Nitrate signaling via Abscisic Acid release from inactive conjugates in Arabidopsis root tips.

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Nitrate is an essential nutrient; as such, plants continuously sense nitrate in the environment, modulating plant growth in response. Root growth is exquisitely sensitive to changes in environmental nitrate, either inhibiting or stimulating growth depending on concentration, location and physiological context. Root branching in response to local nitrate signals had been previously shown to require abscisic acid (ABA) signaling, but the mechanism was unknown. We showed, using a combined immunofluorescence, immunogold and ELISA approach, that after an increase in environmental nitrate, ABA gradually accumulates in the cytoplasm of Arabidopsis root tip cells. We found that nitrate-induced ABA accumulation was preceded by an induction of the *BETA-GLUCOSIDASE 1* (*BG1*) gene, which encodes an enzyme that releases ABA from the inactive ABA glucose ester. Mutants that lack AtBG1 function are unable to stimulate root-tip ABA accumulation in response to a rise in environmental nitrate. We found that ABA strongly induces expression of nitrate-responsive genes, placing ABA signaling squarely within the nitrate signaling pathway in the root, and that the At*bg1* mutant has reduced expression of the nitrate-inducible NITRATE REDUCTASE 1 (*NIA1*) gene under control conditions.

Our immunofluorescence approach revealed that ABA accumulation peaks in the root tip endodermis, cortical/endodermal initial and endodermal daughter cell, with weaker accumulation in the quiescent center. This pattern of accumulation mirrors the expression pattern of SCARECROW (SCR), a transcription factor involved in radial patterning of the root tip that regulates endodermal and quiescent center fate. SCR represses ABA signaling by inhibiting expression of the transcription factors, ABI4 and ABI5. We found that both ABA and nitrate reduce expression of an *SCR:erGFP* reporter gene, suggesting that ABA and nitrate may stimulate ABA-regulated gene expression in the root, by inhibiting an inhibitor, SCR.

We are currently extending this approach to examine ABA localization in the roots of other species, with the intent of using this tool for comparative physiology of different plant taxa.