Expression of the Vacuolar Na^+/H^+ Antiporter Gene (*NHX1*) in Three *Plantago* Species Differing in Salt Tolerance

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Abstract

We have isolated full-length cDNA clones of the *NHX1* gene – encoding the vacuolar Na^+/H^+ antiporter – from three *Plantago* species (*P. crassifolia*, *P. coronopus* and *P. major*) differing in salt tolerance. A phylogenetic tree of their deduced protein sequences and those of NHX1 antiporters previously isolated from other species suggested the presence of some structural features characteristic of the proteins of salt tolerant taxa. The expression patterns of the *NHX1* genes in the presence of salt correlated with the relative degree of salt tolerance of the three species: no induction was detected in the least tolerant *P. major* – and in the glycophyte *A. thaliana* – and the highest and quickest induction was observed in the most tolerant *P. crassifolia*. These data support the hypothesis that the NHX1 antiporters play a functional role in the mechanisms of salt tolerance in plants.

Keywords: *NHX1, Plantago coronopus, P. crassifolia, P. major, salinity.*

Introduction

A general mechanism used by plants to cope with salt stress is based on the so-called 'ion compartmentalisation hypothesis'; that is, the sequestration of toxic ions (Na^+, Cl^-) in the vacuole to avoid their deleterious effects in the cytoplasm. Several ion transporters are responsible for his process, including the tonoplast Na^+/H^+ antiporters, encoded by the *NHX1* genes, which pump Na^+ ions into the vacuole. In several cases it has been shown that these genes are transcriptionally activated in the presence of salt, and that they improve salt tolerance when overexpressed in transgenic plants. Yet, despite these evidences, a general contribution of the *NHX1* to plant resistance to high salinity remains to be demonstrated. Finding a positive correlation between the relative salt tolerance of genetically related species and the level of expression/activity of the corresponding NHX1 antiporters would be in favour of a functional role of *NHX1* in salt tolerance mechanisms.

Aims and objectives

The aim of this work was to determine the patterns of salt-induced *NHX1* expression in three *Plantago* species adapted to different natural habitats, and correlate them with their relative degree of salt tolerance, to support the hypothesis mentioned above. The *AtNHX1* gene from *Arabidopsis thaliana* was used as external control.

Materials and methods

The isolation of full-length cDNAs corresponding to the *NHX1* genes of *P. crassifolia*, *P. coronopus* and *P. major*, by homology with previously cloned homologues from other species, was carried out making use of RT-PCR and RACE-

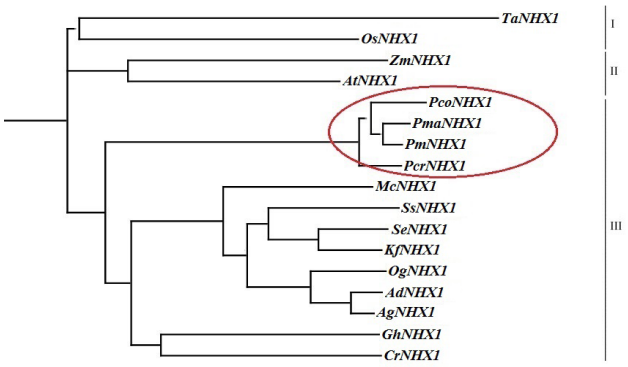

Fig. 1 Neighbour-joining phylogenetic tree of Na^+/H^+ antiporter protein sequences. Clustalx and TreeView, were used to do multiple sequence alignment and generate the phylogenetic tree.

PCR techniques. The relative levels of expression of the gene, in plants of the three *Plantago* species treated with 400 mM NaCl for different times, was checked by qRT-PCR; the actin and tubulin genes were used as internal standards.

Results and Discussion

The protein sequences of the *Plantago* NHX1 antiporters, deduced from the cloned cDNAs, were aligned with those of several homologues previously isolated from other plants; these sequences were then used to generate the phylogenetic tree shown in Fig. 1. The *Plantago* proteins were found to group closer to those of other halophytes (group III in Fig. 1), even non-related taxonomically; this suggested the presence of some structural features in the protein (not yet identified), characteristic of salt tolerant species, thus supporting its possible role in salt tolerance.

The relative salt tolerance of the selected species (*P. crassifolia* > *P. coronopus* > *P. major*) was estimated from their distribution in nature

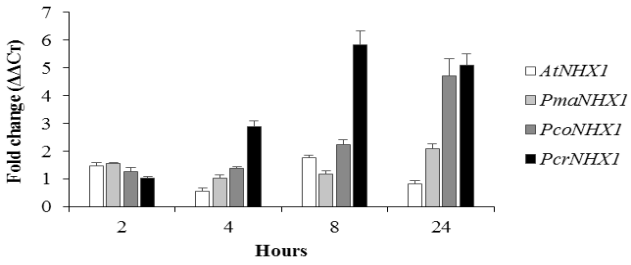

Fig. 2 Fold change ($\Delta\Delta C_T$), in the expression of *NHX1* after application of 400 mM NaCl to *A. thaliana* (*AtNHX1*), *P. major* (*PmaNHX1*), *P. coronopus* (*PcoNHX1*) and *P. crassifolia* (*PcrNHX1*). For each species, the control ('1') corresponded to the mRNA level at time 0, when the salt treatment was started.

and their relative inhibition of growth by NaCl (not shown). The relative levels of *NHX1* expression in salt-treated plants (400 mM NaCl) and the kinetics of induction correlated with the salt tolerance of the investigated taxa (Fig. 2). In *P. crassifolia*, the most tolerant taxon, *NHX1* mRNA levels increased ca. 5-fold during the first 8 h of salt treatment – with respect to the control at time 0 – and then remained more or less constant at least until 24 h (the longest time tested). Similar levels were reached in *P. coronopus*, although the induction of *NHX1* expression in the presence of salt was slower. Finally, in the most sensitive species, *P. major* – as well as in the glycophyte *A. thaliana* – no induction of *NHX1* expression by NaCl was detected in these experiments.

Conclusion

These results support the contribution of the *NHX1* antiporter to plant salt tolerance mechanisms, and confirm the usefulness of performing comparative studies in genetically related species with different levels of tolerance to investigate those mechanisms.