The epigenetic regulator ULTRAPETALA1 suppresses de novo root regeneration from Arabidopsis leaf explants

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ABSTRACT
Plants have the potency to regenerate adventitious roots from aerial organs after detachment. In Arabidopsis thaliana, de novo root regeneration (DNRR) from leaf explants is triggered by wounding signaling that rapidly induces the expression of the ETHYLENE RESPONSE FACTOR (ERF) transcription factors ERF109 and ABR1 (ERF111). In turn, the ERFs promote the expression of ASA1, an essential enzyme of auxin biosynthesis, which contributes to rooting by providing high levels of auxin near the wounding side of the leaf. Here, we show that the loss of the epigenetic regulator ULTRAPETALA1 (ULT1), which interacts with Polycomb and Trithorax Group proteins, accelerates and reinforces adventitious root formation. Expression of ERF109 and ASA1 was increased in ult1 mutants, whereas ABR1 was not significantly changed. Cultivation of explants on media with exogenous auxin equates adventitious root formation in wild-type with ult1 mutants, suggesting that ULT1 negatively regulates DNRR by suppressing auxin biosynthesis. Based on these findings, we propose that ULT1 is involved in a novel mechanism that prevents overproliferation of adventitious roots during DNRR.

Introduction
Specific plant cells such as pericycle-like cells are pluripotent,¹ which allows plants to regenerate from detached organs into a whole plant body. This regeneration ability is widely used in commercial plant propagation.² In tissue culture, two types of regeneration systems can be distinguished, de novo shoot regeneration and de novo root regeneration (DNRR).³ While shoot regeneration requires the supply of exogenous cytokinin,⁴ DNRR can occur without exogenous hormone treatment in Arabidopsis leaf explants.⁵ In the detached leaves, auxin production and transport are required for its dynamic accumulation near the cutting site.⁶⁷ Subsequently, WUSCHEL RELATED HOMEBOX11 (WOX11) responds to the auxin maximum, which upregulates LATERAL ORGAN BOUNDARIES-DOMAIN 16 (LBD16) and, in turn, induces adventitious root formation.⁸

Directly after leaf detachment, the phytohormone jasmonate induces the expression of a subset of APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) transcription factors including ERF109 and ABSCISIC ACID REPRESSOR1 (ABR1/ERF111), which can directly activate the auxin biosynthesis gene ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1 (ASA1).⁹¹⁰¹¹ On the other hand, the miR156-targeted SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors, SPL2, SPL10, and SPL11, suppress auxin accumulation and therefore root regeneration by repressing ERF109 and ABR1.¹¹ In addition, epigenetic factors contribute to the regulation of DNRR.¹² For instance, the H3K36me3 methyltransferase SET DOMAIN GROUP 8 (SDG8) is required for the activation of ASA1 by ERF109.¹³ Hence, the accumulation of auxin at the future rooting site is the key event in the gene regulatory network of transcription and epigenetic factors, which controls DNRR from detached organs such as leaves (reviewed in Jing et al., 2020).¹⁴ ULTRAPETALA1 (ULT1) encodes a SAND domain protein that restricts shoot meristem size, flower organ number, and flowering time.¹⁵¹⁶ ULT1 interacts with ARABIDOPSIS TRITHORAX 1 (ATX1) that functions as Trithorax Group factor.¹⁷¹⁸ Meanwhile, ULT1 also interacts with EMBRYONIC FLOWER 1 (EMF1), a Polycomb group protein associated with the repression marks H3K27me3 and H2Aub and known to regulate development-related genes.¹⁹ Twenty-one of the direct targets of ULT1 is ABSCISIC ACID-SENSITIVE 3 (ABI3).²⁰ A recent study showed that ABI3 plays a role in DNRR from Arabidopsis callus cells.²¹ We therefore address here the question whether ULT1 is also involved in root regeneration.

Results and discussion
ULT1 is required to regulate both cell division rate and auxin signaling in the root apical meristem.²² Whether ULT1 contributes to DNRR is still unknown. To address this question, we examined rooting rate and root regeneration capacity by culturing leaf explants on B5 medium without exogenous hormone treatment in the dark.⁵ In a time course, the rooting rate increased earlier in ult1-3 mutants than in wild-type, indicating that ULT1 decelerates adventitious root formation (Figure 1a-c).
Although the rooting rate of ult1-3 and wild-type leaf explants almost converged over time, the rooting capacity remained significantly higher in ult1-3 mutants (Figure 1c,d).

ABI3, which is a direct target of ULT1, is known to play an essential role in DNRR. To test whether increased expression of ABI3 could promote adventitious root formation in ult1-3 mutants, we performed a comparative expression analysis in leaf explants 30 min after detachment. Although the expression of ABI3 was more variable in ult1-3 mutants, ABI3 mRNA levels were not significantly different from wild-type (Figure 1e), and therefore, ABI3 is likely not responsible for the increased rooting rates in ult1-3.

The transcription factors, SPL2, SPL10, and SPL11, suppress DNRR with age by inhibiting wound-induced auxin biosynthesis. This raises the possibility that ULT1 inhibits root regeneration by direct or indirect activation of these SPLs. To test this hypothesis, we examined the expression levels of SPL2, SPL10, and SPL11, but no significant changes of expression could be detected (Figure 1e). Next, we asked whether ULT1 could restrict DNRR via repression of ERF109 and ABR1. We found the expression of ERF109 but not ABR1 significantly increased (Figure 1e). ERF109 binds directly to the promoter of ASA1 and YUC2, which encode key enzymes in auxin biosynthesis. The expression of ASA1 but not YUC2 was increased in ult1-3 leaf explants compared to wild-type (Figure 1e), suggesting that ULT1 confines DNRR by direct or indirect repression of ERF109, which limits auxin biosynthesis via repression of ASA1 in wild-type. To support this hypothesis, we examined the rooting rate and rooting capacity in the presence of exogenous auxin (Figure 1f-g) so that endogenous auxin biosynthesis was no longer the limiting factor of DNRR. The addition of IAA led to increased rooting in both ult1-3 and wild-type, and their rooting rate and capacity were eventually equalized corroborating the idea that accelerated and enhanced adventitious root formation is mainly caused by increased auxin levels in ult1-3 mutants.

Overproliferation of adventitious roots would decrease the survival rate of detached aerial organs. Here, we showed that ULT1 limits adventitious root formation by repressing ERF109, which seems independent of SPL2, 10, and 11.11 Hence, ULT1 could be part of a novel mechanism preventing in parallel to the SPL module overproliferation of adventitious roots in leaf explants by inhibiting auxin bursts in response to wounding (Figure 1h). Since ULT1 interacts with the Polycomb protein EMF1, the repression of ERF109 could be direct. However, ERF109 is not an H3K27me3 target in leaves but is targeted by

Figure 1. Loss of ULT1 promotes DNRR. (a-b) Leaf explants of wild-type (Col-0) and ult1-3 mutants, 14 d cultured on B5 media. (c) Rooting rates of Col and ult1-3 leaf explants in a time course. (d) Rooting capacity after 15 d cultured on B5 media. (e) Relative gene expression levels in Col and ult1-3 leaves, 30 min after detachment. (f) Rooting rates of Col and ult1-3 leaf explants after 10 d cultured on B5 media with and without IAA. (g) Rooting capacity after 11 d cultured on B5 media. (h) Conceptional model; JA, jasmonate. (c-g) Asterisks indicate significance, p-value ≤ 0.05; ns indicates no significance (Student t-test).
H2Aub.\textsuperscript{17,20} Future studies might check the binding of ULT1 and EMF1 and the levels of the Polycomb repressive mark H2Aub at the ERF109 gene locus. Another possibility is that ULT1 as TrxG factor\textsuperscript{14,16} activates a transcriptional repressor of ERF109.

**Methods**

*Arabidopsis* wild-type (Col-0) and *ult1-3* mutants\textsuperscript{13} were used in this study. Growth conditions of plants and culture conditions of leaf explants for DNRR followed the protocols described earlier.\textsuperscript{5} All leaf explants used in this study were from the first pair of rosette leaves of 14-d-old seedlings and cultivated under dark conditions. For auxin treatments, 1 mM IAA was added to the B5 rooting medium (continuous treatment after detachment). The rooting rate is represented by the percentage of explants with regenerated roots at a given time point,\textsuperscript{7} while the regenerative capacity is represented by the percentage of leaf explants with different number of regenerated roots.\textsuperscript{11} For each experiment, three to five plates with 11 explants of each genotype were used. With the exception of *ABI3* (*N* = 3), all expression data represent four biological replicates. Total RNA preparation, cDNA synthesis, and real-time RT-qPCR including *ABI3\textsuperscript{21}, SPL10\textsuperscript{22}, SPL11\textsuperscript{22}, SPL15\textsuperscript{22}, ERF109\textsuperscript{11}, ABR1\textsuperscript{11}, ASA1\textsuperscript{14}, YUC2\textsuperscript{9}, YUC6\textsuperscript{3}, and cLF4A primers\textsuperscript{24,25} have been previously described.\textsuperscript{24,26} Furthermore, we used primers 5'-GACAATTAAGTGACGCAAGCC-3' and 5'-GTTCGCCCTAAGTCTTCTTCTC-3' for *YUC4* in the gene expression analysis. In this study, all values represent the mean ± standard error.

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**Disclosure statement**

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**Authors’ contribution**

All authors performed the experiments. RMX conceived and initiated the work. QX and RMX wrote the manuscript.

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