Insight

Adjusting plant nutrient acquisition to fluctuating availability: transcriptional co-regulation of the nitrate and phosphate deprivation responses in roots

Uwe Ludewig*, Emil Vatov, Dominik Hedderich and Benjamin Neuhäuser

Institute of Crop Science, Nutritional Crop Physiology, University of Hohenheim, Fruwirthstr. 20, D-70593 Stuttgart, Germany
*Correspondence: u.ludewig@uni-hohenheim.de

This article comments on:

Safi A, Medici A, Szonarski W, Martin F, Clement-Vidal A, Marshall-Colon A, Ruffel S, Gaymard F, Rouached H, Leclercq J, Coruzzi G, Lacombe B, Krouk G. 2021. GARP transcription factors repress Arabidopsis nitrogen starvation response via ROS-dependent and -independent pathways. Journal of Experimental Botany 72, 3894–3914.

A family of NIGT1/HHO-type transcriptional repressors activate phosphate deprivation responses via inhibition of upstream repressors. At the same time, these repress the nitrate deprivation response upon nitrate provision; their loss triggers an increase of nitrate uptake and plant growth. Safi et al. (2021) link the function of NIGT1/HHOs with reactive oxygen species (ROS) signalling under variable nitrate supply, but it remains puzzling how ROS integrate into nutrient-specific signalling cascades.

Native plant ecosystems are often nutritionally co-limited, especially in the two macroelements nitrogen (N) and phosphorus (P) (Fay et al., 2015). Substantial biomass increases are only expected when both elements are added, indicating synergisms between them. On evolutionary scales, nutritional deficiencies were the rule, rather than the exception, and modern heavily fertilized crops carry relics of this in their genetics. Different plant species have different traits related to the acquisition of inorganic phosphate (P), one of the least soil-mobile nutrients that is easily sorbed and fixed to clay particles and nitrate (a highly mobile nutrient in soil), but common responses to low P and N exist, such as the anthocyanin production in leaves and the investment into roots, which results in a higher root/shoot ratio, mediated by phytohormone-driven redirection of photoassimilates. Nutritional interactions occur with other elements as well; for example, molybdenum and iron are linked with nitrate reduction, whereas P mobilization mechanisms often co-solubilize iron, zinc, and manganese (Marschner, 2011).

Function of NIGT1/HHOs in the P deprivation response and the link with nitrate

NIGT (NITRATE-INDUCIBLE GARP-TYPE TRANSCRIPTIONAL REPRESSOR) genes, also known as HRS1 (HYPERSENSITIVITY TO LOW P-Elicited PRIMARY ROOT SHORTENING 1) and HHO (HRS1 HOMOLOG) genes coordinate nitrate and P responses in Arabidopsis (Kiba et al., 2018; Maeda et al., 2018; Ueda et al., 2020b, c, Wang et al., 2020). Their names already indicate that they were identified in different nutritional contexts (Fig. 1).

NIGT1/HHO genes act as positive regulators of the P starvation response. In brief, the master upstream regulators PHOSPHATE STARVATION RESPONSE 1–4 (PHR1–PHR4) bind to cis-elements in their promoters and activate them. In the presence of P, PHRs form inactive complexes with SPX proteins (named after SYG1/Pho81/XPR1); the latter apparently sense the cellular P status. Under P, deficiency, PHRs are released and target cis-elements in promoters of P uptake-, recycling-, and morphological adaptation-associated genes (Fig. 1). NIGT1/HHOs enhance transcription of P uptake transporters (Wang et al., 2020) and repress SPX promoters, while PHRs moderately activate NIGT1/HHO genes (Maeda et al., 2018).

NIN-LIKE PROTEIN 6/7 (NLP6/7) transcription factors, master regulators of the nitrate response, promote nitrate uptake/assimilation genes and NIGT1 expression (Fig. 1).
NIGT1/HHOs dimerize with each other and with more distant HHO proteins; this determines specificity and affinity for targets (Ueda et al., 2020b).

In a feedback loop, NIGT1/HHO proteins repress nitrate uptake upon P_i deprivation, and vice versa. P_i uptake is repressed in the absence of nitrate, as SPX promoters are then not repressed by NIGT1/HHOs (Ueda et al., 2020c). Safi et al. showed that double and quadruple nigt1/hho mutants had elevated high affinity nitrate uptake concomitant with the de-repression of high affinity nitrate transporters. This correlates with an improved growth under controlled conditions with sufficient P_i (Safi et al., 2021). Wang et al. (2020) also previously reported an improvement of low affinity uptake in hho mutants, probably due to NRT1.1. One should, however, keep in mind that Col-0, the genotype used in these studies, is quite inefficient using nitrogen compared with other Arabidopsis accessions (Chardon et al., 2010; Menz et al., 2018). Even though there is functional diversification within the NIGT1/HHO family among monocots and dicots (Ueda et al., 2020a), the transcriptional co-repression of nitrate influx by P_i deficiency seems to be conserved in maize (Wang et al., 2020), and P_i starvation control by nitrate is conserved in wheat and rice (Medici et al., 2019). Still, extrapolation to field-grown crops should be done with caution, as biomass increases under control conditions rarely translate into the field. The quadruple nigt1/hho mutant was impaired in P_i uptake under P deficiency, but not when sufficient P was available (Ueda et al., 2020c). Benefits in nitrate nutrition are thus compromised by negative effects on P acquisition, and crops that fail to adjust to low P_i are undesirable, even with improved nitrate acquisition. It is possible that complex feedback-regulated networks also help plants to explore temporal variations in nutritional availability (e.g. in field environments). As long as the underlying element is not rate limiting for growth, Arabidopsis profits from temporal reduction of external P_i, compared with static supply (Fig. 2).

Connecting nutrient deprivation/starvation responses with ROS

Potassium, N, or P_i deprivation are known to increase H_2O_2 in distinct areas of Arabidopsis roots; this and the misregulation of starvation-responsive nitrate and phosphate transporter genes in an NADPH oxidase mutant (atrbohC) provided some initial hints of ROS involvement in nutrient-specific responses (Shin et al., 2020).
Enforcement of the transcriptional nitrate starvation response by glutaredoxins (Jung et al., 2018; Ehrary et al., 2020) and inhibition by chemical ROS scavenging point to an involvement of ROS homeostasis under fluctuating nutrient conditions. This is supported by Safi et al. (2021), who conclude that 86% of the direct (repressed) targets of HRS1 in protoplasts were dependent on the nitrate context (then HRS1 acted mainly as a repressor of heat shock genes). In complex targeted gene categories, the redox metabolism was over-represented. H$_2$O$_2$ production was not compromised in a quadruple nigt1/hho mutant, but HRS1/HHO1 overexpression suppressed H$_2$O$_2$ evolution upon nitrate deprivation. ROS signalling must not reach toxic levels within cells (Miller et al., 2018) and may participate in the initiation of the response, rather than as an unwanted by-product of long-term N starvation due to insufficient production of proteins involved in scavenging. Furthermore, NIGT1/HHO genes are highly specific to nitrate, rather than ammonium. Rapid responses within hours to nitrate deprivation are completely absent when ammonium is removed from the nutrient solution, and transcriptional changes are seen before nitrate levels drop in the tissue (Menz et al., 2016). We therefore associate the NIGT1/HHO repressors and ROS with nitrate deprivation, rather than with N starvation responses (which occur later). Interestingly, the mRNA of HRS1 declined quickly without nitrate, but was stabilized by NO$_3^-$, pointing to post-transcriptional control (Menz et al., 2016). It may be interesting to ask if there is a link to ROS by carrying out in silico analysis using other datasets from previous transcriptomic studies, for example on AtNIGT1.2/AtHHO2 overexpressors (Kiba et al., 2018; Wang et al., 2020).

**Nutritional co-regulation is highly complex**

Further crosstalk between N and P might depend on LATERAL ORGAN BOUNDARY DOMAIN transcription factors (LBD37–LBD39), another group of redundant repressors of the N deprivation response that are highly up-regulated by diverse N sources, preferentially nitrate. Their main function is to repress anthocyanin biosynthesis in nitrate-sufficient conditions. Their transcripts are repressed in –N, leading to anthocyanin coloration of shoots (and ROS scavenging) in severely –N-stressed plants (Rubin et al., 2009). LBD transcripts may be repressed in –P (Rubin et al., 2009) and less anthocyanin accumulated in the nigt1/hho quadruple mutant with sufficient N (Ueda et al., 2020c), suggesting crosstalk with LBDs in –P (Fig. 1).

In summary, NIGT1/HHOs are central hubs in the cross-talk between nitrate and phosphate deprivation responses. How NIGT1/HHOs co-regulate these nutritional responses...
in crop roots and whether these can be targeted to generate more nutrient use-efficient crops is an interesting task for the future. Overexpression of various nitrate transporters increased nitrate acquisition and yield apparently independently of P, not only in laboratory studies, but even in the field (e.g. Wang et al., 2018), supporting the added value of studying these genes and proteins at a fundamental level.

**Keywords:** Nitrate deprivation, nitrogen starvation, phosphate deprivation, reactive oxygen species.

**References**

Chardon F, Barthélémy J, Daniel-Vedele F, Masclaux-Daubresse C. 2010. Natural variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with limiting and ample nitrogen supply. Journal of Experimental Botany 61, 2293–2302.

Ehrary A, Rosas M, Carpinelli S, Davalos O, Cowling C, Fernandez F, Escobar M. 2020. Glutaredoxin AtGRXS8 represses transcriptional and developmental responses to nitrate in *Arabidopsis thaliana* roots. Plant Direct 4, e00227.

Fay PA, Probe SM, Harpole WS, et al. 2015. Grassland productivity limited by multiple nutrients. Nature Plants 1, 1–5.

Jung JY, Ahn JH, Schachtman DP. 2018. CC-type glutaredoxins mediate plant response and signaling under nitrate starvation in *Arabidopsis*. BMC Plant Biology 18, 281.

Kiba T, Inaba J, Kudo T, et al. 2018. Repression of nitrogen starvation responses by members of the Arabidopsis GARP-type transcription factor NIGT1/HRS1 subfamily. The Plant Cell 30, 925–945.

Maeda Y, Konishi M, Kiba T, Sakuraba Y, Sawaki N, Kurai T, Ueda Y, Sakakibara H, Yanagisawa S. 2018. A NIGT1-centred transcriptional cascade regulates nitrate signalling and incorporates phosphorus starvation signals in Arabidopsis. Nature Communications 9, 1376.

Medici A, Szponarski W, Dangeville P, et al. 2019. Identification of molecular integrators shows that nitrogen actively controls the phosphate starvation response in plants. The Plant Cell 31, 1171–1184.

Marschner P. 2011. Marschner’s mineral nutrition of higher plants, 3rd edn. London: Academic Press.

Menz J, Li Z, Schulze WX, Ludewig U. 2016. Early nitrogen-deprivation responses in Arabidopsis roots reveal distinct differences on transcriptome and (phospho-)proteome levels between nitrate and ammonium nutrition. The Plant Journal 88, 717–734.

Menz J, Range T, Trini J, Ludewig U, Neuhäuser B. 2018. Molecular basis of differential nitrogen use efficiencies and nitrogen source preferences in contrasting Arabidopsis accessions. Scientific Reports 8, 1–11.

Miller G, Couto J, Shulaev V, Mittler R. 2018. Reactive oxygen signaling in plants. Annual Plant Reviews 33, 189–201.

Rubin G, Tohge T, Matsuda F, Saito K, Scheible WR. 2009. Members of the LBD family of transcription factors repress anthocyanin synthesis and affect additional nitrogen responses in Arabidopsis. The Plant Cell 21, 3567–3584.

Safi A, Medici A, Szponarski W, et al. 2021. GARP transcription factors repress Arabidopsis nitrogen starvation response via ROS-dependent and -independent pathways. Journal of Experimental Botany 72, 3894–3914.

Shin R, Berg RH, Schachtman DP. 2005. Reactive oxygen species and root hairs in Arabidopsis root response to nitrogen, phosphorus and potassium deficiency. Plant & Cell Physiology 46, 1350–1357.

Ueda Y, Ohtsuki N, Kadota K, Tezuka A, Nagano AJ, Kadowaki T, Kim Y, Miyao M, Yanagisawa S. 2020a. Gene regulatory network and its constituent transcription factors that control nitrogen deficiency responses in rice. New Phytologist 227, 1434–1452.

Ueda Y, Nosaki S, Sakuraba Y, Miyakawa T, Kiba T, Tanokura M, Yanagisawa S. 2020b. NIGT1 family proteins exhibit dual mode DNA recognition to regulate nutrient response-associated genes in Arabidopsis. PLoS Genetics 16, e1009197.

Ueda Y, Kiba T, Yanagisawa S. 2020c. Nitrate-inducible NIGT1 proteins modulate phosphate uptake and starvation signalling via transcriptional regulation of SPX genes. The Plant Journal 102, 448–466.

Wang W, Hu B, Yuan D, et al. 2018. Expression of the nitrate transporter gene OsNRT1.1A/OsNPF6.3 confers high yield and early maturation in rice. The Plant Cell 30, 639–651.

Wang X, Wang HF, Chen Y, Sun MM, Wang Y, Chen YF. 2020. The transcription factor NIGT1.2 modulates both phosphate uptake and nitrate influx during phosphate starvation in Arabidopsis and maize. The Plant Cell 32, 3519–3534.