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SHORT COMMUNICATION

Further insights into the role of NIN-LIKE PROTEIN 7 (NLP7) in root cap cell release

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ABSTRACT

The root cap protects the root from environmental stress and senses gravity. Cells of the last layer of the root cap are shed in a developmentally programmed process. We previously showed that the transcription factor NIN-LIKE PROTEIN7 (NLP7) regulates root cap cell release likely through regulation of *CELLULASE5* (*CEL5*). Here we provide a supplement to that work. We hypothesized that the *nlp7* mutant has defects in additional root cap functions. We find that neither gravity sensing nor expression of a root cap cell identity marker is altered in *nlp7* but that expression of another cellulase, *CEL3*, is upregulated. We conclude that NLP7 control of root cap cell release is largely independent of gravity sensing and root cap cell identity.

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Columella; cell release; NLP7; NIN transcription factors; root cap

Roots are key for plant health and fitness, as they uptake water and nutrients, anchor plants in soil, and protect plants against soil borne pathogens. The root cap, a specialized tissue at the tip of the root, is critical for a root's ability to navigate the below-ground environment. The root cap protects the root meristem,[1] particularly from biotic stress,[2] senses gravity and mechanical obstacles,[3,4] and is important for hydrotropism.[5,6] The Arabidopsis root cap is composed of primarily two distinct cell types, the lateral root cap and the columella root cap (COL). In Arabidopsis, the central part of the root cap is composed of rectangular COL cells. As COL cells mature, new cells are produced from COL initials while cells in the last layer of the root cap are released from the root, in a process that keeps the COL a stable size.[7]

In most plant families other than the Brassicaceae, the outermost cells of the root cap are known as border cells (BCs), and are released as single cells.[1] However, in several species of Brassicaceae, border cells are attached to one another even after they are released from the root cap and are called Border Like Cells (BLCs). BCs and BLCs secrete mucilaginous substances with antimicrobial compounds and secondary metabolites that defend the root against soil-borne pathogens. The mucilage also plays an important role in biotic stress tolerance.[8-11] The mucilage produced by BCs and BLCs may reduce mechanical resistance as the root grows through soil.[1] The release of BLC depends on the action of enzymes that modify components of the cell wall including cellulases and pectin methylesterases.[12-15] Mutants deficient in cell- wall- homogalacturonan (HG) shows defects in the release of BLCs, with single cells released from the root cap instead of an intact layer.[12,14,15] BLC release is also influenced by degradation of cellulose by cellulases, as a mutant defective in *CELLULASE5* (*CEL5*) shows a sticky root cap.[13,15]

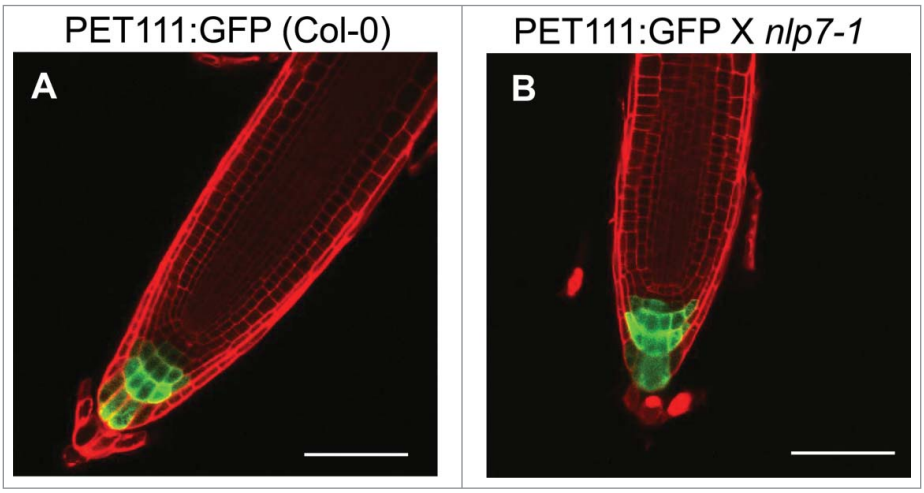

Figure 1. The columella root cap marker PET111 has similar expression in both Col-0 and *nlp7-1*. (A) PET111 expression in Col-0, (B) PET111 X *nlp7-1*. Scale bar = 100 μm.

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Figure 2. *NLP7* does not regulate the gravity sensing mechanism of the root cap. (A) Measurement of root curvature angle 24 h after gravity treatment in WT and *nlp7-1* mutant plants. No significant difference ($P > 0.05$) was observed between WT and *nlp7-1*. N = 18 or more roots. (B) and (C) Root caps of WT (B) and *nlp7-1* (C) stained with 1% Lugol's solution to stain for starch. Images taken at 100X. Scale bar = 20 μm.

We have recently shown that the transcription factor NIN-LIKE PROTEIN 7 (NLP7), previously characterized as a regulator of nitrate signaling,[16-18] modulates BLC release in Arabidopsis.[15] Mutations in *NLP7* lead to the release of BLCs as single cells and to the upregulation of several cell wall modifying enzymes including *CELLULASE5*.[15] The *nlp7* BLC release phenotype is dependent upon a functional *CEL5*, because the *nlp7-1cel5* double mutant releases BLCs as an intact layer.

We hypothesized that due to the defects in root cap cell release, the *nlp7-1* mutant would have other defects in the root cap, such as altered gravity sensing or COL cell identity. Here we show that gravity sensing is not altered in the *nlp7-1* mutant. In addition, expression of a root cap cell identity marker in the *nlp7-1* background is similar to wild type. Finally, we show that another cellulase, *CEL3*, is upregulated in roots of *nlp7-1*, but that mutations in *CEL3* do not lead to altered root cap layers, possibly due to functional redundancy.

To examine whether columella root cap identity was altered, we crossed the previously characterized PET111:GFP enhancer trap line[19] with *nlp7-1*. PET111:GFP is expressed in columella root cap cells[19] and is frequently used as a marker for this cell type.[20-22] Examination of 7 day old roots of *nlp7-1* PET111:GFP revealed a similar expression pattern as that of wild type (WT) PET111:GFP (Fig. 1A, B). This suggests that although there is a reduction in the COL root cap layers in *nlp7-1*,[15] the COL cellular identity is not affected.

We next asked whether root cap physiological functions were defective in *nlp7-1*. Apart from protecting the developing meristem, one major function of the root cap is sensing gravity. COL cells contain specialized starch grains called amyloplasts that play a crucial role in the gravity sensing mechanism of root cap. To examine a role for *NLP7* in gravity sensing, we tested the effect of gravity on the *nlp7-1* mutant. We grew WT and *nlp7-1* seedlings vertically on agar plates for 7 days, and the plates were then turned 180 degrees. We measured the angle of root curvature of the WT and *nlp7-1* 24 hours after gravistimulation (Fig. 2A). As shown in Fig. 2A, no significant difference in the angle of root curvature between WT and *nlp7-1* was observed after gravity treatment. Next, we examined the amyloplast content of the *nlp7-1* mutant. Starch staining with 1% Lugol solution showed that COL cells of *nlp7-1* contain amyloplasts with starch (Fig. 2B, C). These results indicate that *NLP7* does not affect the gravity sensing mechanism of columella root cap cells.

Together, these data suggest that the role of NLP7 in root cap cell release is independent of columella cell identity and gravity sensing. To identify additional cell wall enzymes with expression that may be modulated by NLP7, we focused on another gene in the *CELLULASE* family. We chose to focus on *CEL3* (*At1g71380*) because it is highly expressed in the COL and lateral root caps.[23] We studied the transcript levels of *CEL3* in wild type and the *nlp7-1* mutant and found that *CEL3* expression is 2.5 fold higher in the *nlp7-1* mutant (Fig. 3A), which is similar to the expression level of *CEL5* in *nlp7-1*.[15] Although a defect in *CEL5* leads to additional root cap layers,[15] examination of the *cel3-1* mutant (Salk_057689) revealed no difference in the number of root cap layers between WT and *cel3-1* (Fig. 3B), possibly due to redundancy in the *CELLULASE* gene family.

We conclude that NLP7 has a distinct role in root cap cell release through the regulation of cell wall degrading enzymes and does not appear to function in columella root cap cell identity or gravity sensing.

Materials and methods

Plant materials and growth conditions

The PET111 enhancer trap line[19] in wild type Columbia (Col-0) was crossed to the *nlp7-1* mutant (Salk_026134).

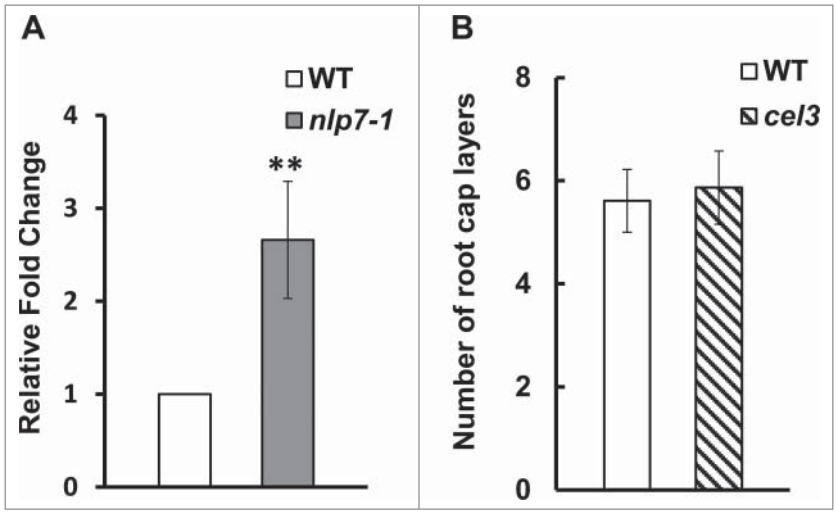

Figure 3. *CEL3* is expression is upregulated in *nlp7-1* mutant but mutations in *CEL3* do not lead to a sticky root cap layer phenotype. (A) Quantitative PCR showing upregulation of *CEL3* in *nlp7-1* mutant. Error bars show standard deviation between three independent biological replicates. $P < 0.05$, (B) Number of root cap layers from columella initials to the last layer of border like cells. No significant difference was observed in the number of root cap layers between WT and the *cel3-1* mutant. N = 20 roots per genotype.

Homozygous *nlp7-1* seedlings expressing the PET111 marker were sterilized in 50% bleach and stratified at 4°C for 48 h. Seeds were plated on 1X Murashige and Skoog (MS, Caisson Labs), pH 5.7 with 1% sucrose and 1% agar. Seedlings were grown vertically in a growth chamber at 22 – 23°C with 16 hour day length. Roots were observed at 7 days after germination with confocal microscopy as previously described.[15]

Homozygous seedlings of the *cel3-1* mutant (Salk_057689) were sterilized and grown as above. The root cap was viewed with confocal microscopy at 7 days after germination as previously described. Root cap layers were counted as previously described.[15]

Lugol staining

Seedlings were grown on MS as above. At 5 days after germination, roots were stained for 1 minute in a 1% Lugol solution and placed immediately on a slide with Visikol (Visikol Inc, NJ, USA) as the mounting and clearing agent. Images were captured on an Olympus BX43 upright microscope under 100X magnification.

Gravistimulation assay

Seeds of WT (Col-0) and *nlp7-1* were sterilized, stratified and plated on MS as above. Seedlings were grown vertically in a growth chamber. At 5 days after germination, plates were turned 180°. Plates were scanned 24 hours after turning and the angle between the primary root and the root hook formed from gravistimulation was measured using ImageJ.

Quantitative PCR

Seedlings were grown on MS as above. At 5 days after germination, RNA was extracted from whole roots as in.[15] cDNA synthesis and qPCR were as in.[15] Primers for *CEL3* were: CEL3-qRT-F: 5′CAAGGTCAGCGATCTGGTCCTCTT 3′ and CEL3-qRT-R: 5′GGTGGTAAACGCCATTGGTAAATTG 3′ and the primer efficiency was evaluated with a cDNA dilution series. Relative expression was calculated relative to *AT1G13320*,[24] using the delta-delta Ct method as in.[15] Three biological replicates, each with three technical replicates, were performed.

Disclosure of interest

The authors report no conflict of interest.

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