PCP
PLANT & CELL PHYSIOLOGY

Callus Initiation from Root Explants Employs Different Strategies in Rice and Arabidopsis

Fu Guo[1], Haidao Zhang[1], Wu Liu[2], Xingming Hu[3], Ning Han[1], Qian Qian[3], Lin Xu[2,*] and Hongwu Bian[1,*]

[1]Institute of Genetic and Regenerative Biology, Key Laboratory for Cell and Gene Engineering of Zhejiang Province, College of Life Sciences, Zhejiang University, Hangzhou 310058, China
[2]National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China
[3]State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China
*Corresponding authors: Lin Xu, E-mail, xulin01@sibs.ac.cn; Fax, +86-21-54924015; Hongwu Bian, E-mail, hwbian@zju.edu.cn; Fax, +86-571-88206535.
(Received February 26, 2018; Accepted May 8, 2018)

Callus formation in tissue culture follows the rooting pathway, and newly formed callus seems to be a group of root primordium-like cells. However, it is not clear whether there are multiple mechanisms of callus initiation in different species and in different organs. Here we show that the *OsIAA11*-mediated pathway is specifically and strictly required for callus initiation in the lateral root (LR) formation region of the primary root (PR) but not for callus initiation at the root tip or the stem base in rice. *OsIAA11* and its Arabidopsis homolog *AtIAA14* are key players in lateral rooting. However, the *AtIAA14*-mediated pathway is not strictly required for callus initiation in the LR formation region in Arabidopsis. LRs can be initiated through either the *AtIAA14*-mediated or *AtWOX11*-mediated pathway in the Arabidopsis PR, therefore providing optional pathways for callus initiation. In contrast, *OsIAA11* is strictly required for lateral rooting in the rice PR, meaning that the *OsIAA11* pathway is the only choice for callus initiation. Our study suggests that multiple pathways may converge to *WOX5* activation during callus formation in different organs and different species.

Keywords: AtIAA14 • Callus • OsIAA11 • Rice • Root • WOX11.

Abbreviations: AR, adventitious root; ARF7/19, AUXIN RESPONSE FACTOR7/19; CIM, callus-inducing medium; DAC, days after culture; DAE, days after excision; LBD16, LATERAL ORGAN BOUNDARIES DOMAIN16; LR, lateral root; PR, primary root; qRT–PCR, quantitative reverse transcription–PCR; WOX5, WUSCHEL-RELATED HOMEOBOX5; WOX11, WUSCHEL-RELATED HOMEOBOX11.

Introduction

Plants have powerful regenerative abilities, which have been widely applied in agricultural technologies (Knizewski et al. 2008, Sugimoto et al. 2011, Su and Zhang 2014, Xu and Huang 2014, Ikeuchi et al. 2016, Kareem et al. 2016, Lup et al. 2016, Birnbaum and Roudier 2017). In tissue culture, when stimulated by a high level of auxin, detached explants form a pluripotent callus, from which roots and shoots may regenerate (Sugimoto et al. 2011, Xu and Huang 2014). Recent studies have suggested that callus formation follows the rooting pathway, and newly formed callus cells are likely to be a group of fast-dividing root primordium-like cells (Che et al. 2007, Atta et al. 2009, Sugimoto et al. 2010, Fan et al. 2012, He et al. 2012, Liu et al. 2014, Yu et al. 2017, Liu et al. 2018).

The ability to form callus in tissue culture varies in diverse species. For example, mature leaves of the dicot Arabidopsis (*Arabidopsis thaliana*) readily form callus, while the mature region of leaves in the monocot rice (*Oryza sativa*) is extremely unresponsive to tissue culture (Hu et al. 2017). Not all somatic cells are competent to form callus; only a group of adult stem cells, i.e. regeneration-competent cells, may initiate regeneration (Che et al. 2007, Atta et al. 2009, Sugimoto et al. 2010, Liu et al. 2014). In Arabidopsis, the procambium and some vascular parenchyma cells in leaves and the xylem-pole pericycle cells in roots may serve as regeneration-competent cells, while in rice the bundle sheath and some immature vascular cells in leaves and the phloem-pole pericycle cells in roots may serve as regeneration-competent cells (Hu et al. 2017). These competent cells are responsible not only for callus initiation but also for root primordium initiation during adventitious root (AR) or lateral root (LR) formation (Liu et al. 2014, Hu and Xu 2016, Hu et al. 2017, Sheng et al. 2017). The different differentiation statuses of these regeneration-competent cells result in diverse regenerative abilities in Arabidopsis and rice (Hu et al. 2017).

In Arabidopsis, there are at least two rooting pathways that may contribute to post-embryonic root system formation: the *WUSCHEL-RELATED HOMEOBOX11* (*AtWOX11*)-mediated and *AtIAA14-AUXIN RESPONSE FACTOR7/19* (*AtIAA14–AtARF7/19*)-mediated rooting pathways (Sheng et al. 2017, Ge et al. 2018). The *AtWOX11*-mediated rooting pathway contributes to AR formation from leaf explants or adventitious LR formation from the primary root (PR) in response to wounding or environmental signals (Sheng et al. 2017, Ge et al. 2018). Using AR formation from leaf explants as an example, auxin first activates *AtWOX11* expression during the fate transition from regeneration-competent cells to root founder cells, and then *AtWOX11* initiates the fate transition from root founder cells to root primordium cells via activation of *WUSCHEL-RELATED*

Plant Cell Physiol. 59(9): 1782–1789 (2018) doi:10.1093/pcp/pcy095, Advance Access publication on 16 May 2018, available FREE online at www.pcp.oxfordjournals.org

HOMEOBOX5 (*AtWOX5*) and *LATERAL ORGAN BOUNDARIES DOMAIN16* (*AtLBD16*) (Liu et al. 2014, Hu and Xu 2016, Sheng et al. 2017, Xu 2018). Co-expression of *AtWOX5* and *AtLBD16* may be a specific marker of root primordium cells (Xu 2018). The *AtIAA14–AtARF7/19*-mediated pathway (also known as the non-*AtWOX11*-mediated pathway) is required for acropetal LR formation (Ge et al. 2018) following an oscillatory root cap-derived auxin flux signal (Fukaki et al. 2002, Okushima et al. 2007, Lee et al. 2009, Peret et al. 2009, Moreno-Risueno et al. 2010, Goh et al. 2012, Lavenus et al. 2013, Van Norman et al. 2014, Xuan et al. 2015, Xuan et al. 2016). Degradation of AtIAA14 upon auxin signaling releases AtARF7/19 to activate *AtLBD16*, and probably also *AtWOX5*, for LR primordium initiation (Fukaki et al. 2002, Okushima et al. 2007, Lee et al. 2009, Goh et al. 2012).

In rice, there are also two pathways to initiate roots in the post-embryonic stage, i.e. the *OsWOX11*-mediated and the *OsIAA11*-mediated pathways. *OsWOX11* is the *AtWOX11* homolog in rice and is required for AR (crown root) formation but not LR formation (Zhao et al. 2009). *OsIAA11* in rice is the closest homolog of *AtIAA14* in Arabidopsis. The *Osiaa11* mutant, which has mutations in domain II of the OsIAA11 protein and interrupts the auxin signaling pathway by constitutively suppressing ARF activity, is defective in generating LRs (Zhu et al. 2012). However, ARs (crown roots) could be formed in the *Osiaa11* mutant, suggesting that the *OsIAA11*-mediated pathway is specifically required for LR formation.

Callus formation borrows the rooting pathway and newly formed callus seems to be a group of root primordium-like cells which specifically express *WOX5* and *LBD16* (Sugimoto et al. 2010, Fan et al. 2012, Liu et al. 2014, Hu et al. 2017, Lee et al. 2017, Liu et al. 2018). However, it is not clear whether there are different mechanisms of callus initiation in different species and in different organs. In this study, we show that the *OsIAA11*-mediated pathway is strictly required for callus initiation from the rice LR formation region of PR explants, whereas the *AtIAA14*-mediated pathway is not strictly required for callus initiation in the LR formation region in Arabidopsis. Thus, rice and Arabidopsis employ different strategies for callus initiation from root explants.

Results and Discussion

Callus initiation strictly requires the *OsIAA11*-mediated pathway in the lateral root formation region of rice root explants

To analyze the mechanism of callus initiation from rice root explants, we first carried out phenotype analysis using PRs from 7-day-old wild-type Kasalath and *Osiaa11* mutant plants (Zhu et al. 2012). The 7-day-old PRs were at the growing stage, and almost no LRs could be observed by eye in the wild type (**Fig. 1A**). We cultured the detached PRs on callus-inducing medium (CIM) to induce callus formation and on N6 medium without exogenous hormones as a control. At 14 d after culture (DAC) on N6 medium, the wild-type PRs formed many LRs (**Fig. 1B**), while the *Osiaa11* PRs had no LRs (**Fig. 1F, G**), which was consistent with previous reports that *OsIAA11* is required for LR formation (Zhu et al. 2012). At 14 DAC on CIM, the wild-type PR explants formed callus in both the LR formation region and the root tip region (**Fig. 1C–E**), while the root explants from the *Osiaa11* mutant PR formed callus only in the root tip region, not in the LR formation region (**Fig. 1H–J**). No callus was found in the *Osiaa11* LR formation region during a prolonged culture period at 20 DAC (Supplementary Fig. S1). Therefore, callus formation strictly required the *OsIAA11*-mediated lateral rooting pathway in the LR formation region, but not in the root tip region.

We then tested expression levels of *OsWOX5*, which is a marker gene of newly formed callus, in root explants from the wild type and the *Osiaa11* mutant. *OsWOX5* was highly up-regulated in both the LR formation region and the root tip region in wild-type root explants at 2 DAC on CIM, while it was up-regulated in the root tip region but not in the LR formation region of the *Osiaa11* root explants (**Fig. 1K, L**; Supplementary Fig. S2). This suggests that the establishment of the callus cell fate is strictly dependent on the *OsIAA11*-mediated pathway in the LR formation region but not in the root tip region.Quantitative reverse transcription–PCR (qRT–PCR) results showed that *OsWOX11* was up-regulated in the root tip region of PRs in rice (Supplementary Fig. S3). *OsWOX11* was also up-regulated in the LR formation region of PRs from *Osiaa11* in rice (Supplementary Fig. S4). However, the up-regulation of *OsWOX11* was not sufficient to induce callus initiation in the LR formation region in the *Osiaa11* background.

The *AtIAA14*-mediated pathway is not strictly required for callus initiation in the lateral root formation region of Arabidopsis root explants

We next analyzed the role of *AtIAA14* in callus initiation from Arabidopsis root explants. We cultured PRs from 7-day-old wild-type Col-0 and *Atiaa14* mutant plants. The 7-day-old PRs had almost no LRs in the wild type or *Atiaa14* at this stage (**Fig. 2A, F**). The PRs were cultured on CIM to induce callus formation and on N6 medium without exogenous hormones as a control. The wild-type PRs developed many LRs on N6 medium at 14 DAC (**Fig. 2B**), while the *Atiaa14* PRs did not produce LRs (**Fig. 2G**), suggesting that *AtIAA14*, like its rice homolog *OsIAA11*, is critical for LR initiation (Fukaki et al. 2002). Interestingly, both the wild-type and *Atiaa14* root explants formed callus in the LR formation region and the root tip region, although the callus mass was relatively smaller in *Atiaa14* than in the wild type (**Fig. 2C, H**). A prolonged culture period led to better growth of the callus mass at 20 DAC (Supplementary Fig. S5). *AtWOX11* was up-regulated in the LR formation region of both Col-0 and *Atiaa14* PRs (Supplementary Fig. S6). Taken together, this suggested that, in contrast to the case in rice, the *AtIAA14*-mediated lateral rooting pathway is not strictly required for callus initiation in the LR formation region of Arabidopsis.

To confirm this finding at the molecular level, we analyzed the expression levels of the callus marker gene *AtWOX5* in root

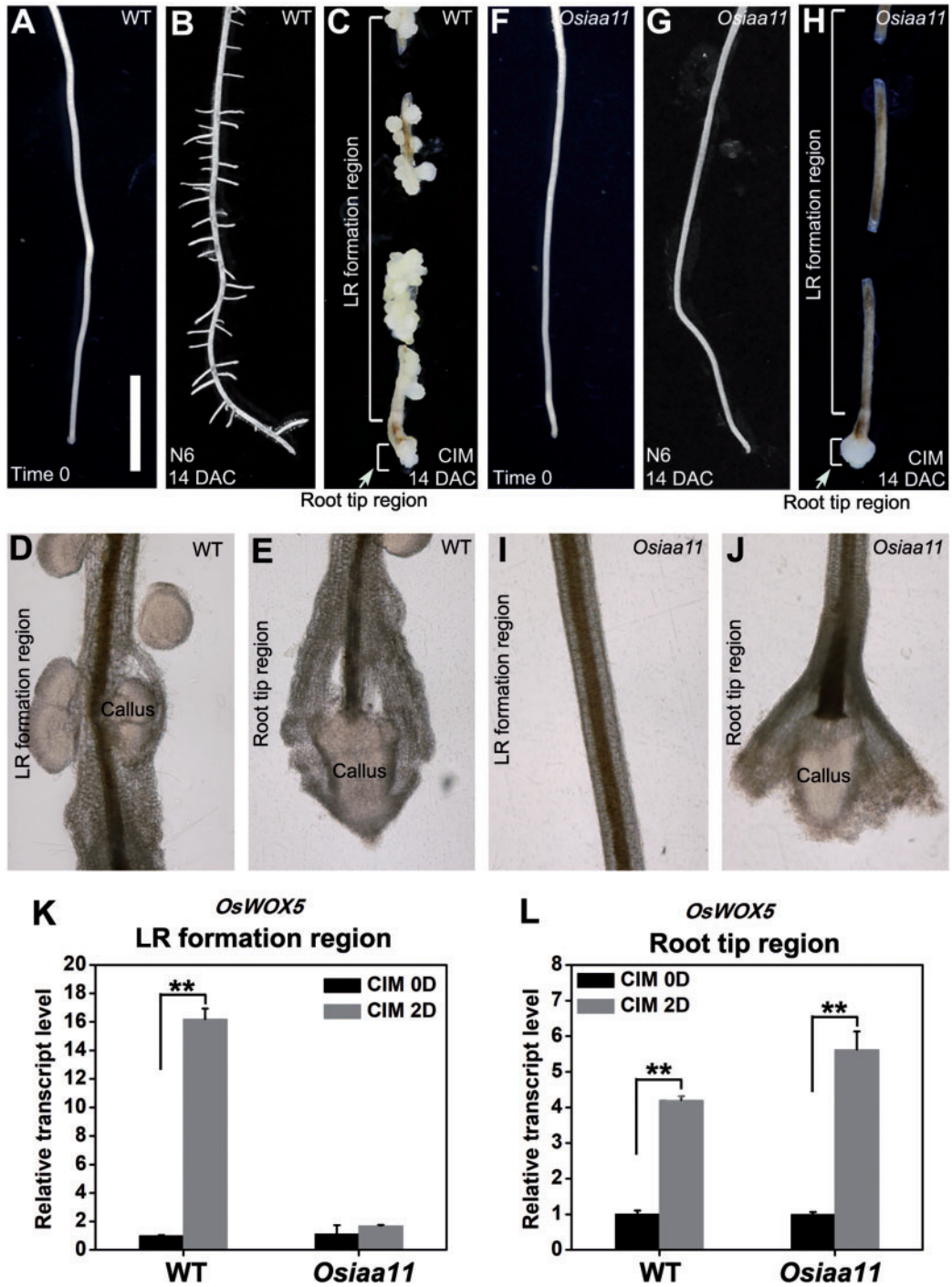

Fig. 1 Callus formation from rice primary roots. (A) PR from 7-day-old wild-type rice cultured on MS medium before tissue culture. (B) LR formation from wild-type rice after 14 d on N6 medium. (C) Callus formation from wild-type rice PRs after 14 d on CIM. The PR explants were cut into approximately 5 mm pieces before culture. (D, E) Close-up of callus formation on CIM at 14 DAC in the LR formation region (D) and the root tip region (E) of wild-type rice PRs. (F) PR from 7-day-old *Osiaa11* rice cultured on MS medium before tissue culture. (G) No LR formation from *Osiaa11* PRs cultured on N6 medium for 14 d. (H) Callus formation in the root tip region but not in the LR formation region from *Osiaa11* PRs cultured on CIM for 14 d. The PR explants were cut into approximately 5 mm pieces before culture. (I, J) Close-up of *Osiaa11* PR explants on CIM at 14 DAC in the LR formation region (I) and the root tip region (J). (K, L) Relative transcript levels of *OsWOX5* in the wild type and *Osiaa11* in the LR formation region (K) and root tip region (L). Bars indicate the SE from three biological replicates. Two asterisks indicate significant differences (Student's test, $P < 0.01$). Values from time 0 (0 D) PRs were arbitrarily fixed at 1.0. Scale bars = 5 mm in (A–C, F–H), 50 μm in (D, E, I, J).

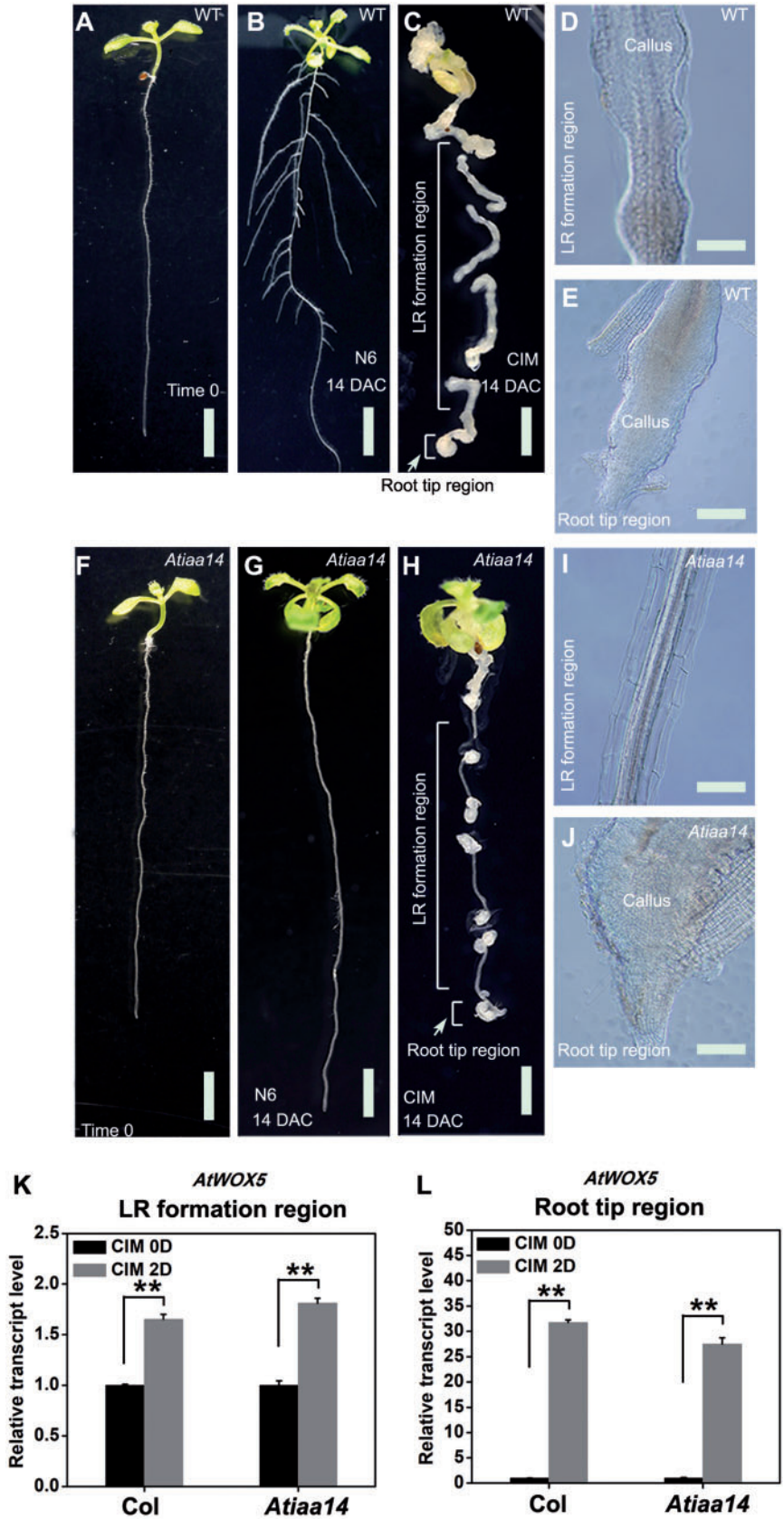

Fig. 2 Callus formation from Arabidopsis primary roots. (A) PR from 7-day-old wild-type Arabidopsis cultured on MS medium before tissue culture. (B, C) LR formation after 14 d on N6 medium (B) and callus formation after 14 d on CIM (C) from wild-type Arabidopsis PRs. The PR explants in (C) were cut into approximately 5 mm pieces before culture. (D, E) Close-up of callus formation on CIM at 14 DAC in the LR formation region (D) and the root tip region (E) of wild-type Arabidopsis PRs. (F) PR from a 7-day-old *Atiaa14* mutant cultured on MS medium

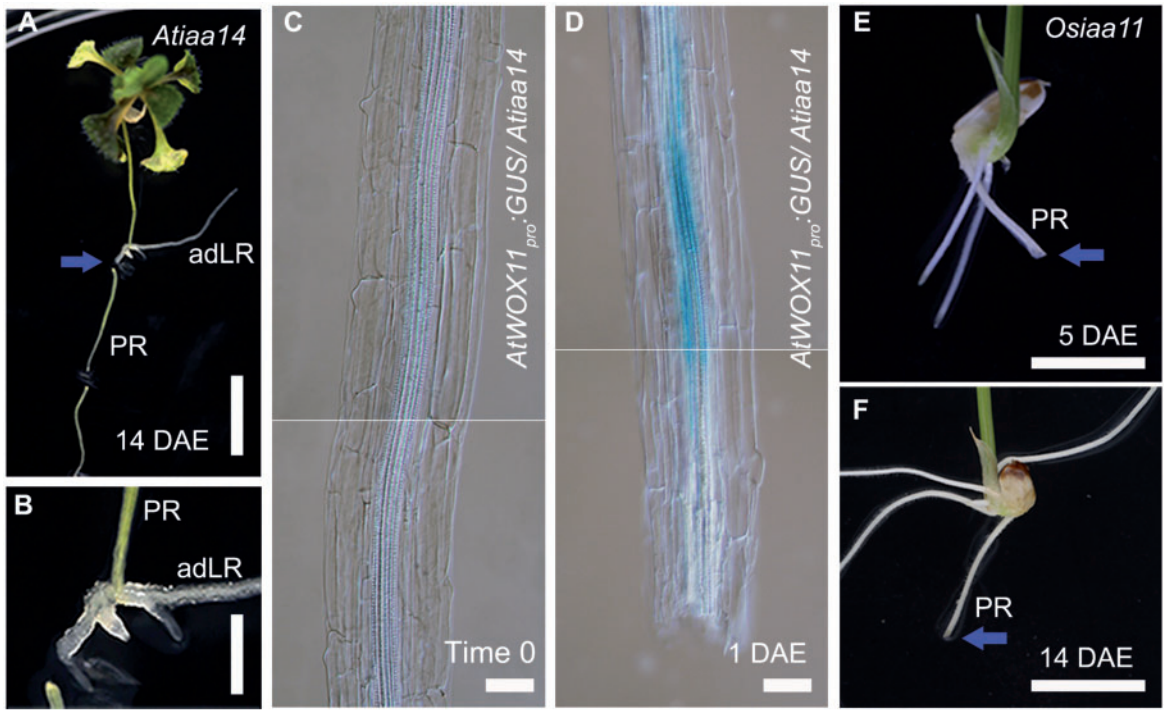

Fig. 3 Rooting abilities of primary roots of Arabidopsis and rice. (A, B) Formation of adventitious LRs on a wounded PR (arrow) of *Atiaa14* at 14 DAE. (B)A close-up of the wound site in (A). (C, D) GUS staining of *AtWOX11*$_{pro}$*:GUS* in intact (C) or wounded (D) PRs of *Atiaa14* at 1 DAE. (E, F) No LR formation was found on wounded primary roots (arrow) of *Osiaa11* at 5 DAE (E) and 14 DAE (F). Scale bars = 5 mm in (A), 50 μm in (C, D) and 1 mm in (B, E, F).

explants from the wild type and the *Atiaa14* mutant. *AtWOX5* was highly up-regulated in both the LR formation region and the root tip region in the wild type and the *Atiaa14* mutant at 2 DAC on CIM (**Fig. 2K, L**). Therefore, the cell fate transition to callus could be accomplished in both the wild type and the *Atiaa14* mutant.

Overall, the major difference between *OsIAA11* and *AtIAA14* is their roles in callus formation in the LR formation region, suggesting that callus formation in the LR formation region is controlled by different mechanisms in rice and Arabidopsis.

Different rooting capacities of rice and Arabidopsis primary roots upon wounding

We thought that the different strategies of callus initiation in the LR formation region in rice and Arabidopsis might be due to the different LR formation mechanisms in the two species. Our previous study suggested that the Arabidopsis PR can produce two types of LRs, i.e. acropetal LRs and adventitious LRs (Sheng et al. 2017, Ge et al. 2018). When Arabidopsis is grown vertically on the medium, LR formation usually follows the *AtIAA14–AtARF7/19* pathway to initiate the acropetal LR primordium; however, when Arabidopsis PRs are wounded, the *AtWOX11*-mediated rooting pathway can be used for adventitious LR initiation. We tested whether the *AtWOX11*-mediated rooting pathway functioned in the *Atiaa14* mutant by cutting the PRs and culturing the wounded PRs on N6 medium. The results showed that the wounded *Atiaa14* PRs could produce adventitious LRs upon wounding (**Fig. 3A, B**). *WOX11* expression was induced in the wound site at 1 d after excision (DAE) (**Fig. 3C, D**).

We then tested whether the *OsWOX11*-mediated rooting pathway could be used in rice PRs to produce LRs. We cut *Osiaa11* PRs and cultured the wounded PRs on N6 medium. The results showed that the wounded *Osiaa11* PRs were not able to produce roots at the wound sites (**Fig. 3E, F**). Therefore, *OsIAA11* is the sole pathway for LR initiation in rice PRs. This can explain why callus formation is strictly dependent on *OsIAA11* in the LR formation region of rice.

Callus formation at the stem base of the *Osiaa11* mutant

Although the *Osiaa11* mutant was defective in LR initiation, it had the ability for AR (crown root) formation (**Fig. 4A, B, D, E**)

Fig. 2 Continued
before tissue culture. (G) No LR formation after 14 d on N6 medium. (H) Callus formation in the LR formation region and the root tip region from *Atiaa14* PR explants cultured on CIM for 14 d. The PR explants were cut into approximately 5 mm pieces before culture. (I, J) Close-up of *Atiaa14* PR explants on CIM at 14 DAC in the LR formation region (I) and the root tip region (J). (K, L) Relative transcript levels of *AtWOX5* in wild-type Arabidopsis and the *Atiaa14* mutant in the LR formation region (K) and root tip region (L). Bars indicate the SE from three biological replicates. Two asterisks indicate significant differences (Student's test, $P < 0.01$). Values from time 0 (0 D) PRs were arbitrarily fixed at 1.0. Scale bars = 3 mm in (A–C, F–H), 50 μm in (D, E, I, J).

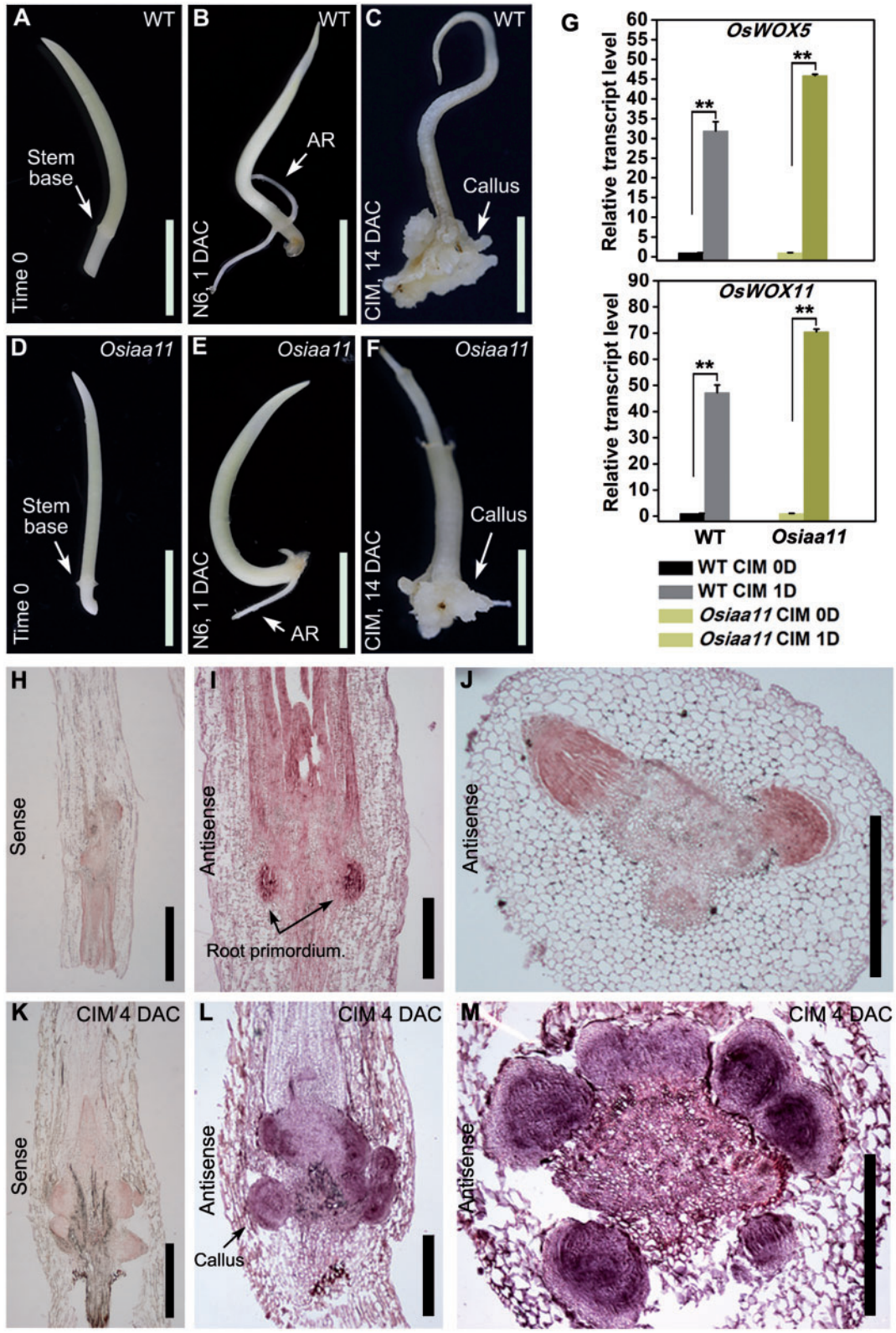

Fig. 4 Callus formation at the stem base in rice. (A) The explant of wild-type rice stem base from the 3-day-old seedling grown in the dark. (B) AR formation from the wild-type rice stem base after 1 d on N6 medium. (C) Callus formation from the wild-type rice stem base after 14 d culture on CIM. (D) The explant of the *Osiaa11* rice stem base from the 3-day-old seedling grown in the dark. (E) AR formation from the *Osiaa11* rice stem base after 1 d on N6 medium. (F) Callus formation from the *Osiaa11* rice stem base after 14 d culture on CIM. (G) Relative transcript levels of *OsWOX5* and *OsWOX11* in the stem base region of the wild type or *Osiaa11*. Bars indicate the SE from three biological replicates. Two asterisks indicate significant differences (Student's test, $P < 0.01$). Values from time 0 (0 D) were arbitrarily fixed at 1.0. (H–M) Longitudinal (H, I, K, L) and transverse (J, M) sections of the wild-type stem base, showing in situ hybridization of *OsWOX5*. Seedlings grown in the dark for 3 d were used to observe AR primordium formation (H–J). Those seedlings were then cultured on CIM for 4 d to observe callus formation (K–M). (H) and (K) are sense controls. Scale bars = 5 mm in (A–F), 1 mm in (H, K) and 500 μm in (I, J, L, M).

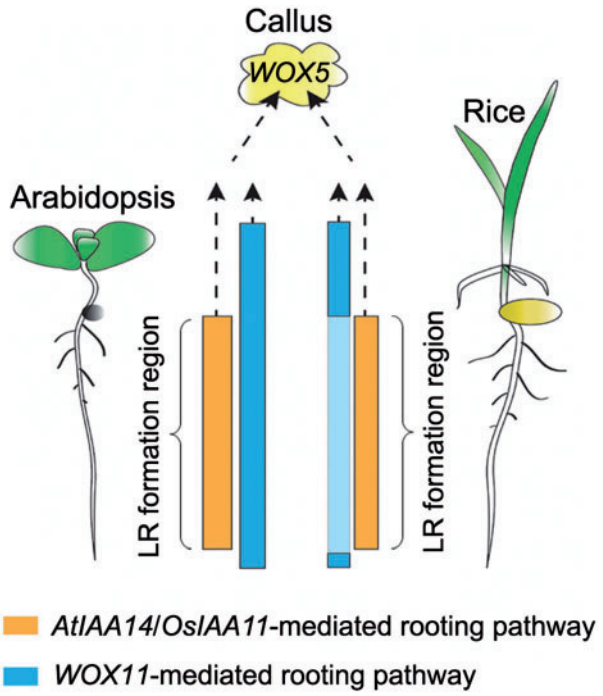

Fig. 5 Model of root and callus formation in Arabidopsis and rice.

(Zhu et al. 2012). We then tested the callus formation ability in the stem base of *Osiaa11*. When the excised shoots were cultured on CIM for 14 d, callus was produced in the crown root initiation region on the stem bases of both the wild type (**Fig. 4C**) and *Osiaa11* (**Fig. 4F**).

The qRT–PCR results showed that the expression levels of *OsWOX5* and *OsWOX11* increased significantly in the stem bases of the wild type and *Osiaa11* cultured on CIM for 1 d (**Fig. 4G**). In situ hybridization results showed that *OsWOX5* expression was detected in the crown root primordium and callus (**Fig. 4H–M**). These data indicated that the mutation in *OsIAA11* does not affect the AR and callus initiation in the stem base of rice.

Conclusion

Overall, the strategies for callus initiation in the LR formation region are different: in rice, *OsIAA11* is strictly required for callus initiation, but *AtIAA14* is not indispensable in Arabidopsis. This is due to differences in LR initiation between rice and Arabidopsis. In Arabidopsis, LR formation can occur via two alternative pathways: the *AtIAA14–AtARF7/19*-mediated pathway for acropetal LRs developing from PRs and the *AtWOX11*-mediated pathway for adventitious LRs initiated upon wounding (Ge et al. 2018). In rice, LR formation from PRs can only occur via the *OsIAA11*-mediated pathway. The different rooting mechanisms in the LR formation region of PRs result in different callus initiation strategies in Arabidopsis and rice (see the model in **Fig. 5**). However, because *Osiaa11* and *Atiaa14* are both gain-of-function mutants, currently we cannot exclude the possibility that the repression of auxin signaling by mutated *AtIAA14* is weaker than that of *OsIAA11*. Further analysis to reveal why *OsWOX11* cannot function to produce LRs in rice PRs could improve our understanding of the evolution of root system formation and regenerative abilities in dicots and monocots.

Materials and Methods

Plant material

Kasalath (*Oryza sativa* L. ssp. *indica*) was used as the rice wild type, and Columbia-0 (Col-0) was used as the Arabidopsis wild type. The *Osiaa11* mutant (in the Kasalath background) was previously described (Zhu et al. 2012). *Atiaa14* and *AtWOX11*$_{pro}$*:GUS* were also described previously (Fukaki et al. 2002, Liu et al. 2014, Shang et al. 2016).

Tissue culture and in situ hybridization

For root-derived callus induction, sterile rice seeds were grown on Murashige and Skoog (MS) medium in a growth chamber with a 16 h light, 28°C/8 h dark, 24°C cycle. Sterilized seeds of Arabidopsis were sown on B5 medium (Gamborg B5 basal medium with 0.5 g l^{-1} MES, 3% sucrose and 0.8% agar, pH 5.7) and grown at 24°C under a 16 h light/8 h dark photoperiod. PRs were used for incubation of LRs or callus. MS medium and N6 medium were described previously (Murashige and Skoog 1962, Chu et al. 1975). Callus induction was performed on CIM (N6 basal medium with 10 μM 2,4-D, 0.5 g l^{-1} MES, 3% sucrose and 0.4% phytagel, pH 5.8). In situ hybridization was performed according to our previous method (Hu et al. 2017).

qRT–PCR

RNA extraction and qRT–PCR were performed as previously described (Guo et al. 2016), using the following gene-specific primers: 5′-ACCGGCTCATGACA TGCTAC-3′ and 5′-ATACCGGACCTTGTCCACCT-3′ for *OsWOX5*; 5′-ACCACT TCGACCGCCACTACT-3′ and 5′-ACGCCTAAGCCTGCTGGTTT-3′ for *OsUbiqutin*; 5′-ATGTTTGGGCAGGACGTGAT-3′ and 5′-GGAAGTAGCTCTC GCCCATC-3′ for *OsWOX11*; 5′-ACAATAACGGAGGAACGGGG-3′ and 5′-TG TTGGAGTTCTAAGACCGGC-3′ for *AtWOX5*; and 5′-TGAGCCTTCCTTGATG ATGCT-3′, 5′-GCACTTGCGGCAAATCATCT-3′ for *AtUbiqutin*. The qRT–PCR results are shown as relative expression levels normalized against the expression of *OsUbiqutin* and *AtUbiqutin*.

Supplementary Data

Supplementary data are available at PCP online.

Funding

This work was supported by the National Natural Science Foundation of China [grant Nos. 31771776, 31630007 and 31422005)]; the China Agriculture Research System [grant CARS-05]; the National Basic Research Program of China [973 Program, grant No. 2014CB943500]; the Chinese Academy of Science (CAS) [Key Research Program (grant No. QYZDB-SSW-SMC010) and the Strategic Priority Research Program 'Molecular Mechanism of Plant Growth and Development' (grant No. XDPB0403)].

Acknowledgments

We thank Dr. Chuanzao Mao (Zhejiang University, China) and Dr. Yuxin Hu (Institute of Botany, Chinese Academy of Sciences, China) for providing plant materials, and Hua Wang for

providing technical assistance in the in situ hybridization experiment. F.G., H.Z. and W.L. performed most of the experiments; F.G., X.H., H.B. and L.X. designed the experiments and analyzed the data; N.H. and Q.Q. supervised the experiments; H.B. and L.X. conceived the project and wrote the article with contributions of all the authors; H.B. supervised and complemented the writing.

Disclosures

The authors have no conflicts of interest to declare.

References

Atta, R., Laurens, L., Boucheron-Dubuisson, E., Guivarc'h, A., Carnero, E., Giraudat-Pautot, V., et al. (2009) Pluripotency of Arabidopsis xylem pericycle underlies shoot regeneration from root and hypocotyl explants grown *in vitro*. *Plant J.* 57: 626–644.

Birnbaum, K.D. and Roudier, F. (2017) Epigenetic memory and cell fate reprogramming in plants. *Regeneration (Oxf)* 4: 15–20.

Che, P., Lall, S. and Howell, S.H. (2007) Developmental steps in acquiring competence for shoot development in *Arabidopsis* tissue culture. *Planta* 226: 1183–1194.

Chu, C.C., Wang, C.C., Sun, C.S., Hsu, C., Yin, K.C. and Chu, C.Y. (1975) Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Sci. Sin.* 16: 659–688.

Fan, M., Xu, C., Xu, K. and Hu, Y. (2012) LATERAL ORGAN BOUNDARIES DOMAIN transcription factors direct callus formation in *Arabidopsis* regeneration. *Cell Res.* 22: 1169–1180.

Fukaki, H., Tameda, S., Masuda, H. and Tasaka, M. (2002) Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of Arabidopsis. *Plant J.* 29: 153–168.

Ge, Y., Fang, X., Liu, W., Sheng, L. and Xu, L. (2018) Adventitious lateral rooting: the plasticity of root system architecture. *Physiol. Plant.* doi: 10.1111/ppl.12741.

Goh, T., Joi, S., Mimura, T. and Fukaki, H. (2012) The establishment of asymmetry in *Arabidopsis* lateral root founder cells is regulated by LBD16/ASL18 and related LBD/ASL proteins. *Development* 139: 883–893.

Guo, F., Han, N., Xie, Y., Fang, K., Yang, Y., Zhu, M., et al. (2016) The miR393a/target module regulates seed germination and seedling establishment under submergence in rice (*Oryza sativa* L.). *Plant Cell Environ.* 39: 2288–2302.

He, C., Chen, X., Huang, H. and Xu, L. (2012) Reprogramming of H3K27me3 is critical for acquisition of pluripotency from cultured *Arabidopsis* tissues. *PLoS Genet.* 8: e1002911.

Hu, B., Zhang, G., Liu, W., Shi, J., Wang, H., Qi, M., et al. (2017) Divergent regeneration-competent cells adopt a common mechanism for callus initiation in angiosperms. *Regeneration (Oxf)* 4: 132–139.

Hu, X. and Xu, L. (2016) Transcription factors WOX11/12 directly activate *WOX5/7* to promote root primordia initiation and organogenesis. *Plant Physiol.* 172: 2363–2373.

Ikeuchi, M., Ogawa, Y., Iwase, A. and Sugimoto, K. (2016) Plant regeneration: cellular origins and molecular mechanisms. *Development* 143: 1442–1451.

Kareem, A., Radhakrishnan, D., Sondhi, Y., Aiyaz, M., Roy, M.V., Sugimoto, K., et al. (2016) *De novo* assembly of plant body plan: a step ahead of Deadpool. *Regeneration (Oxf)* 3: 182–197.

Knizewski, L., Ginalski, K. and Jerzmanowski, A. (2008) Snf2 proteins in plants: gene silencing and beyond. *Trends Plant Sci.* 13: 557–565.

Lavenus, J., Goh, T., Roberts, I., Guyomarc'h, S., Lucas, M., De Smet, I., et al. (2013) Lateral root development in *Arabidopsis*: fifty shades of auxin. *Trends Plant Sci.* 18: 450–458.

Lee, H.W., Kim, N.Y., Lee, D.J. and Kim, J. (2009) LBD18/ASL20 regulates lateral root formation in combination with LBD16/ASL18 downstream of ARF7 and ARF19 in *Arabidopsis*. *Plant Physiol.* 151: 1377–1389.

Lee, K., Park, O.S. and Seo, P.J. (2017) *Arabidopsis* ATXR2 deposits H3K36me3 at the promoters of *LBD* genes to facilitate cellular dedifferentiation. *Sci. Signal.* 10: eaan0316.

Liu, J., Hu, X., Qin, P., Prasad, K., Hu, Y. and Xu, L. (2018) The *WOX11–LBD16* pathway promotes pluripotency acquisition in callus cells during de novo shoot regeneration in tissue culture. *Plant Cell Physiol.* 59: 739–743.

Liu, J., Sheng, L., Xu, Y., Li, J., Yang, Z., Huang, H., et al. (2014) *WOX11* and *12* are involved in the first-step cell fate transition during de novo root organogenesis in *Arabidopsis*. *Plant Cell* 26: 1081–1093.

Lup, S.D., Tian, X., Xu, J. and Perez-Perez, J.M. (2016) Wound signaling of regenerative cell reprogramming. *Plant Sci.* 250: 178–187.

Moreno-Risueno, M.A., Van Norman, J.M., Moreno, A., Zhang, J., Ahnert, S.E. and Benfey, P.N. (2010) Oscillating gene expression determines competence for periodic *Arabidopsis* root branching. *Science* 329: 1306–1311.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 80: 662–668.

Okushima, Y., Fukaki, H., Onoda, M., Theologis, A. and Tasaka, M. (2007) ARF7 and ARF19 regulate lateral root formation via direct activation of *LBD/ASL* genes in *Arabidopsis*. *Plant Cell* 19: 118–130.

Peret, B., De Rybel, B., Casimiro, I., Benkova, E., Swarup, R., Laplaze, L., et al. (2009) *Arabidopsis* lateral root development: an emerging story. *Trends Plant Sci.* 14: 399–408.

Shang, B., Xu, C., Zhang, X., Cao, H., Xin, W. and Hu, Y. (2016) Very-long-chain fatty acids restrict regeneration capacity by confining pericycle competence for callus formation in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 113: 5101–5106.

Sheng, L., Hu, X., Du, Y., Zhang, G., Huang, H., Scheres, B., et al. (2017) Non-canonical *WOX11*-mediated root branching contributes to plasticity in *Arabidopsis* root system architecture. *Development* 144: 3126–3133.

Su, Y.H. and Zhang, X.S. (2014) The hormonal control of regeneration in plants. *Curr. Top. Dev. Biol.* 108: 35–69.

Sugimoto, K., Gordon, S.P. and Meyerowitz, E.M. (2011) Regeneration in plants and animals: dedifferentiation, transdifferentiation, or just differentiation? *Trends Cell Biol.* 21: 212–218.

Sugimoto, K., Jiao, Y. and Meyerowitz, E.M. (2010) *Arabidopsis* regeneration from multiple tissues occurs via a root development pathway. *Dev. Cell.* 18: 463–471.

Van Norman, J.M., Zhang, J., Cazzonelli, C.I., Pogson, B.J., Harrison, P.J., Bugg, T.D., et al. (2014) Periodic root branching in *Arabidopsis* requires synthesis of an uncharacterized carotenoid derivative. *Proc. Natl. Acad. Sci. USA* 111: E1300–E1309.

Xu, L. (2018) *De novo* root regeneration from leaf explants: wounding, auxin, and cell fate transition. *Curr. Opin. Plant Biol.* 41: 39–45.

Xu, L. and Huang, H. (2014) Genetic and epigenetic controls of plant regeneration. *Curr. Top. Dev. Biol.* 108: 1–33.

Xuan, W., Audenaert, D., Parizot, B., Moller, B.K., Njo, M.F., De Rybel, B., et al. (2015) Root cap-derived auxin pre-patterns the longitudinal axis of the *Arabidopsis* root. *Curr. Biol.* 25: 1381–1388.

Xuan, W., Band, L.R., Kumpf, R.P., Van Damme, D., Parizot, B., De Rop, G., et al. (2016) Cyclic programmed cell death stimulates hormone signaling and root development in *Arabidopsis*. *Science* 351: 384–387.

Yu, J., Liu, W., Liu, J., Qin, P. and Xu, L. (2017) Auxin control of root organogenesis from callus in tissue culture. *Front. Plant Sci.* 8: 1385.

Zhao, Y., Hu, Y., Dai, M., Huang, L. and Zhou, D.X. (2009) The WUSCHEL-related homeobox gene *WOX11* is required to activate shoot-borne crown root development in rice. *Plant Cell* 21: 736–748.

Zhu, Z.X., Liu, Y., Liu, S.J., Mao, C.Z., Wu, Y.R. and Wu, P. (2012) A gain-of-function mutation in *OsIAA11* affects lateral root development in rice. *Mol. Plant* 5: 154–161.