

XXIII Meeting of the Spanish Society of Plant Physiology/XVI Spanish Portuguese Congress of Plant Physiology

Pamplona, 26-28 June 2019

**HCO<sub>3</sub><sup>-</sup> enrichment causes cytosolic NO<sub>3</sub><sup>-</sup> efflux in *Posidonia oceanica* leaf cells**

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*Posidonia oceanica* (L.) Delile is a seagrass, the only group of vascular plants to colonize the marine environment. Seawater is an extreme yet stable environment, characterized by high salinity, alkaline pH and low availability of essential nutrients, such as nitrate or phosphate. In addition, in aquatic environments the supply of CO<sub>2</sub> for the photosynthesis is limited by diffusion and therefore many aquatic plants use HCO<sub>3</sub><sup>-</sup> as the inorganic carbon source for photosynthesis. Our previous results have shown that Na<sup>+</sup>-dependent transport systems operate on the plasma membrane of *P. oceanica* mesophyll leaf cells for the high-affinity NO<sub>3</sub><sup>-</sup>, Pi or amino acids uptake (Rubio *et al.*, 2018). In addition, a direct transport of HCO<sub>3</sub><sup>-</sup> driven by H<sup>+</sup> has been found in this species that provides inorganic carbon for photosynthesis and could be a significant component of a carbon concentrating mechanism in this species (Rubio *et al.*, 2017). Interestingly, this HCO<sub>3</sub><sup>-</sup> direct uptake caused the efflux of chloride from the cytosol, probably through S-type anion channels, pointing that other anions could also be removed from the cytosol (Rubio *et al.* 2017). This hypothesis could be relevant in the case of NO<sub>3</sub><sup>-</sup>, since the decrease of cytosolic NO<sub>3</sub><sup>-</sup> in response to HCO<sub>3</sub><sup>-</sup> enrichment could limit N-assimilation. Here we analyse the effect of HCO<sub>3</sub><sup>-</sup> increase on NO<sub>3</sub><sup>-</sup> uptake and cytosolic homeostasis in *P. oceanica*. Enrichment of natural seawater with 3 mM HCO<sub>3</sub><sup>-</sup> evokes the ongoing decrease of cytosolic NO<sub>3</sub><sup>-</sup>, from 5.7 ± 0.2 to 4.8 ± 0.7 mM after 40 min of treatment. On the other hand, the incubation of *P. oceanica* leaf pieces in natural seawater enriched with 3 mM HCO<sub>3</sub><sup>-</sup> for 30 min causes an initial increase of NO<sub>3</sub><sup>-</sup> concentration in the medium. Maximum efflux (21 nmol NO<sub>3</sub><sup>-</sup> g<sub>FM</sub><sup>-1</sup> min<sup>-1</sup>) occurs within the first minute of incubation. Then, external NO<sub>3</sub><sup>-</sup> is depleted from the medium at lower net uptake rate (4 ± 0.7 nmol NO<sub>3</sub><sup>-</sup> g<sub>FM</sub><sup>-1</sup> min<sup>-1</sup>) than the value observed in non HCO<sub>3</sub><sup>-</sup>-enriched natural seawater (10 ± 0.3 nmol NO<sub>3</sub><sup>-</sup> g<sub>FM</sub><sup>-1</sup> min<sup>-1</sup>). These results also fit the hypothesis that natural seawater HCO<sub>3</sub><sup>-</sup> enrichment causes the nitrogen loose from *P. oceanica* leaf. Thus, the chronic diminution of cytosolic NO<sub>3</sub><sup>-</sup> concentration could impair nitrogen assimilation and would contribute to the N biomass dilution expected under elevated inorganic carbon (Taub and Wang, 2008).

**References.**Rubio *et al.*, 2018. *Int. J. Mol. Sci.*, 19, 1570; doi:10.3390/ijms19061570Rubio *et al.*, 2017. *Plant Cell Environ.*, 40: 2820–2830. doi:10.1111/pce.13057Taub and Wang, 2008. *J Integr Plant Biol.* 50: 1365-1374. doi: 10.1111/j.1744-7909.2008.00754.x

Acknowledgements &amp; Funding (Times, 10, justified)

Spanish MINECO, projects BFU2017-85117-R and BIO2016-81957-REDT