## Na<sup>+</sup>-dependent NO<sub>3</sub><sup>-</sup> uptake in leaf cells of the seagrass *Posidonia* oceanica L. Delile

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Posidonia oceanica (L.) Delile is an endemic Mediterranean seagrass of recognized ecological significance and, as other seagrasses, this species has secondarily adapted to live in the marine environment. In this alkaline medium with a high  $\mathrm{Na}^+$  concentration (0.5 M), the high inwardly directed electrochemical potential gradient for sodium is used in the seagrass Zostera marina to energize the uptake of nitrate<sup>1</sup> and phosphate that usually occur at concentration below 10  $\mu\mathrm{M}$ . Here we summarize several evidences for the operation of a sodium-dependent high-affinity nitrate transport system at the plasma membrane of the mesophyll leaf cells of *P. oceanica*.

Leaf cells of *P. oceanica* possess a H<sup>+</sup>-ATPase as a primary pump, exhibit a plasma membrane potential ( $E_m$ ) of -174  $\pm$  10 mV and show reduced Na<sup>+</sup> permeability. The addition of micromolar nitrate concentrations induces membrane depolarizations that show saturation kinetics. Curve fitting of the values renders a semisaturation constant ( $K_m$ ) of 21.3  $\pm$  6.6  $\mu$ M and a maximum depolarization ( $D_{max}$ ) of 7  $\pm$  1 mV. In dark conditions,  $D_{max}$  decreases by fifty percent but no significant effect is observed on the  $K_m$  value. On the other hand, nitrate induced depolarizations show sodium dependence. The depolarizations induced by 100  $\mu$ M NO<sub>3</sub> in media containing increasing Na<sup>+</sup> concentrations (from 0 to 250 mM) show saturation kinetics, rendering a  $K_m$  value of 16  $\pm$  5 mM Na<sup>+</sup>. Moreover, the depolarization induced by 100  $\mu$ M NO<sub>3</sub> is accompanied by a simultaneous increase of cytosolic sodium, measured by Na<sup>+</sup>-sensitive microelectrodes, of 0.4  $\pm$  0.2 mM above the resting cytosolic sodium concentration (17  $\pm$  2 mM).

Finally, nitrate uptake rates, measured in depletion experiments, decreases by 50% and 80% in dark conditions and in the absence of Na<sup>+</sup>, respectively, compared with control conditions (0.5 M Na<sup>+</sup> and light).

All together, these results strongly suggest that NO<sub>3</sub> uptake in *P. oceanica* leaf cells is mediated by a high-affinity nitrate carrier that uses Na<sup>+</sup> as the driving ion.

<sup>&</sup>lt;sup>1</sup> Rubio et al. (2005). J. Exp. Bot, 412: 613-622. Project Funding: CTM 2011-30356. (MEC)