

The effects of the combination of salinity and excess boron on the water relations of tolerant tomato (*Solanum lycopersicum* L.) cv. Poncho Negro, in relation to aquaporin functionality

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Abstract

As elevated levels of boron (B) are accompanied by conditions of excessive salinity with drastic consequences for crops, it is crucial to find a crop that is tolerant to these conditions. In this work, the interaction between salinity and excess B with respect to aquaporin-mediated changes by blockade of mercury and water relations were studied as well as the osmotic adjustment of the plants. The treatments, for tomato 'Poncho Negro' cultivated hydroponically in a controlled environment chamber, were control (75 and 150 mM) NaCl and/or 5 mg L⁻¹ or 20 mg L⁻¹ B. Hydraulic conductance (L₀) of detached exuding root systems exhibits large variations in response to abiotic stimuli. No additive (synergic) effects of B and salinity were observed. Under salinity, the plants increased their turgor, compensating for the decrease in the leaf water potential through the reduction in the leaf osmotic potential by the accumulation of soluble sugars and proline. The involvement of Hg²⁺-insensitive aquaporins or the osmotic gradient as the main force for water flow through the apoplastic pathway must be contemplated. Finally, all the data reveal the tomato cv. Poncho Negro to be a germplasm of agronomic interest and a good alternative for cultivation areas with high content of salts and the excess B of the soil and irrigation water.

Additional key words: boric acid; plant water relations; root hydraulic conductance; tomato Poncho Negro.

Resumen

Efectos de la combinación de salinidad y exceso de boro en las relaciones hídricas del tomate (*Solanum lycopersicum* L.) tolerante cv. Poncho Negro y su relación con la funcionalidad de las acuaporinas

En muchas ocasiones niveles elevados de boro (B) van acompañados de condiciones de excesiva salinidad, cuyas consecuencias pueden ser drásticas para los cultivos, por lo que es fundamental encontrar variedades que puedan tolerar estas condiciones. En este trabajo, se estudió cómo la interacción entre la salinidad y el exceso de B afecta a la actividad de las acuaporinas y a las relaciones hídricas, así como al ajuste osmótico de las plantas. Se cultivó en hidroponía tomate cv Poncho Negro en una cámara de crecimiento con ambiente controlado con los tratamientos control, NaCl (75 y 150 mM) y/o B (5 ó 20 mg L⁻¹). No se observó ningún efecto aditivo (sinergia) del exceso de B y la salinidad. Las plantas aumentaron su turgencia en condiciones salinas, compensando así la disminución del potencial hídrico foliar, a través de la reducción de su potencial osmótico por la acumulación de azúcares solubles y prolina. La participación de acuaporinas insensibles al Hg²⁺ o del gradiente osmótico, como la principal fuerza impulsora del flujo del agua, a través de la vía apoplástica, deben ser contemplados. Finalmente, todos los datos revelan que el tomate cv. Poncho Negro puede ser un germoplasma de interés agronómico y una buena alternativa para cultivar en condiciones de alto contenido de sales y exceso de B del suelo y del agua de riego.

Palabras clave adicionales: ácido bórico; conductancia hidráulica de las raíces; relaciones hídricas; tomate Poncho Negro.

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Introduction

Most of the salt-affected land in the world has arisen from natural causes, via the accumulation of salts over long periods of time in arid and semi-arid zones (Rengasamy, 2002). The Lluta valley in northern Chile is characterised by high levels of salinity and an excessive presence of boron (B), in both cultivated soils and irrigation waters. Since these features greatly limit the cultivation of many crops, farmers can only grow tolerant crops in the valley, meaning that there is less diversity in local agriculture. One of these tolerant crops, that can be exposed to both salinity and B toxicity stress, simultaneously, is the variety of tomato, *Solanum lycopersicum* L., called 'Poncho Negro' by farmers (Díaz, 2008). Its behaviour, according to studies conducted to date, is similar in some characteristics to that of the ecotype *Zea mays* L. *amylacea* 'Luteño' (Bastías *et al.*, 2004a,b; Martínez-Ballesta *et al.*, 2008).

Salts on the outside of roots have an immediate effect on cell growth and associated metabolism; toxic concentrations of salts take time to accumulate inside plants before they affect plant function. Hence, salinity affects crop growth, yield and productivity (Tester and Davenport, 2003; Munns and Tester, 2008). Plant salt tolerance itself is a complex trait (Shi *et al.*, 2000). The current notion is that the defence mechanisms of plants against stress conditions are tightly associated with their growth habits, and hence every claim of tolerance enhancement needs to be tested on a crop-yield basis, coupled with its economic significance from an agricultural point of view (Sade *et al.*, 2009). However, despite the large number of attempts to improve the abiotic stress tolerance of commercial crop plants through genetic engineering, no major progress has been made (Flowers, 2004; Passioura, 2007).

Boron is an essential micronutrient involved in maintaining cell wall structure and function, enzyme activation, nucleic acid metabolism and carbohydrate transport (Loomis and Durts, 1992; Power and Woods, 1997). In excess, it causes negative physiological effects, including reduced root cell division, decreased shoot and root growth, lower stomatal conductance (Lovatt and Bates, 1984; Nable *et al.*, 1990), a decrease in leaf chlorophyll and inhibition of photosynthesis (Ghanati *et al.*, 2002). The resistance to B toxicity of tolerant varieties of different plant species was shown to be related to their ability to reduce the intracellular concentration of B by active efflux from the roots, thereby maintaining a lower root B concentration (Stangoulis

et al., 2001; Hayes and Reid, 2004; Reid *et al.*, 2004, 2007; Cervilla *et al.*, 2007). Supporting this idea, functional B efflux transporters have been reported in *Arabidopsis thaliana* (Takano *et al.*, 2002) and *Hordeum vulgare* L. (Sutton *et al.*, 2007); these have a higher capacity to provide B tolerance.

While salinity and B stresses and their independent effects on plant growth and yield have been well investigated (Bernstein, 1975; Gupta *et al.*, 1985; Munns and Termaat, 1986; Nable *et al.*, 1990), our understanding of plant responses to the combination of salinity and B is inconclusive. The fact that B accumulation was decreased in various studies by a range of different salts, including NaCl, CaCl₂ and Na₂SO₄, reinforces the role of osmotic effects in reducing B toxicity. Alternatively, Bastías *et al.* (2004a) measured the physiological effects of salinity and B on *Zea mays* and found that water relations, growth and chlorophyll content were affected mainly by the salinity. They suggested that B absorption, via passive diffusion, could depend on the sensitivity of the plants to salt stress, which, in turn, depends on aquaporin functionality. Increasing the NaCl concentration in the irrigation water has been shown to decrease the rate of B uptake (Alpaslan and Gunes, 2001; Ben-Gal and Shani, 2002; Edelstein *et al.*, 2005; Yermiyahu *et al.*, 2007, 2008).

It is well known that water flow across cellular membranes is important for plant growth, under both favourable and adverse environmental conditions (Yang *et al.*, 2007). The discovery of aquaporins gave a new insight into the mechanism of trans-membrane water transportation, provided a solid molecular basis for the fast and reversible regulation of trans-membrane water transport and supported the idea that such high water permeability might be required for certain physiological process (Maurel, 1997; Maurel and Chrispeels, 2001). Aquaporins, or major intrinsic proteins (MIPs), are channel-forming membrane proteins with the extraordinary ability to combine a high flux across biological membranes with a high specificity for water (Zhao *et al.*, 2008). These membrane integral proteins form water-conducting channels, which is considered responsible for the variable conductivity of root systems (Javot and Maurel, 2002; Bramley *et al.*, 2009). There are two highly-important aspects of plant aquaporins (Maurel and Chrispeels, 2001). One is their tremendous diversity in plants that can be explained in part by their presence in multiple sub-cellular compartments (Chrispeels and Agre, 1994; Takata *et al.*, 2004) and the other is the fact that some aquaporins are multi-

functional channel proteins, allowing some small, neutral solutes, such as CO₂, urea, B and hydrogen peroxide, to move across cellular membranes (Dordas and Brown, 2001; Hachez *et al.*, 2006). Recently, evidence indicating the involvement of aquaporins in plant responses to environmental stresses has been provided, and different regulation patterns were detected under different stress conditions (Yu *et al.*, 2005; Vandeleur *et al.*, 2009). Although aquaporins are considered to play a regulatory role in water transport, attempts to utilise them to improve crop tolerance of abiotic stresses have yielded contradictory results (Sade *et al.*, 2009).

Therefore, the aim of this work was to determine the effect of the interaction between salinity and excess boron on the water status of a tolerant tomato 'Poncho Negro' which also is grown in the Lluta valley (northern Chile), in relation to aquaporin functionality. More specifically, the inhibition by HgCl₂ of water transport across the roots was used to indicate the proportion of the total water flow which occurs *via* aquaporins. In addition, proline and soluble sugars were determined in order to characterise the osmotic adjustment of the plants in these conditions.

Material and methods

Plant material and culture conditions

The experiment was conducted in environmental controlled chamber. Seeds of *Solanum lycopersicum* L. cv. Poncho Negro were planted after germination into individual pots (2L) containing a mixture of perlite and vermiculite 1:1 (v/v), in a greenhouse at 30°C. After this, plants were transferred to a controlled-environment chamber at a day/night temperature of 28/18°C, a day/night relative humidity of 45/85 % and a 16-h photoperiod. The photon flux density was 350-400 μE m⁻² s⁻¹. After 7 d under these conditions, plants were grown hydroponically in aerated Hoagland nutrient solution: 6 mM KNO₃, 4 mM Ca(NO₃)₂, 1 mM NH₄H₂PO₄, 1 mM MgSO₄, 50 μM KCl, 25 μM H₃BO₃, 2 μM MnSO₄, 2 μM ZnSO₄, 0.5 μM CuSO₄, 0.5 μM (NH₄)₂MoO₄ and 20 μM Fe-EDDHA.

Treatments

Five days before measuring or harvesting, the following treatments were applied: 0 mM (Control),

75 mM NaCl, 150 mM NaCl, 5 mg L⁻¹ B (B5), 20 mg L⁻¹ B (B20), 75 mM NaCl+B5, 150 mM NaCl+B5, 75 mM NaCl+B20 and 150 mM NaCl+B20. The plants were 32 days-old when the experiments were carried out. Measurements and harvesting were all performed in the middle of the light period.

Root hydraulic conductance (L₀)

The hydraulic conductance (L₀) of roots was measured by applying pressure to the roots in a Scholander chamber (Jackson *et al.*, 1996). Sap was collected for a different time at each pressure, in Eppendorf tubes. Sap flow, J_v, was expressed in mg (g root FW)⁻¹ h⁻¹ and plotted against pressure, the slope being the L₀ value. In order to study the effect of the blocking agent (HgCl₂) on the aquaporins (Maggio and Joly, 1995; Carvajal *et al.*, 1996), L₀ was measured in control, B-, NaCl- and B+NaCl-treated plants. Then, 50 μM HgCl₂ was supplied in the nutrient solution of the same roots for 10 min. Plants were then transferred to a fresh nutrient solution prior to measuring L₀ again. Afterwards, 5 mM Dithiothreitol (DTT) was added to the nutrient solution in order to remove the Hg. In all the steps of the experiment, L₀ was determined as described above.

Stomatal conductance (G_s), transpiration rate (E)

The gas exchange characters were measured on fully-expanded leaves between 11.00 and 12.00 a.m., using a portable photosynthesis system (LI-6400, LICOR, and Lincoln, NE-USA) with an infra-red gas analyser (IRGA). Measurements were carried out at natural relative humidity (45-60%) and temperature (20-27°C) in the greenhouse.

Water relations parameters

A Scholander pressure chamber was used for the measurement of water potential (Ψ_w) (Martínez-Ballesta *et al.*, 2004). Young, fully-expanded leaves (4 replicates) were used for each treatment.

For osmotic potential (Ψ_π) measurements, four homogeneous, young, fully-expanded leaves were placed in Eppendorf tubes with holes at the bottom and frozen rapidly at -20°C for 4 h. These tubes were then centri-

fused twice into assay tubes, at $4,000 \times g$ for 4 min (4°C), using a HERMLE 2300K (Germany) centrifuge, in such a way that all sap was extracted from samples. The osmotic potential of the leaf sap was calculated, after measuring sap osmolarity with an automatic freezing-point depression osmometer (Digital Osmometer, Roebbling, Messtechnik, Berlin), by the Van't Hoff equation (Nobel, 1991):

$$\Psi_{\pi} = -nRT$$

where n = mOsmol of sap, $R = 0.083$ and T = temperature ($^{\circ}\text{K}$).

Turgor potential (Ψ_p) was calculated as the difference between leaf water potential and osmotic potential.

Proline content and soluble sugar content

The proline content of salt-stressed and control plants were determined using the method of Bates *et al.* (1973). Proline was extracted from leaf samples (20 mg FW) according to Weimberg (1987) with minor modifications. For proline measurement, reaction with ninhydrin was carried out and the absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined as $\mu\text{mol/g DW}$ using a standard curve prepared with dilutions from a $50 \mu\text{g mL}^{-1}$ proline stock solution.

The soluble sugars were extracted from 20 mg FW of leaf tissue with 80% ethanol and determined colorimetrically at 625 nm using the Anthrone method (Irigoyen *et al.*, 1992).

Experimental design and data analysis

All experimental described were repeated three time independently with six replications each time. A completely randomized design was used. The experimental data were analyzed by ANOVA and the differences were compared by employing the Duncan test with a significance of $p < 0.05$ using the SPSS software (SPSS version 15.0, 2006).

Results

Determination of L_0

Root hydraulic conductance, L_0 , determined using the Scholander chamber, showed no significant diffe-

rence between the control and the B5 and 75 mM NaCl-treated plants (Fig. 1a and 1b). The B20 treatment gave the highest L_0 values (Fig. 1c). However, plants treated with 150 mM NaCl or B (5 or 20 mg L^{-1}) + (75 or 150 mM) NaCl showed significant and similar decreases compared with control plants. The L_0 of plants treated with B20, 75 mM NaCl or B20 + (75 or 150 mM) NaCl was lower after 10 min of the $50 \mu\text{M HgCl}_2$ addition, although there were only significant differences in the 75 mM NaCl treated plants. However, control plants, B5, B5 + (75 or 150 mM) NaCl (Fig. 1b) and 150 mM NaCl treated plants were only affected slightly by HgCl_2 . The addition of 5 mM DTT restored L_0 to the initial values in all treatments.

Stomatal conductance and transpiration rate responses

Stomatal conductance (G_s), measured at midday, showed significant decreases in treatment B5, but significant increases in B20-treated plants (Fig. 2a). G_s values were reduced by the addition of NaCl, with respect to the non-salt-treated plants. However, there were no significant differences among plants treated with (75 or 150 mM) NaCl or NaCl + B.

The transpiration rate (E) showed similar results to stomatal conductance (Fig. 2b). Control plants and B20 showed higher E values compared with B5 treated plants. Whereas salinity application decreased E with regard to control plants, the transpiration rate values of the NaCl and the B + NaCl treated plants did not differ. G_s and E showed similar behaviour to L_0 and correlations between L_0 and E ($R^2 = 0.9797$) and between L_0 and G_s existed ($R^2 = 0.6199$) in the tomato Poncho Negro (data not shown).

Water relations

The leaf water potential (Ψ_w) in control plants, 75 mM NaCl + B0, + B5 and + B20 and in 150 mM NaCl + B0 and + B5 treated plants was similar and without significant differences (Fig. 3a). However, in B5, B20, 150 mM NaCl + B5 and + B20 treated plants, a significant decrease in Ψ_w was observed with regard to control plants. Leaf osmotic potential (Ψ_{π}) showed significant decreases in plants treated with NaCl or B + NaCl, but, again, B5 and B20 treated plants did not show significant differences in their Ψ_{π} values relative to the control (Fig. 3b). For leaf turgor potential (Ψ_p),

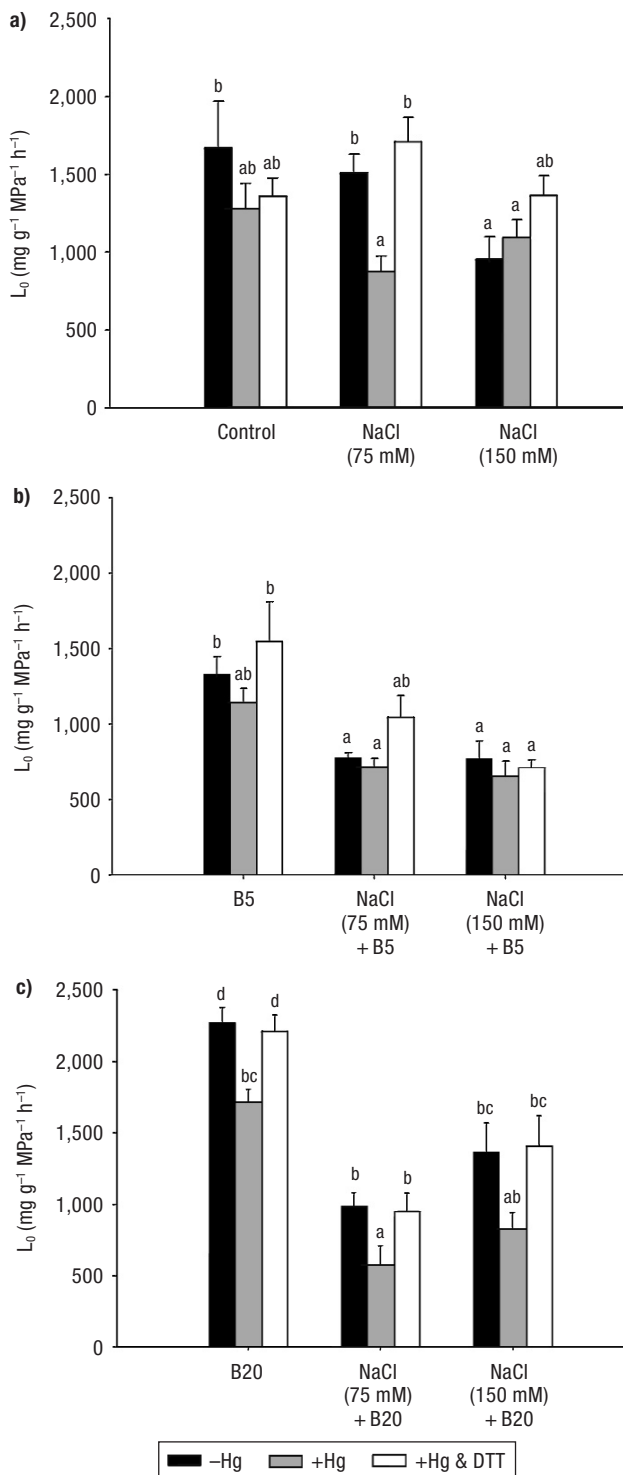


Figure 1. Root hydraulic conductance of tomato plants grown in the following treatments with NaCl (control, 0 mM; 75 mM; 150 mM) and B (B0, control; B5, 5 mg L⁻¹; and B20, 20 mg L⁻¹), before and after a 10 min treatment with 50 mM HgCl₂ and a subsequent treatment with 5 mM DTT. Values represent the mean of six samples ± SE. Bars with the same letter are not significantly different according to the LSD at $p < 0.05$.

there were no changes in the values between control and B5 or B20 treated plants (Fig. 3c). The highest values were observed in 75 mM NaCl, B20 + 75 mM NaCl and B5 + 150 mM NaCl treated plants.

Soluble sugar content

The soluble sugar content in the leaf tissue was increased significantly only by salinity at 75 and 150 mM NaCl (Fig. 4a). However, the soluble sugar content in control plants, B5, B20, B5 and B20 + (75 and 150 mM) NaCl treated plants remained constant and markedly lower than in the NaCl treated plants.

Proline accumulation

Salinity increased significantly the proline concentration in the leaf tissue (Fig. 4b), although the effect

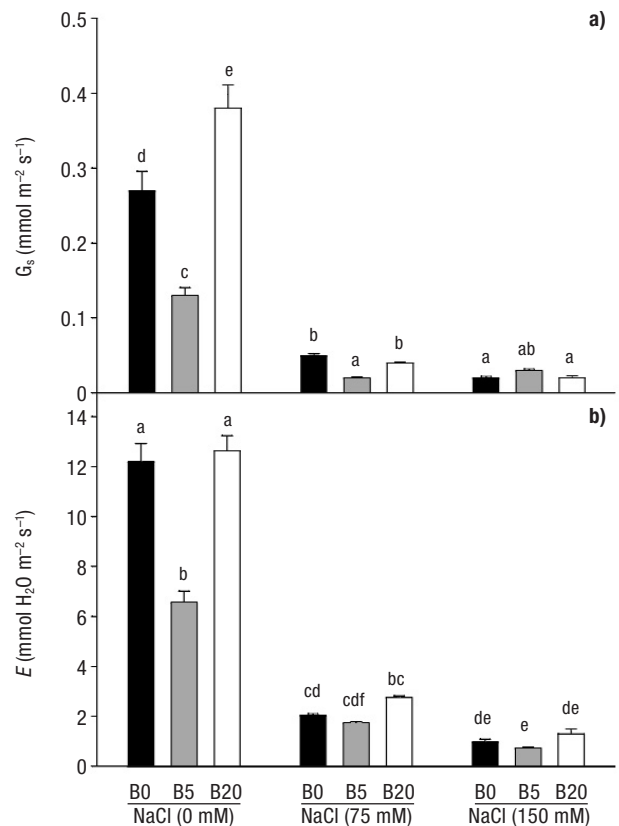


Figure 2. Stomatal conductance (G_s) and transpiration rate (E) of tomato leaves grown in the following treatments with NaCl (control, 0 mM; 75 mM; 150 mM) and B (B0, control; B5, 5 mg L⁻¹ and B20, 20 mg L⁻¹). Values represent the mean of six samples ± SE. Bars with the same letter are not significantly different according to the LSD at $p < 0.05$.

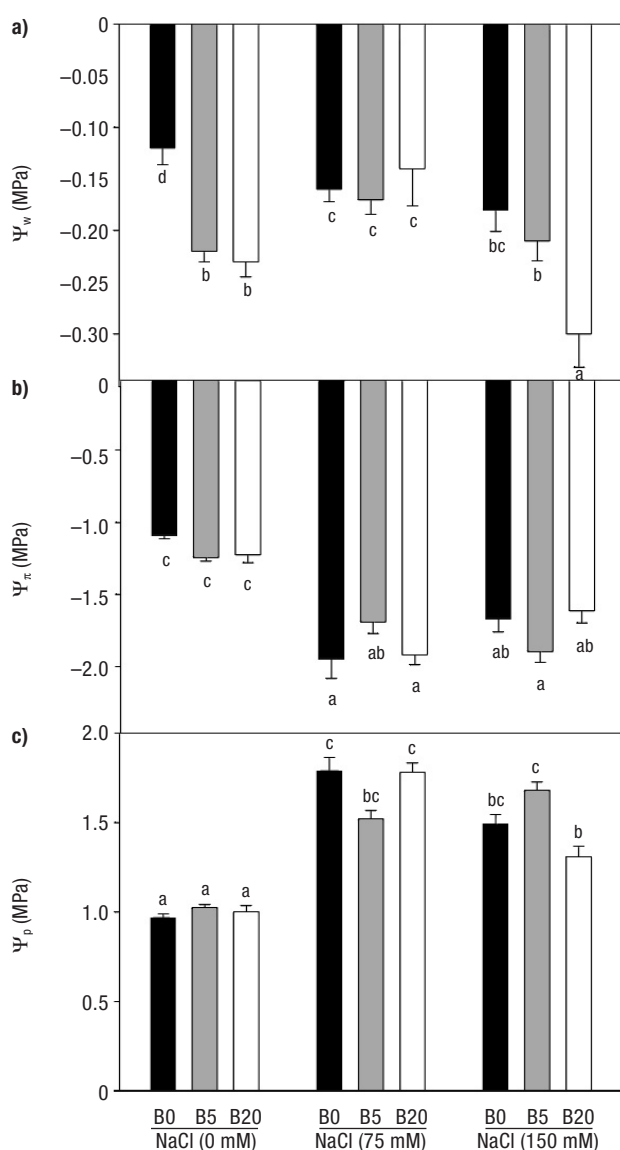


Figure 3. Leaf water potential (Ψ_w), leaf osmotic potential (Ψ_π) and leaf turgor potential (Ψ_p) of tomato leaves grown in the following treatments with NaCl (control, 0 mM; 75 mM; 150 mM) and B (B0, control; B5, 5 mg L⁻¹ and B20, 20 mg L⁻¹). Values represent the mean of six samples \pm SE. Bars with the same letter are not significantly different according to the LSD at $p < 0.05$.

varied among the different treatments, but the addition of boron alone had no effect on the proline concentration. Thus, in B5 and B20 treated plants, the proline amount showed no changes relative to the control, and the values were significantly lower than for the rest of the B and NaCl combinations. The highest value was observed in 150 mM NaCl + B20 treated plants. However, all the 75 mM NaCl + B0 and + B20 treated plants and the 150 mM NaCl + B0 and + B5 treated

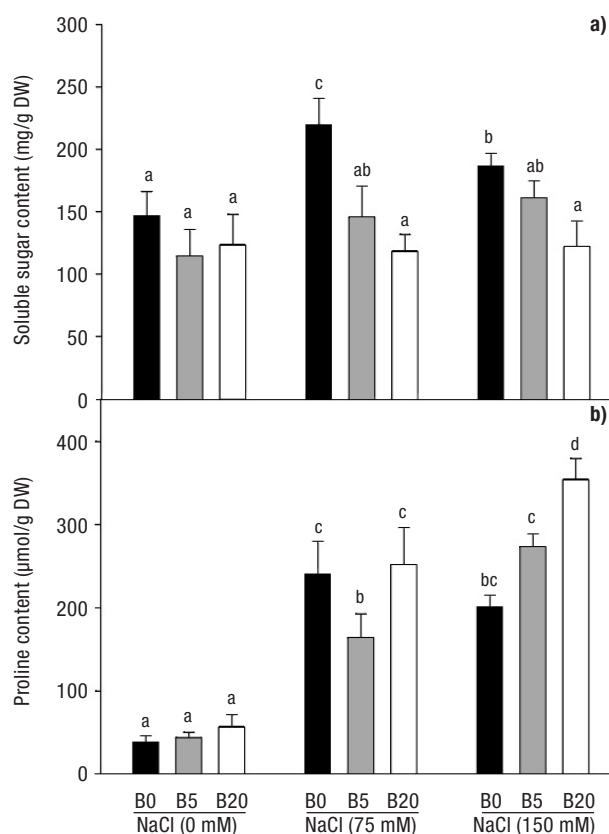


Figure 4. Soluble sugar and proline concentrations of leaves of tomato plants grown in the following treatments with NaCl (control, 0 mM; 75 mM; 150 mM) and B (B0, control; B5, 5 mg L⁻¹ and B20, 20 mg L⁻¹). Values represent the mean of six samples \pm SE. Bars with the same letter are not significantly different according to the LSD at $p < 0.05$.

plants did not differ significantly. While, 75 mM NaCl + B5 plants showed lower values.

Discussion

It is well known that high levels of NaCl reduce the root hydraulic conductance (L_0) of plants; this may be due to the hyperosmotic stress and ionic imbalance caused by apoplastic accumulation of Na⁺ and Cl⁻, or it could be related closely to a decrease in the activity or concentration of aquaporins in the root plasma membrane (Azaizeh and Steudle, 1991; Carvajal *et al.*, 1999; Martínez-Ballesta *et al.*, 2000). However, in the present work, the overall L_0 values of the detached roots of plants treated with NaCl, in the presence or absence of B, were moderately decreased. Aquaporin-mediated water transport is among the initial targets of NaCl in roots (Zhang and Tyerman, 1999; Carvajal

et al., 2000; Martínez-Ballesta *et al.*, 2000). The fact that L_0 was slightly affected by $HgCl_2$, a reagent commonly used to inhibit aquaporin-mediated water transport (Chrispeels and Maurel, 1994; Wan and Zwiazek, 1999; Javot and Maurel, 2002) in all the treatments suggests that in these plants changes in L_0 were not mainly an aquaporin-mediated response or were mediated by Hg^{2+} -insensitive aquaporins. In addition, the greater inhibition by Hg^{2+} in treated plants, compared with control plants, could be an indication that different aquaporin isoforms may operate under different environmental conditions (Qi *et al.*, 2009) or that in treated plants the expression or number of aquaporins was modified compared with the non-treated plants (Bastías *et al.*, 2004a; Martínez-Ballesta *et al.*, 2008).

In addition, it has been reported that, at high external B concentrations, considerable B transport occurs through the plasma membrane aquaporins, and a specific MIP has been described (Dordas *et al.*, 2000; Dordas and Brown, 2001; Wimmer *et al.*, 2003). Also recently, two different aquaporins (HvPIP1;3 and HvPIP1;4) have been described as B transporters although their expression has not responded to B nutritional status, but expressed under toxicity conditions (Fitzpatrick and Reid, 2009). Thus, B uptake across the plasma membrane, by permeation through the lipid membrane and aquaporins, may be influenced greatly by the plant tolerance to salinity, through the associated changes in L_0 (Bastías *et al.*, 2004b; Martínez-Ballesta *et al.*, 2008). The observed decrease in L_0 caused by salinity would have then, as a consequence, coupled effects on root B uptake and B transport from root to shoot (Martínez-Ballesta *et al.*, 2008).

That changes in root L_0 are likely to be among the initial signals triggering stomatal closure and reducing transpiration rates in response to soil stress factors (Wan and Zwiazek, 1999; Voicu and Zwiazek, 2004) is consistent with our observed reductions of G_s and E by salinity (Figs. 2a and 2b). However, the combined effect of salinity and B in tomato Poncho Negro seems to be an antagonistic interaction rather than an additive effect. The effect of B and salinity at toxic levels generally implies an antagonistic relationship regarding growth and yield (Yermiyahu *et al.*, 2008), giving an inhibition and showing that the toxic effect on growth and yield was less severe for combined B toxicity and salinity than what would be expected if the effects of the individual factors were additive. This has been observed previously in maize plants (Bastías *et al.*, 2004a,b). Therefore, these authors showed a less-toxic

effect of the B and salinity combination on plant growth than what would be expected if the effects of the separate factors were additive. In any case, stomatal closure and/or maintenance of root L_0 (defined as the steady-state root exudation rate under specified root pressure) and water absorption could be important mechanisms of acclimation to salinity and/or excess boron by tolerant genotypes. This has been demonstrated by the fact that the tomato Poncho Negro, moderately tolerant of high salinity and excess B, grows in the Lluta valley in northern Chile under these conditions.

Therefore, our results suggest that the influx of water in tomato Poncho Negro may be determined by the extent to which water flow predominates in the apoplastic and symplastic pathways according to the composite model (Steudle and Heydt, 1997), where the relative hydraulic conductivity of the pathways and the relative magnitudes of the hydrostatic and osmotic gradients drive the water transport and vary under the stress conditions (Steudle and Heydt, 1997; Bramley *et al.*, 2009). Thus, this effect was higher in NaCl-treated plants, where the regulation by the stomatal conductance was reduced. These results agree with those reported by Vandeleur *et al.* (2009), indicating that, at low transpiration, osmotic gradients may become more significant and that there should be a large difference in the reflection coefficients between the cell-to-cell and apoplastic pathways.

Plants grown under salt stress accumulate osmolytes that, in turn, reduce cellular osmotic potential and ensure continued water uptake and cell turgor maintenance (Blum, 1996). In this sense, Ψ_π is approximately ten times higher than Ψ_w , even in control plants, supporting that water flow should occur mainly through osmotic gradients. In this experiment, the reductions observed in Ψ_w (Fig. 3a) when the treatments were applied, were compensated by reductions in the Ψ_π (Fig. 3b), which had a significant effect on turgor (Fig. 3c). The increase in the turgor during the treatment application indicates an osmotic adjustment, which was well correlated with the increase in the soluble sugars and especially the proline of the leaves of tomato Poncho Negro (Fig. 4). An increased proline level is a common response of plants to stress treatments (Alpaslan and Gunes, 2001; Karabal *et al.*, 2003) and, in our experiment, proline concentrations were increased significantly by NaCl and NaCl + B treatment, but the increase was different depending on the salinity treatment and B treatment. In addition, the soluble sugar concentration was not altered in plants grown with a

high availability of B under saline conditions, indicating that the contribution of other osmotic compounds or electrolytes to the osmotic adjustment must be considered and that their synthesis must be influenced by the presence of B. However, the fact that B addition to NaCl plants reduced the soluble carbohydrates to control values is an interesting point that could be explained by any effect on photosynthetic activity or carbohydrate partition. This aspect together with the effect on proline accumulation, indicate a complex interaction of B with the metabolism that should be investigated in further experiments.

In summary, the present study shows that the tomato Poncho Negro maintained water uptake, probably due to the increase of osmotic rather than hydrostatic gradients, through reductions in the transpiration rate and accumulation of salts in the roots that allowed the roots to take up water. Therefore, the contribution of aquaporins to the membrane water permeability under stress and non-stress conditions, in terms of expression and/or abundance, needs to be clarified and the role of each aquaporin isoform must be considered, since the hydraulic conductance was not affected by mercury in these plants. But, investigation into the function and regulation of aquaporins in poorly-explored physiological contexts (Maurel *et al.*, 2008), and into effects such as stomatal regulation, will be required. The plants were able to adjust osmotically, increasing turgor, and no toxic effects of salinity were observed. All these data show tomato Poncho Negro, from the Lluta valley (northern Chile), to be a germplasm with agronomic interest that was obtained without the use of genetic manipulation, that has not undergone strong human-imposed selective pressures and which yields well in the field.

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