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# CHARACTERIZATION OF A NITRATE HIGH-AFFINITY TRANSPORT SYSTEM IN BRACHYPODIUM

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## Introduction

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).** Using phylogenetic studies, seven *NRT2* orthologues and two *NRT3* orthologues were found in *Brachypodium distachyon* (Figure 1) [1].

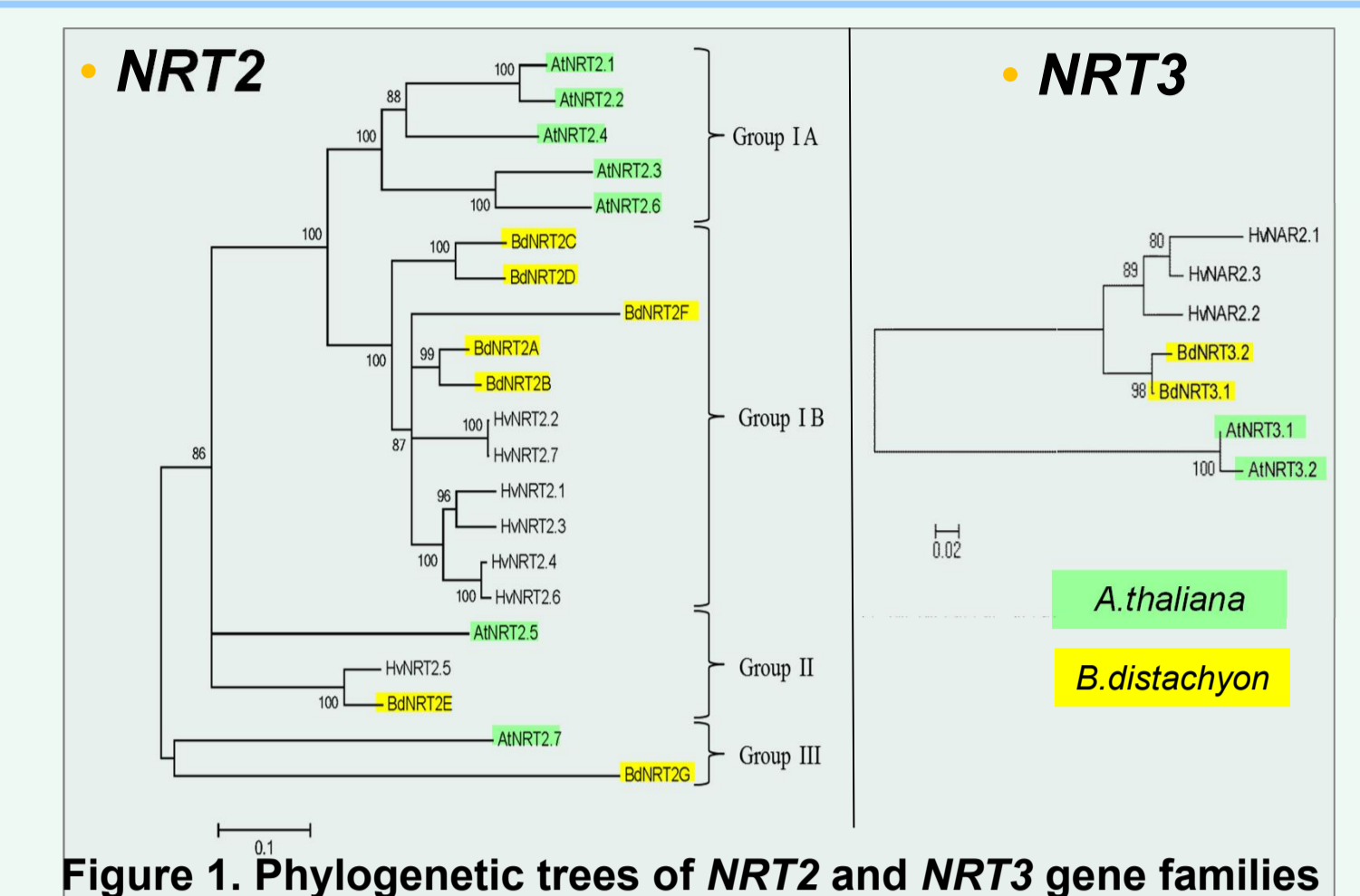


Figure 1. Phylogenetic trees of *NRT2* and *NRT3* gene families

## 1a) *BdNRT2A/2B* and *BdNRT3.2* expressions are induced in response to nitrate

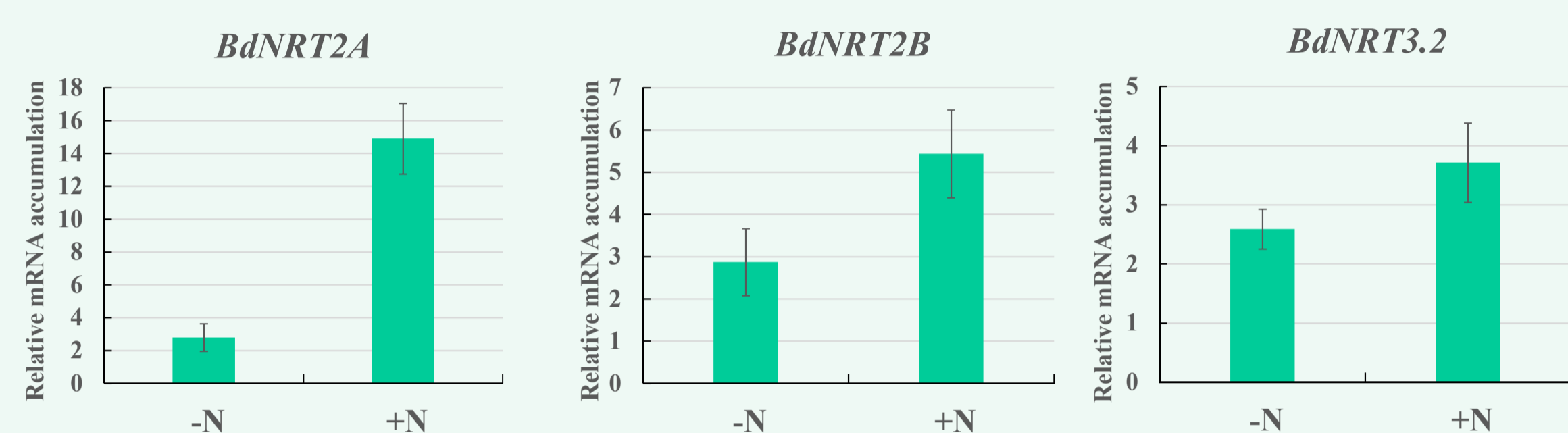


Figure 2. Expression profiles of *BdNRT2A/B* and *BdNRT3.2* genes

Real-time RT-PCR performed on roots of 22 day old plants grown in hydroponic on 0.1 mM NO<sub>3</sub><sup>-</sup> for 17 days then on 0 mM NO<sub>3</sub><sup>-</sup> for 4 days

- and then 0 mM (-N)
- or 1 mM NO<sub>3</sub><sup>-</sup> for 3 h (+N).

## 1b) *BdNRT2A/2B* and *BdNRT3.2* expressions in response to nitrate availability fits with the characteristics of a HATS activity [2]

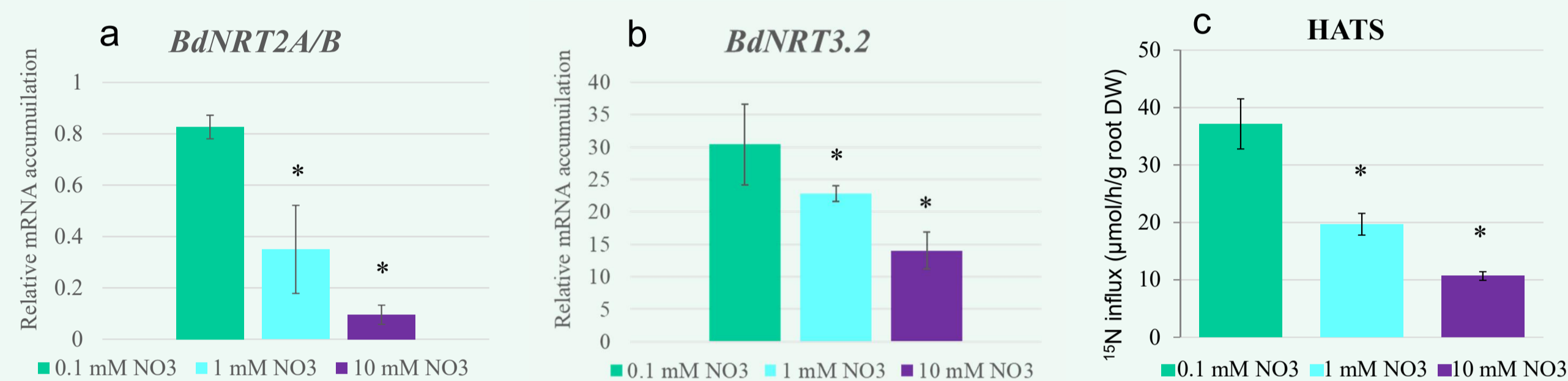


Figure 3. Expression profiles of *BdNRT2A/B* and *BdNRT3.2* genes and HATS activity

Real-time RT-PCR performed on roots of 17 day old plants grown in hydroponic on 0.1; 1 or 10 mM NO<sub>3</sub><sup>-</sup> (a,b).

- <sup>15</sup>N influx measurements into roots (0.2mM <sup>15</sup>NO<sub>3</sub><sup>-</sup> for 5 min) (HATS), (c).

*BdNRT2A/2B* and *BdNRT3.2* gene expressions were enhanced by low nitrate supply in root in correlation with the HATS activity, and thus are good candidates for HATS activity in Brachypodium.

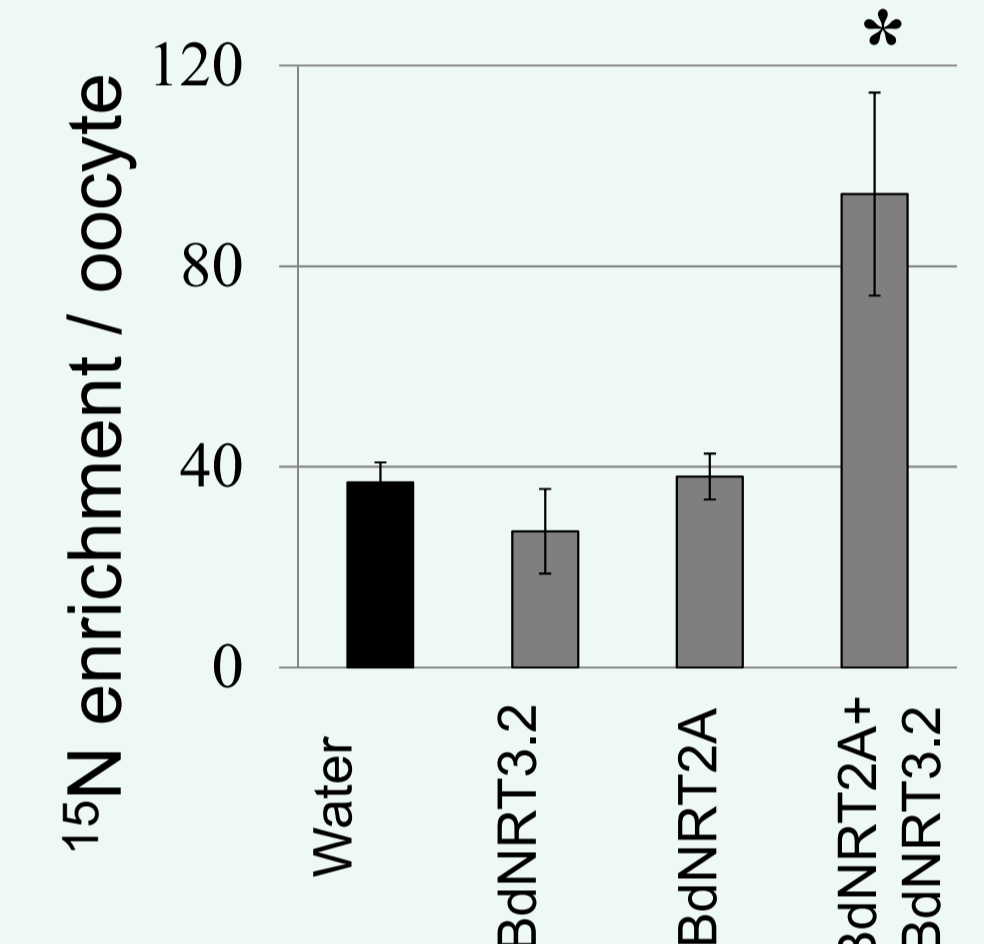
## 3) Co-expression of *BdNRT2A* and *BdNRT3.2* is required for an effective nitrate transport in the heterologous expression system *Xenopus oocytes*

Functional interaction between *BdNRT2A* and *BdNRT3.2* was shown by NO<sub>3</sub><sup>-</sup> uptake measurements in a heterologous expression system.

Only *Xenopus oocytes* co-injected with *BdNRT2A* and *BdNRT3.2* were able to transport NO<sub>3</sub><sup>-</sup>.

Figure 5. <sup>15</sup>NO<sub>3</sub><sup>-</sup> transport measurements in *Xenopus oocytes*.

*Xenopus laevis* oocytes were injected with nuclease-free water, *BdNRT2A*, *BdNRT3.2* or a co-injection of *BdNRT2A* and *BdNRT3.2* cRNA. After three days, oocytes were incubated in a solution enriched with 0.5 mM Na<sup>15</sup>NO<sub>3</sub> (atom% <sup>15</sup>N: 98%) and the Na<sup>15</sup>N enrichment of individual oocytes was measured after 16h.



## 2) *BdNRT2A* has a major role in NO<sub>3</sub><sup>-</sup> uptake in low N availability (HATS). Growth and HATS activity are lowered in Brachypodium mutants defective in *BdNRT2A*

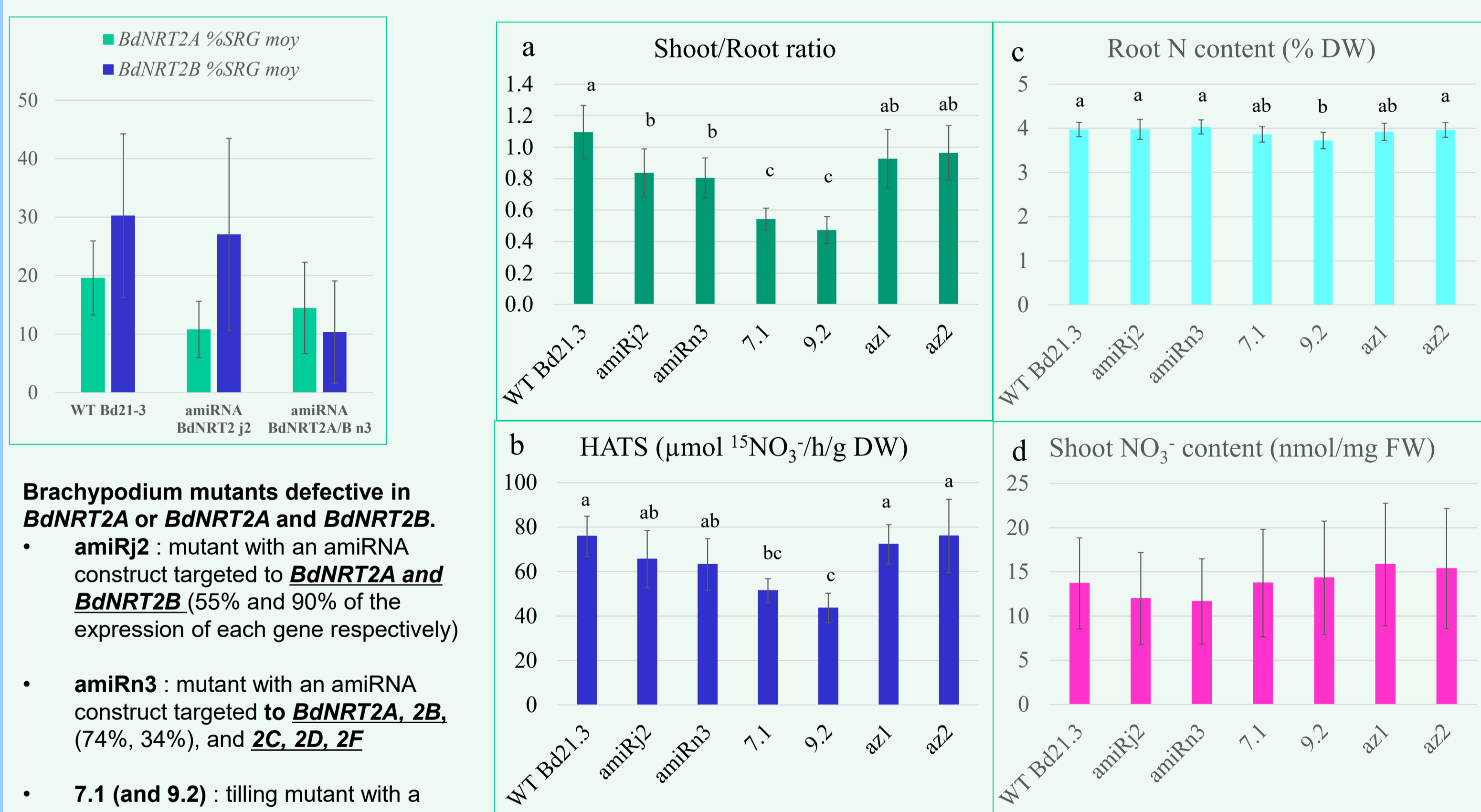


Figure 4. Phenotype of Brachypodium mutants defective in *BdNRT2A*.

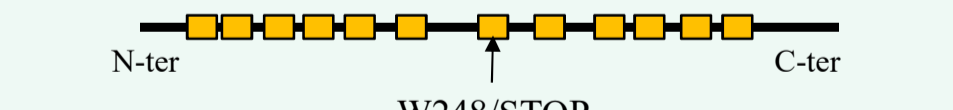
- (a) Shoot/root biomass ratio of 3 week-old plants of Brachypodium grown in hydroponics with 0.2 mM NO<sub>3</sub><sup>-</sup>.  
 (b) <sup>15</sup>NO<sub>3</sub><sup>-</sup> (0.2 mM) influx measured during 5 min uptake (HATS).  
 (c) N content in roots  
 (d) Nitrate content in shoots

**Brachypodium mutants defective in *BdNRT2A* or *BdNRT2A* and *BdNRT2B*.**

- *amiRj2*: mutant with an amiRNA construct targeted to *BdNRT2A* and *BdNRT2B* (55% and 90% of the expression of each gene respectively)

- *amiRn3*: mutant with an amiRNA construct targeted to *BdNRT2A*, *2B*, (74%, 34%), and *2C*, *2D*, *2F*

- *7.1* (and *9.2*): tilling mutant with a STOP codon (W248) in *BdNRT2A*



- *az1* and *az2*: two azygote lines of the *BdNRT2A* tilling mutant

## 4) Species specificity of *BdNRT2A/BdNRT3* interaction: *BdNRT2A* is targeted to plasma membrane in presence of *BdNRT3.2* but not *AtNRT3.1*

*NRT2* targeting to plasma membrane requires a protein-protein interaction with *NRT3*. Transient expression experiments in *atnrt2.1-2* protoplasts showed that *BdNRT2A* interact with *BdNRT3.2* but not with *AtNRT3.1*.

A Ser specifically conserved in the C-terminal extension of *NRT2.1* in monocotyledons has been identified as responsible of the *NRT2/NRT3* interaction in Barley [3].

Site directed mutagenesis of *BdNRT2A* Ser454 mimicking (or not) a constitutive phosphorylation status of this Ser (S454D or S454A respectively) was used to study the species specificity of the interaction.

The results showed that Ser454 is not sufficient to explain the species specificity of the interaction.

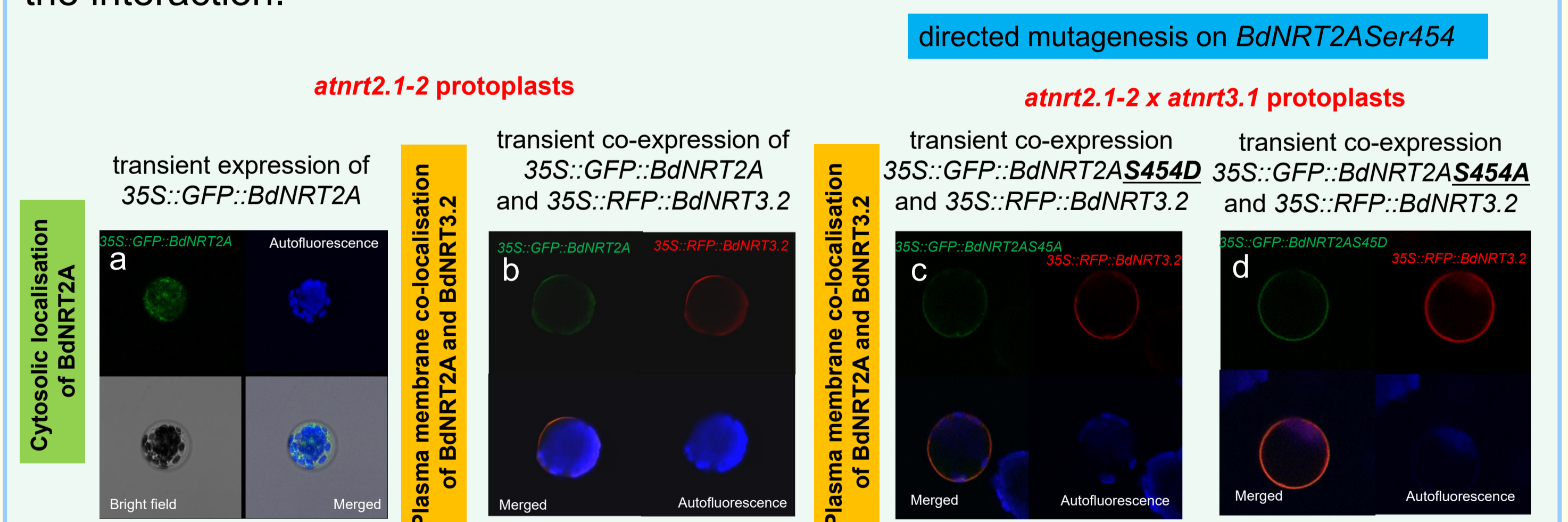


Figure 6. Subcellular localisation of *BdNRT2A* in Arabidopsis protoplasts observed with confocal imaging.

## Conclusion

Using various approaches, these results show that *BdNRT2A* and *BdNRT3.2* are the main component of the HATS activity of NO<sub>3</sub><sup>-</sup> (up to 30-40%) in Brachypodium and allow an optimal growth in low N conditions. The functional interaction between *BdNRT2A* and *BdNRT3.2* seem species specific.

## References

- [1] 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) 5683-5696.
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- [3] Ishikawa S, Yuka Ito, Sato Y, Fukaya Y, Takahashi M, Morikawa H, Ohtake N, Ohya T, Sueyoshi K. 2009 Two-component high-affinity nitrate transport system in barley: Membrane localization, protein expression in roots and a direct protein-protein interaction. Plant Biotechnology 26, 197–205.

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