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Are root hydraulic conductivity responses to salinity controlled by aquaporins in broccoli plants?

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Abstract

Broccoli (*Brassica oleracea* L. var. *Italica*) is a recognised health-promoting vegetable, which is moderately sensitive to salinity. In this study, the primary response of broccoli plants (cv. Marathon) to salinity has been characterised. For this, leaf water relations, nutrient composition, root hydraulic conductivity (L_0) and the effect of mercury (an aquaporin blocker) on L_0 were determined for plants grown with 0, 20, 40, 60, 80 or 100 m*M* NaCl for 2 weeks. During the 2 weeks of treatment, the plants showed a two-phase growth response to salinity. During the first phase (1 week), growth reduction was high, probably related to water stress as no osmotic adjustment occurred and reductions of L_0, the mercury effect and Gs were observed. After 2 weeks, the growth reduction could have resulted from internal injury caused by Na^+ or Cl^-, since osmotic adjustment was achieved and water relations plus the mercury effect were re-established to a high degree, indicating high aquaporin functionality. The fact that aquaporin functionality fits well with the overall water relations response is very relevant, since the two-phase adaptation to salinity may imply two types of aquaporin regulation.

Abbreviations: DTT – dithiotreithol; Gs – stomatal conductance; L_0 – root hydraulic conductivity; $\Psi\pi$ – osmotic potential; Ψ_τ, – turgor potential; Ψw – water potential; RGR – relative growth rate

Introduction

Water and dissolved salts are essential to plant growth, but water re-use and high evaporation rates in arid or semi-arid regions concentrate the salts as salinisation occurs. Salinity is one of the most severe and insidious limitations to crop growth because it is intricately related to water and nutrient uptake into the plant and because its effects at low and moderate concentrations are very ubiquitous (Shannon et al., 1994). The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of the soil solution (water stress), (2) nutritional imbalance, (3) specific ion effects (salt stress), or (4) a combination of these factors (Marschner, 1995; Shannon, 1998). All of these cause adverse pleiotropic effects on plant growth and development at physiological and biochemical levels (Munns, 2002) and at the molecular level (Mansour, 2000; Tester, 2003; Winicov, 1998).

The concentrations at which these effects take place differ with the genotype, growth stage, environmental interactions and ion species. Native plant species have adapted to an incredibly wide range of saline environments, but crop plants, with a few exceptions, grow best with fairly low

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concentrations of salts in the root medium. In general, salt concentrations higher than 45 m*M* NaCl can decrease yields of many crops (Shannon et al., 1994). Despite a great deal of research into salinity tolerance of plants, mainly on water relations, photosynthesis and accumulation of various inorganic ions and organic metabolites (Munns, 1993, 2002), the metabolic sites at which salt stress damages plants and, conversely, the adaptive mechanisms utilised by plants to survive saline stress are still not well understood. The mechanisms of salt tolerance are so complex that variation occurs not only amongst species but, in many cases, also among cultivars within a single species (Ashraf, 2002; Greenway and Munns, 1980). Plants adapt to stress by different mechanisms, including changes in morphological and developmental patterns as well as physiological and biochemical processes (Zhu, 2001). Such mechanisms may start beyond the physical barriers of the plant cell itself (Suhayda et al., 1990). Other mechanisms may operate to pump specific ions back out of the cytosol or into vacuoles (Hasegawa et al., 2000). Many other components of the shoot, such as stomata, leaf mesophyll structure, epicuticular waxes, leaf colouration and leaf shape, may play significant roles in water relations, as a response to salinity stress (Shannon et al., 1994).

The discovery of aquaporins in plants has resulted in a paradigm shift in the understanding of plant water relations (Maurel and Chrispeels, 2001). These proteins provide an organism with the possibility to accelerate water movement across membranes, but diffusion will still occur in parallel. Furthermore, the ability to increase or decrease the water permeability of a cell seems to justify the enormous effort in expressing large amounts of these proteins (Schäffner, 1998). Therefore, the responses to stress in plants have been related to aquaporins. Although the hydraulic conductivity of tissues could be regulated by changing the level of specific aquaporins, regulation could also occur by changing the activity of the proteins. Salinity possibly affects water channel function via both gene expression and biochemical changes in the water channel protein (Carvajal et al., 1999; Martinez-Ballesta et al., 2000). Differences in the regulation of aquaporin function may reflect the tolerance to stress in plants (Carvajal et al., 2000). It has been shown that $HgCl_2$ blocks the flow of water through aquaporins in plant roots and that the flow is restored by reducing agents (Carvajal et al., 1996; Maggio and Joly, 1995). Assuming that aquaporins close upon treatment with $HgCl_2$, previous results indicate that, in control plants, about 85% of water transport across the plasma membrane is mediated by specific water channels. The remaining 15% of the hydraulic conductivity would then reside in the lipid bilayer, in other proteinaceous arrays such as ion pumps, channels or other transport systems for specific solutes, or in non-Hg sensitive water channels (Bastías et al., 2004, Martínez-Ballesta et al., 2003b).

Broccoli (*Brassica oleracea* L. var. *Italica*) is a recognised health-promoting vegetable and one of the most important vegetables produced in the Southeast of Spain. Broccoli is moderately sensitive to salinity, although it has higher tolerance than other common vegetables such as lettuce, onion, maize and carrot (Shannon and Grieve, 1999). Our aim in this study was to relate water uptake, water transport and growth during the primary response of broccoli plants (cv. Marathon) to salinity. As it has been reported that aquaporins accounts for variations in hydraulic conductance for metabolically active root regions (North et al., 2004), in the present paper, we study water relations of the whole plant in response to salinity in relation to the functionality of the root aquaporins. For this, leaf water relations, root hydraulic conductivity and Na^+ and Cl^- concentration were determined after 1 and 2 weeks of applying different concentrations of NaCl (0, 20, 40, 60, 80 and 100 m*M*). Also, the involvement of aquaporins in this response, in terms of Hg sensitivity, was analysed.

Materials and methods

Plant material and growth conditions

Broccoli seeds (*Brassica oleracea* var. *italica*, cv. Marathon) were pre-hydrated with aerated, de-ionised in water for 12 h and germinated in vermiculite, at 28 °C in an incubator, for 2 days. They were then transferred to a controlled-environment chamber with a 16-h light–8-h dark cycle and air temperatures of 25 and 20 °C, respectively. The relative humidity (RH) was 60% (day) and 80% (night) and photosynthetically-active

radiation (PAR) was 400 μmol m^{-2} s^{-1}, provided by a combination of fluorescent tubes (Philips TLD 36 W/83, Germany and Sylvania F36 W/GRO, USA) and metal halide lamps (Osram HQI.T 400 W, Germany). After 5 days, the seedlings were placed in 15-L containers with continuously-aerated Hoagland (Hoagland and Arnon, 1938) nutrient solution: KNO_3 (3 m*M*), $Ca(NO_3)_2$ (2 m*M*), $NH_4H_2PO_4$ (0.5 m*M*), $MgSO_4$ (0.5 m*M*), KCl (50 μM), H_3BO_3 (25 μM), $MnSO_4$ (2 μM), $ZnSO_4$ (2.0 μM), CuSO4 (0.5 μM), H_2MoO_4 (0.5 μM), Fe-EDDHA (ethylendiamino-di(o-hydroxyphenylacetic) acid) (20 μM). The solution was replaced completely every week. After 21 days (when plants were 26 days-old), plants were treated with 0, 20, 40, 60, 80 or 100 m*M* NaCl, corresponding to electrical conductivities of 2, 4, 6, 8, 10 and 12 dS m^{-1}, respectively. Water potential (Ψ_w), osmotic potential (Ψ_π), turgor potential (Ψ_τ), root hydraulic conductance (L_0), stomatal conductance, relative growth rate (RGR) and Na^+ and Cl^- concentrations were measured after 7 and 14 days of the treatments, when plants were 32 and 39 days-old, respectively. Measurements and harvesting were all performed in the middle of the light period.

Measurement of relative growth rate (RGR)

Plants were harvested and fresh weight was determined across the intervals 0 to 7 days of the treatments and 0 to 14 days (Hunt et al., 2002). RGR (g g^{-1} day^{-1}) was calculated according to the following equation (Fisher, 1921):

$$\mathrm{RGR} = (\mathrm{Ln}W_2 - \mathrm{Ln}W_1)/(t_2 - t_1)$$

W, fresh weight at different times (g); *t*, time (day).

Stomatal conductance (g_s)

Adaxial stomatal conductance of leaves (more stomata were detected on the adaxial surface of the leaves, data not shown) was measured using a portable porometer (AP4 porometer, Delta-T Devices). Measurements were made on the most recent fully-expanded leaves.

Water potential (Ψ_w)

A Scholander pressure chamber was used for the measurement of water potential. Leaves were put inside the pressure chamber so that the petiole was the only part of the plant in contact with the external environment. The chamber was closed and the pressure was increased until a drop of xylem sap appeared; this negative pressure was considered the water potential of the leaf. For determination, four homogeneous, young, fully-expanded leaves were used for every treatment.

Osmotic potential (Ψ_π)

Leaves were put in Eppendorf tubes with holes at the bottom and rapidly frozen with liquid nitrogen. These tubes were then centrifuged twice into assay tubes, at $4000 \times g$ for 4 min (4 °C), using a Hettich-Universal32R 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, Roebling, Berlin), by the van't Hoff equation:

$$\Psi_\Pi = -nRT$$

where n = mOsmol, R = 0.083 and $T = t^a$ (K).

Turgor potential (Ψ_τ)

This parameter was calculated by the difference between Ψ_w and Ψ_π.

Root hydraulic conductance (L_0)

Hydraulic conductance (L_0) of roots was measured by pressurising the roots using the Schölander chamber (Jackson et al., 1996). For this, the aerial parts of the plant were removed, leaving the base of the stem, which was sealed with silicone grease, into a tapered glass tube. The plant was placed into a pressure chamber, with the same nutrient solution that it was grown in, and a gradual increase of pressure (from 0.1 to 0.5 MPa) was applied to detached roots. The range of the generated sap flows included a flow equivalent to the whole-plant transpiration flow. In the pressure chamber, it is assumed that there is a balance between the negative pressure in the xylem and the pressure that forces water from the cells into the vessels. Sap was collected in Eppendorf tubes and weighed on a precision balance.

Sap flow, J_V, was expressed in mg $(g_{rootFW})^{-1}$ h^{-1} and plotted against pressure (MPa), the slope being the L_0 value (mg $(g_{rootFW})^{-1}$ h^{-1} MPa^{-1}).

Mineral content analysis

Leaves and roots collected 7 and 14 days after starting the salt treatments were dried at 65 °C for 5 days. For sodium, chemical analyses were carried out after a HNO_3–$HClO_4$ (2:1) digestion. Sodium concentrations, determined by atomic absorption spectrometry (Perkin-Elmer ICP 5500, Norwalk, CT, USA), were measured for extract aliquots diluted with a $LaCl_3$ + CsCl solution. For chloride concentrations, a Dionex D-100 ion chromatograph, with an ionpac AS 124-4 mm (10–32) column and an AG 14 (4 × 50 mm) guard column, was used. The flow rate was adjusted to 1 mL min^{-1}, with an eluent composition of 0.5 mM Na_2CO_3 and 0.5 mM $NaHCO_3$. Chloride concentrations were measured with Chromeleon/Peaknet 6.40 chromatography software, by comparing peak areas with those of known standards.

Mercury inhibition of root hydraulic conductivity

In order to study the effect of the blocking agent ($HgCl_2$) on the aquaporins (Carvajal et al. 1996; Maggio and Joly, 1995), L_0 was measured in control and NaCl-treated plants. Then, $HgCl_2$ (50 μM) was supplied in the nutrient solution of the same roots for 15 min. Plants were then transferred to a fresh nutrient solution prior to measuring L_0 again. Afterwards, DTT (2 mM) was added to the nutrient solution, in order to capture the Hg, and L_0 was measured a third time in the same roots. In all the steps of the experiment, L_0 was determined as described above. The results are presented as the percentage of inhibition of L_0 compared with the first measurement of L_0.

Data analysis

Data were analysed statistically, using the SPSS 7.5 software package, by ANOVA and by Tukeý́s Multiple Range Test, to determine differences between means, and by the CORR procedure for correlation analysis.

Results

The relative growth rate (RGR) decreased progressively as the nutrient solution EC increased, after both 1 and 2 weeks of treatment application. After 2 weeks, RGR values were lower than after the first week, but the decrease due to NaCl treatment was significantly less than after 1 week, as shown by the values of the slope of the regression analysis ($P < 0.001$) (Figure 1).

Root hydraulic conductance (Figure 2) showed a sharp decrease with increasing nutrient solution EC, more so after 1 week than after 2 weeks; thus, the slope of the regression line for 1 week was 50 % higher (−158.25) than for 2 weeks (−104.72), being significantly different for $P < 0.05$.

Water potential showed a progressive decrease as salt concentration increased, during both weeks of measurement (Figure 3). However, at 20, 40 and 60 mM NaCl, Ψ_w was more or less constant, while at higher concentrations (80 and 100 mM NaCl) the decrease was greater. In the case of osmotic potential, after 1 week of treatment, there was an increase at 40 mM NaCl compared with the control. However, there were no significant differences with the rest of the salinity treatments. After 2 weeks of treatment, there was a slight and progressive decrease of Ψ_π with increasing concentration of NaCl. Turgor potential, after 1 week of treatment, showed a strong decrease for the 40 mM NaCl treatment, which was maintained for the higher NaCl concentrations. However, after 2 weeks of treatment, the turgor potential remained constant with all salinity treatments.

The decrease observed for stomatal conductance (Figure 4) was again higher after 1 week than after 2 weeks, the slope of the regression line at 1 week being 25% higher than at 2 weeks ($P < 0.1$).

The percentage inhibition of L_0 by Hg was measured after $HgCl_2$ (50 μM) addition to the nutrient solution (Figure 5); values of L_0 prior to Hg application were similar to those shown in Figure 2. It can be appreciated that there was a sharp decrease of the inhibition as the concentration of NaCl increased from 0 to 40 mM, for both weeks of measurement. However, the inhibition remained very low and stable from 60 to 100 mM NaCl. Lower values of inhibition were observed after 2 weeks for all the treatments. Inhibition for

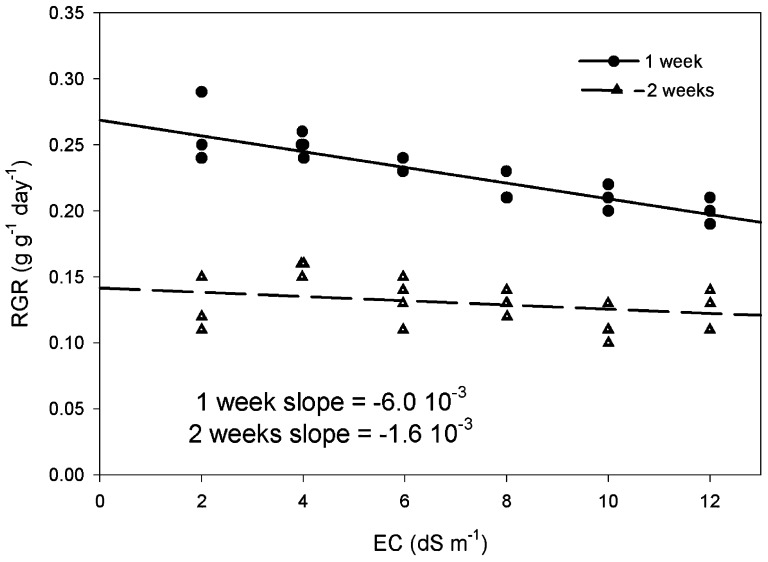

Figure 1. Measurement of the relative growth rate (RGR) of broccoli plants grown under different NaCl treatments (2, 4, 6, 8, 10 and 12 dS m^{-1}), after 1 and 2 weeks. Each point represents an individual value. Lines represent the regression analysis for each week.

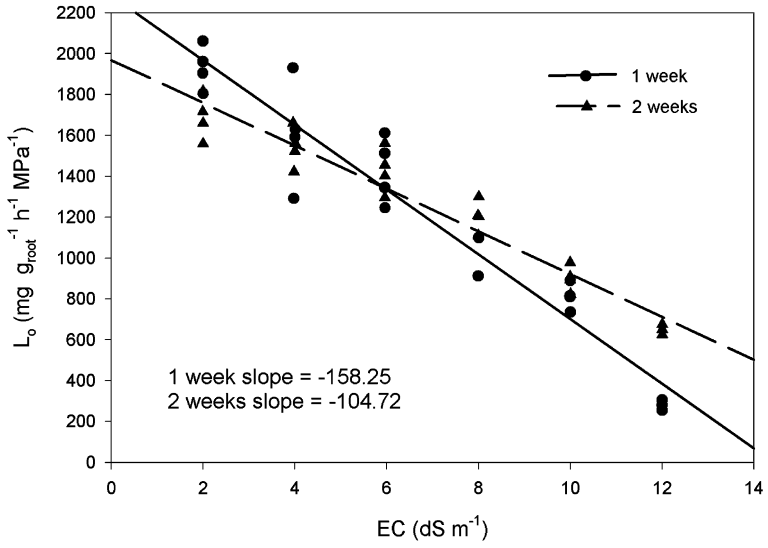

Figure 2. Root hydraulic conductance (L_0) of broccoli plants grown under different NaCl treatments (2, 4, 6, 8, 10 and 12 dS m^{-1}), after 1 and 2 weeks. Each point represents an individual value. Lines represent the regression analysis for each week.

control and salinity-treated plants, when it occurred, was reduced to zero after DTT application, indicating that initial L_0 values were restored.

After 1 week of treatment, the concentration of sodium was increasing progressively as external NaCl increased, similarly for leaves and roots (Figure 6). After 2 weeks, sodium concentrations increased to a higher extent than after the first week, in leaves from 20 m*M* NaCl and in roots from 40 m*M* NaCl, compared with controls. However, in roots, no significant differences were found between concentrations after 1 and 2 weeks. After both 1 and 2 weeks, chloride, like sodium, increased progressively with the level of the salinity treatment. However, only after

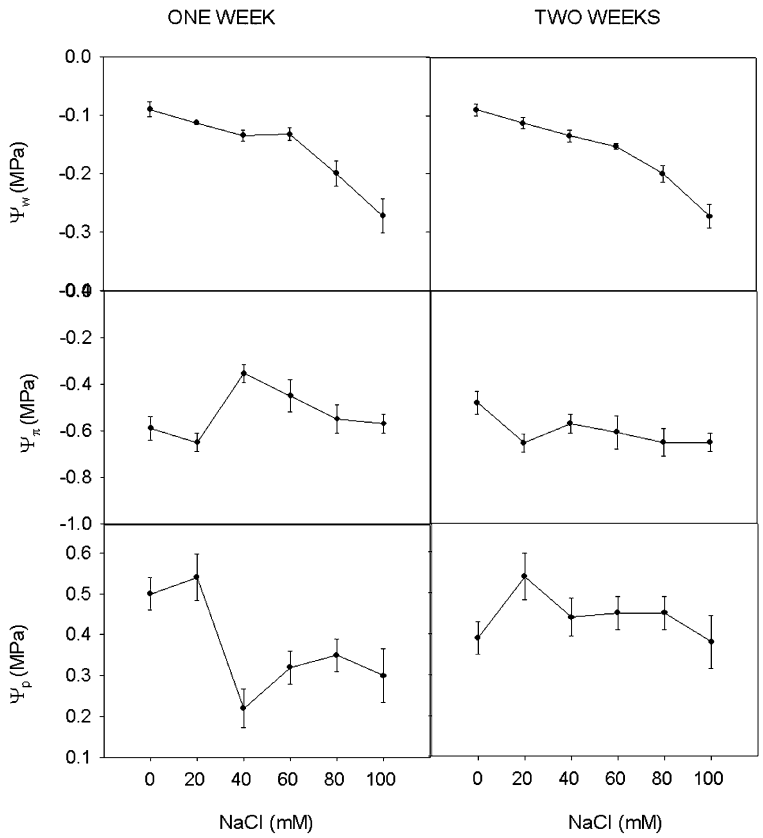

Figure 3. Water, osmotic and turgor potential (Ψ_w, Ψ_π, Ψ_τ) of broccoli plants grown under different treatments (control, 20, 40, 60, 80, 100 mM NaCl), after 1 and 2 weeks. Each point represents the mean of four samples ± SE.

2 weeks of the salinity treatments was the sodium concentration greater in leaves than in roots.

Discussion

It has been reported that reductions in growth depend on the period of time over which the plants have grown in saline conditions, leading to the hypothesis that, on most occasions, there is a two-phase growth decrease in response to salinity (Munns, 2002). The first phase of growth reduction is quickly apparent, and is due to the salt outside the roots. It is essentially a water stress or osmotic phase. Then, there is a second phase of growth reduction, which takes time to develop and results from internal injury due to salts accumulating in transpiring leaves (Kawasaki et al., 2001). Broccoli plants showed this biphasic response, the decrease in growth probably due to water and osmotic stress occurring after 1 week and in proportion to the level of salinity. After 2 weeks, the growth decrease due to salinity was lower than after 1 week. However, the amounts of Na^+ and Cl^- accumulated were higher. This could indicate that Na^+ and Cl^- were compartmentalised in the vacuole (Munns, 1993), producing only a slight reduction in growth for all the treatments over 40 mM NaCl. During the second week, control plants also had a decreased growth rate, showing a different phase of growth, which could also explain the lower effect of salinity treatments. In other plants, growth has been related to plant water status (Carvajal et al., 1999; Cerdá et al., 1979). In our plants, the two phases of growth seem to be related to water uptake and transport. Furthermore, the regulation of aquaporin functionality could be the key for the movement of water through the plant.

Water relations in plants are affected by salinity (Hasegawa et al., 2000). The presence of salt

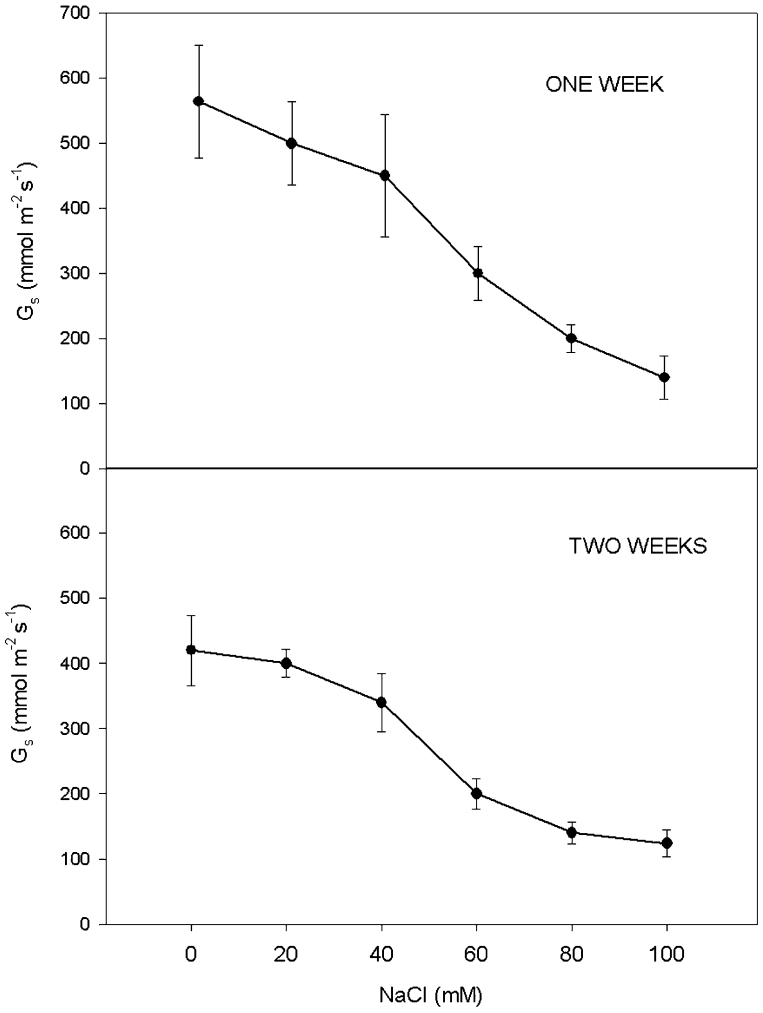

Figure 4. Stomatal conductance of broccoli plants grown under different treatments (control, 30, 60 m*M* NaCl), after 1 and 2 weeks. Each point represents the mean of four samples ± SE.

decreases the water potential of the medium, so plants have problems with respect to absorption of water. In order to compensate for the negative values of the nutrient solution, plants have to decrease their water potential; this involves a decrease of the osmotic potential, to maintain turgor and achieve osmotic adjustment (Blum et al., 1996). In our experiment, only after 2 weeks, the decrease in osmotic potential maintained the turgor potential for all the treatments. The fact that turgor potential decreased in control plants during the second week, compared with the first week, could also be related to the different phase of growth that was observed in this week. One important issue regarding osmotic adjustment is that the metabolic costs of including salts and of their intracellular compartmentation are relatively small in relation to those of the synthesis of organic solutes for osmotic adjustment (Raven, 1985; Yeo, 1983). The fact that the concentrations of Na^+ and Cl^- in leaves were higher than in roots leads us to suggest that the broccoli plants achieved osmotic adjustment with inorganic ions, which produced a low decrease in growth. However, the degree of damage due to ion accumulation in the longer term has to be assessed. As we reported previously (Carvajal et al., 1999), the decrease of aquaporin functionality in saline conditions seems to be related to the high concentration of Na^+ or Cl^-

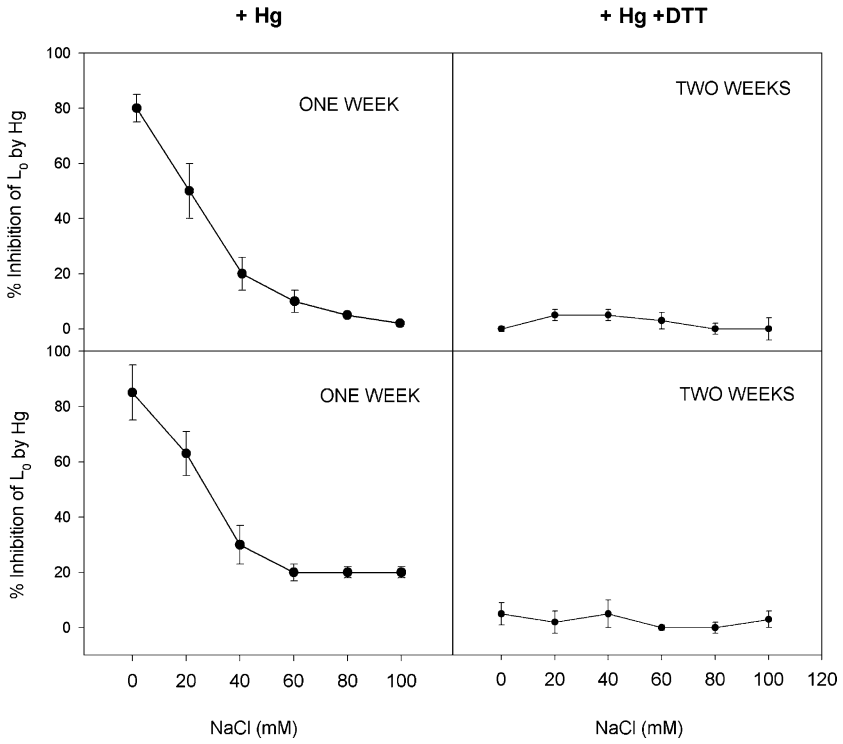

Figure 5. Percentage inhibition of L_0 by Hg. Measurement of the variation of root hydraulic conductance (L_0) of broccoli plants grown under different treatments (control, 20, 40, 60, 80, 100 m*M* NaCl), after 1 and 2 weeks. Inhibition was calculated after addition of $HgCl_2$ (50 μM) to the nutrient solution and after subsequent addition of DTT (2 m*M*). Each point represents the mean of four samples $\pm$SE.

in the cells rather than to the osmotic effect. Therefore, once again, it has to be assumed that ions were accumulated in vacuoles, in order to decrease the negative effect on aquaporins during the second week.

Any signal of stress can affect the water balance of plants. In order to prevent this, plants close stomata and the water lost by transpiration in this way is decreased, and so the risk of dehydration is decreased. It has been suggested that a decline in photosynthesis, to some extent, occurred through stomatal closure in broccoli (Ashraf, 2001). So, the fact that, after 2 weeks, the decrease in the stomatal conductance was less than after 1 week could suggest a relationship between growth and stomatal opening in all plants (control and treated). The movement of water through plants can be regulated at several points in the pathway from the nutrient solution to the leaf surface. From our previous experiments, we observed that aquaporins dominate the symplastic movement of water though the plant (Carvajal et al., 1996). Therefore, at least when stresses are applied via the nutrient solution, changes in root hydraulic conductance produce further alteration in stomatal conductance, giving the root the main role in plant water uptake.

The effects of increased salinity on L_0 have been widely reported (Azaizeh and Steudle, 1991; Carvajal et al., 1999; Munns and Passioura, 1984; Shalhevet et al., 1976) and it has been suggested that they are due to the high concentrations of Na^+ and Cl^- in the cytoplasm, that reduce the water transport through the plasma membrane (Carvajal et al., 1999). Our experiments showed a sharp decrease of L_0 with salt treatments, but to a lesser extent after 2 weeks of measurement (Figure 2). This could be related to the results obtained for the osmotic adjustment (Figure 3) and for the stomatal conductance (Figure 4), meaning a relatively higher water uptake and transport in treated plants after 2 weeks. In previous experiments, it was

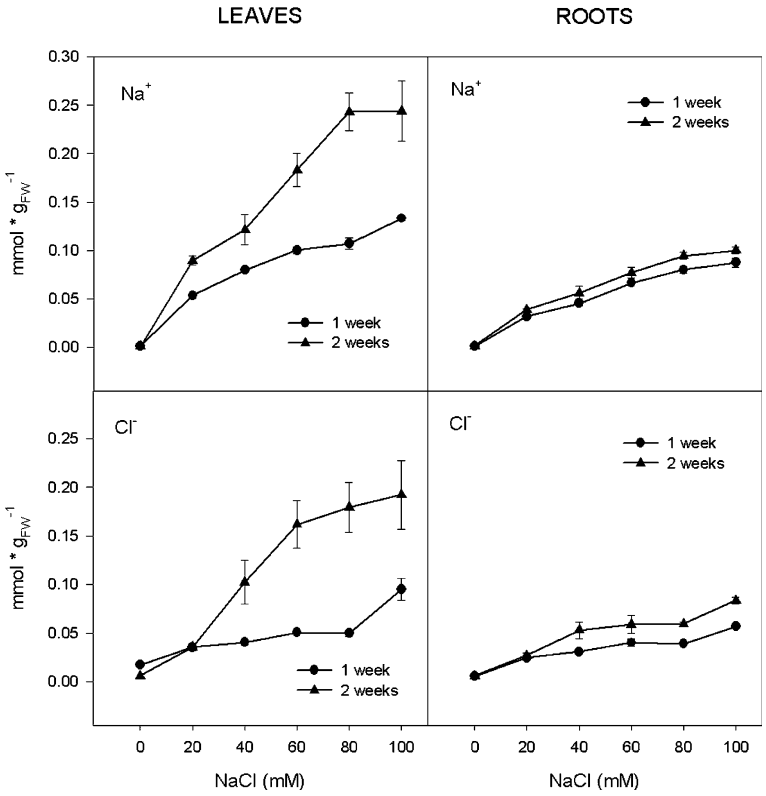

Figure 6. Chloride and sodium concentrations of broccoli plants (leaves and roots) grown under different treatments (control, 20, 40, 60, 80, 100 m*M* NaCl), after 1 and 2 weeks. Each point represents the mean of four samples ± SE.

observed that large reductions in the root hydraulic conductance of salt-stressed plants were closely related to the decrease in the functionality or concentration of aquaporins in root plasma membrane (Carvajal et al., 1999; Martínez-Ballesta et al., 2003a).

Our results for Hg inhibition of L_0 (Figure 5) suggest that root aquaporins could be related with water uptake and transport. Heavy metal ions, such as Hg^{2+}, are known to inhibit the water transport activity of the tonoplast (Maurel et al., 1993) and plant plasma membrane aquaporins (Kammerloher et al., 1994), although some plasma membrane aquaporins are mercury-insensitive (Daniels et al., 1994). Using site-directed mutagenesis, it has been shown that Hg^{2+} binds to cysteine residues in or near the aqueous pore of the aquaporins, thereby inhibiting water transport (Agre et al., 1998; Daniels et al., 1996). The assumption that the addition of a heavy metal solution specifically blocks aquaporins was supported by the fact that the swelling of water channel-expressing oocytes could be strongly reduced by a specific concentration of mercury chloride (Preston et al., 1992). To account for these results, it has been concluded that heavy metal ions specifically block aquaporins and, consequently, could indicate the significance of these proteins in whole plant or cellular water transport. Although this conclusion seems to be reasonable, it is necessary to bear in mind that the pharmacology of mercurials includes numerous secondary effects (Patra and Sharma, 2000; Schütz and Tyerman, 1997). However, 0.1 m*M* $HgCl_2$ did not significantly reduce root respiration during the initial hour of treatment in aspen seedlings (Wan and Zwiazek, 1999) and in *A. thaliana*, there were no significant differences in oxygen consumption during the 10 min of Hg treatment (Martínez-Ballesta et al., 2003a), suggesting that the mercuric inhibition of root water flow was probably not due to metabolic inhibition. In other experiments, it has been reported that H^+-ATPase activity was inhibited by Hg

(Martínez-Ballesta et al., 2003b), suggesting a nutrient imbalance. However, the fact that the flux of K^+ into the xylem was not altered (Carvajal et al., 1999; Maggio and Joly, 1995) indicates that the low Hg^{2+} concentration (50 μM) and duration of exposure (10 min) used only affected water transport through aquaporins in roots (Patra and Sharma, 2000). Therefore, assuming that the flux of water through aquaporins was blocked by Hg in our broccoli plants, we can estimate aquaporin functionality in NaCl-treated plants. So, the fact that there was a high reduction of the percentage inhibition by Hg in NaCl-treated plants indicates that as NaCl increased, the number of putative aquaporins or their functionality decreased. These results are in accordance with previous results for pepper plants (Martínez-Ballesta et al., 2003a). In the same way, the inhibition was higher during the second week than during the first week, from 40 to 100 mM NaCl, suggesting that the number or functionality of putative aquaporins was higher during the second week.

Concentrations of Na^+ and Cl^- were observed to be in the same range, and their responses to the different treatments were similar, showing an almost linear increase with salinity in leaves and roots. These results indicate that the resistance of broccoli plants to salinity involves minimisation of the concentration of salt in the cytoplasm (Binzel et al., 1988; Munns, 2002). Our results suggest that root hydraulic conductance in relation to aquaporin functionality could depend on both osmotic adjustment and ionic toxicity. However, deeper studies will have to be done to elucidate how this occurs.

In conclusion, broccoli plants are moderately tolerant to salt stress. During the first 2 weeks of treatment, they showed a two-phase growth response to salinity. During the first phase (1 week), growth reduction was high, probably related to water stress. After 2 weeks, the lesser growth reduction could have resulted from internal injury due to Na^+ or Cl^-, since osmotic adjustment was achieved and water relations were re-established relatively well. In addition, it is likely that specific toxicity of the Na^+ or Cl^- ions was responsible for the decrease in aquaporin functionality in NaCl-treated plants (Martinez-Ballesta et al., 2000). However, the facts that after 2 weeks, the aquaporin functionality was higher than after 1 week and that the Na^+ and Cl^- concentrations were higher during this week suggest that these ions were compartmentalised into the vacuole. The fact that aquaporin functionality fits well with the overall water relations response is very relevant, since the two-phase process of adaptation to salinity may imply two types of aquaporin regulation (closing during the first phase and opening during the second phase). This complicates the whole picture concerning the regulation of the different aquaporins during the response of plants to stresses.

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