated from the crosses between *moc1* and Minghui 63 (*indica*).

Mapping and cloning of *MOC1*

MOC1 was mapped primarily with Simple Sequence Length Polymorphism (SSLP) and Restriction Fragment Length Polymorphism (RFLP) markers[25], using 280 F_2 mutant plants. The *MOC1* locus was further placed within a 20-kb region between the markers 17-3 and 12-2 by using 2,010 F_2 mutant plants and newly developed molecular markers (Supplementary Table 1). The candidate gene was amplified from both the *moc1* and wild-type genomic DNA using primers MOC1F1 and MOC1R3 (5′-TCGTTGTAGTAGCTCT GGTG-3′ and 5′-CTAACTAGAGATCGAGTAGC-3′), and the PCR products were sequenced directly.

Complementation test

A 3.2-kb genomic DNA fragment containing the entire *MOC1* coding region, the 1,534-bp upstream sequence and the 316-bp downstream sequence, was inserted into the binary vector pCAMBIA1300 to generate a transformation plasmid pC8247. A control plasmid, pC8247S, containing a 3′ truncated *MOC1* gene that encodes the first 283 amino-acid residues, was also constructed. The two binary plasmids were introduced into *Agrobacterium tumefaciens* LBA4404 and the *moc1* mutant was transformed as reported previously[26]. Fifteen independent transgenic lines were obtained for pC8247 and four for pC8247S, respectively. Each transgenic line included 10–100 sibling plants.

Subcellular localization

MOC1–GFP fusion was made by replacing the CaMV35SΩ region with a 2.9-kb DNA fragment that contained the entire *MOC1* coding region and 1.5-kb upstream sequence in the CaMV35SΩ-sGFP (S65T)-NOS-3′ cassette vector[27]. The construct was sequenced to verify the in-frame fusion and no nucleotide mutations. The *MOC1–GFP* fusion gene was subcloned into the binary vector pCAMBIA1300 and then transformed into wild-type rice plants. The tip of the transgenic rice plant stem was sectioned longitudinally, stained for 30 min with 2 μg ml^{-1} propidium iodide (PI) (in 30 mM 2-(N-morpholino)-ethanesulphonic acid and 100 mM mannitol, pH 5.9), and visualized with a confocal microscope (Bio-Rad MRC 1024).

Histology and *in situ* hybridization

Shoot apexes of rice seedlings at the four-leaf stage were fixed with formalin–acetic acid–alcohol (FAA) fixative solution at 4 °C overnight followed by dehydration steps and then embedded in paraffin (Paraplast Plus, Sigma). The tissues were sliced into 7–10-μm sections with a microtome (Leica RM2145), affixed to microscope slides, and stained with Safranin O and Fast Green (Fisher). Sections were observed under bright-field through a microscope (Leica DMR) and photographed using a Micro Color charge-coupled device (CCD) camera (Apogee Instruments).

RNA *in situ* hybridization was performed as described previously[28]. The 3′ ends of *MOC1*, *OsTB1* and *OSH1* were subcloned into pBluescript SK(+) vector and used as templates to generate sense and antisense RNA probes. Digoxigenin-labelled RNA probes were prepared using a DIG Northern Starter Kit (Cat. No. 2039672, Roche) according to the manufacturer's instructions. The slides were observed under bright-field and epifluorescence at the same time with a microscope (Leica DMR) and photographed using a 3CCD colour video camera (DXC-390P, Sony).

RNA extraction and analysis

Total RNA extraction was performed as described previously[29]. The first-strand cDNA was synthesized from total RNA and used as RT–PCR templates. RT–PCR primers for *OSH1* were 5′-GAGATTGATGCACATGGTGTG-3′ and 5′-ATTAGCAGCAGCAAGAGTAGC-3′, for *OsTB1* were 5′-AAGTTCTTCGCGCTCCAGGA-3′ and 5′-AACGCCATGATCACGT CGCT-3′, and for actin were 5′-TCATGAAGATCCTGACGGAG-3′ and 5′-AACAGCTCC TCTTGGCTTAG-3′. Rapid amplification of cDNA ends (RACE)–PCR was performed with the 5′/3′ RACE Kit (Roche) according to the manufacturer's instructions.

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1. Li, Y.-H. *Morphology and Anatomy of Grass Family Crops* 138–142 (Shanghai Science and Technology Press, Shanghai, 1979).
2. Hanada, K. in *Science of the Rice Plant* Vol. 1 *Morphology* (eds Matsuo, T. & Hoshikawa, K.) 222–258 (Food and Agriculture Policy Research Center, Tokyo, 1993).
3. Hanada, K. in *Science of the Rice Plant* Vol. 2 *Physiology* (eds Matsuo, T., Kumazawa, K., Ishii, R., Ishihara, K. & Hirata, H.) 61–65 (Food and Agriculture Policy Research Center, Tokyo, 1995).
4. Iwata, N., Takamure, I., Wu, H.-K., Siddinq, E. A. & Rutger, J. N. List of genes for various traits (with chromosome and main literature). *Rice Genet. Newsl.* **12,** 61–93 (1995).
5. Schumacher, K., Schmitt, T., Rossberg, M., Schmitz, G. & Theres, K. The *Lateral suppressor* (*Ls*) gene of tomato encodes a new member of the VHIID protein family. *Proc. Natl Acad. Sci. USA* **96,** 290–295 (1999).
6. Pysh, L. D., Wysocka-Diller, J. W., Camilleri, C., Bouchez, D. & Benfey, P. N. The GRAS gene family in *Arabidopsis*: sequence characterization and basic expression analysis of the *SCARECROW-LIKE* genes. *Plant J.* **18,** 111–119 (1999).
7. Di Laurenzio, L. *et al.* The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. *Cell* **86,** 423–433 (1996).
8. Helariutta, Y. *et al.* The *SHORT-ROOT* gene controls radial patterning of the *Arabidopsis* root through radial signaling. *Cell* **101,** 555–567 (2000).
9. Bolle, C., Koncz, C. & Chua, N. H. PAT1, a new member of the GRAS family, is involved in phytochrome A signal transduction. *Genes Dev.* **14,** 1269–1278 (2000).
10. Peng, J. *et al.* The *Arabidopsis GAI* gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* **11,** 3194–3205 (1997).
11. Silverstone, A. L., Ciampaglio, C. N. & Sun, T. The *Arabidopsis RGA* gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. *Plant Cell* **10,** 155–169 (1998).
12. Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M. & Matsuoka, M. The gibberellin signaling pathway is regulated by the appearance and disappearance of *SLENDER RICE1* in nuclei. *Plant Cell* **14,** 57–70 (2002).
13. Peng, J. *et al.* 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **400,** 256–261 (1999).
14. Richards, D. E., Peng, J. & Harberd, N. P. Plant GRAS and metazoan STATs: one family? *Bioessays* **22,** 573–577 (2000).
15. Smith, H. M., Hicks, G. R. & Raikhel, N. V. Importin α from *Arabidopsis thaliana* is a nuclear import receptor that recognizes three classes of import signals. *Plant Physiol.* **114,** 411–417 (1997).
16. Stirnberg, P., van De Sande, K. & Leyser, H. M. *MAX1* and *MAX2* control shoot lateral branching in *Arabidopsis*. *Development* **129,** 1131–1141 (2002).
17. Katayama, T. Analytical studies of tillering in paddy rice. *J. Imp. Agric. Exp. Stn Jpn* **1,** 327–374 (1931).
18. Yan, J.-Q., Zhu, J., He, C.-X., Benmoussa, M. & Wu, P. Quantitative trait loci analysis for the developmental behavior of tiller number in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **97,** 267–274 (1998).
19. Sato, Y. *et al.* A rice homeobox gene, *OSH1*, is expressed before organ differentiation in a specific region during early embryogenesis. *Proc. Natl Acad. Sci. USA* **93,** 8117–8122 (1996).
20. Doebley, J., Stec, A. & Hubbard, L. The evolution of apical dominance in maize. *Nature* **386,** 485–488 (1997).
21. Lukens, L. & Doebley, J. Molecular evolution of the teosinte branched gene among maize and related grasses. *Mol. Biol. Evol.* **18,** 627–638 (2001).
22. Hubbard, L., McSteen, P., Doebley, J. & Hake, S. Expression patterns and mutant phenotype of *teosinte branched 1* correlate with growth suppression in maize and teosinte. *Genetics* **162,** 1927–1935 (2002).
23. Schmitz, G. *et al.* The tomato *Blind* gene encodes a MYB transcription factor that controls the formation of lateral meristems. *Proc. Natl Acad. Sci. USA* **99,** 1064–1069 (2002).
24. Otsuga, D., DeGuzman, B., Prigge, M. J., Drews, G. N. & Clark, S. E. *REVOLUTA* regulates meristem initiation at lateral positions. *Plant J.* **25,** 223–236 (2001).
25. Harushima, Y. *et al.* A high-density rice genetic linkage map with 2275 markers using a single F_2 population. *Genetics* **148,** 479–494 (1998).
26. Hiei, Y., Ohta, S., Komari, T. & Kumashiro, T. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J.* **6,** 271–282 (1994).
27. Niwa, Y., Hirano, T., Yoshimoto, K., Shimizu, M. & Kobayashi, H. Non-invasive quantitative detection and applications of non-toxic, S65T-type green fluorescent protein in living plants. *Plant J.* **18,** 455–463 (1999).
28. Fobert, P. R., Coen, E. S., Murphy, G. J. & Doonan, J. H. Patterns of cell division revealed by transcriptional regulation of genes during the cell cycle in plants. *EMBO J.* **13,** 616–624 (1994).
29. Hu, Y.-X., Bao, F. & Li, J. Promotive effect of brassinosteroids on cell division involves a distinct CycD3-induction pathway in *Arabidopsis*. *Plant J.* **24,** 693–701 (2000).

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The genes *orthodenticle* and *hunchback* substitute for *bicoid* in the beetle *Tribolium*

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In *Drosophila*, the morphogen Bicoid organizes anterior patterning in a concentration-dependent manner by activating the transcription of target genes such as *orthodenticle* (*otd*)[1] and *hunchback* (*hb*), and by repressing the translation of *caudal*[2,3]. Homologues of the *bicoid* gene have not been isolated in any organism apart from the higher Dipterans[4–7]. In fact, head and thorax formation in other insects is poorly understood. To elucidate this process in a short-germband insect, I analysed