Integrating N signals and root growth: the role of nitrate transceptor NRT1.1 in auxin-mediated lateral root development

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Plants modify their root system architecture (RSA) to efficiently acquire nutrients from the environment. Nitrate (NO$_3^-$) is an essential nutrient that elicits changes in RSA through the action of the NO$_3^-$ transceptor NRT1.1. Maghiaoui et al. (2020) have demonstrated that NRT1.1 modulates lateral root growth in response to NO$_3^-$ by regulating both auxin biosynthesis and downstream auxin transport during lateral root development.

Dynamic changes in RSA in response to nitrogen availability

The plant root system is highly dynamic; various external stimuli from the soil environment dictate root growth strategies to optimize uptake of water and nutrients. This spatiotemporal, structural arrangement of plant root biomass is referred to as root system architecture (RSA). RSA encompasses such traits as the primary (or embryonic) root length, the length and density of lateral (post-embryonic) roots, and the angles at which these roots grow (Osmont et al., 2007). One way in which plants adjust RSA is through the action of auxins, a major class of phytohormones regulating plant growth, particularly the development of lateral root primordia (Benková et al., 2003). Auxin gradients within these root tissues are coordinated by the action of auxin influx and efflux transporters (Petrášek and Friml, 2009).

RSA must have high plasticity because essential nutrients are heterogeneously distributed throughout the soil profile in patches and gradients (Giehl and von Wirén, 2014). Of these nutrients, nitrogen (N) has been of particular interest because it has a significant and complex effect on RSA. For example, N starvation has been shown to severely inhibit primary root growth and development of lateral root primordia (Araya et al., 2014), but mild N deprivation induces lateral root growth as part of a ‘foraging’ mechanism used by the plant to acquire N (Krouk et al., 2010; Gruber et al., 2013). However, there remains a need for research to understand how N affects the underlying signaling processes—such as auxin transport—in governing RSA.

The main form of N that many plant species preferentially take from the soil is inorganic nitrate (NO$_3^-$) (Nacy et al., 2013). In Arabidopsis, NO$_3^-$ uptake is facilitated by the NRT2 and NPF families of NO$_3^-$ transporters, which in general are known to have high and low affinity for NO$_3^-$, respectively (Kiba and Krapp, 2016). However, one key NPF member, NRT1.1 (NPF6.3), uniquely exhibits dual affinity for NO$_3^-$ and has been implicated in not only NO$_3^-$ transport but also NO$_3^-$-responsive control of root development (Tsay et al., 1993; Liu and Tsay, 2003; Remans et al., 2006; Krouk et al., 2010; Bouguyon et al., 2015). Specifically, NRT1.1 serves as a transporter and receptor (transceptor) of NO$_3^-$ (Ho et al., 2009) as well as an auxin transporter in the lateral root primordia when NO$_3^-$ supply is low or absent (Krouk et al., 2010; Bouguyon et al., 2015; Box 1). Due to these multiple roles, research characterizing the role of NRT1.1 in regulating RSA provides critical insight into novel molecular pathways in which plants modulate root growth in response to environmental signals.

NRT1.1 acts as an integrator of NO$_3^-$ signaling and auxin biosynthesis and transport

In their work published in this issue, Maghiaoui et al. (2020) have demonstrated additional roles of NRT1.1 as an integrator
optimizing auxin signaling pathways, highlighting the importance of this transceptor in modulation of RSA. In order to determine the effect of NO$_3^-$ on known families of auxin transporters, the authors analyzed the expression levels of key auxin transporters in wild-type seedlings and the chl1-5 mutant line lacking NRT1.1 expression by growing them with or without NO$_3^-$ supplementation. Their results show that gene and protein expression of various PIN, ABCB, and AUX/LAX auxin transporters are N responsive but not in an NO$_3^-$ specific or NRT1.1-dependent manner, as re-supplementation of glutamine restores their expression in the chl1-5 mutant.

However, the one exception to these trends was LAX3, an auxin influx transporter that imports auxin into cortical cells overlying the lateral root primordia, which leads to loosening of the cell wall and allows the primordia to emerge as they develop (Swarup et al., 2008). LAX3 gene and protein expression as well as gene expression of LBD29, an upstream regulator of LAX3 (Porco et al., 2016), were found to be repressed in the wild type but not in the chl1-5 mutant in the absence of NO$_3^-$ supplementation. Additionally, there was no recovery of LAX3 expression by glutamine. Further evidence indicates that a key auxin biosynthetic gene, TAR2, is also suppressed by NRT1.1.
under NO$_3^-$ deprivation. TAR2 had previously been implicated in controlling root development as mild N deficiency induces TAR2 expression, which in turn promotes auxin accumulation and lateral root emergence for N foraging; however, this function was not characterized in relation to NRT1.1 (Ma et al., 2014). Maghiaoui et al. (2020) thus propose that repression of LAX3 and TAR2 gene expression occurs through a putative upstream signaling module involving NRT1.1, and this prevents the growth of lateral root primordia under low NO$_3^-$ availability. These results also emphasize how NO$_3^-$ specifically acts to regulate auxin transport and lateral root development, supporting previous findings from Krouk et al. (2010).

In summary, Maghiaoui et al. (2020) demonstrate that NRT1.1 acts as an integrator for NO$_3^-$–derived signals on two sides of auxin signaling, biosynthesis and transport, during the development of the lateral root primordia. These results add to our knowledge of the NO$_3^-$ transceptor NRT1.1 and open up questions about NO$_3^-$ as a specific signaling molecule modulating auxin distribution in a multifaceted manner—primarily affecting basipetal auxin transport within the primordia, and additionally required for controlling gene expression of an auxin influx transporter at overlaying cortical cells and a key auxin biosynthetic gene in the vasculature (Box 1). It remains to be investigated how NRT1.1 transmits the signal and impacts on auxin signaling in other aspects of RSA.

**Future perspectives: the impact of N and auxin on RSA**

There are multiple avenues for further research that will deepen our understanding of RSA development in response to inorganic N in soils. Plants have the capacity to take up and utilize N in the form of ammonium (NH$_4^+$) in addition to NO$_3^-$ (Hachiya and Sakakibara, 2017). NH$_4^+$ uptake is mediated by the AMT family of transporters (Yuan et al., 2007). NH$_4^+$ availability has been shown to elicit changes in RSA in a distinct manner, promoting higher order branching of lateral roots in contrast to NO$_3^-$–driven mechanisms, which may reflect the differences between accumulation of these N sources within the soil profile (Lima et al., 2010; Kiba and Krapp, 2016). Thus, NH$_4^+$ could also be exerting changes in auxin transport or biosynthesis pathways to modulate RSA. Additionally, there is evidence that NRT1.1 plays a role in Arabidopsis response to NH$_4^+$ toxicity, suggesting that NO$_3^-$ and NH$_4^+$ response mechanisms may partially overlap (Jian et al., 2018). The interaction of these two forms of inorganic N and how they regulate lateral root development could be a promising area for further research.

Another direction of inquiry would be to determine whether NRT1.1–dependent auxin signaling differs depending on local or homogenous NO$_3^-$ availability. In Arabidopsis, NRT1.1 has been implicated in lateral root proliferation in response to local NO$_3^-$ supply (Remans et al., 2006; Mounier et al., 2014). It would be interesting to see whether the pathways involving LAX3 and TAR2 elucidated in the study by Maghiaoui et al. (2020) are conserved when plants are experiencing local supplementation of NO$_3^-$ and how this could affect development of primordia located in distal parts of the root. A subsequent extension of this research would be to study the potential impact of this pathway on development of lateral root primordia with relevance to higher order branching of lateral roots, as has been observed with a local supply of NH$_4^+$ (Lima et al., 2010).

This research provides novel insight into regulation of auxin transport, biosynthesis, and signaling pathways modulating RSA, and further highlights a key question in understanding the integration of plant development and the NO$_3^-$ environment: what other types of developmental processes could be regulated by proteins with versatile functions like the NO$_3^-$ transceptor NRT1.1? The mechanisms highlighted in this study may also have interesting implications for other plant species beyond dicots, for example OsNRT1.1B in Oryza sativa, given its essential role in increasing the N use efficiency (Hu et al., 2015). Extension of these concepts into an agriculturally relevant plant species will allow researchers to bridge basic and applied research approaches.

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