Role of tonoplast H^+-pyrophosphatase and Na^+/H^+ antiporter in salt tolerance of *Populus euphratica* Oliv.

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INTRODUCTION

Efficient exclusion of Na^+ excess from the cytoplasm and vacuolar Na^+ accumulation are the main mechanisms for the adaptation of plants to salt stress. *P. euphratica* is the only tree species that occurs naturally in semiarid areas. It is a halophytic plant, tolerating salt and drought stress, and has been used as a model to study plant defense mechanisms against salt stress. The aim of this work was to contribute to the elucidation of mechanisms underlying salt tolerance in *P. euphratica* using heterotrophic cell suspensions as a biological model. Tonoplast vesicles were purified from cells grown in medium containing different salt concentrations and used for the determination of the activity of proton pumps and Na^+/H^+ exchanger.

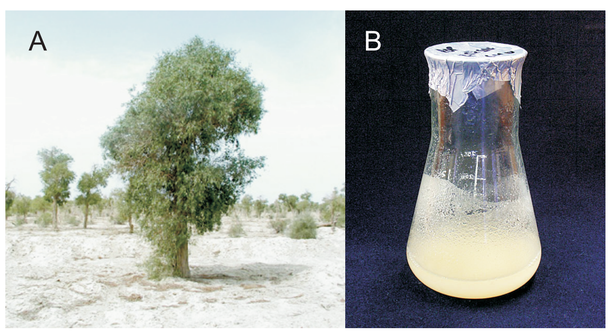


Figure 1. *Populus euphratica* in the deserts of western China (A) and cell suspension culture from calli as a model system to study salt stress defence mechanisms (B).

RESULTS

Growth in batch cultures with NaCl

Figure 2 depicts the growth of *P. euphratica* cell suspensions in MS medium with 2.5% sucrose in the presence and absence of 150 mM NaCl. Cells grown with 150 mM NaCl exhibited the same maximal specific growth rate (μ_{max}= 0.2 day^{-1}) than those grown without salt, however, the lag phase was longer.

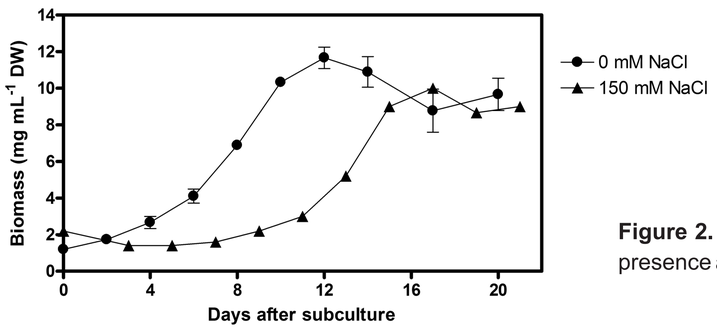
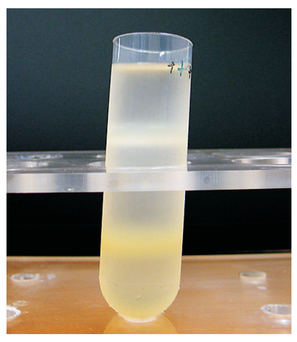


Figure 2. Growth of *P. euphratica* cultured cells in the presence and absence of NaCl.

Preparation of tonoplast vesicles



Cells were homogenised in a Polytron at 4ºC. After removal of the mitochondrial fraction, microsomal fraction was obtained by centrifugation at 100,000x*g*, resuspended and layered onto a discontinuous sucrose gradient (32/46%). After centrifugation, tonoplast fraction was collected from the top of 32% layer of the gradient (Figure 3) and sedimented at 180,000x*g*.

Figure 3. *Populus euphratica* plasma membranes ( ) and vacuolar membranes ( ) after the fractionation procedure.

Activity of tonoplast proton pumps

In *P. euphratica* suspension cultured cells, V-PPase (pyrophosphatase) seems to be the main tonoplast H^+ pump (Figure 4). Activity was as follows: H^+ pumping K_m, 3.4 µM and V_{max}, 960 %F min^{-1} mg^{-1} protein; PPi hydrolysis K_m, 44 µM and V_{max}, 94 nmol PPi min^{-1} mg^{-1} protein (Figure 5 and Figure 6). Saturating substrate concentration of V-PPase (0.1 mM PPi) is about 10-fold lower than V-ATPase (1 mM ATP).

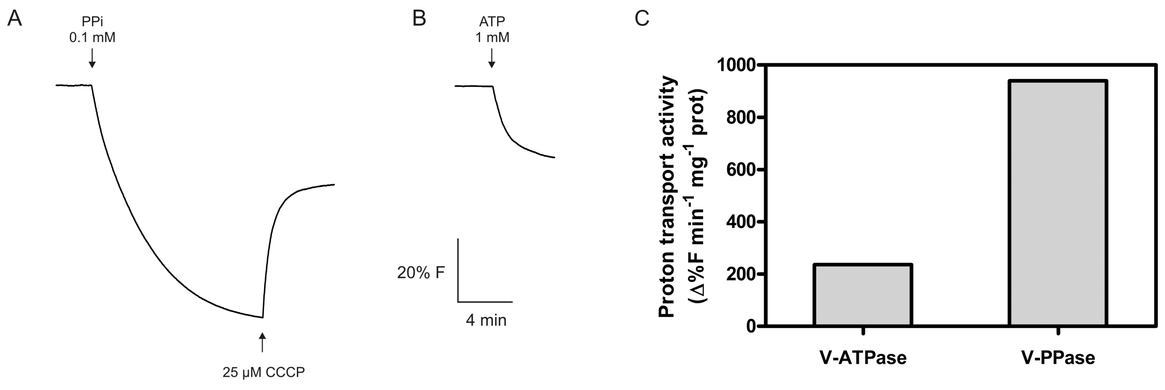


Figure 4. Proton pumping activity of V-PPase (A) and V-ATPase (B) in tonoplast vesicles from *P. euphratica* cultured cells. The accumulation of protons inside the vesicles was determined by measuring the fluorescence quenching of ACMA (Façanha and de Meis, 1998).

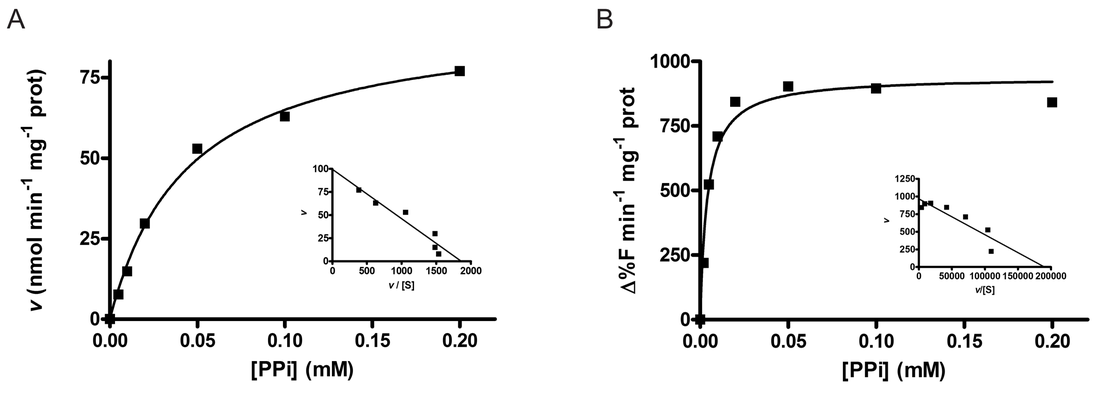


Figure 5. Kinetic analysis of *P. euphratica* V-PPase. Initial velocities of PPi hydrolysis (A) and H^+ pumping (B).

When cells were cultivated in the presence of up to 150 mM salt, V-ATPase hydrolysis activity increased but not V-PPase activity (Figure 6).

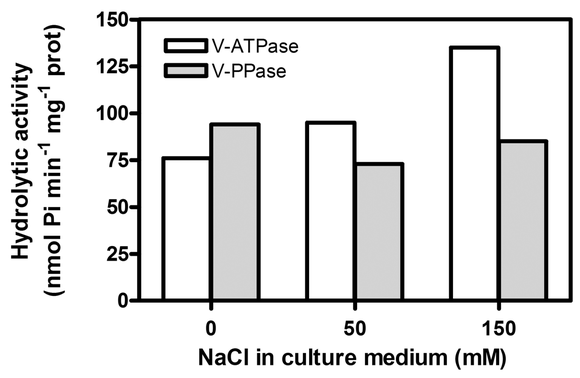


Figure 6. Hydrolysis activity (V_{max}) of V-PPase and V-ATPase in tonoplast vesicles from *P. euphratica* suspension cells cultivated in the absence and presence of salt.

NaCl behaved as an uncompetitive inhibitor of V- PPase (Figure 7). V_{max} of H^+ pumping decreased according to an exponential inhibition kinetics: k_i = 4.6 M^{-1}; C_{50} = 158 mM.

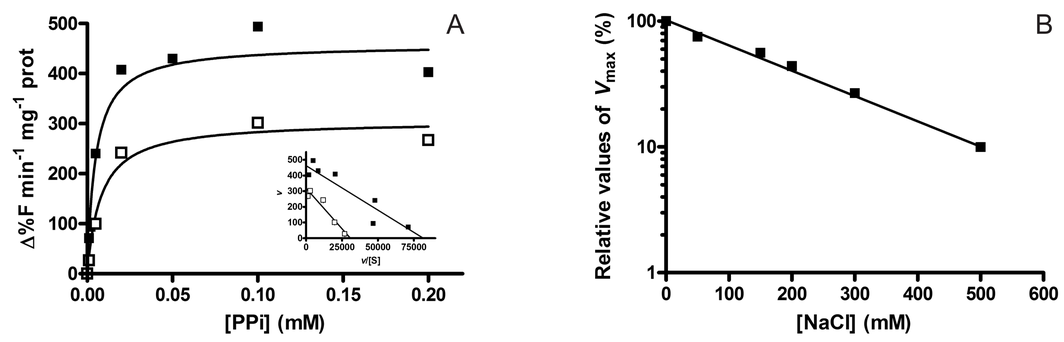


Figure 7. Initial velocities of proton pumping of *P. euphratica* V-PPase in the absence (■) and presence (□) of 100 mM NaCl (A). Inhibition of V_{max} of proton pumping by NaCl in the assay medium (B).

Activity of Na^+/H^+ exchanger

The activity of the tonoplast Na^+/H^+ exchanger, monitored as the ability of Na^+ to dissipate an established pH gradient, was negligible in cells grown without salt, however, the increase of salt in the culture medium up to 150 mM promoted a 8-fold increase of the exchanger activity (Figure 8).

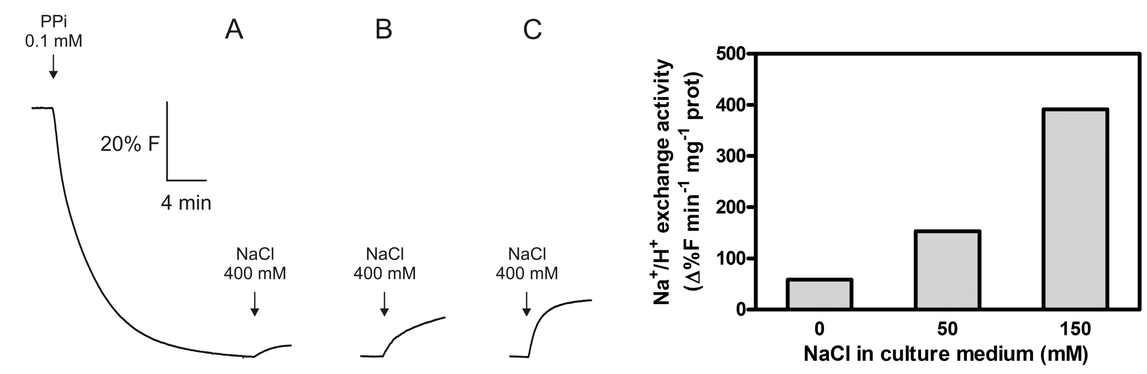


Figure 8. Plasma membrane Na^+/H^+ exchange activity in tonoplast vesicles isolated from *P. euphratica* suspension cells cultivated in the absence (A) and presence of 50 mM (B) and 150 mM (C) of NaCl.

As the exchanger AtNHX1 from *Arabidopsis* (Venema *et al.*, 2002), *P. euphratica* vacuolar Na^+/H^+ exchanger catalyses low affinity Na^+ transport (K_m 100 mM); Li^+ is also a substrate for this antiporter (Figure 9).

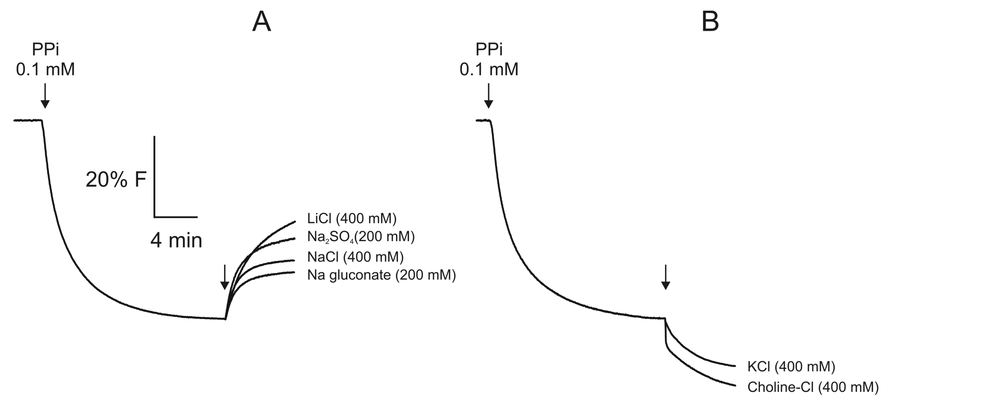


Figure 9. Ion specificity of tonoplast H^+-coupled exchange activity in salt treated *P. euphratica* suspension cultured cells.

As shown in Figure 9, Cl^- is also able to accumulate in the vacuole following its electrochemical gradient, the depolarizing effect causing an increase of proton pumping.

Concluding remarks

- Two electrogenic H^+ pumps, V-ATPase and V-PPase, generate and maintain the electrochemical gradient across the vacuolar membrane of *P. euphratica*.
- V-PPase is the predominant H^+ pump in vacuoles of *P. euphratica*, since its H^+ pumping activity is much higher than that of V-ATPase. Also, the V_{max} of its activity is achieved at 10-fold lower substrate concentration when compared to that of V-ATPase.
- Na^+/H^+ exchange activity is negligible in cells grown without salt; activity is induced abruptly when suspension cells were grown with salt, indicating the important role of the antiporter in Na^+ detoxification.

References

- Façanha, A.R., de Meis, L. 1998. Reversibility of H^+-ATPase and H^+-Pyrophosphatase in tonoplast vesicles from maize coleoptiles and seeds. *Plant Physiol* 116: 1487-1495.
- Venema, K., Quintero, F. J., Pardo, J. M. and Donaire, J. P. 2002. The *Arabidopsis* Na^+/H^+ exchanger AtNHX1 catalizes low affinity Na^+ and K^+ transport in reconstituted liposomes. *J Biol Chem* 277: 2413-2418.

Acknowledgements: Paulo Silva is supported by FCT, grant ref. SFRH/BD/13460/2003
Carlos Conde is supported by FCT, grant ref. SFRH/BD/10689/2002