

CHARACTERIZATION OF A NITRATE HIGH-AFFINITY TRANSPORT SYSTEM IN *BRACHYPODIUM DISTACHYON*

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Nitrogen is an essential element for growth and plant development. Improving the nitrogen use efficiency of crops is a priority for the future in order to limit the fertilizer use and environmental impacts. An efficient nitrate uptake system contributes to improve the nitrogen use efficiency of crops in conditions of low N availability. The High-Affinity nitrate Transport System (HATS) in plants is active in low range of external nitrate and is mediated by a two-component system (the high affinity transporters NRT2 associated to a partner protein NRT3).

In *Brachypodium*, the model plant for C3 cereals, seven *BdNRT2* and two *BdNRT3* have been identified (Girin *et al.* 2014). Expression of the *BdNRT2* and *BdNRT3* genes in response to nitrate availability fits with the characteristics of the HATS components already described in other species (David, Girin *et al.* 2019). Likewise, co-expression of *BdNRT2A* and *BdNRT3.2* is required for an effective nitrate transport in the heterologous expression system *Xenopus* oocytes. Functional interaction between *BdNRT2A-GFP* and *BdNRT3.2-RFP* has been observed at the plasma membrane in *Arabidopsis* protoplasts transiently expressing both constructs, while a cytosolic localization was observed when *BdNRT2A-GFP* or *BdNRT3.2-RFP* were transiently expressed separately, suggesting a monocotyledonous/dicotyledonous specificity for the functional interaction. Site directed mutagenesis on a Ser residue of the C-terminal extension of *BdNRT2A* specifically conserved in monocotyledonous plants and mimicking a constitutive phosphorylation status of this Ser was used to study the species specificity of the NRT2/NRT3 interaction. Finally, ¹⁵NO₃ influx measurements with *Brachypodium* mutants, defective in *BdNRT2A* expression, confirmed that *BdNRT2A* is a major contributor of the HATS in *Brachypodium*.

Girin T, David LC, Chardin C, Sibout R, Krapp A, Ferrario-Méry S, Daniel-Vedele F. 2014 *Brachypodium*: a promising hub between model species and cereals. *J Exp Bot.* 65(19):5683-96.

David LC, Girin T, Fleurisson E, Phommabouth E, Mahfoudhi A, Citerne S, Berquin P, Daniel-Vedele F, Krapp A, Ferrario-Méry S. 2019 Developmental and physiological responses of *Brachypodium distachyon* to fluctuating nitrogen availability. *Sci Rep.* 9(1):3824.