

online



**Biología de Plantas**  
2021

XXIV Meeting of the Spanish Society of Plant Biology  
XVII Spanish Portuguese Congress on Plant Biology  
BP 2021  
7<sup>th</sup>- 8<sup>th</sup> July 2021

## **EFFECT OF SEAWATER INORGANIC CARBON ENRICHMENT ON NO<sub>3</sub><sup>-</sup> TRANSPORTER AND REDUCTION ENZYME GENE EXPRESSION IN *ZOSTERA MARINA* LEAVES**

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**Abstract:** CO<sub>2</sub> concentration has increased over the past 200 years and is expected to continue rising. In seawater, the increase of dissolved CO<sub>2</sub> has led to a decrease in pH, changing the inorganic carbon chemical speciation to increase HCO<sub>3</sub><sup>-</sup>. Seagrasses are the only vascular plants that colonized the marine environment, developing the capacity to use bicarbonate as a source of carbon for photosynthesis (Beer *et al.*, 2002). In *Posidonia oceanica* the direct uptake of HCO<sub>3</sub><sup>-</sup> through a H<sup>+</sup> symporter and its subsequent dehydration renders CO<sub>2</sub> for photosynthesis, but alters cytosolic anion homeostasis promoting the cytosolic nitrate leak (Rubio *et al.*, 2017; Rubio *et al.*, 2020). Therefore, under increasing dissolved HCO<sub>3</sub><sup>-</sup> conditions the cytosolic NO<sub>3</sub><sup>-</sup> diminution could induce the N biomass impoverishment shifting the expression pattern of nitrate transporters in seagrasses. *Zostera marina* genome, the first fully sequenced from a marine angiosperm, reveals lost of important vascular plants innovations but less than 20% as specific gene families (Olsen *et al.*, 2016). Thus, key physiological adaptations to thrive in seawater, characterized by a low concentration of NO<sub>3</sub><sup>-</sup>, seem to be due to molecular changes of the same family genes rather than the speciation of pre-existing genes. This could be the case for the high-affinity nitrate transporters, sodium driven high-affinity transport unique among vascular plants (Rubio and Fernández, 2019). In this work we have analysed the gene expression levels related to cytosolic nitrate homeostasis, including transporters and enzymes, in leaves of *Z. marina* plants incubated in high HCO<sub>3</sub><sup>-</sup> or in the absence of N. Overall gene analysed, expression levels of *ZosmaNTR2*, the only gene quoted as a high-affinity nitrate transporter belonging to NRT2 family in *Z. marina* genome (Rubio *et al.*, 2019), as well as its regulatory protein *ZosmaNAR2*, were induced in both conditions. Interestingly, expression levels of such genes were higher than the obtained for *ZosmaCHL1* (*ZosmaNPF6.3*), which expression was lower in N-replete plants, but did not vary in response to HCO<sub>3</sub><sup>-</sup>. These results suggest that the induction of the expression of the NO<sub>3</sub><sup>-</sup> high-affinity transporter *ZosmaNRT2* and its regulatory protein *ZosmaNAR2* are the main response for the cytosolic NO<sub>3</sub><sup>-</sup> decrease in both N-deficiency or in C-enrichment.

**Key words:** *Zostera marina*, HCO<sub>3</sub><sup>-</sup> enrichment, *ZosmaNRT2* expression levels.

**Acknowledgments:** GLOCOMA (FEDER-UCA 18-107243) participated by LR and JAF. JD-G is beneficiary of the PhD contract FPU18/03300

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