Sci. Bull. (2016) 61(22):1728–1731
DOI 10.1007/s11434-016-1185-9

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Letter

TAA family contributes to auxin production during de novo regeneration of adventitious roots from *Arabidopsis* leaf explants

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Received: 1 September 2016 / Revised: 3 October 2016 / Accepted: 9 October 2016 / Published online: 28 October 2016

Abstract Many differentiated plant organs have the ability to regenerate into a new plant after detachment via de novo organogenesis. During de novo root organogenesis from *Arabidopsis thaliana* leaf explants, wounding first induces endogenous auxin production in mesophyll cells. Auxin is then polar transported to, and accumulates in, regeneration-competent cells near the wound to trigger the cell-fate transition. The TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA) family proteins and the YUCCA (YUC) family proteins catalyze two successive biochemical steps in auxin biogenesis, and YUCs have been shown to be involved in auxin production in mesophyll cells during de novo root organogenesis. In this study, we show that the TAA family is also required for adventitious rooting. Inhibition of TAA blocked adventitious root formation from leaf explants. Intriguingly, whereas *YUC1* and *YUC4* have been shown to be highly induced by wounding, *TAA* genes retained consistent expression levels before and after leaf detachment. Therefore, we suggest that TAAs and YUCs are both required for auxin biogenesis in leaf explants, but they play different roles in regeneration. While YUC1 and YUC4 function in response to wounding to catalyze the rate-limiting step in auxin biosynthesis, TAAs probably serve as abiding and basal enzymes during de novo root organogenesis from leaf explants.

Keywords De novo root organogenesis · *TAA1* · *TAR2* · Plant regeneration · Auxin biogenesis · *Arabidopsis*

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De novo root organogenesis is the process in which adventitious roots regenerate from wounded or detached plant organs [1]. Using a simple method of culturing *Arabidopsis thaliana* leaf explants on B5 medium without added hormones [2], endogenous auxin was shown to be the critical hormone controlling cell fate transition during adventitious rooting [3, 4]. Wounding first induces the production of auxin primarily in the mesophyll cells of the leaf explant. The auxin is then polar transported into regeneration-competent cells, i.e., procambium and vascular parenchyma cells, near the wound. The regeneration-competent cells retain a high auxin level to trigger the cell fate transition [3].

The production of auxin in mesophyll cells is an early event in de novo root organogenesis. The main natural auxin, indole-3-acetic acid (IAA), is biosynthesized

primarily via two successive chemical reactions. The amino acid tryptophan (Trp) is first converted into indole-3-pyruvate (IPA) in a reaction catalyzed by the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA) family of Trp aminotransferases [5, 6]. Then, IPA is converted into IAA by the YUCCA (YUC) family of flavin-containing monooxygenases [7, 8]. Our previous study showed that *YUC1* and *YUC4* are expressed in response to wounding in leaf explants within 4 h after culture (HAC). Blocking of YUCs resulted in defective adventitious rooting, suggesting that the YUC-mediated IPA-to-IAA transition is required in this process. In this study, we analyzed the role of the TAA-catalyzed Trp-to-IPA transition in de novo root organogenesis.

Arabidopsis thaliana Columbia-0 (Col-0) was used as the wild type in this study. The *weak ethylene insensitive 8-1* (*wei8-1*) and *tryptophan aminotransferase related 2-2* (*tar2-2*) mutants and $DR5_{pro}$:*GUS* were described previously [5, 9]. The plant growth conditions and culture conditions for de novo root organogenesis from leaf explants were described previously [2]. In this study, we cultured leaf explants in light conditions to avoid the influence of darkness on auxin biogenesis. The L-kynurenine (Kyn) (Cat. K8625, Sigma, USA) treatment was carried out as previously described [10].

GUS staining was performed as previously described [2, 3] and observed under a Nikon Eclipse 80i light microscope (Nikon, Tokyo, Japan).

For qRT-PCR and RT-PCR analyses, RNA extraction, reverse transcription, PRC and quantitative PCR were performed as described previously [3] using the following primers: 5′-CGCACTCTCTTCACTAGCC-3′ and 5′-GCATCGCTCTCTTTCACAAC-3′ for *TAA1*; 5′-GCAAGATTGAAGAAGACG-3′ and 5′-CCTGATTTGAGGCAGTCTG-3′ for *TAR2*; 5′-CGTGTGTTGGTTCCTAGAGC-3′ and 5′-GCCACCTCTATGTCCTTCAC-3′ for *TAR1*; 5′-CGATGTCGGAGCTATGTCTC-3′ and 5′-CTGTACAAGTTTATTACTTCG-3′ for *YUC1*; and 5′-TGGCATCA(T/C)ACTTTCTACAA-3′ and 5′-CCACCACT(G/A/T)AGCACAATGTT-3′ for *ACTIN*. The qRT-PCR results are relative expression levels, which were normalized against those produced by the primers for *ACTIN*.

We first tested the rooting ability of *Arabidopsis* leaf explants from wild type and TAA family mutants at 8 or 12 d after culture (DAC) on B5 medium in light conditions [2]. Wild-type Columbia-0 (Col-0) leaf explants formed adventitious roots (Fig. 1a, d). In contrast, leaf explants from the *TAA1* mutant allele *wei8-1* showed severely defective adventitious rooting, and the *wei8-1 tar2-2* double mutant leaf explants were almost unable to regenerate adventitious roots under our culture conditions (Fig. 1b, d). Addition of 1 μmol/L IAA in B5 medium could partly rescue the rooting defect of *wei8-1 tar2-2* leaf explants. Among a total of 15 *wei8-1 tar2-2* leaf explants analyzed, 7 formed adventitious roots with IAA treatment. We also evaluated whether the TAA protein inhibitor Kyn [10] affected the regenerative capacity of leaf explants. When B5 medium was supplemented with Kyn, regeneration from wild-type leaf explants was severely blocked (Fig. 1c, e). These findings suggested that the TAA family is involved in de novo root organogenesis.

Next, we monitored the patterns of auxin activity during de novo root organogenesis using the auxin response reporter $DR5_{pro}$:*GUS* [9]. The results showed that, compared with the level of $DR5_{pro}$:*GUS* staining in leaf explants from wild type on B5 medium, the level of $DR5_{pro}$:*GUS* staining was reduced in both mesophyll and vascular cells of the leaf explants cultured on B5 medium containing Kyn (Fig. 1f, g). These observations confirmed that the TAA family is required for auxin production.

Finally, we analyzed the transcript levels of the TAA family during de novo root organogenesis. The results of qRT-PCR and RT-PCR analyses showed that there were high transcript levels of TAA family genes in leaves at time 0 and that the transcript levels remained high after leaf detachment (Fig. 1h, i). These data suggest that the transcriptional levels of TAA family genes are not dependent on wounding. Currently we cannot exclude the possibility that the TAA family could be regulated at the protein level by wounding. It is also not clear whether TAA family genes are spatially regulated by wounding, although their transcript levels are not as sensitive as *YUC1* and *YUC4* to wounding. In addition, TAA family genes act in response to dark or shade treatment, and this might also be involved in regeneration [4–6].

Overall, our data revealed that the TAA family is required for auxin production during de novo root organogenesis from leaf explants. The TAA family proteins and YUC family proteins catalyze two successive steps during auxin biogenesis. However, the two families of genes might act differently in response to wounding. *YUC1* and *YUC4* are highly induced by wounding after detachment of leaf explants (Fig. 1h) [4], while TAA family genes are not. Therefore, we propose that during de novo root organogenesis, the TAA family continuously produces the intermediate product IPA, and the YUC family responds to wound signals to catalyze a rate-limiting step in IAA production.

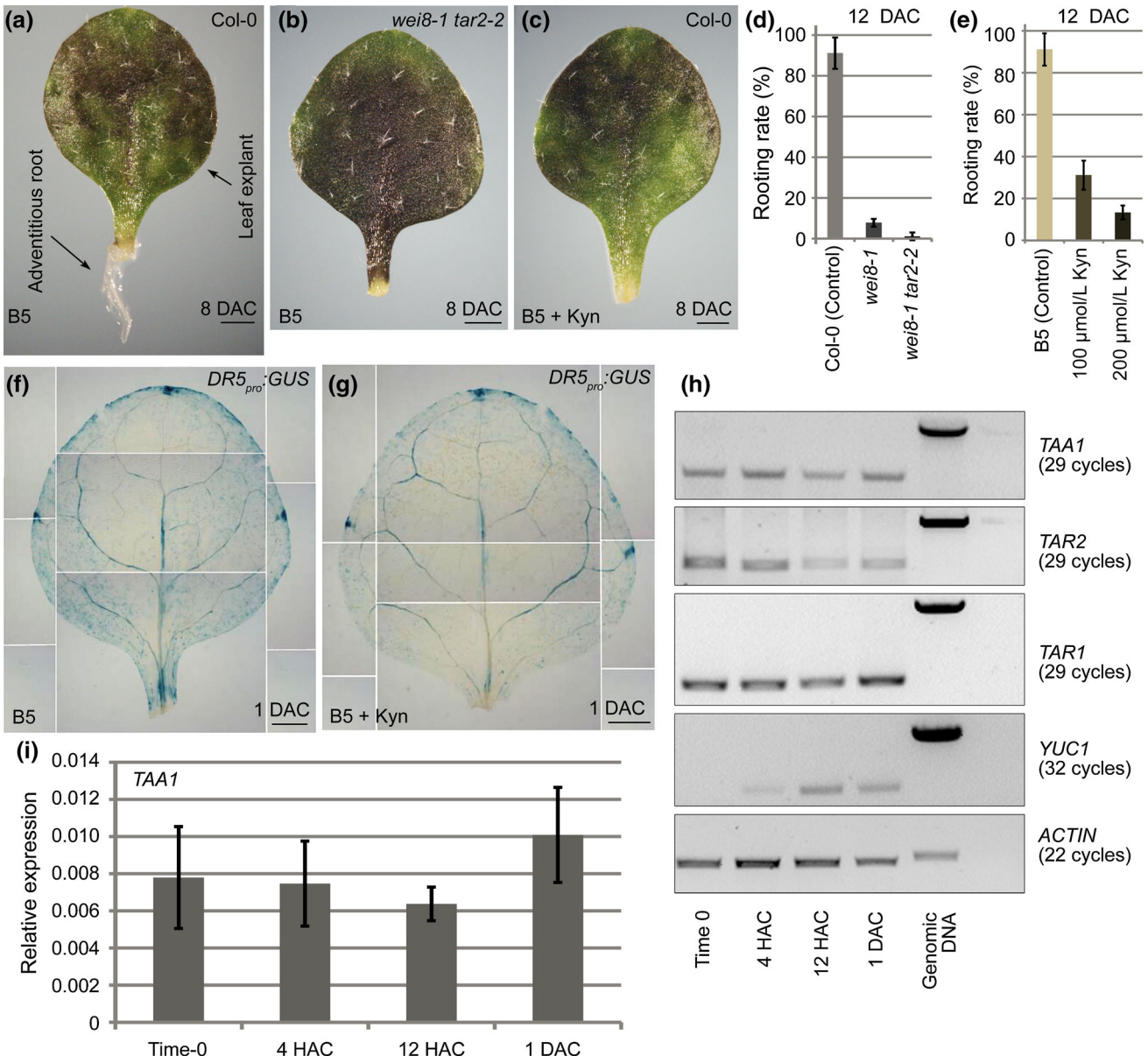

Fig. 1 (Color online) TAA family is required for de novo root organogenesis in *Arabidopsis*. Leaf explants from wild-type Col-0 (**a**) and double mutant *wei8-1 tar2-2* (**b**) cultured on B5 medium at 8 DAC. **c** Wild-type Col-0 leaf explant cultured on B5 medium with 200 µmol/L Kyn at 8 DAC. **d** Rooting rate of leaf explants (percentage of leaf explants with regenerated adventitious roots) from wild-type Col-0, *wei8-1* single mutant, and *wei8-1 tar2-2* double mutant at 12 DAC cultured on B5 medium. **e** Rooting rate of wild-type Col-0 leaf explants at 12 DAC on B5 medium containing different concentrations of Kyn. Experiment was performed together with that shown in (**d**), using the same control. Bars in (**d**, **e**) show SD with three biological replicates; $n = 30$ in each replicate. GUS signals in leaf explants from $DR5_{pro}$:*GUS* reporter line at 1 DAC on B5 medium (**f**) or B5 medium containing 200 µmol/L Kyn (**g**). Images in (**f**, **g**) show small parts of the same leaf explant, because entire explant could not be captured in a single microscope field. **h** RT-PCR analysis of *TAA* and *YUC1* transcript levels in Col-0 leaf explants at time 0, 4 HAC, 12 HAC, and 1 DAC on B5 medium. Note that *YUC1* transcripts were barely detected at time 0 but strongly induced after leaf explant detachment, while *TAA1*, *TAR2* and *TAR1* transcripts were detected at all tested time points. *ACTIN* was internal control gene. **i** qRT-PCR analysis of *TAA1* transcript levels in Col-0 leaf explants cultured on B5 medium at time 0, 4 HAC, 12 HAC, and 1 DAC. Bars in (**i**) show SEM with three biological replicates, and each biological replicate was performed with three technical replicates. Scale bars, 1 mm in (**a**–**c**) and 500 µm in (**f**, **g**)

Acknowledgements We thank T. J. Guilfoyle and ABRC for *Arabidopsis* seeds used in this work. This work was supported by the National Basic Research Program of China (973 Program, 2014CB943500/2012CB910503), the National Natural Science Foundation of China (91419302/31422005) and Youth Innovation Promotion Association CAS.

Conflict of interest The authors declare that they have no conflict of interest.

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