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Surviving hypoxia: aquaporin-like protein NIP2;1 mediates lactic acid transport

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Stress due to hypoxia or low oxygen is one of the many problems encountered by plants inhabiting marshy, submerged, or inadequately aerated soils. Poor oxygen availability wreaks havoc on cellular respiration, resulting in a severe energy crisis and ultimately leading to flood-adaptive changes at the molecular, metabolic, and developmental levels (Voesenek and Bailey-Serres, 2013; Voesenek and Bailey-Serres, 2015). Immediately following hypoxia exposure, mitochondrial ATP declines due to the lack of NADH oxidation to NAD^+ (Schmidt et al., 2018). In addition, the accumulation of toxic metabolites and cytosol acidification pose threats to plant survival under hypoxia.

To mitigate these effects, plants have evolved adaptive molecular mechanisms, such as increasing the flux of glycolysis to produce ATP and promoting fermentation to regenerate NAD^+ (Drew, 1997). However, enhanced fermentation comes with a price: lactate/lactic acid, the end product of lactic acid fermentation, causes acidosis and toxicity if allowed to accumulate within plant tissues. Lactic acid levels in Arabidopsis (*Arabidopsis thaliana*) roots may increase up to 14-fold within 2 h of hypoxia induction (Mustroph et al., 2014). To reduce toxicity and survive hypoxia, plants excrete a portion of excess lactic acid via roots into the rhizosphere (Dolferus et al., 2008). The precise mechanisms underlying lactic acid efflux during hypoxia and its subsequent effects on lactic acid homeostasis remain largely unknown.

Among the hypoxia-triggered core genes that confer plant survival, the expression levels of *Nodulin 26-like Intrinsic Protein 2;1* (*NIP2;1*) rapidly increase within short periods of submergence (Choi and Roberts, 2007). NIP2;1 belongs to a land plant-specific subfamily of aquaporins (membrane proteins that transport water) with the classic "hourglass" structure (Figure 1) but functionally diversified to transport many additional substrates (Roberts and Routray, 2017). Arabidopsis NIP2;1 was shown to selectively transport lactic acid in African clawed frog (*Xenopus*) oocytes (Choi and Roberts, 2007). However, functional evidence of NIP2;1 as a lactic acid transporter *in planta* has been lacking.

In this issue of *Plant Physiology*, Beamer et al. (2021) demonstrate the function of Arabidopsis NIP2;1 as a lactic acid channel that promotes plant survival under hypoxic stress. To investigate changes in *NIP2;1* transcript levels, the authors conducted time-course gene expression assays on roots and shoots of Arabidopsis seedlings after hypoxia challenge. *NIP2;1* expression levels in the roots increased up to 1,000-fold within 2–4 h of hypoxia induction, compared to about 40-fold in the shoots, indicating the predominance of NIP2;1 in root tissues. Following a similar pattern, transgenic plants showed enhanced GUS staining throughout the root within 4 h of hypoxic treatment (Figure 1). In contrast, *NIP2;1* expression levels were extremely low in the hypoxic roots of a T-DNA insertion mutant (*nip2;1*) that performed poorly under hypoxia stress, indicated by chlorosis and high seedling mortality. The susceptibility of *nip2;1* was confirmed by strongly reduced yield of photosystem II under hypoxia, indicating a drop in photosynthetic efficiency.

Microscopy analyses of *nip2;1* plants complemented with *NIP2;1 promoter::NIP2;1-GFP* confirmed enhancement in NIP2;1-GFP protein levels with predominant localization in the plasma membrane upon hypoxia (Figure 1). Consistent with the proposed function of NIP2;1 as a lactic acid channel, the roots of *nip2;1* mutant seedlings under hypoxia accumulated significantly higher levels of lactic acid compared to wild type. In addition, experiments using a pH-sensitive dye showed the inability of *nip2;1* to excrete lactic acid and thereby acidify the growth media. Gene expression assays of *nip2;1* revealed differential expression patterns of transcripts encoding pyruvate and lactate metabolic enzymes. In particular, the levels of *glycolate oxidase3* (*GOX3*), which does not respond to hypoxia, were downregulated in the *nip2;1*

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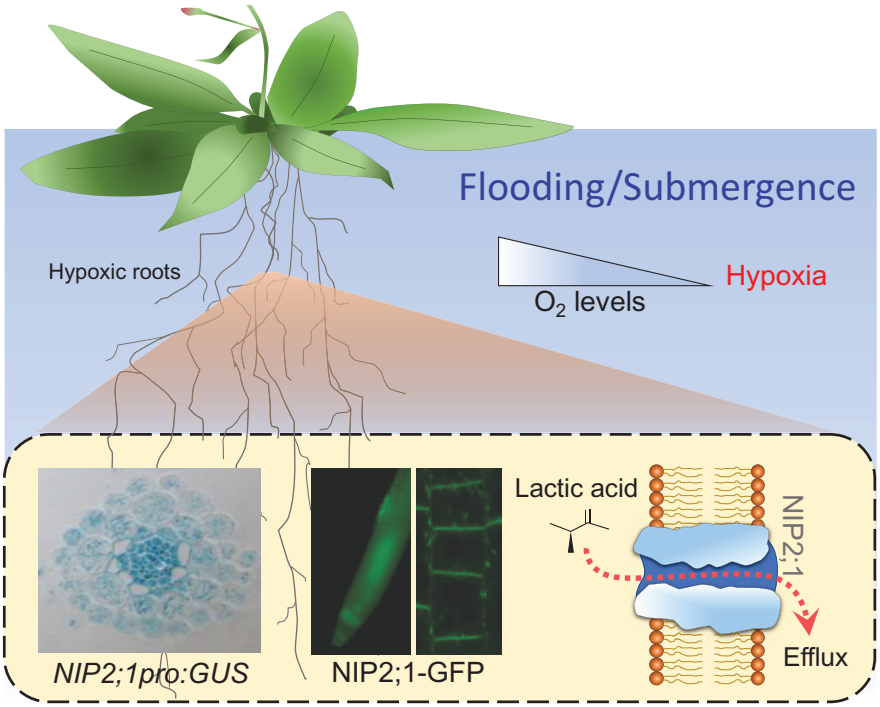

Figure 1 NIP2;1 functions as a lactate efflux channel. Flooding or submergence induces hypoxia stress in plant roots. Transgenic Arabidopsis plants expressing *NIP2;1 promoter:GUS* show enhanced GUS staining 3–4 h after hypoxia induction. Within 6 h of hypoxia, NIP2;1-GFP accumulates in roots of the complemented line (*NIP2;1promoter::NIP2;1-GFP nip2;1*). Confocal microscopy reveals predominant localization of NIP2;1-GFP in the plasma membrane of root cells. A schematic representing the membrane-bound NIP2;1 with the canonical "hourglass" structure is also provided. Adapted from various figures of Beamer et al. (2021).

mutant. As GOX3 functions in peroxisomes converting lactate back to pyruvate, this result suggests the involvement of NIP2;1 in lactic acid homeostasis.

Characterization of Arabidopsis NIP2;1 as a lactate-specific efflux channel is a crucial step in understanding the molecular mechanisms that alleviate lactic acid toxicity during hypoxia stress. The enhancement of *NIP2;1* in shoots, albeit to a lesser extent compared to roots, suggests NIP2;1 also functions in shoot tissues. Since lactic acid accumulation under hypoxia is predominant in roots (Mustroph et al., 2014), NIP2;1 expression in shoots may have additional unknown functions. As a bi-directional transporter, NIP2;1 may facilitate the recovery of a portion of lactic acid secreted to the intercellular spaces or excreted to the rhizosphere. Additionally, NIP2;1 may mediate intracellular lactic acid transport between membrane-bound organelles. Localization of a fraction of NIP2;1-GFP at the internal membranes supports this hypothesis. The regulatory mechanisms that trigger rapid enhancement in *NIP2;1* transcript levels during hypoxia and the decline after recovery requires detailed investigation. As phosphorylation affects preferential trafficking of aquaporins to membranes, future research may address the possibility of NIP2;1 phosphorylation. The lactate efflux mediated by NIP2;1 may also affect lactic acid homeostasis that triggers ethanolic fermentation under prolonged hypoxia. Besides NIP2;1, similar proteins in the aquaporin subfamily may possess lactate-specific functions vital in hypoxia survival. In-depth knowledge of the signaling functions and homeostasis of lactic acid in plants may aid in engineering flood-resilient crop plants in the future.

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