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Can I have some light and sugar with my nitrate?

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Nitrate is an essential nutrient for plants, however nitrate availability varies in the soil. In order to effectively take up nitrogen plants have to forage for nitrate by adjusting the growth of their root system and by expressing nitrate transport proteins that take up nitrate at different concentrations. Nitrate uptake must be balanced with carbon fixation and production of sugars by photosynthesis, which depends on light intensity. Light signaling has a clear positive effect on the expression of nitrate transporters of the NRT2 family (Lejay et al., 1999; Lejay et al., 2003), which form the high-affinity nitrate uptake system that transports nitrate when the concentration in the soil is low. In this issue of *Plant Physiology*, Ruffel et al. (2021) reveal how light fine tunes nitrate uptake by the NRT2 family to balance the production of sugars with the need for nitrate.

The NRT2 family of nitrate transporters has four members that are expressed in the root and take up nitrate: NRT2.1, NRT2.2, NRT2.4, and NRT2.5. NRT2.1 and NRT2.2 transport nitrate at low soil concentrations, while NRT1.1, the other main nitrate uptake transporter, does so at high concentrations (O'Brien et al., 2016). NRT2.4 and NRT2.5 are the highest affinity nitrate transporters and can transport nitrate at very low concentrations (<50 μM). Therefore, NRT2.4 and NRT2.5 are expressed more constantly than NRT2.1 and NRT2.2. NRT2.1 is induced by low nitrate in the soil and by the light environment of the shoot. These mechanisms of NRT2.1 induction have been well characterized separately (Lejay et al., 1999; Chen et al., 2016); however, these factors never occur in isolation and it is important to study the combined effect of the light and nitrate environment on the regulation of nitrate uptake.

To do so, Ruffel et al. conducted micro-array expression analysis experiments where light intensity and nitrate availability varied in strength and duration. They also tested the effect of light in a CO_2-containing and CO_2-free atmosphere to separate light effects from photosynthesis effects. In all conditions, NRT2.1 was differentially regulated by light, low nitrate, and the availability of sugars.

Next, Ruffel et al. searched for genes co-regulated with NRT2.1 and used those genes to construct a gene regulatory network. Within that network they found three transcription factors, MYC1, TGA3, and bHLH93, that acted as main hubs, indicating those three factors combined can regulate almost all genes in the network. These three transcription factors had not been previously identified as players in the regulation of nitrate uptake. Therefore, the authors wanted to validate the function of these transcription factors in regulating nitrate uptake in different light regimes. Both MYC1 and TGA3 were upregulated by light in combination with low nitrate, while bHLH93 was mainly upregulated by light and only in an atmosphere containing CO_2. Knockout mutants of these transcription factors had reduced light-dependent nitrate uptake, showing their functional importance. But how do they regulate nitrate uptake?

MYC1, TGA3, and bHLH93 potentially bind promoters of all NRT2 genes; however, evidence indicated they preferentially regulated NRT2.4 and NRT2.5 and to a lesser extent NRT2.1 and NRT2.2. In knockout mutants of *MYC1*, *TGA3*, and *bHLH93*, expression levels of *NRT2.4* and *NRT2.5* were reduced. Furthermore, TGA3 and MYC1 directly bound the *NRT2.4* and *NRT2.5* promoters. Thus, Ruffel et al. uncovered that combined low nitrate and high light can induce *MYC1* and *TGA3* activity and light and sugar production can induce *bHLH93*. Induction of these three transcription factors then leads to induced expression of *NRT2.4* and *NRT2.5*, helping to initiate nitrate uptake (Figure 1).

The uptake of nitrate is an essential process for plant growth and requires fine-tuning. Ruffel et al. used three

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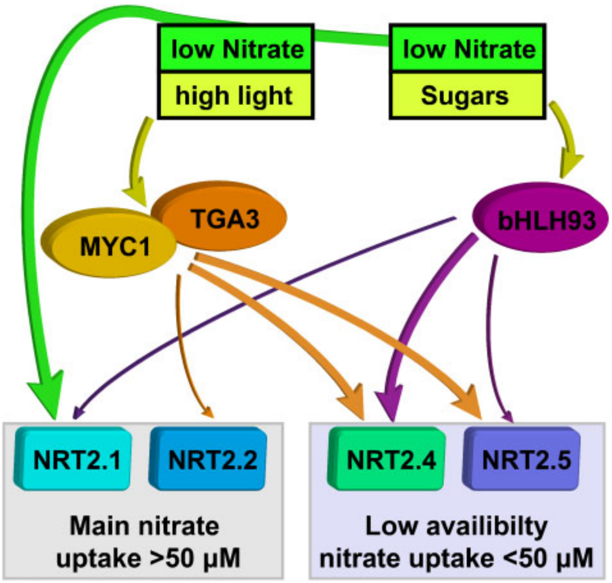

Figure 1 MYC1, TGA3, and bHLH93 regulate light-dependent nitrate uptake. Low nitrate, combined with light and/or sugars, can induce expression of MYC1, TGA3, and bHLH93, which in turn induce the expression of nitrate uptake transporters of the NRT2 family. The thickness of the arrows indicates the relative strength of induction. Low nitrate alone can induce NRT2.1, but NRT2.4 and NRT2.5 are strongly regulated via MYC1, TGA3, and bHLH93 action. NRT2.4 and NRT2.5 transport nitrate at very low concentrations, when NRT2.1 cannot yet do so. When a small level of nitrate is taken up, NRT2.1 is induced and takes over the bulk of the high affinity nitrate uptake. Figure based on Ruffel et al. (2021, Figure 7).

different intensities of light to study this fine-tuning; however, they did not vary light quality, which can also influence gene expression. Ruffel et al. found that the transcription factor HY5 was also involved in regulating light-dependent nitrate uptake, but chose not to focus on it. HY5 regulates NRT2.1 expression and is induced by light and by different light qualities, such as increased vegetation shade (Chen et al., 2016; van Gelderen et al., 2018). The versatility and broad developmental role of HY5 make it a very potent integrator of both light and nitrate signaling.

The subtle approach by Ruffel et al. shows there is more to be discovered to nitrate signaling when also accounting for light signaling. Even though years of research have identified key players in the regulation of nitrate uptake, the transcription factors MYC1, TGA3, and bHLH93 had not been previously identified in its regulation. This study shows that by carefully selecting the experimental conditions and then using that data to construct a gene regulatory network, scientists can find additional regulatory factors that have eluded others and discover how the integration of different signals work. This approach may also be useful for other combinations of (a)biotic influences and highlights that integration of different factors in real plant life is an important next step in plant molecular genetics.

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