


ADDENDUM



The TARANI/ UBIQUITIN PROTEASE 14 protein is required for lateral root development in Arabidopsis

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ABSTRACT

In our article published in *Plant Physiology*, we had reported *tarani* (*tni*) mutant in Arabidopsis, in which poly-ubiquitin hydrolysis is adversely affected, shows pleiotropic phenotypic defects including fewer lateral roots due to the stabilization of several AUX/IAAs and reduced auxin response. *TNI* encodes UBIQUITIN-SPECIFIC PROTEASE14 that maintains normal auxin response through ubiquitin recycling. Fewer lateral roots observed in *tni* could be due to defects in their primordia initiation or subsequent elongation post-initiation. Here we have tested this by marking the lateral root primordia with *pCycB1;1::CycB1;1(DB):GUS* reporter and counting the number of lateral root at various stages development of as a marker of lateral root primordium. The results suggest that TNI/UBP14 is required for LRP development, and a reduction in TNI activity causes a delay in LRP initiation and consequently shorter lateral roots in the *tni* seedlings.

ABBREVIATIONS: LRP, lateral root primordium; XPP, xylem pole pericycle; LRFC, lateral root founder cells

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Lateral root organogenesis is a post-embryonic developmental event that commences with the de-differentiation of a pair of xylem pole pericycle (XPP) cells in the basal meristem of the primary root.^{1,2} Following the commitment to de-differentiation, the XPP cells re-enter into cell cycle to specify lateral root founder cells (LRFCs). Thus, reactivation of cell cycle is the hallmark for lateral root initiation. An auxin maxima and robust auxin signaling in the LRFC are pre-requisites for re-initiating division of these cells.³ The activated LRFC eventually undergoes several rounds of anticlinal and periclinal divisions and form the lateral root primordium (LRP).^{1,4} LRPs, which are confined within the epidermal layer of the primary root, form the emerged lateral roots when they come out of the epidermis by disrupting it.⁴ The G2→M transition marker *CycB1;1*, a B-type cyclin, is strongly expressed in all the stages of lateral root development including the very first division of the LRFC and is widely used as a marker for lateral root initiation.^{5,6} Besides, several other cell-cycle regulators, including the D-type cyclins *CYCD2;1*, *CYCD3;1* and *CYCD4;1*, are required for cell division in the LRPs.⁷ *ICK2/KRP2* [INTERACTOR OF CYCLIN DEPENDANT KINASE 2/ KINASE INHIBITORY PROTEIN (KIP) RELATED PROTEIN], a negative regulator of the G1→S phase transition, destabilizes *CYCD2;1* and inhibits the formative division in LRP.⁸

Cell cycle check points are tightly regulated by auxin and perturbation in auxin biosynthesis, transport or signaling affects lateral root initiation.^{6,9,10} Auxin activity in LRPs reduces *ICK2/KRP* level to promote cell division in a *CYCD2;1*-dependant manner.⁸ Gain-of-function mutations that stabilize AUX/IAAs, including those in *iaa3/shy2*, *iaa28*,

iaa18, *iaa14/slr* dominant mutants, exhibit defects in lateral root initiation, emergence, or elongation.^{11–14} This suggests that ubiquitin-mediated turn-over of AUX/IAA repressors by the 26S proteasome is crucial for auxin-dependent lateral root development.^{1,3,10}

We previously reported that the *tarani* (*tni*) mutant in Arabidopsis, in which poly-ubiquitin hydrolysis is adversely affected, shows pleiotropic phenotypic defects including fewer lateral roots due to the stabilization of several AUX/IAAs and reduced auxin response.^{15,16} *TNI* encodes UBIQUITIN-SPECIFIC PROTEASE14 that maintains normal auxin response through ubiquitin re-cycling [16, 17, 18 and 19]. Fewer lateral roots observed in *tni* could be due to defects in their initiation or growth post-initiation. We have tested this by using the *pCycB1;1::CycB1;1(DB):GUS* reporter as a marker of lateral root primordium. The *pCycB1;1::CycB1;1(DB):GUS* line was crossed with *tni* and a double homozygous line of *pCycB1;1::CycB1;1(DB):GUS tni* genotype was established. The *pCycB1;1::CycB1;1(DB):GUS* signal was detected at various stages of the actively dividing LRPs in both Col-0 and *tni* seedlings (Figure 1z). The GUS-stained LRPs were counted in these seedlings at 5, 9 and 11 days after germination (DAG) and their average numbers were compared. The results show that, though fewer LRPs were observed in *tni* seedlings at 5 DAG compared to Col-0, this difference was not sustained at later stages of development (Figure 1b). This suggests a delay in LRP initiation in *tni*, perhaps accounting for a reduction in the emerged lateral root number reported earlier.¹⁶ A delay in LRP initiation is expected to result in shorter lateral roots especially at the early stages of seedling growth. We tested this by measuring the cumulative length of all lateral roots in a seedling

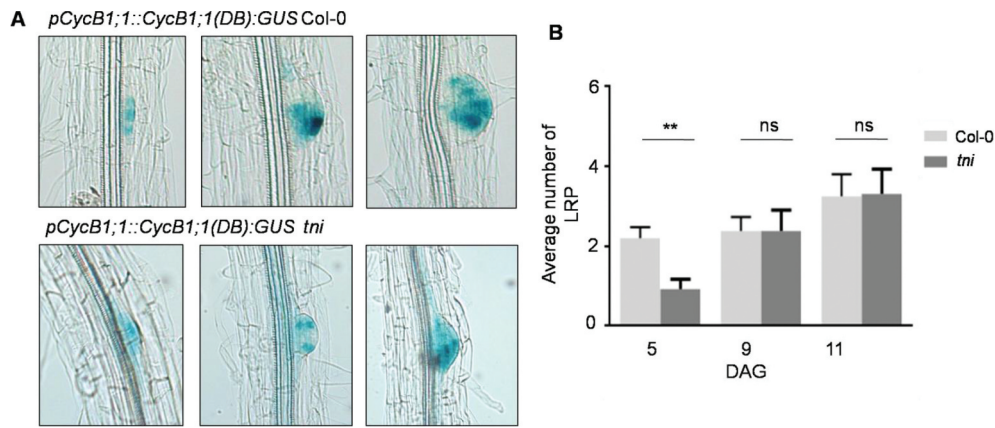


Figure 1. Comparison of the lateral root initiation in Col-0 and *tni*. (a) Light microscopic images of Col-0 (upper panel) and *tni* (lower panel) lateral root primordia (LRP) at various stages of initiation (from left to right) expressing the *CYC*B1;1:*GUS* signal (blue) in actively dividing cells at 5–9 days after germination (DAG). (b) The average number (N = 8–15) of LRPs per seedling at the indicated DAG. The *pCycB1;1::CycB1;1(DB):GUS* reporter was used as a marker to visualize LRPs. The error bars represent standard error of mean. Unpaired Student's *t*-test was performed for significance analysis. ** denotes $p = .0021$; ns, not significant ($p = .9876$ and 0.9458 on 9 and 11 DAG, respectively).

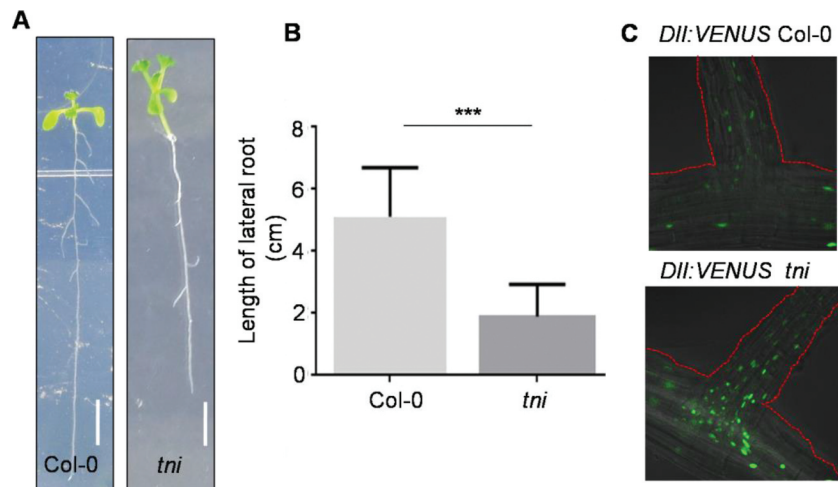


Figure 2. Reduced length of *tni* lateral roots. (a, b) 9 day-old seedlings showing the lateral roots (a) and their cumulative lengths per seedling (b). Scale bars, 5 mm. N = 33 (Col-0) and 34 (*tni*). Error bars represent standard deviation. Unpaired Student's *t*-test was performed for significance test. *** denotes $p < .0001$. (c) *DII:VENUS* signal in the lateral roots of 9 day-old *DII:VENUS* Col-0 and *DII:VENUS tni* seedlings. The red dotted lines highlight the outline of lateral roots.

and comparing the values between Col-0 and *tni*. At 9 DAG, the length of lateral roots was nearly 2.5-fold shorter in *tni* than in Col-0 (Figure 2a and 2b). As auxin is required for lateral root elongation,³ impaired lateral root growth in *tni* could be due to reduced auxin signaling imposed by the stabilization of the AUX/IAA repressor proteins.^{10,16} We tested this by comparing the signal strength of *DII:VENUS* (*DII* denotes domain II of AUX/IAAs), a widely used inverse reporter of auxin activity,¹⁸ between Col-0 and *tni*. Distinctly increased fluorescence signal was detected in the *tni* lateral roots compared to Col-0 (Figure 2c), suggesting reduced auxin activity in the mutant LRPs. Stabilization of multiple AUX/IAA proteins may have resulted in reduced auxin response in *tni* lateral roots.¹⁶ Taken together, these results suggest that TNI/UBP14 is required for LRP development, and a reduction in TNI activity causes a delay

in LRP initiation and consequently shorter lateral roots in the *tni* seedlings. Further work is required to identify the specific targets of TNI that mediate this process.

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