RESEARCH PAPER

Functions for rice RFL in vegetative axillary meristem specification and outgrowth

Gauravi M. Deshpande, Kavitha Ramakrishna*, Grace L. Chongloi* and Usha Vijayraghavan†

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India

* These authors contributed equally to this work.
† To whom correspondence should be addressed. E-mail: uvr@mcbl.iisc.ernet.in or uvr123@gmail.com

Received 5 September 2014; Revised 5 February 2015; Accepted 6 February 2015

Abstract

Axillary meristems (AMs) are secondary shoot meristems whose outgrowth determines plant architecture. In rice, AMs form tillers, and tillering mutants reveal an interplay between transcription factors and the phytohormones auxin and strigolactone as some factors that underpin this developmental process. Previous studies showed that knockdown of the transcription factor gene RFL reduced tillering and caused a very large decrease in panicle branching. Here, the relationship between RFL, AM initiation, and outgrowth was examined. We show that RFL promotes AM specification through its effects on LAX1 and CUC genes, as their expression was modulated on RFL knockdown, on induction of RFL:GR fusion protein, and by a repressive RFL–EAR fusion protein. Further, we report reduced expression of auxin transporter genes OsPIN1 and OsPIN3 in the culm of RFL knockdown transgenic plants. Additionally, subtle change in the spatial pattern of IR4 DR5:GFP auxin reporter was observed, which hints at compromised auxin transport on RFL knockdown. The relationship between RFL, strigolactone signalling, and bud outgrowth was studied by transcript analyses and by the tillering phenotype of transgenic plants knocked down for both RFL and D3. These data suggest indirect RFL–strigolactone links that may affect tillering. Further, we show expression modulation of the auxin transporter gene OsPIN3 upon RFL:GR protein induction and by the repressive RFL–EAR protein. These modified forms of RFL had only indirect effects on OsPIN1. Together, we have found that RFL regulates the LAX1 and CUC genes during AM specification, and positively influences the outgrowth of AMs though its effects on auxin transport.

Key words: Auxin transport, axillary meristem specification, CUC1, CUC2, LAX1, Oryza sativa, RFL.

Introduction

The aerial architecture of plants is determined by the activity of the shoot apical meristem (SAM) and axillary meristems (AMs) formed in the axils of lateral primordia. The outgrowth of vegetative AMs as shoots branch reiterates the primary shoot developmental pattern and thus gives the plant a bushy architecture. The activity of AMs is dynamically influenced by genetic and environmental factors. In rice, vegetative AMs give rise to tillers and this process involves two key steps: first, the initiation of AMs at leaf axils of juvenile plants that have compact nodes and very limited, if any, internode elongation; and second, the regulated outgrowth of these buds as tillers, a process coincident with internode elongation that occurs along with the transition of the apical meristem to flowering.

Studies on rice mutants with altered tillering have identified a number of genes important for AM initiation and/or the outgrowth of tiller buds (Pautler et al., 2013; Liang et al., 2014). The moc1 (monoculm1) mutant produces a main culm with a limited number of, or no, side tillers. In these plants, flowering transition is normal but panicles have a reduced number of rachis branches and spikelets (Li et al., 2003). The orthologues of MOCI are tomato LATERAL SUPPRESSOR (LS) and...
Arabidopsis LATERAL SUPPRESSOR (LAS), and these all encode a member of the GRAS family of plant-specific transcriptional regulators (Li et al., 2003). Rice LAX PANICLE1 (LAX1) encodes a basic helix-loop-helix (bHLH) transcription factor, and mutants in this affect AM development. Meristematic cells are specified in lax1 mutants but they fail to fully progress to form AMs (Oikawa and Kyoizuka, 2009).

The outgrowth of the axillary buds is influenced by hormonal signals and by environmental cues like plant density, nutrient availability, and light. Studies on more axillary growth (max) Arabidopsis branching mutants, the dwarf (d) group of rice tillering mutants, and ramosus (rms) mutants in pea identified the inhibitory role of strigolactones (Stirnberg et al., 2002; Gomez-Roldan et al., 2008; Umehara et al., 2008). Amongst the genes involved in this pathway, MAX3/D17/HIGH TILLERING DWARF1 and MAX4/D10 encode biosynthetic enzymes (Zou et al., 2006; Arite et al., 2007), while MAX1, a CYTOCHROME P450 (CYP) superfamily member of the CYP711 clade, is involved in later stages of strigolactone biosynthesis (Booker et al., 2005). The MAX2/D3 gene encodes a protein member of the F-box family that forms the substrate recognition subunit of the Skp, Cullin, and F-box (SCF) ubiquitin E3 ligases that mediate regulated protein degradation. In rice, some additional genes, D14, D27 and D53, are also known that encode factors involved in strigolactone biosynthesis, protein–protein interactions, or signalling (Arite et al., 2009; Lin et al., 2009; Jiang et al., 2013).

Phytohormones that influence the outgrowth of AMs are auxin and cytokinin. Auxin synthesized at the growing tip of the plant is transported basipetally by the polar auxin transport stream (PATS). The direction of auxin movement through tissues is determined by the asymmetric cellular localization of members of the PINFORMED (PIN) family of transporters (Wisniewska et al., 2006). Further, at the cellular level, PIN genes are regulated by transcriptional control and the dynamic subcellular localization of PIN proteins. The serine/threonine protein kinase PINOID (PID) regulates PIN localization. The rice counterparts OsPIN1 and OsPID are key factors for polar auxin transport (Xu et al., 2005). Recent studies in Arabidopsis elucidate some mechanistic details underlying auxin, apical dominance, and shoot branching. One model proposes that strigolactones act downstream of auxin and directly inhibit axillary bud outgrowth, possibly by regulating meristem activity (Brewer et al., 2009; Brewer et al., 2013). Other studies with Arabidopsis max mutants show that strigolactones may control the outgrowth of axillary buds by modulating polar auxin transport itself. These studies propose that, depending on polar auxin transport status, strigolactones may mediate the release of auxin from buds into the stem and thereby determine the number of buds to be activated (Crawford et al., 2010; Shinohara et al., 2013).

The rice LEAFY orthologue RICE FLORICULA/LEAFY (RFL), also called ABBERRANT PANICLE ORGANIZATION 2 (APO2), is expressed at high levels in very young branching panicles (Kyozuka et al., 1998; Prasad et al., 2003); before this, in juvenile vegetative plants, RFL is expressed in the axes of leaves (Rao et al., 2008). The latter expression pattern of RFL and the reduced tillering phenotype seen on knockdown of RFL (Rao et al., 2008) prompted this study. Here, we probed the relationship between RFL and other factors that control AM development. The expression status of key transcription factor genes, auxin transporters and components in the strigolactone pathway gave an insight on the interplay between RFL and some of these key downstream factors. Our data suggest that RFL could prompt tiller specification and outgrowth by its cumulative effects on meristematic genes such as LAX1 and CUP SHAPED COTYLEDON1 (CUC1), and effects on auxin transport by regulation of OsPIN3.

Materials and methods

Plant materials

Rice (Oryza sativa L. var. japonica) TP309 embryogenic calli were used to raise transgenic plants via Agrobacterium-mediated transformation as described by Prasad et al. (2001).

RT-PCR and real-time PCR

For expression analysis in dsRNAiRFL, UbiRFL(asi)-1, pUGN RFL:GR or I2B:RFL-EAR transgenic plants, RNA was isolated by the Trizol method (TRI reagent, Sigma) as per the manufacturer’s instructions. 2 µg of total RNA was used for cDNA synthesis using MMLV reverse transcriptase (Fermentas) and oligo dT20 as the reverse primer. 25 ng cDNA was used for each qPCR with 5 µM gene-specific primers and Sensimix SYBR (Bioline). For calculating the fold change in the expression of genes in dsRNAiRFL, UbiRFL(asi)-1, UGN RFL:GR or I2B:RFL-EAR axillary tissues, transcript levels in wild-type and transgenic axillary tissues were first normalized to the levels of actin, taken as the normalizing control, to obtain Ct values. The 2-ΔΔCt values were then calculated to determine the fold change of expression as described by Yadav et al. (2007), with the standard error calculated from six replicates derived from two biological samples. Primers used are listed in Supplementary Table S1. For validating the transgenic status of dsRNAiRFL or Ubi amiR RFL transgenic plants, RNA was isolated by the Trizol method (TRI reagent, Sigma) as per the manufacturer’s instructions from leaf tissues and reverse transcription reactions were performed. Wild-type tissues were used as negative controls. 50 ng cDNA was used as a template for PCR reactions with transcript-specific primers.

Histological analysis and microscopy

Rice culms containing SAM with leaf sheaths were fixed in formaldehyde/acetic acid/alcohol, stained with 1% eosin in 90% alcohol, and dehydrated through a graded ethanol series. The tissues were then embedded in Paraplast Plus (Sigma) and 8 µM sections prepared for bright-field microscopy (Zeiss Axioskop2, Germany). Images of the vegetative shoot in control or transgenic axillary tissues, transcript levels in wild-type and transgenic axillary tissues were first normalized to the levels of actin, taken as the normalizing control, to obtain Ct values. The 2-ΔΔCt values were then calculated to determine the fold change of expression as described by Yadav et al. (2007), with the standard error calculated from six replicates derived from two biological samples. Primers used are listed in Supplementary Table S1. For validating the transgenic status of dsRNAiRFL or Ubi amiR RFL transgenic plants, RNA was isolated by the Trizol method (TRI reagent, Sigma) as per the manufacturer’s instructions from leaf tissues and reverse transcription reactions were performed. Wild-type tissues were used as negative controls. 50 ng cDNA was used as a template for PCR reactions with transcript-specific primers.

1-Naphthylphthalamic acid treatment

For 1-Naphthylphthalamic acid (NPA) treatment, wild-type and UbiRFL(asi)-1 seeds were sterilized with 70% ethanol followed by...
40% bleach, and were then washed thoroughly with sterile water. These seeds were then inoculated on plates using 1/2MS media with varying concentration of NPA (Sigma). The plates were incubated vertically during seedling growth for 10 days in 16h/8h light/dark cycles. The seminal root length was determined for five plants taken for each treatment.

Dexamethasone-based induction of RFLΔGR
15-day-old T1 seedlings of pUGNRFL-GR-2 and control wild-type seedlings of similar age were treated with 10 μM dexamethasone or 0.1% ethanol as a mock control. Five plants per treatment were grouped to form two biological replicates and the treatment regime was for 9 h. RNA was prepared from culm tissues of these groups of plants (wild type and pUGNRFL-GR-2) by the Trizol method (TRI reagent, Sigma) as per the manufacturer’s instructions.

Results
AM development in RFL knockdown plants
To examine tillering defects on knockdown of RFL, a binary construct dsRNAiRFL expressing hairpin RNAs against RFL was used (Rao et al., 2008). We generated 14 independent transgenic lines and 14 control wild-type regenerated plants were raised in parallel. Leaf tissues in dsRNAiRFL transgenics were used to confirm their transgenic status by testing for the presence of hairpin RNAs against RFL. All 14 lines were positive (Supplementary Figure S1) and hence were taken for phenotypic analysis. The status of AMs in two of these plants (dsRNAiRFL-13 and dsRNAiRFL-14) was examined in eosin-stained sections of the vegetative culm in 15-day-old hardened plants. As compared to a culm section of a tissue culture-regenerated wild-type plant of similar age, the culm in dsRNAiRFL-13 had not initiated any AMs (Fig. 1B, compare with the wild type in 1D). In the dsRNAiRFL-14 line, buds were greatly reduced in number and their outgrowth was compromised (Fig. 1C). The remaining 12 lines were allowed to develop further until flowering transition of their shoot apical meristem had occurred and a young panicle of 0.2 cm had formed. The time taken for this developmental transition was delayed in the RNAi lines (Supplementary Figure S1), as is expected for loss-of-RFL-function plants (Rao et al., 2008; Ikeda-Kawakatsu et al., 2012). Further, a lower number of visible axillary buds were noted as compared to the bud number in tissue culture-regenerated wild-type plants (Fig. 1A). At an age when apical meristems are converted to ~0.2 cm panicles, six dsRNAiRFL lines (dsRNAiRFL-1, dsRNAiRFL-3, dsRNAiRFL-4, dsRNAiRFL-8, dsRNAiRFL-10, and dsRNAiRFL-12) displayed defects in AM specification and outgrowth (Fig. 1E). Six other lines (dsRNAiRFL-2, dsRNAiRFL-5, dsRNAiRFL-6, dsRNAiRFL-7, dsRNAiRFL-9, and dsRNAiRFL-11) had poor growth of axillary buds (Fig. 1F). These data were compared to bud numbers in 14 tissue culture-regenerated wild-type plants of similar developmental age (Fig. 1A and G). We also generated a knockdown of RFL by the ubiquitous expression of an artificial microRNA. Sixteen of these amiR-RFL lines, confirmed for the presence of the transgenic T-DNA, were studied phenotypically (Supplementary Figure S1). These lines displayed delayed transition to flowering as they took ~100 days as opposed to ~64 days in tissue culture-regenerated wild-type plants (Supplementary Figure S1). Also, the amiR-RFL plants had fewer visible tillers than tissue culture-regenerated wild-type plants (Fig. 1A).

Reduction of RFL activity alters expression status of AM development genes
To explore the relationship between the expression status of RFL and pathways known to support AM specification and outgrowth, transcript levels for some other key transcription factors and signalling pathways involved in AM development were examined. Auxillary meristem tissues were dissected from eight transgenic lines of dsRNAiRFL knockdown plants and used to generate two biological RNA pools. Similar numbers of control tissue culture-regenerated wild-type plants were dissected in parallel to generate control AM RNA samples. In culm tissues from knockdown plants, we noted about 7-fold RFL downregulation as compared to transcript levels in wild-type tissues (Fig. 2A). LAX PANICLE1 (LAX1), a bHLH transcription factor and orthologue of maize BARRENSTALK1 (BA1), is expressed in cells at the margins between the shoot apical meristem and peripheral regions from where new primordia emerge. The lax1 mutants fail to specify AMs (Komatsu et al., 2003). We observed a 2-fold downregulation of LAX1 transcripts in the culm of RFL RNAi plants (Fig. 2A). These results support the anatomical analyses of RFL knockdown plants presented earlier, which showed reduced or no buds in these transgenics. Transcript levels for rice MOCI, which plays an important role in the initiation of AMs, was increased about 3-fold as compared to the levels in wild-type culm tissues. The rice homeobox gene ORYZA SATIVA HOMEBOX1 (OSHI) serves as a molecular marker for meristematic cells and it is the closest homologue of Arabidopsis STM1. Expression of OSH1 in rice AMs precedes the expression of LAX1 (Okawa and Kyoizuka, 2009). In RFL knockdown plants, OSH1 transcript levels were unaffected in the culm tissues (Fig. 2A). Another homeobox gene, OsKNAT1 (OSHI5), normally expressed in leaf axils (Sentoku et al., 1999), was also unaffected (Fig. 2A). The CUC1, CUC2, and CUC3 proteins, which belong to the NAC domain family, function redundantly to establish boundaries between SAM and emerging primordia (Aida et al., 1997; Vroemen et al., 2003). More recently, functions for CUC3 in AM initiation have been reported in Arabidopsis (Raman et al., 2008). Hence, we examined the expression status of these genes in rice culms from wild-type and RFL knockdown plants. As CUC1 and CUC2 share significant functional overlap, and are both regulated by miR164, a set of primers to detect their combined levels was used, while CUC3 was measured independently. We found reduced transcript levels for both CUC1 and CUC3 genes in RFL knockdown tissues (Fig. 2A).

After the specification of AMs, their outgrowth is influenced by strigolactone and auxin-signalling pathways. We investigated the expression status of strigolactone biogenesis genes HTD1 and D10, encoding CAROTENOID CLEAVAGE DIOXYGENASE 7 and 8, respectively. We also determined transcript levels for D3, encoding F-box/
**Fig. 1.** Plants with RFL knockdown show a defect in AM specification and outgrowth. (A) Number of visible tillers in dsRNAiRFL, amiR RFL and wild-type (WT) flowering plants with 0.2 cm panicles (n = 12 for dsRNAiRFL; n = 16 for wild type and amiR RFL). The error bars represent the standard error of the mean. (B–D) Eosin-stained longitudinal sections passing through the shoot apical meristem of hardened 15-day-old plants. The status of AMs in dsRNAiRFL-13 (B), dsRNAiRFL-14 (C), and control wild type (D) are shown with black arrows indicating axillary buds. Scale bars: 100 μm. (E–F) Representative display of AM status in plants after their transition to flowering: dsRNAiRFL-1 (E); dsRNAiRFL-2 (F); and a tissue culture-regenerated wild-type plant (G). The leaves and leaf sheaths were removed to show the tiller buds. **, P < 0.01.

LRR-repeat protein involved in hormone perception. The transcript levels of HTDI were not significantly altered in RFL knockdown tissues (changes greater than 1.5-fold were taken as significant). However, nearly 4-fold upregulation in transcript levels of D10 and D3 genes occurred in the culm of RFL knockdown plants as compared to wild-type tissues (Fig. 2B). To understand if the effects of RFL on expression status of these genes are discernible in plants at earlier developmental stages, we studied culm tissues from juvenile plants for transcript levels of LAX1, D3, D10, and OsTBI genes. For these experiments we utilized seed-germinated transgenics with a partial knockdown of RFL achieved through
expression of antisense RNA against RFL (Rao et al., 2008). We took this approach as strong knockdown of RFL leads to much reduced flower number and seed set (Rao et al., 2008; Ikeda-Kawakatsu et al., 2012). Seedlings from the UbiRFL (as)-I line were grown and we dissected the culm both from plants 15 and 30 days post-germination and from plants that had undergone the transition to flowering. Similarly staged wild-type tissues were also collected. With comparative analysis, we found transcript levels of LAX1 were affected even in 15-day-old RFL partial knockdowns, a defect which persisted in 30-day-old seedlings and in the culm of plants that were flowering. On the other hand, changes in expression levels of D3 and D10 were obvious in 30-day-old plants and in plants that had made a transition to flowering (Fig. 3A–C). A slight increase in OsTB1 transcript levels was discernible only in plants that had undergone the transition to flowering (Fig. 3C). Interestingly, OsTB1 expression is unaffected by strigolactone addition (Minakuchi et al., 2010). These data suggest that RFL may influence OsTB1 by alternative mechanisms. Together, these data on regulators of AM specification and outgrowth indicate an early developmental defect in AM specification with progressive deregulation in strigolactone synthesis and perception in plants that have compromised RFL activity.

Interaction of RFL, strigolactone signalling, and bud outgrowth

Transcripts from the D3 gene are expressed in a wide range of tissues including leaves, vegetative culm (with SAM and AMs), roots, young panicles (0.1–0.3 cm) and dissected AMs (Supplementary Figure S3). The tissue distribution of Arabidopsis MAX2 transcripts has been studied using promoter–reporter fusions, and activity was restricted to meristematic and vascular tissues of juvenile and adult plants (Shen et al., 2007). In rice, RFL transcripts are spatially and temporally regulated. It is expressed in the vegetative culm and dissected axillary tissues, and its transcripts are dynamically regulated in developing panicles. Transcripts are not detected in mature leaves and roots (Supplementary Figure S3; Kyoizuka et al., 1998; Rao et al., 2008). To examine any relationship between RFL and the strigolactone pathway, we carried out knockdown of D3 in plants that were otherwise wild type and in plants that were knocked down for RFL (Fig. 4A). Transgenic lines that harboured a single copy of the integrated T-DNA (dsRNAiRFL, Ubi amiR D3, or amiR D3–dsRNAiRFL; Supplementary Figure S4) were analysed phenotypically. Five independent single-copy T-DNA lines with artificial microRNA-driven knockdown of D3 were obtained. These plants had increased tillering (Fig. 4D) and reduced plant height as compared to the control wild-type regenerated plants (Fig. 4B, Supplementary Figure S5) indicating lowered strigolactone pathway activity. These phenotypes partially mimic, with lesser severity, the effects of a T-DNA insertion at the D3 locus (Ishikawa et al., 2005). In this context we examined the consequences of RFL knockdown. Six independent transgenic lines with single-copy T-DNA expressing amiRNA against D3 and hairpin RNAs against RFL were studied for their tillering phenotype. These plants had an average of only one active tiller, a significant reduction when compared to an average five active tillers in lines with knockdown of only D3 (Fig. 4E, F). The number of active tillers in these amiR D3–dsRNAiRFL plants was comparable but not statistically equivalent to that in RFL knockdown plants (Fig. 4F). Thus, RFL functions, through other interacting pathways, are important for tiller outgrowth even when strigolactone activity is dampened. To investigate the likelihood of a causal link between strigolactone and auxin transport proteins during tiller outgrowth, we studied OsPIN1 and OsPIN3 transcript levels in wild-type plants treated with GR24, a synthetic analogue of strigolactones. We observed that plants of three developmental stages (15 and 30 days, and after flowering transition) treated with GR24 had reduced levels of OsPIN1 transcripts in vegetative culm tissues, but this had no effect on OsPIN3 transcript levels (Supplementary Figure S5A and E). Taken together, our data show that strigolactones can influence OsPIN1 expression status, but the role played by RFL in this interaction requires further investigation.

RFL knockdown plants are compromised for auxin transport

Recent studies indicate that strigolactone regulates bud outgrowth by altering the PATS (Shinohara et al., 2013). The
PIN family of auxin efflux carriers plays a predominant role in the PATS. Their intracellular localization determines the direction of auxin flow and is also critical for the positioning and initiation of a variety of lateral meristems and organ primordia (Reinhardt et al., 2000). In panicles of RFL knockdown plants our earlier studies on gene expression profiles showed that the auxin efflux facilitator OsPIN3-like (AK101504) was downregulated (Rao et al., 2008). Here, we investigated the expression levels of OsPIN1 and OsPIN3 in...
the culm tissues of RFL knockdown plants (dsRNAiRFL) after their transition to flowering. We detected downregulation of OsPIN1 expression by about 5-fold and of OsPIN3 by 7-fold (Fig. 5A). To understand if the effects of RFL on expression status of these PIN genes also occur in young plants we examined the transcript levels of OsPIN1 and OsPIN3 in culm tissues of seed-germinated RFL partial knockdown Ubi:RFL(as)-1 plants at different developmental stages. Comparative expression analysis was done using plants 15 and 30 days post-germination and in partial knockdown plants that had undergone the transition to flowering. We detected lowered transcript levels for OsPIN1 and OsPIN3 expression at all three growth stages (Fig. 5B), confirming the regulatory effect of RFL on their expression from early stages of AM outgrowth. To assess whether altered transcript levels for these auxin transporters influence hormone transport, we studied the effects of NPA, an inhibitor of auxin efflux pumps in root growth (Reed et al., 1998). The effects on root growth in wild-type and RFL partial knockdown Ubi:RFL(as)-1 plants were assessed. We observed that even without NPA treatment, seminal root length in RFL partial knockdown seedlings was significantly reduced as compared to wild-type seedlings. Wild-type seedlings subjected to NPA treatment show a statistically significant reduction in seminal root length, even at the lowest concentration of NPA used (Fig. 5C). On exposure to increasing concentrations of NPA, the roots of partial knockdown plants have a subtle reduction in root length that is statistically insignificant as compared to untreated partial knockdown plants (Fig. 5D). These data suggest compromised auxin transport in RFL partial knockdown plants, hence we examined the activity of the synthetic auxin-responsive reporter IR4 DR5:GFP in wild-type and RFL knockdown plants. This reporter is often used as a read-out for the spatially regulated buildup of auxin maxima that follows auxin transport by PIN proteins (Benkova et al., 2003; Gallavotti et al., 2008). In young regenerated wild-type plantlets with the IR4 DR5:GFP reporter cassette, which are expected to have a robust polar auxin transport system, we observed high levels of GFP fluorescence at the compact basal nodes, which contain the SAM and AMs (Supplementary Figure S6D). In contrast, dsRNAiRFL plantlets with a single T-DNA knockdown cassette and the IR4 DR5:GFP reporter have diffuse GFP fluorescence throughout the plantlet (Supplementary Figure S6E), indicating poor basipetal auxin flow. Also, optical sections of shoot meristems in IR4 DR5:GFP transgenics show spatially restricted auxin maxima (Fig. 5F). On the other hand, a diffuse pattern of GFP activity is detected in apices of RFL knockdown plants (Fig. 5G). Further, we note that dsRNAiRFL culm tissues have a slight increase in OsIAA7 and OsIAA20 transcript levels (Fig. 5E). As the expression of these rice genes is auxin responsive (Jain et al., 2006; Itoh et al., 2008), our observations could reflect a deranged auxin buildup in RFL knockdown tissues.

We exploited the close phylogenetic relationship of AtPIN2, OsPIN2, and OsPIN3 (Vieae et al., 2013) to detect OsPIN2/ OsPIN3 proteins (Cho et al., 2013) using cross-reacting antibodies (Supplementary Figure S6F; Supplementary Methods). Immunohistochemistry of wild-type culm tissues allowed the detection of signals in the vegetative SAM, and vascular strands in the leaf sheath and axillary buds (Supplementary Figure S6G–J). A subtle reduction is seen in the immunoreactivity of amiR-RFL shoot apices (Supplementary Figure S6I–K) and vascular tissues (Supplementary Figure S6G–J). These observations are supported by the reduced efficiency of 3H-IAA basipetal transport in stem and root tissues of amiR-RFL plants (Supplementary Table S2; Supplementary Methods). Together, these data suggest that the reduced expression of OsPIN1 and OsPIN3, and the subtle defects in vascular tissues, contribute to weakened auxin transport in RFL partial knockdown plants.

RFL modulates the expression of LAX1, CUC1, and OsPIN3

To ascertain the ability of RFL to regulate the expression of LAX1 and other AM development genes that were affected on knockdown of RFL, we created transgenics that express modified versions of the RFL protein. A binary construct, pUGNRFL:GR, where the T-DNA segment harbours an expression cassette for production of RFL protein with a C-terminal translational fusion to the GR domain, was made (Fig. 6A; Supplementary Methods). The fusion protein is chemically inducible in these transgenic plants. Under mock conditions, the RFL:GR protein would be cytoplasmically sequestered, while on dexamethasone treatment nuclear translocation and the downstream effects due to overexpression of RFL would occur. We raised 15 plants and used the T1 seeds from pUGNRFL:GR-2 for further experimental analysis. Five plants were grouped to form two biological replicates and this was done for each of the 9h treatment regimes, i.e. with ethanol as the mock or with dexamethasone as the inducer. Wild-type plants were similarly mock or dexamethasone treated. DNA prepared from culm tissues of these groups of plants (wild type and pUGNRFL:GR -2) were used to determine expression levels of candidate genes involved in AM development. We noted about a 2-fold increase in the transcript levels for LAX1 (Fig. 6C) and CUC1 genes on the ectopic induction of RFL:GR. On the other hand, induced overexpression of RFL:GR had no effect on transcript levels for MOC1 and CUC3. Importantly, OsPIN3 expression was elevated up to 4-fold in culm tissues of pUGNRFL:GR-2 plants that were dexamethasone treated (Fig. 6C), while no changes were noted in OsPIN1 expression levels. The modulation in expression status of genes in the strigolactone signalling pathway was also examined, and here we studied effects on D3, D10, and OsTBI1 genes. We detected no significant change in their transcript levels on induction of RFL.

In a complementary approach, we undertook studies where the consequences of expressing a repressive form of RFL on gene expression in culm tissues could be examined. For these experiments we created a set of transgenic plants where the cis regulatory elements from the RFL locus (Prasad et al., 2003) would drive the expression of RFL protein C-terminally tagged with the EAR transcription repressor motif (Fig. 6B; Hiratsu et al., 2003; Supplementary Methods). Twelve transgenic plants, confirmed for the I2B:RFL-EAR T-DNA, were
taken for comparative expression analysis with control wild-type regenerated plants. We observed that the transcript levels for \textit{LAX1}, \textit{CUC1}, \textit{CUC3}, \textit{OsTB1}, \textit{OsPIN1}, and \textit{OsPIN3} were reduced in the culm tissues of these plants that express the dominant repressive form of \textit{RFL} (Fig. 6D). Together, these data show that \textit{RFL} may directly modulate the transcript levels for these genes.
levels of LAX1, CUC1 and OsPIN3 in the culm, whereas the effects on strigolactone pathway genes and OsPIN1 are probably indirect effects.

Discussion

In this study, we examined the influence of rice RFL on factors that control specification and outgrowth of vegetative AMs. Our findings indicate that defects in AM development that occur on RFL knockdown arise from its regulatory effects first during specification of these meristems and later during their outgrowth as a tiller. Our data place meristem specification transcription factors LAX1 and CUC downstream of RFL. Additionally, the positive regulatory effect of RFL on auxin transporters in the culm could affect outgrowth of AMs.

RFL regulates genes important for AM specification

The LAX1 gene is required for development of primary branches, secondary branches, and lateral spikelets of the rice panicle (Komatsu et al., 2001). All these branch meristems are formed at the axes of bracts (Itoh et al., 2005). While nearly all mutant alleles of LAX1 show panicle-branching defects (Komatsu et al., 2001), they have variable effects on tillering. The strong lax1-2 null mutant, with reduced tillering, showed LAX1 functions are relevant to all AMs (Oikawa and Kyozuka, 2009). In young vegetative tissues RFL transcripts are detected in leaf axils and in young AMs (Rao et al., 2008). This expression domain encompasses cells that express LAX1 mRNA and cells with the LAX1 protein (Oikawa and Kyozuka, 2009). We found a reduction of RFL activity caused persistent reduction in AM development even after plants had undergone the transition to flowering. Therefore, we examined the relationship between RFL and LAX1 in the culm of plants at different developmental stages. We show that in the culm, LAX1 transcript levels are co-related with those of RFL. Further, our data from gene expression effects in transgenics with repressive or inducible RFL show modulation in LAX1 transcript levels. Recent studies on the rice lax2 mutant indicate that multiple pathways contribute to AM development, as the lax1 lax2 double mutants have synergistic tillering defects (Tabuchi et al., 2011). While LAX2, a novel nuclear protein, does not affect expression of LAX1, it is an interacting partner proposed to contribute to the AM functions of LAX1. Our findings imply a probable direct activating role for RFL in AM development that acts, in part, through attaining appropriate LAX1 expression levels. These findings are analogous to the role ascribed to a maize AT-hook protein, BAF1 that, along with unknown factors, was required for threshold levels of BA1 (maize LAX1 orthologue) expression (Galavotti et al., 2011). BAF1 thereby contributed to AM initiation in maize either upstream of, or parallel to, BA1. Arabidopsis LFY has a predominant role in conferring floral meristem (FM) identity (Weigel et al., 1992; Wagner, 2009; Moyroud et al., 2010) with its functions in AMs being unclear until recently. The latter functions were uncovered with the new LFY_HARA allele with only partial defects in FM identity (Chahtane et al., 2013). This mutant allele showed that LFY can promote growth of vegetative AMs through its direct target REGULATOR OF AXILLARY MERISTems1 (RAX1), encoding an R2R3 MYB domain factor (Chahtane et al., 2013). These functions for Arabidopsis LFY and RAX1 in AM development are parallel to, and redundant with, the pathway regulated by LATERAL SUPPRESSOR (LAS) and REGULATOR OF AXILLARY MERISTEM FORMATION1 (ROXI). The
latter gene activities are largely functional only in AMs and not FMs (Greb et al., 2003; Yang et al., 2012). Interestingly, ROX1 is orthologous to rice LAX1 and we find that LAX1 expression levels in flowering panicles and vegetative AMs is dependent on the expression status of RFL (Rao et al., 2008; also this study). Our data also show elevated transcript levels for MOCI, the homologue of Arabidopsis LAS, in plants downregulated for RFL. However, we found that induction of RFL:GR and expression of the repressive RFL–EAR protein did not significantly alter MOCI transcript levels. Therefore, we postulate that in RFL knockdown plants the changes in MOCI transcript levels arise due to indirect consequences. Further, as MOCI is subjected to post-translational and post-transcriptional control (Lin et al., 2012; Xu et al., 2012), understanding any relationship between RFL and MOCI requires further studies. Thus, we speculate that the multiple pathways that control Arabidopsis AM development, i.e. LFY-dependent and LFY-independent mechanisms, are closely linked in rice.

In Arabidopsis, CUC2 and CUC3 genes, in addition to their role in shoot meristem formation and organ separation, play a role in AM development possibly by defining a boundary for the emerging AM. These functions for the Arabidopsis CUC genes are routed through their effects on LAS and by mechanisms that are independent of LAS (Hibara et al., 2006; Raman et al., 2008). A relationship between Arabidopsis LFY and CUC genes in AMs has not yet been defined, but the direct regulatory effects of LFY on CUC2 in FMs is known (Winter et al., 2011). Our data show modulation in RFL activity leads to corresponding expression changes in CUC1, CUC2, and CUC3 gene expression in the culm. Further, we note overlapping expression domains for rice RFL, OsNAM1/CUC2, and OsCUC3 in leaf axils (Hibara and Nagato, 2006; Rao et al., 2008). Thus, during AM development, the meristem functions of RFL and CUC genes are related.

RFL regulates AM outgrowth by positively influencing auxin transport

The outgrowth of axillary buds is also influenced by hormonal signals and external cues such as planting density, nutrient availability, and light (Shimizu-Sato and Mori, 2001; Beveridge et al., 2003; Leyser, 2003; Brewer et al., 2013). Studies of Arabidopsis max, pea rms, and rice dwarf strigolactone mutants provide two mutually non-exclusive models that explain the general relationships between auxin, strigolactone, and bud outgrowth, but details of temporal and spatial dynamics of these links are unclear. One model postulates that strigolactones inhibit bud outgrowth by altering gene expression with possible direct effects on AMs (Brewer et al., 2009; Brewer et al., 2013). Computational modelling of long-distance auxin transport and decapitation experiments with pea support this model, and suggest that strigolactone may affect bud meristem cell division downstream of BRC1, a TCP-domain transcription factor (Brewer et al., 2009; Dun et al., 2012; Renton et al., 2012). Other recent reports using Arabidopsis show that strigolactones inhibit bud outgrowth by depleting plasma membrane-localized PIN transporters (Crawford et al., 2010; Shinohara et al., 2013). These findings form the mechanistic basis for the second model, which proposes that strigolactone inhibits bud outgrowth depending on the overall auxin transport status of the plant, with bud outgrowth requiring active export of auxin.

Here, we have shown that transgenic plants compromised for RFL function have reduced levels of OsPIN1 and OsPIN3 transcripts and diffuse auxin-sensitive reporter activity in the SAM (Fig. 5). Supporting this are immunolocalization data that hint at a mild reduction in OsPIN2/OsPIN3 cross-reactivity in RFL knockdown plants, further supported by impaired auxin transport in the plants (Fig. 5; Supplementary Figure S6; Supplementary Table S2). Our findings are consistent with a relationship between RFL and auxin transport in vegetative culms during bud outgrowth, with probable direct effects on OsPIN3. We cannot exclude the effects of RFL on transporters such as those of the ABCB family (Multani et al., 2003; Knöller et al., 2010). Other likely players downstream of RFL could be factors like AXR1 (Stirnberg et al., 1999); their contributions remain to be tested. The indirect regulatory effects of RFL on bud-specific transcription factor genes such as OsTBI could also contribute to outgrowth.

Overall, our studies indicate an important role for RFL in vegetative AM specification and perhaps on the outgrowth of axillary buds.

Supplementary material

Supplementary Table S1. Primers used in this study.

Supplementary Table S2. PATS assay in wild-type and amiR–RFL plants.

Supplementary Figure S1. Expression of hairpin loop RNA in dsRNAiRFL plants detected by RT-PCR analysis of transgenic leaf tissues.

Supplementary Figure S2. Transcript levels for D3 and RFL in various rice tissues.

Supplementary Figure S3. Copy number determination for T-DNAs in Ubi amiRD3, dsRNAiRFL and amiR D3-dsRNAiRFL lines.

Supplementary Figure S4. Plant height phenotype for dsRNAiRFL, Ubi amiRD3, amiR D3-dsRNAiRFL, and wild-type plants.

Supplementary Figure S5. Effect of GR24 on transcript levels of OsPIN1 and OsPIN3 in wild-type plants of three developmental growth stages.

Supplementary Figure S6. Live imaging of GFP reporter activity driven by an auxin-responsive promoter in young dsRNAiRFL and wild-type plantlets; and immunohistochemistry for OsPIN2/OsPIN3 in histological sections of wild-type and amiR RFL culm tissues.

Supplementary Methods. These methods are for the design of various constructs; live imaging; genomic DNA isolation and determination of T-DNA copy number; immunoblotting and immunohistochemistry; GR24 treatment; dexamethasone-based induction; and PATS assay.
Acknowledgements

Dr Nagasree Rao is acknowledged for generating *Usb RFL*(as)-1 and *pUGNRFLΔGR-2* seeds. This work was funded by grant support from the Department of Biotechnology, Government of India to UVR for rice functional genomics. Infrastructure support for microscopy and real-time PCR from the Grant-in-aid Programme to IISc from Department of Biotechnology, GOI, is also acknowledged. We thank the reviewers for their valuable comments on this work. Senior Research Fellowships to GD from the Indian Institute of Science and to GLC from the Council for Scientific and Industrial Research are acknowledged. A DBT Research Associateship supported KR. We thank Mr Prabhu and Ms Shipra Goel for care of transgenic plants and Ms Samrajay and Ms Pannaga Girish for their help with confocal imaging.

References

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M. 1997. Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. The Plant Cell 9, 841–857.

Arite T, Iwata H, Ohshima K, et al. 2007. DWF10, an RMS1/MAX5/DAD1 ortholog, controls lateral bud outgrowth in rice. The Plant Journal 51, 1019–1029.

Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyozuka J. 2009. d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. Plant Cell Physiology 50, 1416–1424.

Benkova E, Michniewicz M, Sauer M, Teichmann T, Seifertova D, Jürgens G and Friml J. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115, 591–602.

Beveridge CA, Weller JL, Singer SR, Hofer JM. 2003. Axillary meristem development. Budding relationships between networks controlling flowering, branching, and phloem/parenchyma differentiation. Plant Physiology 131, 927–934.

Booker J, Sieberer T, Wright W, et al. 2005. MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. Developmental Cell 8, 443–449.

Brewer B, Koltai H, Beveridge CA. 2013. Diverse roles of strigolactones in plant development. Molecular Plant 6, 18–28.

Brewer PB, Dun EA, Ferguson BJ, Rameau C, Beveridge CA. 2009. Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and Arabidopsis. Plant Physiology 150, 482–493.

Chahatane H, Vachon G, Le Masson M, et al. 2013. A variant of LEAFY reveals its capacity to stimulate meristem development by inducing RAX1. The Plant Journal 74, 678–693.

Cho SH, Yoo SC, Zhang H, Pandeya D, Koh HJ, Hwang JY, Kim GT, Paek NC. 2013. The rice narrow leaf2 and narrow leaf3 loci encode WUSCHEL-related homeobox 3A (*OstWOX3A*) and function in leaf, spikelet, tiller and lateral root development. New Phytologist 198, 1074–1084.

Crawford S, Shinhoara N, Sieberer T, Williamson L, George G, Hepworth J, Muller D, Domagalska MA, Leyser O. 2010. Strigolactones enhance competition between shoot branches by dampening auxin transport. Development 137, 2905–2913.

Dun A, Germain A, Rameau C, Beveridge CA. 2012. Antagonistic action of strigolactone and cytokinin in bud outgrowth control. Plant Physiology 158, 487–498.

Gallavotti A, Malcomber S, Gaines C, Stanfield S, Whipple C, Kellogg E, Schmidt RJ. 2011. BARREN STALK FASTIGIATE1 is an AT-hook protein required for the formation of maize ears. The Plant Cell 23, 1756–1771.

Gallavotti A, Yang Y, Schmidt RJ, Jackson D. 2008. The relationship between auxin transport and maize branching. The Plant Journal 147, 1913–1923.

Gomez-Roldan V, Femras S, Brewer PB, et al. 2008. Strigolactone inhibition of shoot branching. Nature 455, 189–194.

Greb T, Clarens O, Schäfer E, Muller D, Herrero R, Schmitz G, Theres K. 2003. Molecular analysis of the LATERAL SUPPRESSOR gene in Arabidopsis reveals a conserved control mechanism for axillary meristem formation. Genes and Development 17, 1175–1187.

Hibara K, Karim MR, Takada S, Taoka K, Furutani M, Aida M, Tasaka M. 2006. Arabidopsis CUP-SHAPED COTYLEDON3 regulates postembryonic shoot meristem and organ boundary formation. The Plant Cell 18, 2946–2957.

Hibara K, Nagato Y. 2006. OsVAM and OsCUC3 are expressed specifically in organ boundaries. Rice Genetics Newsletter 23, 96–97.

Hiratsu K, Matsui K, Koyama T, Ohme-Takahashi M. 2003. Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in Arabidopsis. The Plant Journal 34, 733–739.

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

Ishikawa S, Maekawa M, Arite T, Onishi K, Takamure I, Kyozuka J. 2005. Suppression of tiller bud activity in tillering dwarf mutants of rice. Plant Cell Physiology 46, 79–86.

Itoh JI, Hibara KI, Sato Y, Nagato Y. 2008. Developmental role and auxin responsiveness of class III homeodomain leucine zipper gene family members in rice. Plant Physiology 147, 1960–1975.

Itoh J, Nonomura K, Ikeda K, Yamaki S, Inukai Y, Yamagishi H, Kitano H, Nagato Y. 2005. Rice plant development: from zygoate to spikelet. Plant Cell Physiology 46, 23–47.

Jain M, Kaur N, Garg R, Thakur JK, Tyagi AK, Khurana JP. 2006. Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (*Oryza sativa*). Functional and Integrative Genomics 6, 47–59.

Jiang L, Liu X, Xiong G, et al. 2013. DWF3 s3 acts as a repressor of strigolactone signalling in rice. Nature 504, 401–405.

Knöller AS, Blakeslee JJ, Richards EL, Peer WA, Murphy AS. 2010. Brachytyci2/3InBAC31 functions in IAA export from intercalary meristems. Journal of Experimental Botany 61, 3689–3696.

Komatsu K, Maekawa M, Ujije S, Satake Y, Furutani I, Okamoto H, Shimamoto K, Kyozuka J. 2003. LAX and SPA: Major regulators of shoot branching in rice, Proceedings of National Academy of Sciences, USA 100, 11765–11770.

Komatsu M, Maekawa M, Shimamoto K, Kyozuka J. 2001. The LAX1 and FRIZZY PANICLE2 genes determine the inflorescence architecture of rice by controlling rachis-branch and spikelet development. Developmental Biology 231, 364–373.

Kyozuka J, Konishi S, Nemoto K, Izawa T, Shimamoto K. 1998. Down regulation of RFL the FLO/FLY homolog of rice accompanied with panicle and branch initiation. Proceedings of National Academy of Sciences, USA 95, 1979–1982.

Leyser O. 2003. Regulation of shoot branching by auxin. Trends in Plant Science 8, 541–545.

Liang W, Shang F, Lin Q, Lou C, Zhang J. 2014. Tillering and panicle branching genes in rice. Gene 537, 1–6.

Lin H, Wang R, Qian Q, et al. 2009. DWF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. The Plant Cell 21, 1512–1526.

Lin Q, Wang D, Dong H, et al. 2012. Rice APC/C(TE) controls tillering by mediating the degradation of MONOCULM1. Nature Communications 3, 752.

Li X, Qian Q, Fu Z, et al. 2003. Control of tillering in rice. Nature 422, 618–621.

Minakuchi K, Kameoka H, Yasuno N, et al. 2010. FINE CULM1 (FC1) works downstream of strigolactones to inhibit the outgrowth of axillary buds in rice. Plant Cell Physiology 51, 1127–1135.

Moyroud E, Kusters E, Monniaux M, Koes R, Parcy F. 2010. LEAFY blossoms. Trends in Plant Science 6, 346–352.

Multani DS, Briggs SP, Chamberlin MA, Blakeslee JJ, Murphy AS, Johal GS. 2003. Loss of an MDR Transporter in Compact Stalks of Maize *br2* and Sorghum *dw3* mutants. Science 302, 81–83.

Okawa T, Kyozuka J. 2009. Two-step regulation of LAX PANICLE1 protein accumulation in axillary meristem formation in rice. The Plant Cell 21, 1095–1108.
Lateral Bud Formation to Inhibit Lateral Bud Growth in

Shinohara N, Taylor C, Leyser O. 2001. Positive regulator of photomorphogenesis in the stem?

Reed RC, Brady SR, Muday GK. 2001. Ectopic expression of rice Osmads1 reveals a role in specifying the lemma and palea, grass floral organs analogous to sepal.

OsMADS1 reveals a role in specifying the lemma and palea, grass floral organs analogous to sepal. Development Genes and Evolution 211, 281–290.

Raman S, Greb T, Peaucelle A, Blein T, Laufs P, Theres K. 2008. Interplay of miR164, CUP-SHAPED COTYLEDON genes and LATERAL SUPPRESSOR controls axillary meristem formation in Arabidopsis thaliana. The Plant Journal 55, 65–76.

Rao NN, Prasad K, Kushalappa K, Vijayraghavan U. 2008. Distinct regulatory role for RFL, the rice LFY homolog, in determining flower timing and plant architecture. Proceedings of the National Academy of Sciences, USA 105, 3646–3651.

Reed RC, Brady SR, Muday GK. 1998. Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. Plant Physiology 118, 1369–1378.

Reinhardt D, Mandel T, Kuhlemeier C. 2000. Auxin regulates the initiation and radial position of plant lateral organs. The Plant Cell 12, 507–518.

Renton M, Hanan J, Ferguson J, Beveridge CA. 2012. Models of long-distance transport: how is carrier-dependent auxin transport regulated in plants? New Phytologist 194, 704–715.

Sentoku N, Sato Y, Kurata N, Ito Y, Kitano H, Matsuoka M. 2002. The Arabidopsis LATERAL SUPPRESSOR (Las) gene is required for axillary meristem formation in Arabidopsis. The Plant Cell 14, 3677–3689.

Shen H, Luong P, Huq E. 2007. The F-box protein MAX2 functions as a positive regulator of photomorphogenesis in Arabidopsis. Plant Physiology 145, 1471–1483.

Shimizu-Sato S, Mori H. 2001. Control of outgrowth and dormancy in axillary buds. Plant Physiology 127, 1405–1413.

Shinohara N, Taylor C, Leyser O. 2013. Strigolactone can promote or inhibit shoot branching by triggering rapid depletion of the auxin efflux protein PIN1 from the plasma membrane. PLoS Biology 11, e1001474.

Stirnberg P, Chatfield SP, Leyser HMO. 1999. AXR1 Acts after Lateral Bud Formation to Inhibit Lateral Bud Growth in Arabidopsis. Plant Physiology 121, 839–847.

Stirnberg P, van de Sande K, Leyser O. 2002. MAX1 and MAX2 control shoot lateral branching in Arabidopsis. Development 129, 1131–1141.

Tabuchi H, Zhang Y, Hattori S, et al. 2011. LAX PANICLE2 of rice encodes a novel nuclear protein and regulates the formation of axillary meristems. The Plant Cell 23, 3276–3287.

Umehara M, Hanada A, Yoshida S, et al. 2008. Inhibition of shoot branching by new ternopetal plant hormones. Nature 455, 195–200.

Viaene, Delwiche CF, Rensing SA, Friml J. 2013. Origin and evolution of PIN auxin transporters in the green lineage. Trends in Plant Science 18, 5–10.

Vroemen CW, Mordhorst AP, Albrecht C, Kwaaitaal MA, de Vries SC. 2003. The CUP-SHAPED COTYLEDON3 gene is required for boundary and shoot meristem formation in Arabidopsis. The Plant Cell 15, 1563–1577.

Wagner D. 2009. Flower morphogenesis: timing is key. Development Cell 16, 621–622.

Weigel D, Alvarez J, Smyth DR, Yanofsky MF, Meyerowitz EM. 1992. LEAFY controls floral meristem identity in Arabidopsis. Cell 69, 843–859.

Winter CM, Austin RS, Blanvillain-Baufume S, et al. 2011. LEAFY target genes reveal floral regulatory logic, cis motifs, and a link to biotic stimulus response. Developmental Cell 20, 430–443.

Wisniewska J, Xu J, Seifertova D, Brewer PB, Ruzicka K, Biliou I, Rouquie D, Benkova E, Scheres B, Friml J. 2006. Polar PIN localization directs auxin flow in plants. Science 312, 883.

Xu C, Wang Y, Yu Y, Duan J, Liao Z, Xiong G, Meng X, Liu G, Qian Q, Li J. 2012. Degradation of monoculm 1 by APC/C(TAD1) regulates rice tillering. Nature Communications 3, 750.

Xu M, Zhu L, Shou H, Wu P. 2005. A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. Plant Cell Physiology 46, 1674–1681.

Yadav SR, Prasad K, Vijayraghavan U. 2007. Divergent regulatory OsMADS2 functions control size, shape and differentiation of the highly derived rice floret second-whorl organ. Genetics 176, 283–294.

Yang F, Wang Q, Schmitz G, Muller D, Theres K. 2012. The bHLH24/39 protein ROC acts in concert with RAX1 and LAS to modulate axillary meristem formation in Arabidopsis. The Plant Journal 71, 61–70.

Zou J, Zhang S, Zhang W, et al. 2006. The rice HIGH-TILLERING DWARF1 encoding an ortholog of Arabidopsis MAX3 is required for negative regulation of the outgrowth of axillary buds. The Plant Journal 48, 687–698.