**LBD16 and LBD18 acting downstream of ARF7 and ARF19 are involved in adventitious root formation in Arabidopsis**

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**Abstract**

**Background:** Adventitious root (AR) formation is a complex genetic trait, which is controlled by various endogenous and environmental cues. Auxin is known to play a central role in AR formation; however, the mechanisms underlying this role are not well understood.

**Results:** In this study, we showed that a previously identified auxin signaling module, AUXIN RESPONSE FACTOR (ARF) 7/ARF19-LATERAL ORGAN BOUNDARIES DOMAIN (LBD) 16/LBD18 via AUXIN1 (AUX1)/LIKE-AUXIN3 (LAX3) auxin influx carriers, which plays important roles in lateral root formation, is involved in AR formation in Arabidopsis. In aux1, lax3, arf7, arf19, lbd16 and lbd18 single mutants, we observed reduced numbers of ARs than in the wild type. Double and triple mutants exhibited an additional decrease in AR numbers compared with the corresponding single or double mutants, respectively, and the aux1 lax3 lbd16 lbd18 quadruple mutant was devoid of ARs. Expression of LBD16 or LBD18 under their own promoters in lbd16 or lbd18 mutants rescued the reduced number of ARs to wild-type levels. LBD16 or LBD18 fused to a dominant SRDX repressor suppressed promoter activity of the cell cycle gene, Cyclin-Dependent Kinase (CDK) A1;1, to some extent. Expression of LBD16 or LBD18 was significantly reduced in arf7 and arf19 mutants during AR formation in a light-dependent manner, but not in arf6 and arf8. GUS expression analysis of promoter-GUS reporter transgenic lines revealed overlapping expression patterns for LBD16, LBD18, ARF7, ARF19 and LAX3 in AR primordia.

**Conclusion:** These results suggest that the ARF7/ARF19-LBD16/LBD18 transcriptional module via the AUX1/LAX3 auxin influx carriers plays an important role in AR formation in Arabidopsis.

**Keywords:** Arabidopsis thaliana, Adventitious root formation, Auxin response factor, Lateral organ boundaries domain, LBD16, LBD18

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**Background**

Root architecture in higher plants, which is critical for anchorage in soil and the uptake of water and nutrients, is diverse at both the system and anatomical levels [1]. In general, dicotyledonous plants, such as Arabidopsis thaliana, have a primary root that branches to form lateral roots (LRs). In both monocot and dicotyledonous plants, the primary root can develop adventitious roots (ARs) that arise naturally from the aerial organs as an adaptive response to environmental changes, such as flooding and dark-light transitions, or artificially by wounding [2–5]. AR formation is critical for vegetative propagation of elite genotypes in agriculture and is also important for plant survival under a variety of biotic and abiotic stresses [4, 5]. Auxin plays a central role in both LR and AR formation [6]. Although signaling and molecular mechanisms of auxin-regulated primary and LR development are relatively well characterized, our understanding of how auxin regulates AR formation is rudimentary [1, 7–9].

Plant root development is regulated by establishing auxin maxima at the primordium tip through auxin transport [10–13]. During auxin transport, auxin travels acropetally or basipetally over long distances by the combined action of plasma membrane-localized auxin efflux and influx carriers and triggers various regulatory mechanisms along its path [14–17]. Auxin efflux carriers, including two major
transmembrane proteins, i.e., PIN-FORMED (PIN) and ATP-binding cassette subfamily B (ABCB), were shown to be involved in both LR and AR formation in different plant species [10, 18–22]. Studies with the polar auxin transport inhibitor, N-1-naphthylphthalamic acid, showed that PIN1-mediated auxin transport is necessary for AR emergence in rice plants [23]. Auxin together with ethylene positively regulates AR initiation through DIAGEOTROPICA (DGT), which encodes a cyclophilin A-type protein (SICYP) [24, 25]. SICYP1 changes the abundance of PIN efflux carriers at the plasma membrane to modulate polar auxin transport during AR initiation [26–29].

Auxin signaling is regulated by two large protein families: the AUXIN RESPONSE FACTOR (ARF) proteins, which act as the DNA-binding transcriptional regulators of auxin responses, and the Aux/IAA proteins, negative regulators of ARFs [30]. Several ARFs have been identified as playing a role in AR formation in both Arabidopsis and rice. ARF6 and ARF8 have been shown to act as positive regulators of AR initiation in Arabidopsis hypocotyls, whereas ARF17 acts as a negative regulator [31, 32]. These ARFs regulate each other’s expression at the transcriptional and posttranscriptional levels by modulating the homeostasis of miR160, which targets ARF17 and miR167, subsequently targeting both ARF6 and ARF8 [32]. This complex network of transcription factors regulates the expression of three auxin-inducible GRETCHEN HAGEN3 (GH3) genes, encoding acyl-acid-amido synthetases, which are required for fine-tuning AR initiation in the Arabidopsis hypocotyls by modulating jasmonic acid homeostasis [33]. Some studies have indicated that auxin signaling modules involved in LR formation could play a role in AR formation as well. For instance, ARF7 and ARF19 control LR formation as well as AR formation in Arabidopsis [34–38]. OsARF6, which is a rice ortholog of ARF7 and ARF19, controls the initiation of adventitious crown root primordia in rice by activating the expression of CROWN ROOTLESS1/ADVENTITIOUS ROOTLESS1 (CRL1/ARL1), which encodes a LATERAL ORGAN BOUNDARIES DOMAIN (LBD) protein [39–41].

In Arabidopsis, several LBD genes, such as LBD16, LBD18, and LBD19, have been demonstrated to play critical and distinct roles in auxin-regulated LR development [37, 42–49]. It has been shown that the auxin influx carriers AUXIN1 (AUX1) and LIKE-AUX1 3 (LAX3) are required for auxin-responsive expression of LBD16 and LBD18 to control various stages of LR development in Arabidopsis [45, 50, 51]. In the present study, we show that the AUX1/LAX3-ARF7/ARF19-LBD16/LBD18 signaling module is also important for AR formation in Arabidopsis, providing evidence of a common regulatory mechanism being utilized for LR and AR formation during auxin signaling.

Results

Analysis of GUS expression patterns of ProARF2-GUS, ProARF19-GUS, ProLAX3-GUS, ProLBD16-GUS and ProLBD18-GUS during AR development

To gain insights into the function of the LAX3-ARF7/ARF19-LBD16/LBD18 signaling module during AR development, we analyzed GUS expression in ProLBD16-GUS, ProLBD18-GUS, ProARF7/GUS, ProARF19-GUS and ProLAX3-GUS transgenic plants during the early stages of AR formation (Fig. 1). GUS expression was detected in the cotyledon and lower part of the hypocotyl of 3-d-old dark-grown ProLBD16-GUS seedlings at time T0 (Fig. 1a). After transferring these seedlings to the light for 72 h, GUS expression was clearly detected in the early AR primordium in the hypocotyl (Fig. 1b). After 6 d in the light, GUS expression generally increased in both the hypocotyl and root and was detected in the hypocotyl stele tissue near the emerged AR (Fig. 1c). Regarding ProLBD18-GUS, GUS expression was detected only in the hypocotyl of 3-d-old dark-grown seedlings (Fig. 1d): after transferring to the light for 72 h, strong GUS expression was detected in the AR primordium in the hypocotyl (Fig. 1e). In ProARF7-GUS, ProARF19-GUS and ProLAX3-GUS seedlings, GUS expression was detected in both the hypocotyl stele tissue and AR primordium after transferring 3-d-old dark-grown seedlings to the light for 72 h (Fig. 1f–h). These overlapping and distinctive GUS expression patterns in the hypocotyl stele tissue and AR primordium of the GUS reporter transgenic lines indicated that LBD16 and LBD18 may play an overlapping role in early AR primordium development and LBD18 may play a distinctive role in the AR primordium in later developmental stages downstream of ARF7/ARF19-LAX3 during AR development.

AUX1, LAX3, LBD16 and LBD18 are involved in AR formation in Arabidopsis hypocotyls

To determine the roles of auxin influx carriers, AUX1 and LAX3, and two critical LBD transcription factors, LBD16 and LBD18, in AR formation, we measured AR numbers on hypocotyls from single and multiple mutants derived from aux1, lax3, lbd16 and lbd18 (Fig. 2). To induce AR formation in hypocotyls, seeds germinated were etiolated vertically for 3 d and grown in the light for 7 d, and the ARs were counted [32]. The aux1, lax3, lbd16 and lbd18 single mutants showed a significant decrease in AR numbers compared with that of the wild type after transferring 3-d-old dark-grown seedlings to the light for 7 d (49.01, 41.17, 23.52 and 41.17% for aux1, lax3, lbd16 and lbd18, respectively) (Fig. 2). The number of ARs in double mutants, lbd16 lbd18, aux1 lbd16, aux1 lbd18, lax3 lbd16, lax3 lbd18 and aux1 lax3, was further reduced compared with that of their corresponding single mutants (Fig. 2). AR numbers in triple mutants, aux1 lbd16 lbd18 and lax3 lbd16 lbd18,
were further reduced to ~8% compared to that of the wild type, whereas the aux1 lax3 double mutant and aux1 lax3 lbd16 and aux1 lax3 lbd18 triple mutants exhibited 3.5–4% that of the wild-type AR number (Fig. 2). In the quadruple mutant, aux1 lax3 lbd16 lbd18, AR formation could not be detected. These results indicate that auxin influx carriers, such as AUX1 and LAX3, are critical for AR formation and that AUX1, LAX3, LBD16 and LBD18 genes are essential for AR formation. To further confirm the role of LBD16 and LBD18 in AR formation, we generated transgenic Arabidopsis plants expressing LBD16:MYC (ProLBD16:LBD16:MYC/lbd16) or LBD18:HA (ProLBD18:LBD18:HA/lbd18) in lbd16 or lbd18 mutant background under the control of their own promoter, respectively, (Fig. 3a, b), and analyzed AR numbers of these complementation lines. The three different complementation lines selected for AR analysis rescued AR development defects caused by lbd16 or lbd18 mutations to varying degrees (Fig. 3c). ProLBD16:LBD16:Myc/lbd16 (#2–1) and ProLBD18:LBD18:HA/lbd18 (#2–1) transgenic mutant lines showed wild-type levels of AR numbers (Fig. 3c). These results demonstrated that LBD16 and LBD18 play significant roles in AR development.

Both LBD16:SRDX and LBD18:SRDX suppress cell cycle gene promoter activities during induction of AR formation

Previous studies have shown that LBD16 and LBD18 regulate expression of some cell cycle genes during LR initiation [45, 48]. To examine if LBD16 and LBD18 might be involved in the expression of cell cycle genes during AR formation, we used transgenic Arabidopsis
expressing LBD16:SUPERMAN REPRESSIVE DOMAIN X (SRDX) or LBD18:SRDX under the control of their own promoters and harboring Cyclin-Dependent Kinase (CDK)A1:1 or CDKB1:1 in Col-0 or in lbd18 mutants [45]. We found that LBD16:SRDX suppressed GUS expression in the vasculature of the hypocotyl in ProCDKB1;1:GUS transgenic plants, whereas LBD18:SRDX suppressed GUS expression in the primordium and vasculature in the hypocotyl of ProCDKA1;1:GUS transgenic plants (Fig. 4). Suppression of GUS expression in the hypocotyl vasculature of ProCDKB1;1:GUS by LBD16:SRDX or in the AR of ProCDKA1;1:GUS by LBD18:SRDX correlates with preferential expression of LBD16 or LBD18 in the hypocotyl stele tissue or only in the AR primordium, respectively. We also noted that LBD16:SRDX completely blocked AR formation in the hypocotyl in the light, whereas LBD18:SRDX did not block AR formation (Fig. 4). These results indicated that LBD16 and LBD18 might be involved in the expression of some cell cycle genes during AR development at distinctive stages. However, further quantitative and functional analyses are necessary to clarify the direct involvement of LBD16 and LBD18 in regulating cell cycle gene expression during AR development.

**LBD16 and LBD18 act downstream of ARF7 and ARF19 to regulate AR development**

During LR development, LBD16 and LBD18 are transcriptionally regulated downstream of ARF7 and ARF19 during auxin signaling [34, 37, 52]. We thus investigated whether the same transcriptional module functions during AR formation in a light-dependent manner. To this end, we analyzed expression of LBD16 and LBD18 in hypocotyls of arf7 and arf19 single mutants and an arf7 arf19 double mutant at different time points during the early stages of AR formation by RT-qPCR analysis (Fig. 5). At T0, which is the etiolated stage of seedlings (just before transferring to light), the expression of LBD16 and LBD18 was unchanged in all three mutants compared with that of the wild type (Fig. 5). After transferring to the light for 72 h (T72L), the expression of LBD16 and LBD18 was enhanced in the wild type as well as in all mutant plants. However, light-induced expression of LBD16 and LBD18 was significantly reduced in arf7 (by 48.5 and 45.6%, respectively) and arf19 mutants (20.9 and 33.3%, respectively) compared with that of the wild type (Fig. 5a). LBD16 and LBD18 expression were further reduced in arf7 arf19 double mutants (by 66.6 and 55.9%, respectively), compared with that of the wild type (Fig. 5b), indicating that ARF7 and ARF19 regulate LBD16 and LBD18 expression during AR development after the dark-light transition.

In a previous study, overexpression of LBD16 or LBD18 in arf7 arf19 mutant under the control of the Cauliflower mosaic virus (CaMV 35S) promoter resulted in induction of notable LR formation within 12 d, demonstrating that LBD16 and LBD18 can independently function downstream of ARF7 and ARF19 to control LR development [37, 42]. As reported in previous studies [37, 42], the LR phenotype of the arf7 arf19 mutant was rescued by LBD16 or LBD18 overexpression to some extent (Fig. 6b, top panel). We next examined whether overexpression of LBD16 or LBD18 in arf7 arf19 mutants could cause ectopic initiation of ARs, but could not detect any AR formation even after 2 weeks of plant
growth in the light (Fig. 6b, bottom panel). This result suggested that unlike LR initiation, \textit{LBD16} and \textit{LBD18} require additional factors produced by ARF7 and ARF19 to induce AR initiation.

Previous studies have shown that \textit{ARF6} and \textit{ARF8} act as positive regulators of AR formation [31, 32]. Thus, we tested if \textit{ARF6} and \textit{ARF8} could control the expression of \textit{LBD16} and \textit{LBD18} for AR formation. The time-course response of \textit{LBD16} and \textit{LBD18} expression in \textit{arf6} and \textit{arf8} mutants after treatment with auxin indole-3-acetic acid was analyzed using RT-qPCR, but no alteration in the expression of \textit{LBD16} or \textit{LBD18} in \textit{arf6} and \textit{arf8} mutant backgrounds was observed compared with that of the wild type (Additional file 1: Figure S1), suggesting that \textit{ARF6} and \textit{ARF8} regulate AR formation through a distinct pathway, independent of \textit{LBD16} and \textit{LBD18}, during auxin signaling. Taken together, these results indicated that \textit{LBD16} and \textit{LBD18} expression is regulated downstream of \textit{ARF7} and \textit{ARF19}, but not of \textit{ARF6} and \textit{ARF8}, for AR development.

\textbf{Discussion}

Studies on genetic aspects and hormonal responses of LR and AR formation suggested that LRs and ARs share key elements of genetic and hormonal regulatory networks but with different regulatory mechanisms [1]. While genetic components and molecular signaling pathways during LR development in Arabidopsis have been well characterized...
those involved in AR development are largely unknown. In this work, we showed that the auxin-responsive ARF7/ARF19-LBD16/LBD18 transcriptional module, via AUX1/LAX3 transporters, plays an important role in AR formation in the Arabidopsis hypocotyl.

In Arabidopsis, the developmental processes of LRs are well defined, and consists of the priming of pericycle cells in the basal meristem of the primary root, the first anticlinal asymmetric cell division (initiation), ordered anticlinal and periclinal cell divisions to form a dome-shaped LR primordium, and the emergence of an LR primordium from the primary root [53, 54]. Although the developmental stages of AR formation are not well described in Arabidopsis, AR formation in apple cuttings have been similarly divided into four successive phases: cell dedifferentiation, induction as the beginning of cell division, the outgrowth of a dome-shaped primordium, and the emergence of the AR [55]. In Arabidopsis, ARs initiate from the pericycle cells adjacent to the xylem pole in the hypocotyl, similar to how LRs initiate [56]. ARF proteins, which are involved in LR initiation, were found to regulate AR initiation in Arabidopsis [32, 33], indicating that although AR and LR originate from different organs, similar molecular mechanisms may regulate AR initiation. In rice, CRL1/ARL1, which promotes crown root initiation, positively regulates 277 genes, and among those, it positively regulates many genes homologous to Arabidopsis genes involved in LR formation [57].

In the present study, we showed that the signaling module of AUX1/LAX3-ARF7/ARF19-LBD16/LBD18, which has been shown to regulate LR formation [37, 42, 45, 48], is involved in AR formation (Fig. 7), indicating conservation of developmental processes for AR and LR formation. However, we noted some differences in the roles of each signaling component between LR and AR development. We had previously found that quadruple mutations in lbd16 lbd18 aux1 lax3 nearly abolished the formation of emerged LRs, but barely affected LR primordium density at early developmental stages from I to III [45]. However, we could not detect any AR primordium in this quadruple mutant (Fig. 2). This observation indicates that the LBD16/LBD18 transcriptional module downstream of AUX1/LAX3 is essential for both AR initiation and development, whereas the same signaling module functions redundantly during LR initiation possibly in conjunction with other LBD genes. Moreover, we found that overexpression of LBD16 or LBD18 in the arf7 arf19 mutant could not rescue AR defects, whereas the same approach significantly stimulated LR formation in the arf7 arf19 mutant (Fig. 6) [34, 37], indicating that LBD16 and LBD18 require additional components produced by ARF7 and ARF19 to regulate AR formation. In addition, LBD29 plays an important role in LR formation downstream of ARF7 by directly activating LAX3 expression in response to auxin [47, 49]. LBD18 expression is regulated downstream of LAX3 [45] and LAX3 is involved in AR formation (Fig. 1). Thus, it is most likely that LBD29 also plays a role in AR formation.

We noticed that the complementation lines of the lbd16 or lbd18 mutant generated by expressing LBD16 or LBD18 under the control of their own promoter
exhibited much higher expression levels of each transgene compared with that of the wild-type LBD16 or LBD18, and yet the AR numbers in the complementation lines are comparable to that of the wild-type Col-0 [Fig. 3]. This result indicates that overexpression of a single LBD transcription factor gene regulated downstream of ARF7 is not sufficient to overproduce ARs in transgenic plants.

It has been previously reported that the apical part of argonaute1 (ago1) mutants displays a defect in AR formation, but not in LR development, in response to auxin [31]. AGO1 is one of the components that plays a critical role in the regulation of posttranscriptional gene silencing [58]. ARF17, which is upregulated in ago1 mutants, negatively regulates AR formation by repressing GH3 genes and thus perturbing auxin homeostasis in a light-dependent manner [31]. Together, these results suggest that AR development has both unique and shared components with LR development in Arabidopsis. Identification of unique components, which play critical roles in AR development, aid in the discovery of the distinctive developmental processes between AR and LR development.

Conclusions
The ARF7/ARF19-LBD16/LBD18 transcriptional module via the AUX1/LAX3 auxin influx carriers plays an important role in AR formation in the Arabidopsis hypocotyl, suggesting that a common regulatory mechanism is utilized for LR and AR formation during auxin signaling.

Methods

Plant growth and tissue treatment
Arabidopsis thaliana seedlings were grown and treated as described previously [59]. For treatment with auxin IAA, seedlings were grown in a 16-h photoperiod on a 3 MM Whatman filter paper on top of agar plates at 23°C. The filter paper with seedlings was then transferred to a plate containing IAA at 20 μM and incubated for a given period of time with gentle shaking in the light at 23°C. The light intensity was approximately 120 μmol m⁻² s⁻¹ and was provided by three daylight wavelength color fluorescent bulbs (Kumho Electric Co.).

Plant materials
The Arabidopsis thaliana ecotype Columbia (Col-0) was used in this study. We used the homozygous T-DNA insertion mutant lines lbd16, lbd18, lax3, aux1–21, lbd16 lbd18, aux1 lax3, lax3 lbd16, lax3 lbd18, lax3 lbd16 lbd18, aux1 lbd16, aux1 lbd18, aux1 lbd16 lbd18, aux1 lax3 lbd16, aux1 lax3 lbd18, and aux1 lax3 lbd16 lbd18, aux7, aux19, and aux7 aux19, which were developed in previous studies [37, 45, 51, 52]. We identified arf6–1 (CS24606) and arf8–2 (CS24608) knockout T-DNA insertion mutants from the Arabidopsis Biological Resource Center (ABRC). The ProLBD16:GUS and ProLBD18:GUS transgenic plants were obtained from a previous study [37]. ProLAX3:GUS transgenic seeds were generously provided by Dr. Malcolm Bennett [51]. ProARF7:GUS (CS24633) and ProARF19:GUS (CS24634) transgenic seeds were obtained from the ABRC. To generate ProLBD16:LBD16:4xMyc in the lbd16 mutant background, the promoter region of LBD16, encompassing -1309 to -21 bp relative to the AUG codon, was amplified by PCR using the pfu DNA polymerase with primers harboring the NotI (N-terminus) and Ascl (C-terminus) sites, and was cloned into the pENTR™/SD/
D-TOPO (Invitrogen) vector to yield pENTR™/SD/D-TOPO:ProLBD16. The LBD16 DNA fragment was inserted into the pENTR™/SD/D-TOPO:ProLBD16 plasmid with the Acl restriction site in both the N- and C-terminus to yield a pENTR™/SD/D-TOPO:ProLBD16:LBD16 plasmid. This construct was subcloned into the destination vector pGWB516 (Nakagawa, Shimane University, Japan) by an LR recombination reaction, and was then transformed into the lbd16 mutant by Agrobacterium-mediated transformation, generating ProLBD16:LBD16:4xMyc/lbd16 Arabidopsis. ProLBD18:LBD18:3xHA/lbd18 Arabidopsis generated from a previous study was used [60]. Pro35S:LBD16:GR/arf7 arf19 and Pro35S:LBD18:GR/arf7 arf19 transgenic mutants were generated by crossing arf7−1 arf19−1 (female) with Pro35S:LBD16:GR (male) or Pro35S:LBD18:GR (male) [37]. Homozygous lines were isolated according to genotype, the lack of lateral root phenotype for arf7−1 arf19−1 and by PCR detection of genomic DNA for the LBD16:GR or LBD18:GR transgenes. All mutants and transgenic plants were confirmed via genotyping prior to usage. The primer sequences used in this study are shown in Additional file 2: Table S1.

**AR analysis**

Induction of ARs in the hypocotyl was performed as previously described [32]. After seed sterilization, the seeds were sown on plates, incubated at 4°C for 2 d for stratification, and transferred to the light for several hours to induce germination. Plates were then placed vertically in the dark for 3 d, until the hypocotyls reached approximately 6 mm length, and were then transferred to the light for 7 d before counting the emerged ARs.

**RNA isolation and RT-qPCR analysis**

Following treatment, Arabidopsis plants were immediately frozen in liquid nitrogen and stored at −80°C. For the reverse
transcription quantitative polymerase chain reaction (RT-qPCR) analysis, total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen), and real-time RT-PCR was carried out using a Quantitect SYBR Green RT-PCR kit (Qiagen) in a CFX96™ Real-time system using a C1000™ Thermal cycler (Bio-Rad) as described previously [61]. All RT-qPCR was conducted in triplicate biological replications and subjected to statistical analysis. Analysis of relative gene expression data for determining fold changes was conducted as described previously [60, 62]. Data analysis for determining the copy number of the transcripts and for determination of reaction specificities was performed as described previously [61]. RT-qPCR conditions and primer sequences are shown in Additional file 2: Table S1. The experimental conditions used for RT-qPCR followed MIQE (minimum information for publication of quantitative real time PCR experiments) requirements as described in Additional file 3: Table S2.

Microscopy and histochemical GUS assays
Whole-mount visualization of the seedlings and histochemical assays for GUS activity were conducted as described previously [63].

Statistical analysis
Quantitative data were subjected to statistical analysis for every pair-wise comparison using software for Student’s t-Test (Predictive Analytics Software for Windows version 20.0).

Fig. 7 A model showing the auxin signaling module ARF7/19-LBD16/18 via AUX1/LAX3 auxin influx carriers mediated AR formation in Arabidopsis

Additional files

Additional file 1: Figure S1. Time-course expression of LBD16 and LBD18 in response to auxin in Col-0, arf6 and arf8 mutants. (PDF 110 kb)

Additional file 2: Table S1. Primer sequences and PCR conditions. (XLS 12 kb)

Additional file 3: Table S2. Experimental conditions used in RT-qPCR based on MIQE requirements. (XLSX 10 kb)

Abbreviations
AR: Adventitious root; ARF: Auxin response factor; Aux/IAA: Auxin/indole acetic acid protein; GUS: ß-glucuronidase; LBD/ASL: Lateral organ boundaries domain/asymmetric leaves2-like; RT-qPCR: Quantitative reverse transcription-PCR

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Availability of data and materials
All the data supporting our findings is contained within the manuscript. Constructs and seeds are available upon request from JK.

Authors’ contributions
HWL, CC, SKP, YP, and MJK designed and conducted the experiments and analyzed the data. SKP prepared the manuscript draft. JK conceived the project, designed the experiments, analyzed the data, and wrote the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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