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SHORT COMMUNICATION

Lateral root development differs between main and secondary roots and depends on the ecotype

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ABSTRACT

Root architecture depends on the development of the main root and also on the number and density of lateral roots. Most molecular knowledge about the development of lateral roots was acquired studying primary roots, and it was implied that high order roots follow the same pattern. Recently, we informed that *AtHB23* is differentially regulated in primary and secondary roots. Here we show that *LBD16*, a target of AtHB23, also is differentially regulated; it is expressed in the tip of secondary and tertiary roots but not in primary ones. Moreover, the key hormone auxin exhibits a different distribution pattern in secondary and tertiary roots, according to the reporter *DR5*. Finally, we show that in Col 0 and Ler ecotypes development of secondary and tertiary roots exhibits significant variations. Altogether, we can conclude that different genetic programs govern secondary and tertiary roots development and such processes are dependent on the Arabidopsis genotype.

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Lateral root; LBD16; AtHB23; tertiary root; Col 0-Ler

Introduction

Plant adaptation to soil depends on root architecture and the latter is defined by the length of the main root together with the number and density of lateral roots (LRs). LRs develop from *de novo* meristems dependent on auxin and this process can be repeated several times in higher-order LRs.[1] Notably, most studies were focused on the formation of secondary roots from primary roots and tacitly accepted that the molecular mechanisms involved are subsequently repeated.

Most knowledge about LR development has been acquired in the model dicot *Arabidopsis thaliana*. This process involves transcription factors (TFs) as main players, particularly those from the Lateral organ Boundaries Domain (LBD) and Auxin Response Factors (ARF) families, and auxin as the hormone responsible for the integration of many internal and external signals.[2]

AtHB23 is a homeodomain-leucine zipper (HD-Zip) I TF[3] expressed at the base of the secondary LR primordium and we showed by Chromatin Immunoprecipitation assays (ChIP-qPCR) that it directly controls the expression of the auxin carrier *LAX3* and the TF *LBD16*.[4] LBD16 is a TF associated with the acquisition of LR founder cell polarity and cell cycle activation[5-7] and it is directly modulated by ARF7 SUMOylation in response to water availability.[8]

Using mutant lines, it was revealed that tertiary roots do not develop from secondary roots following the same molecular pathways that the latter from the primary ones because the expression of the HD-Zip I TF AtHB23 differs between secondary and tertiary root development.[4]

Adding complexity to LR developmental process and taking into account that available mutant lines are not always on the same genetic backgrounds, we considered this issue in our studies. Severald evelopmental events may occur via different pathways when comparing two Arabidopsis genotypes. For example, petal development in *kin13A* mutants significantly differs between Columbia (Col 0) and Landsberg *erecta* (Ler) genotypes.[9] A second example is the different response to phosphate starvation[10] and the high difference in the root tip transcriptomes of such genotypes including mRNAs, lncRNAs, and small RNAs.[11] Regarding particularly roots, the size of these organs exhibits a natural variation between Col 0 and Ler accessions when plants are subjected to osmotic stress. In such conditions, the total LR number in Ler plants was significantly higher than in Col 0 seedlings.[12]

The above-mentioned observations make necessary to revise several conclusions about root architecture determination derived only from the study of LR development from primary roots and also consider the genotype in which studies are carried out. This is because these processes are certainly more complex than thought so far. In this manuscript, we contribute with experimental evidence supporting that high order LRs exhibit key-genes differential expression patterns than primary roots. Furthermore, we reveal here that root architecture and development follow different programs in Col 0 and Ler ecotypes.

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Results and discussion

AtHB23 positively regulates auxin distribution in tertiary roots

To further investigate LR development from main and secondary roots, we analyzed the expression of the auxin carriers *AUX1* and *LAX1* and the peak of auxin response (shown by the *DR5* synthetic reporter) in tertiary roots using *prAUX1:GUS*, *prLAX1:GUS*, and *prDR5:GUS* transgenic plants. These analyses were performed by GUS histochemistry[13] both in the WT background as well as in *AtHB23*-silenced plants (Figure 1). The expression of these carriers in secondary roots was previously informed.[14] The assessment of auxin-carrier promoters (*prAUX1* and *prLAX1*) driving *GUS* expression in tertiary roots was almost not affected when the plants were crossed with *amiR23* ones.[4] In contrast to previous reports of what is observed in secondary roots,[4] histochemical analysis of tertiary roots in the above-mentioned crosses indicated that the auxin peak (revealed by DR5 activity) disappeared in tertiary lateral roots primordia and tertiary lateral roots, suggesting a key and different role for AtHB23 in secondary and tertiary roots development.

LBD16 is expressed in the tip of secondary and tertiary roots in contrast with primary roots

It was recently reported that AtHB23 directly regulates *LBD16*.[4] Transgenic plants carrying *LBD16* promoter driving the expression of the reporter *GUS* were previously described,[5] obtained from the ABRC and analyzed by histochemistry. *LBD16* is a deeply characterized gene, including its expression pattern as well as its role in the promotion of LR initiation.[2,6,15–17] Using *prLBD16:GUS* transgenic plants, we investigated *LBD16* expression in secondary and tertiary roots. Surprisingly, we observed GUS staining in the cap as well as in the vascular tissue of lateral root and tertiary lateral roots whereas, and in agreement with the literature, it was not expressed at all in the tip of main roots (Figure 2). This experimental evidence further supports that secondary and tertiary roots follow different molecular programs.

Lateral root development significantly differs between Col 0 and Ler genotypes

We analyzed LR development from main and secondary roots in Col 0 and Ler genotypes and we detected clear differences between them. Main roots are longer in 8-day-old Ler plants than in Col 0 ones whereas secondary roots showed the opposite scenario, i.e. longer in Col 0 than in Ler plants (Figure 3a). Eight-day-old Ler seedlings exhibit more lateral root primordia (LRP) than their Col 0 counterparts whereas no significant differences were detected in this parameter in 14-day-old plants (secondary to tertiary roots; Figure 3b). Similarly, as LRP, the number of LR in the main root was higher in Ler than in Col 0 genotype in 8-day-old seedlings and the opposite was observed in secondary roots in 14-day-old plants (Figure 3c). In agreement with these observations, total more LR were developed from main roots in Ler than in Col 0 individuals whereas those from secondary roots were slightly less

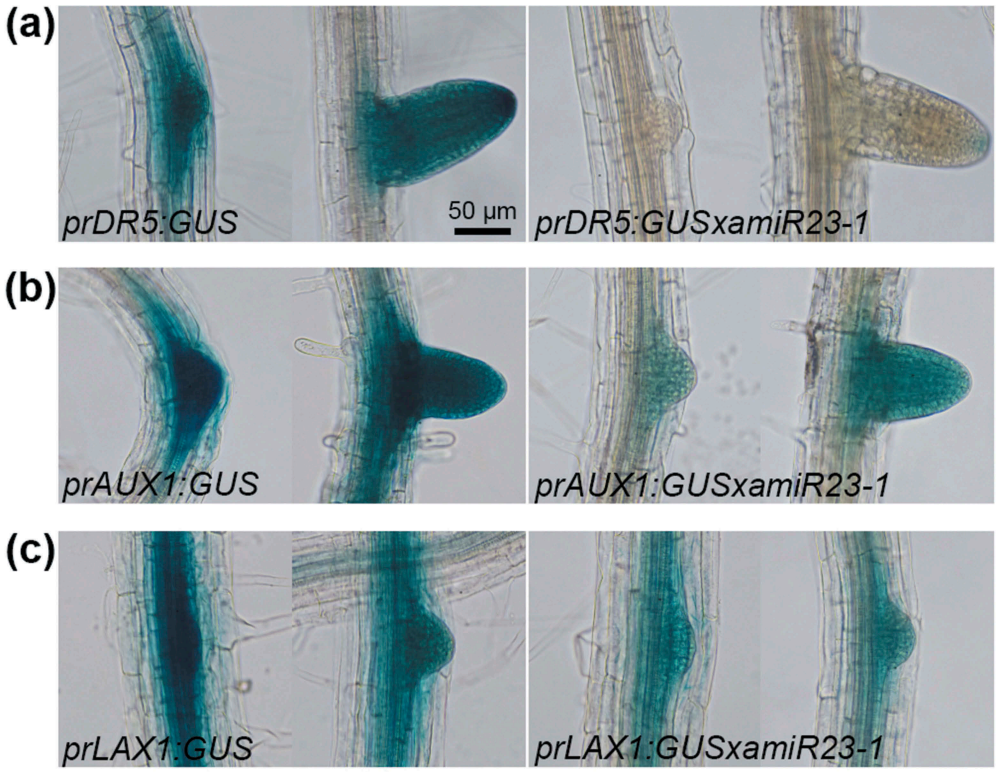

Figure 1. DR5 reporter activity is repressed in the tertiary lateral roots of *AtHB23*-silenced plants. Left panel: (a-c) Histochemistry of GUS in single transgenic (*prDR5:GUS, prAUX1:GUS*, and *prLAX1:GUS*) 15 day-old plants. Right panel: plants described in the left panel crossed with the *amiR23-1* plants. Each picture illustrates a lateral root of each genotype of different order and was carried out with N: 15 per genotype and repeated at least three times. Staining reactions were carried out overnight.Black bars represent 50 µm.

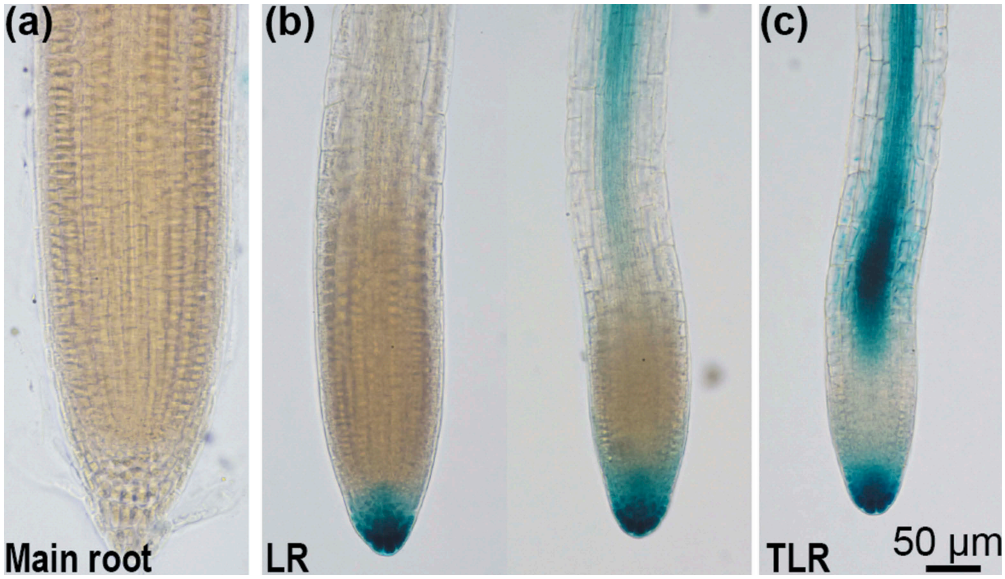

Figure 2. *LBD16* promoter is active in the root tip of secondary and tertiary lateral roots. GUS histochemistry of 15-day-old *prLBD16:GUS* roots grown in control conditions. (a) Tip of the main root; (b) Tips of two secondary roots representing different developmental emergence stages; and (c) tip of the tertiary lateral root. Each picture represents an illustration of each genotype and root order, and was carried out with N: 15 per genotype and repeated at least three times.

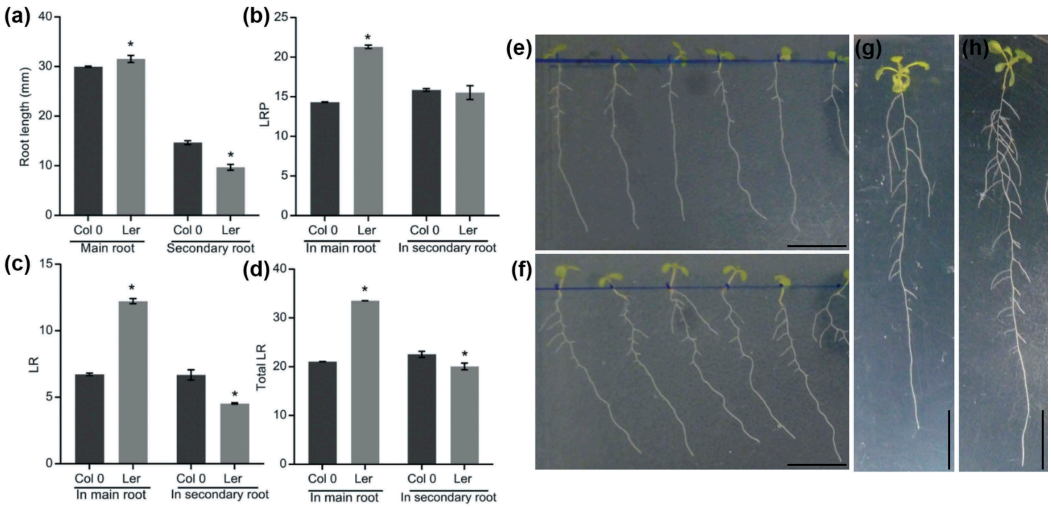

Figure 3. Secondary and tertiary root development differs between Col 0 and Ler ecotypes. (a) Root length of the main root of 8-day-old plants and total lateral secondary roots length of 14-day-old plants. (b) Lateral Root Primordium (LRP) in the main root of 8-day-old plants and in secondary roots of 14-day-old plants. (c) Lateral root (LR) in the main root of 8-day-old plants and in the secondary root of 14-day-old plants. (d) Total Lateral Root (LRP + LR) in the main root of 8-day-old plants and in secondary roots of 14-day-old plants. (e) Col 0 8-day-old seedlings. (f) Ler 8-day-old seedlings. (g) Col 14-day-old seedlings. (h) Ler 14-day-old seedlings. The assays were repeated at least three times with N: 15/genotype. Error bars represent SEM. Asterisks indicate statistical significance determined using the Sidak-Bonferroni method, with alpha = 5.0% (*P < 0.0001). Black bars represent 1 cm.

in the opposite sense (Figure 3d). These observations can be directly visualized in the illustrative pictures of Col 0 and Ler 8- and 14-day-old seedlings shown in Figure 3e,f.

Concluding remarks

It is well known that root architecture is essential for plant adaptation to the soil and environment and there is a vast literature showing that main and lateral roots development is modulated by environmental factors. At the molecular level, architecture plasticity follows complex mechanisms that involve many actors tightly regulated at the transcriptional level. In the present work, we showed another layer of complexity, i.e. LR development, both in main and secondary roots, is differentially modulated in Col 0 and Ler genotypes. Moreover, we added experimental evidence demonstrating that key molecular actors in Col0 behave differently in the development of secondary and tertiary roots. Although tertiary roots development remains poorly studied, it is not so surprising that plant plasticity evolved for soil adaptation with highly regulated programs subjected to natural variation.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

1. Osmont KS, Sibout R, Hardtke CS. Hidden branches: developments in root system architecture. Annu Rev Plant Biol. 2007;58:93–113. doi:10.1146/annurev.arplant.58.032806.104006.
2. Lavenus J, Goh T, Roberts I, Guyomarc'h S, Lucas M, De Smet I, Fukaki H, Beeckman T, Bennett M, Laplaze L. Lateral root development in Arabidopsis: fifty shades of auxin. Trends Plant Sci. 2013;18:450–458. doi:10.1016/j.tplants.2013.04.006.
3. Perotti MF, Ribone PA, Chan RL. Plant transcription factors from the homeodomain-Leucine Zipper family I. Role in development and stress responses. IUBMB Life. 2017;69:280–289. doi:10.1002/iub.1619.
4. Perotti MF, Ribone PA, Cabello JV, Ariel FD, Chan RL. AtHB23 participates in the gene regulatory network controlling root branching, and reveals differences between secondary and tertiary roots. Plant J. 2019;100:224–1236. doi:10.1111/tpj.14511.
5. Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M. ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in Arabidopsis. Plant Cell. 2007;19:118–130. doi:10.1105/tpc.106.047761.
6. Lee HW, Kim NY, Lee DJ, Kim J. LBD18/ASL20 regulates lateral root formationin combination with LBD16/ASL18 downstream of ARF7 and ARF19in Arabidopsis. Plant Physiol. 2009;151:1377–1389. doi:10.1104/pp.109.143685.
7. Goh T, Joi S, Mimura T, Fukaki H. The establishment of asymmetry in Arabidopsis lateral root founder cells is regulated by LBD16/ASL18 and related LBD/ASL proteins. Development. 2012;139:883–893. doi:10.1242/dev.071928.
8. Orosa-Puente B, Leftley N, von Wangenheim D, Banda J, Srivastava AK, Hill K, Truskina J, Bhosale R, Morris E, Srivastava M, et al. Root branching toward water involves post-translational modification of transcription factor ARF7. Science. 2018;362:1407–1410. doi:10.1126/science.aau3956.
9. Fujikura U, Elsaesser L, Breuninger H, Sánchez-Rodríguez C, Ivakov A, Laux T, Findlay K, Persson S, Lenhard M. Atkinesin-13A modulates cell-wall synthesis and cell expansion in Arabidopsis thaliana via the THESEUS1 pathway. PLoS Genet. 2014;10:e1004627. doi:10.1371/journal.pgen.1004627.
10. Reymond M, Svistoonoff S, Loudet O, Nussaume L, Desnos T. Identification of QTL controlling root growth response to phosphate starvation in Arabidopsis thaliana. Plant Cell Environ. 2006;29:115–125. doi:10.1111/j.1365-3040.2005.01405.x.
11. Blein T, Balzergue C, Roulé T, Gabriel M, Scalisi L, Sorin C, Christ A, Delannoy E, Martin-Magniette M-L, Nussaume L, et al. Two ecotype-related long non-coding RNAs in the environmental control of root growth. BioRxiv. 2020. doi:10.1101/579656.
12. Fitz Gerald JN, Lehti-Shiu MD, Ingram PA, Deak KI, Biesiada T, Malamy JE. Identification of quantitative trait loci that regulate Arabidopsis root system size and plasticity. Genetics. 2005;172:485–498. doi:10.1534/genetics.105.047555.
13. Jefferson RA, Kavanagh TA, Bevan MW. GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. Embo J. 1987;6:3901–3907. doi:10.1002/j.1460-2075.1987.tb02730.x.
14. Péret B, Swarup K, Ferguson A, Seth M, Yang Y, Dhondt S, James N, Casimiro I, Perry P, Syed A, et al. AUX/LAX genes encode a family of auxin influx transporters that perform distinct functions during Arabidopsis development. Plant Cell. 2012;24:2874–2885. doi:10.1105/tpc.112.097766.
15. Lavenus J, TatsuakiGoh T, Guyomarc'h S, Hill K, Lucas M, Voß U, Kenobi K, Wilson MH, Farcot E, Hagen G, et al. Inference of the Arabidopsis lateral root gene regulatory network suggests a bifurcation mechanism that defines primordia flanking and central zones. Plant Cell. 2015;27:1368–1388. doi:10.1105/tpc.114.132993.
16. Péret B, de Rybel B, Casimiro I, Benkova I, Swarup R, Laplaze L, Beeckman T, Bennett MJ. Arabidopsis lateral root development: an emerging story. Trends Plant Sci. 2009;4:399–408. doi:10.1016/j.tplants.2009.05.002.
17. Motte H, Beeckman T. The evolution of root branching: increasing the level of plasticity. J Exp Bot. 2019;70:785–793. doi:10.1093/jxb/ery409.