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NITROGEN FORAGING

A pHantastic ammonium response

Hans Motte & Tom Beeckman

Plant roots have to orchestrate their growth pattern to access available nutrients. Root architecture is governed by auxin that locally steers growth and development of lateral roots thereby increasing the uptake capacity. A new mechanism for ammonium acquisition by influencing cellular auxin import has been defined.

Nutrients have to be taken up from the soil but are not always homogenously distributed, which can be challenging for foraging in organisms lacking mobility. To cope with this, plants have developed during evolution a high degree of plasticity in their root system. The foundation of this flexibility lies in the remarkable ability to produce and stimulate the growth of lateral roots where it would be appropriate to take up nutrients. In the condition of overall nutrient levels becoming limiting, plants can respond to a locally enriched availability of nutrients by promoting lateral root development into the nutrient-rich patches. This foraging behavior seems, however, to be dependent on the nutrient form and is best known for phosphate and nitrogen (N).

The primary sources of N in the soil are ammonium and nitrate. Local ammonium supply to Arabidopsis thaliana plants has earlier been reported to increase lateral root initiation and higher-order lateral root branching, whereas the elongation of lateral roots is stimulated mainly by nitrate[1]. In order to respond to the local external availability of these nutrients and because the plant hormone auxin is dominating the entire lateral root formation process, a signaling mechanism is needed that will interconnect N availability with auxin. A key feature of the way auxin regulates root development is based on its distribution pattern within the tissue. In the case of lateral root formation, an auxin response maximum is built up and orchestrates the different developmental phases from the specification of lateral root founder cells to the outgrowth of the primordia. The latter requires a high auxin level in cortical and epidermal cells overlying young primordia, which leads to the loosening of cell walls and facilitates the emergence of lateral root primordia from the parent root. Classically, the auxin distribution pattern is controlled by the activity and differential localization of auxin transport proteins. Indole acetic acid (IAA), the most common naturally occurring auxin, can also diffuse through the membranes when it is present in its protonated form. This typically occurs at low pH, such as in the apoplast (pH ≈5.5).

Here auxin is able to diffuse through the plasma membrane and enter the cytoplasm. In the more basic cytoplasm (pH ≈ 7), auxin becomes deprotonated and is unable to diffuse out of the cell. Most of the growth responses seem to be mainly controlled by the carrier-mediated transport, but in a paper published in Nature Plants, Meier *et al.*[2] report on the existence of a pH dependent auxin mobility that is crucial in the response of roots towards the externally available N form and that has a clear impact on the local branching pattern of the root.

Studying the effect of local nitrate and ammonium on auxin response and root development Meier *et al.* observed, amongst others, that ammonium, but not nitrate, is capable of inducing an auxin response signal in cells overlying newly formed primordia. Considering the acidification ability of ammonium and the possible importance of pH for auxin transport, they questioned a link between pH and ammonium-dependent lateral root formation. Ammonium appeared to have a totally inverse effect on the pH in the intercellular space. This turned out to be essential for ammonium-specific increased auxin transport from the vasculature to the cortex and epidermis layers and thus for lateral root emergence. The ammonium induced acidification was shown to be able to rescue the lateral root emergence defect of *aux1 lax3* double mutants, further supporting an auxin carrier-independent mechanism for auxin import in the cells overlying developing root primordia. Remarkably, artificially lowering the pH was even able to restore lateral root formation defects in an ammonium-uptake defective mutant. Hence, ammonium-dependent lateral root formation seems not to be dependent on a yet unknown ammonium biochemical signaling pathway, but rather on ammonium-induced acidification and subsequent passive radial auxin migration towards the outer root cell tissues (Fig. 1).

In contrast to nitrate signalling, clear insights in ammonium signalling were lacking so far, which hampered the understanding of how ammonium interact with pathways controlling lateral root formation. Ammonium was previously proposed to be a signal on itself with the ammonium transporter AMT1;3 as its putative receptor[3]. This could explain the recovering of the lateral root phenotype of a quadruple ammonium transporter mutant after complementation by AMT1;3, but not by another transporter providing similar ammonium levels[1]. However, further supporting evidence is lacking, and, despite a fast effect of ammonium on gene expression and post-translational modifications of proteins[4,5], no key regulatory signalling components, nor common possible regulatory elements in the promoter regions of ammonium response genes were identified so far. Meier *et al.*[2] now elucidated the ammonium response pathway, at least with respect to lateral root formation: ammonium responses go via pH changes, employing rather a chemical signalling pathway than a classical biochemical signal-receptor-response pathway. The next question would be whether this pathway is generally involved in ammonium responses. The observation that ammonium-specific transcriptional responses could be explained either by pH or by ammonium assimilation products[4], supports this. Furthermore, acidic stress causes two other typical ammonium responses as well: inhibition of root elongation and loss of root gravitropism. Just as for the lateral root phenotype, these ammonium effects on roots could be mimicked by the use of the proton pump activator fusicoccin or could be observed in the *feronia-4* (*fer-4*) mutant that shows enhanced acidification of the apoplast[6,7]. Therefore, ammonium-induced root responses might in general follow a pH-dependent chemical signalling pathway.

N, acquired by plants mainly in forms of nitrate and ammonium from the soil dominates growth and development, and high-yield crop production relies heavily on N fertilization. The mechanisms of root adaptation to altered supply of N forms and concentrations have been intensively studied but the precise mechanism on how these N forms influence the auxin-controlled endogenous root branching process are less-well understood. The paper by Meier et al.[2] is an important step forward towards a better knowledge of mechanisms underlying the different plant responses to nitrate and ammonium, and will help to develop strategies to enhance N use efficiency (NUE), leading to a reduction in fertilizer application.

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Competing interests

The authors declare no competing interests.

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References

1 Lima, J. E., Kojima, S., Takahashi, H. & von Wirén, N. Ammonium triggers lateral root branching in Arabidopsis in an AMMONIUM TRANSPORTER1;3-dependent manner. *The Plant cell* **22**, 3621-3633, doi:10.1105/tpc.110.076216 (2010).

2 Meier, M., Liu, Y., Lay-Pruitt, K. S., Takahashi, H. & von Wirén, N. Auxin-mediated root branching is determined by the form of available nitrogen. *Nature Plants* **X**, X (2020).

3 Liu, Y. & von Wirén, N. Ammonium as a signal for physiological and morphological responses in plants. *Journal of Experimental Botany* **68**, 2581-2592, doi:10.1093/jxb/erx086 (2017).

4 Patterson, K. *et al.* Distinct signalling pathways and transcriptome response signatures differentiate ammonium- and nitrate-supplied plants. *Plant, Cell & Environment* **33**, 1486-1501, doi:10.1111/j.1365-3040.2010.02158.x (2010).

5 Engelsberger, W. R. & Schulze, W. X. Nitrate and ammonium lead to distinct global dynamic phosphorylation patterns when resupplied to nitrogen-starved Arabidopsis

seedlings. *The Plant Journal* **69**, 978-995, doi:10.1111/j.1365-313X.2011.04848.x (2012).

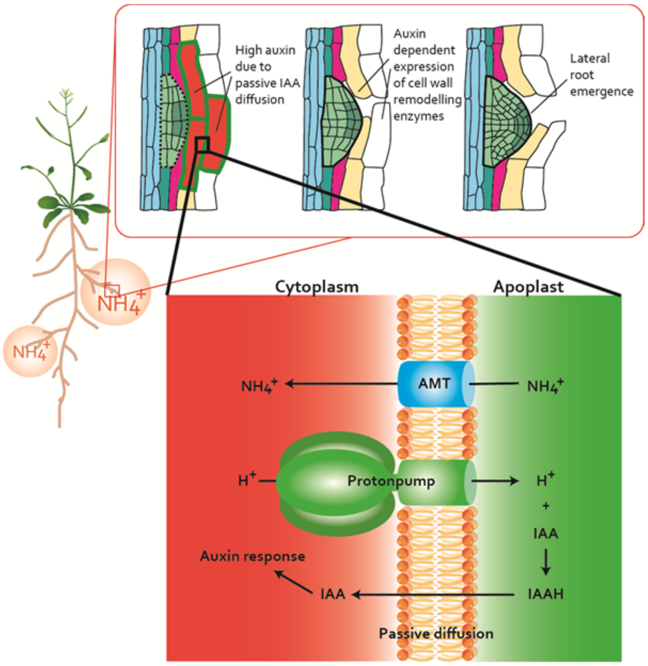
6 Barbez, E., Dünser, K., Gaidora, A., Lendl, T. & Busch, W. Auxin steers root cell expansion via apoplastic pH regulation in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences* **114**, E4884-E4893, doi:10.1073/pnas.1613499114 (2017).

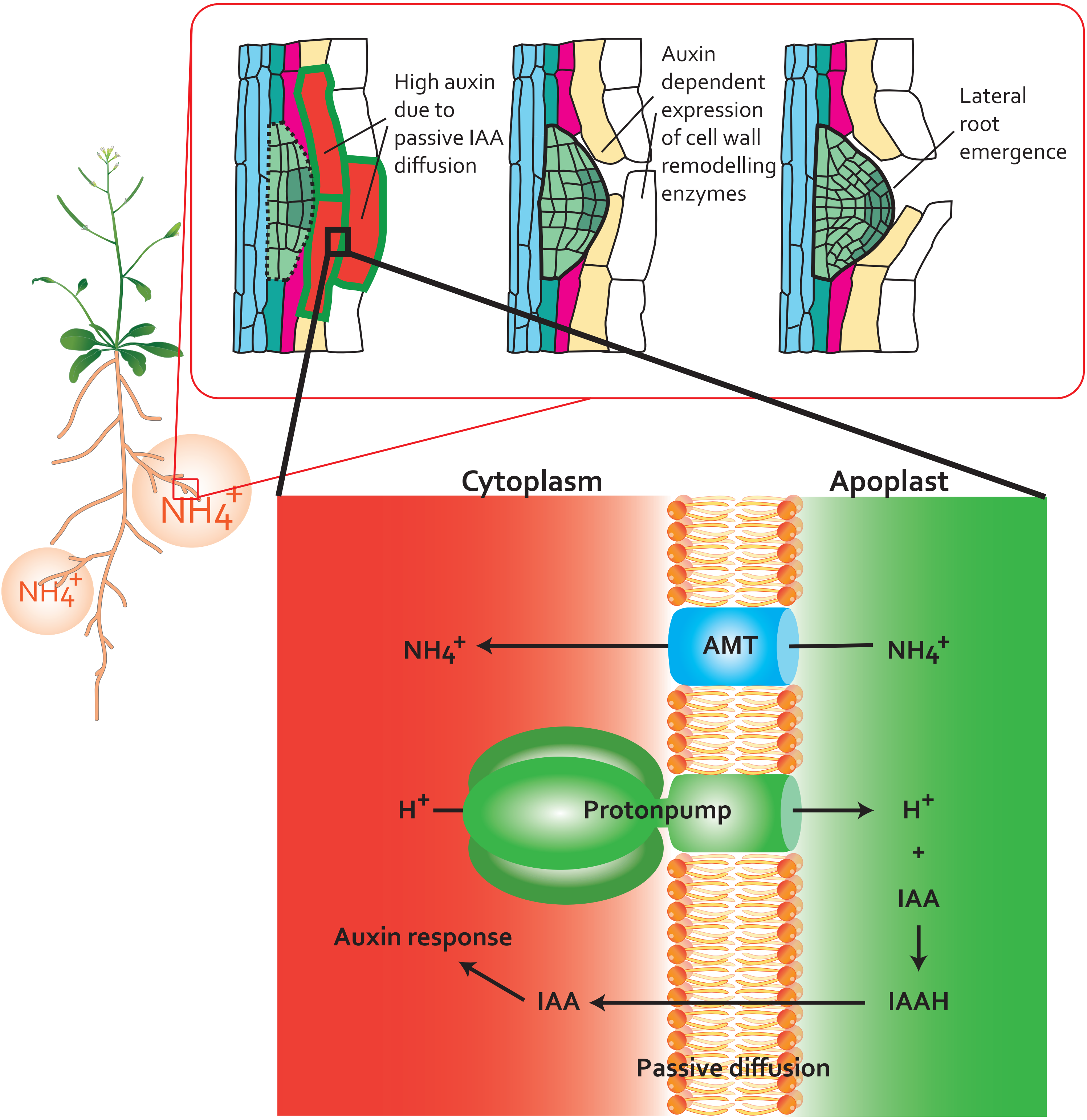
7 Gujas, B., Alonso-Blanco, C. & Hardtke, C. S. Natural *Arabidopsis brx* loss-of-function alleles confer root adaptation to acidic soil. *Current Biology* **22**, 1962-1968, doi:10.1016/j.cub.2012.08.026 (2012).

Figure 1.

Schematic presentation of the mechanism underlying ammonium-induced lateral root emergence as demonstrated by Meier et al[2].

Ammonium is locally taken up by the outer tissue layers of the root, and is transported via AMT ammonium transporters from the apoplast (green) to the cytoplasm (red). This transport stimulates proton release, leading to apoplastic acidification and protonation of IAA into IAAH. IAAH diffuses into cortex and epidermis cells overlaying early stage lateral root primordia. The auxin accumulation induces the expression of genes involved in cell wall loosening. As a result, the mechanical resistance of the outer cell layers decreases, allowing the lateral root emergence. Cells or cell layers are coloured light blue (vasculature), cyan (pericycle), light green (lateral root primordium), green (auxin maximum in lateral root primordium), pink (endodermis), yellow (cortex), white (epidermis), or red (auxin maximum due to IAAH diffusion in cortex and epidermis).




High auxin due to passive IAA diffusion
Auxin dependent expression of cell wall remodelling enzymes
Lateral root emergence
NH_4^+
NH_4^+
Cytoplasm
Apoplast
AMT
NH_4^+
NH_4^+
H^+
Protonpump
H^+
+
IAA
Auxin response
IAA
IAAH
Passive diffusion