

THE PHI THICKENING IN ROOTS OF BROCCOLI PLANTS: AN ACCLIMATION MECHANISM TO SALINITY?

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Although broccoli is moderately tolerant to salt stress, the tolerance mechanism is still unknown. Therefore, in this article, the uptake and transport of nutrients and water in relation to the changes in root anatomy (phi thickening appearance) caused by salinity stress have been studied. The effect of phi thickening in the response of these plants to salinity was studied by comparing two methods of measuring root hydraulic conductance, pressurizing roots and natural exudation, and analyzing the nutrient concentrations in the xylem. The permeability properties of phi thickening were tested by a tracer that moves only via the apoplastic pathway. *Brassica oleracea* L. var. *Italica* plants, grown under different levels of NaCl (0, 40, and 80 mM), showed modifications in the cell wall of the cortical layer bordering the endodermis, such as phi thickenings. The results also showed a decrease of the L_0 and G_S of plants under salinity stress and an ionic imbalance in the xylem sap. Na^+ , Mg^{2+} , SO_4^{2-} , and Cl^- concentrations increased, while Ca^{2+} decreased. The fact that the proportion of apoplastic movement, which was higher when plants were measured with the Scholander chamber than with natural exudation, was lower for NaCl-treated plants whereas the alteration of nutrient uptake was similar suggests that the phi thickenings could be a physical barrier only to apoplastic water transport.

Keywords: broccoli, phi thickenings, root anatomy, root hydraulic conductance, salinity, xylem sap nutrient concentration.

Introduction

Root systems have developed a variety of strategies and mechanisms that enable them to react to stressful changes in their environment. A number of studies have shown alterations in the morphology and fine structure of the root induced by different unfavorable conditions (Peterson 1992; Hose et al. 2001; Enstone et al. 2003). It has been shown that salinity promotes suberization of the hypodermis and endodermis and that the Casparian strip is developed closer to the root tip than in nonsaline roots (Shannon 1997). In cotton seedling roots, the formation of an exodermis, the premature differentiation of protophloem sieve tube members, and the induction of lateral root initiations much closer to the root tip can be induced by salinity (Reinhardt and Rost 1995a, 1995b; Ma and Peterson 2003). In addition, some plant species develop phi thickening in different root tissues, modifications of the midportion of the radial cell walls (Degenhardt and Gimmler 2000) that can form either a uniseriate or a multiseriate layer (Peterson et al. 1981). Phi thickenings were first described in the nineteenth century and consist of nitrocellulose wall deposits that are impregnated with lignin. They usually form on the walls of certain cell layers in the root cortex of some gymnosperms (Haas et al. 1976) and a few families of angiosperms (Peterson et al. 1981; Praktikakis et al. 1998). Although Weerdenburg and Peterson (1983) assumed

that phi thickenings function primarily as supportive structures, their role has been the subject of much speculation and experimentation but is still not clear.

Ion and water homeostasis is the main physiological process that plants need to optimize to maintain growth in saline environments (Munns and Termaat 1986; Hasegawa et al. 2000). The effect of salinity on plants is a stress imposed by the reduction of osmotic potential in the soil solution, nutritional imbalance, specific ion effects, and a combination of these factors (Shannon 1997). These deleterious effects modify growth via effects at the physiological and biochemical levels (Munns 2002) and at the molecular level (Winicov 1998). Osmotic adjustment helps plant cells to withstand salinity, and water deficit conditions generate a gradient of water potential between the plant and the substrate, necessary to maintain water uptake and sufficient turgor for growth (Carvajal et al. 1999, 2000). This adjustment implies the regulation of the intracellular levels of organic compounds, such as proline (Jeschke et al. 1986; Djanaguiraman et al. 2006) or glycine betaine (Verslues et al. 2006), and the compartmentation of ions, such as Mg^{2+} in the cytoplasm and Na^+ , K^+ , and Cl^- in the vacuole (Voetberg and Sharp 1991). Also, the high apoplastic concentrations of Na^+ and Cl^- modify the root hydraulic conductance because of a toxic effect that negatively influences the concentration or functionality of aquaporins (Martínez-Ballesta et al. 2000; Navarro et al. 2000, 2003).

Salinity stress also affects the stomatal conductance (G_S) (Goldstein et al. 1996; Santiago et al. 2000; Djanaguiraman et al. 2006). Bayuelo-Jiménez et al. (2003) reported a decreased G_S in *Phaseolus* plants grown under salinity because of a

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toxic effect of Na^+ and Cl^- ; apart from this, higher concentrations of these ions were capable of reducing CO_2 assimilation. They also mentioned that reduced stomatal conductance decreased the photosynthetic carbon assimilation. Similar results have been reported for rice (Djanaguiraman et al. 2006), amaranth (Omami and Hammes 2006), and mangrove (Suárez and Medina 2006).

Broccoli plants, which have been proven to possess anti-cancer properties, are moderately tolerant to salt stress (López-Berenguer et al. 2006), although the tolerance mechanism is still unknown. Therefore, the objective of this article was to study the uptake and transport of nutrients and water in relation to the changes in root anatomy (phi thickening appearance) produced by salinity stress. For this, leaf and root osmotic potential, stomatal conductance, and root hydraulic conductivity were measured to check the water relations under salinity. The effect of phi thickening in the response of these plants to salinity was studied by comparing two methods of measuring root hydraulic conductance, pressurizing roots and natural exudation, and analyzing the nutrient concentrations in the xylem. The permeability properties related to phi thickening were tested by using a tracer that moves only via the apoplastic pathway.

Material and Methods

Plant Culture

Seeds of broccoli (*Brassica oleracea* cv. Maratón) were pre-hydrated with deionized water, continuously aerated, for 12 h. After this, the seeds were germinated in vermiculite in the dark at 28°C for 2 d. They were then transferred to a chamber with controlled environmental conditions: a 16L : 8D cycle with temperatures of 25° and 20°C and relative humidities of 60% and 80%, respectively. The PAR was 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. After 3 d, the seedlings were placed in 15-L containers with continuously aerated Hoagland nutrient solution: KNO_3 (3.0 mM), $\text{Ca}(\text{NO}_3)_2$ (2.0 mM), KH_2PO_4 (0.5 mM), MgSO_4 (0.5 mM), H_3BO_3 (25.0 μM), MnSO_4 (2.0 μM), ZnSO_4 (2.0 μM), CuSO_4 (0.5 μM), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ (0.5 μM), Fe-EDTA (20.0 μM). The solution was changed every 4 d. After 2 wk, the salinity treatments of 0, 40, and 80 mM NaCl began. Measurements were carried out 1 d, 1 wk, and 2 wk after applying NaCl.

Osmotic Potential ($\Psi\pi$)

The most recent fully expanded leaves of broccoli plants of different treatments were selected, placed in Eppendorf tubes with holes at the bottom, and frozen at -80°C . These tubes were then placed in centrifuge tubes and centrifuged twice at 4000 rpm for 4 min, using a Hettich-Universal 32R centrifuge in such a way that all sap was extracted from the samples. The osmolarity of the leaf sap was measured with an automatic freezing point depression osmometer (Digital Osmometer, Roebbling, Berlin), and the osmotic potential ($\Psi\pi$) was calculated by the van't Hoff equation (Nobel 1991):

$$\Psi\pi = nRT,$$

where n is mosmol, $R = 0.0083$, and T is temperature (K). The $\Psi\pi$ was determined 1 d, 1 wk, and 2 wk after NaCl application.

Root Hydraulic Conductance (L_0)

Root hydraulic conductance was measured by natural exudation and by pressurizing the roots using the Scholander chamber. For natural exudation, the aerial parts of the plants were removed, leaving the base of the stem, which was sealed with silicone grease into a tapered plastic tube. They were then left for a specific amount of time (2 min for control, 8 min for 40 mM NaCl, or 15 min for 80 mM NaCl), and the sap accumulated was collected in Eppendorf tubes. The roots and the Eppendorf tubes were weighed in a precision balance. Sap flow (J_v) was expressed in $\text{mg g (root fresh weight)}^{-1} \text{h}^{-1}$. The osmolarities of the sap flow and the corresponding nutrient solution were measured. The osmotic pressure difference ($\Delta\Psi\pi$) between the sap flow and nutrient solution was calculated according to their osmolarity values. The hydraulic conductance was determined by the equation

$$L_0 = \frac{J_v}{\Delta\Psi\pi} \text{ mg g (root fresh weight)}^{-1} \text{h}^{-1} \text{MPa}^{-1}.$$

As a second method of determining L_0 , roots were pressurized in a Scholander pressure chamber. The aerial parts of the plants were removed, and the stems were put in plastic tubes. The roots were placed into a pressure chamber with the same nutrient solution that they were grown in, and a gradual increase of pressure (from 0.1 to 0.4 MPa) was applied to the detached roots. The sap that was accumulated in this pressure range during a certain time according to the treatment was collected in Eppendorf tubes. The roots and the tubes were weighed in a precision balance. Sap flow (J_v) was expressed in $\text{mg g (root fresh weight)}^{-1} \text{h}^{-1}$ and plotted against pressure (MPa), with the slope being the L_0 value in $\text{mg g (root fresh weight)}^{-1} \text{h}^{-1} \text{MPa}^{-1}$. The measurements, both by natural exudation and pressurizing, were made in the middle of the photoperiod, 2 wk after applying NaCl.

Stomatal Conductance (G_s)

The measurements of G_s were made using a portable porometer (AP4 porometer, Delta-T Devices) in the adaxial stomata (more stomata exist on the adaxial surface of broccoli leaves; López-Berenguer et al. 2006) of the most recent fully expanded leaves during the middle of the photoperiod.

Measurement of the Apoplastic Pathway

Measurements of the apoplastic pathway of water movement were performed using light green dye (light green SF yellowish; Aldrich Chemical, Gillingham, Dorset), which has the ability to move apoplastically but not symplastically (Epel and Bandurski 1990). Dye solution (250 $\mu\text{mol L}^{-1}$) was added 15 min before each treatment, before collecting sap for the natural exudation and pressure chamber methods. The concentration of the dye was determined immediately with a spectrophotometer (Beckman DU-40UV) at 630 nm. The percentage of apoplastic pathway was calculated from the dye concentration in the sap flow. The concentration of dye in the nutrient solution of each treatment was considered to be 100%.

Ion Analysis

For the anion analysis, xylem sap was diluted and injected into a Dionex-D-100 ion chromatograph. An ionpac AS 124-4 mm (10-32) column and AG 14 (4 × 50 mm) guard column were used. The flow rate was 1 mL min⁻¹, with an eluent of 0.5 mM Na₂CO₃/0.5 mM NaHCO₃. The anion concentration was measured with a conductivity detector and quantified with Chromoleon/Peaknet 6.40 software by comparing peak areas with those of known standards. For cation analysis, an ICP plasma analyzer (IRIS Intrepid II XDL, Thermo Electron) was used.

Light Microscopy

Roots sections (3 mm long) were cut at the apex and 3 and 10 cm from the tip and were fixed with 2.5% glutaraldehyde and 3% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 2.5 h. After three 15-min washes with the buffer, the specimens were postfixed in 1% osmium tetroxide in the same buffer for 2 h. After this, three washes with phosphate buffer were performed, and the samples were left overnight at 4°C. All fixed tissues were dehydrated in a graded series of ethanol (35%, 50%, 70%, 96%, and 100%) and then infiltrated, first with propylene oxide and then with propylene oxide and spurr resin mixture. The samples were then immersed in spurr resin overnight at 4°C. Finally, the samples were embedded in spurr resin. Sections 1 μm thick were cut with a Reichert ultramicrotome and mounted on glass slides. Semithin sections were stained with toluidine blue and observed with a Leica DMR light microscope.

Histochemical Stain for Lignin (Phloroglucinol)

Semithin sections were soaked in 1.0% (w/v) phloroglucinol in 25 : 75 (v/v) HCl-ethanol for 10–15 min. The stained sections were observed and photographed with a Leica DMR light microscope.

Data Analysis

Variance analysis and Tukey's HSD test were carried out to determine differences among treatments, using SYSTAT 9.0 software for Windows.

Results

Effect of Salinity on Osmotic Potential

Root and leaf osmotic potential of broccoli plants decreased significantly after adding NaCl to the nutrient solution. The reduction was greater for leaves and roots from plants treated with 80 mM NaCl (fig. 1), with the differences between 40 and 80 mM NaCl being higher in leaves than in roots. The strongest decrease with respect to control plants was observed during the first day of treatment in roots and leaves for both salinity treatments. After that, the decrease was less and was maintained practically constant until the end of the assay.

Effect of Salinity on Hydraulic Conductance

Root hydraulic conductance (L_0) values of broccoli plants for different treatments were measured by natural exudation

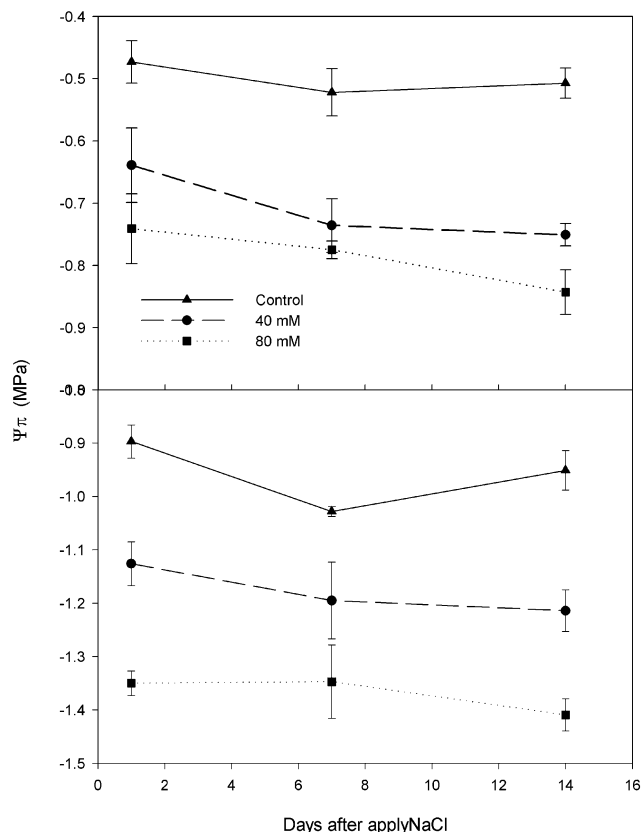


Fig. 1 Osmotic potential (Ψ_{π}) of roots (top) and leaves (bottom) of control and 40 and 80 mM NaCl-treated broccoli plants ($n = 5 \pm SE$).

and by using the Scholander chamber 2 wk after applying NaCl (fig. 2). There were slight differences in the L_0 values between the natural exudation and Scholander chamber methods in control and 40 mM NaCl-treated plants: higher L_0 values were obtained with the pressure chamber. The L_0 , determined by both methods, decreased significantly in plants treated with NaCl, with respect to control plants. This reduction was higher in plants receiving 80 mM NaCl. The L_0 determined by the pressure chamber decreased by 66% and 80% with 40 and 80 mM NaCl, respectively, compared with control plants. The L_0 determined by natural exudation decreased by 45% and 63%, respectively, compared with control plants.

Measurement of Apoplastic and Cell-to-Cell Pathways

The movement of water via the apoplastic pathway was significantly higher when measured by the pressure chamber, with respect to natural exudation, in control plants (table 1). The percentage of water movement via the apoplastic pathway decreased for both methods with increasing NaCl in the nutrient solution. In the absence of NaCl, 75% of water was transported in plants by the cell-to-cell pathway, whereas for plants receiving NaCl, the value was 96%. For the natural exudation technique, 99% of water was transported by the cell-to-cell pathway for all treatments.

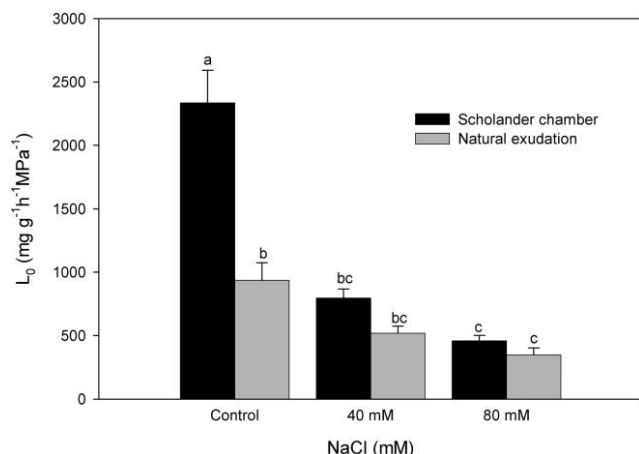


Fig. 2 Root hydraulic conductance (L_0) of control and 40 and 80 mM NaCl-treated broccoli plants, determined using a Scholander chamber or natural exudation 14 d after applying salinity stress ($n = 5 \pm SE$). Columns with the same letters are not significantly different ($P < 0.05$, Tukey test).

Effect of Salinity on Stomatal Conductance

The stomatal conductance (G_s) was measured every 3 d after NaCl application (fig. 3). In control plants, G_s values increased slightly during the first 4 d of measurements. In plants treated with NaCl, the G_s decreased significantly with respect to control plants, with the decrease being greater in plants treated with 80 mM NaCl at all times of measurement.

Xylem Sap Ion Concentrations

The cation concentrations of xylem sap obtained by the natural exudation and Scholander chamber methods were determined in all treatments 2 wk after NaCl application (fig. 4). For the Scholander chamber, the analyzed sap was obtained at a pressure equivalent to transpiration (0.4 MPa). The K^+ concentration was much higher than other cations, and it was much higher when the xylem sap was extracted by natural exudation. Nevertheless, there was no significant difference in the K^+ concentration between treatments separately comparing both methods. The Na^+ , Ca^{2+} , and Mg^{2+} concentrations increased significantly after applying NaCl when the xylem sap was extracted by the Scholander chamber. There were no significant differences in the Mg^{2+} concentrations between control and NaCl-treated plants when the xylem sap was extracted by natural exudation; the Ca^{2+} concentration decreased significantly, and the Na^+ concentration increased.

The analysis of anion concentrations in xylem sap (fig. 5) showed that Cl^- and SO_4^{2-} increased significantly after applying NaCl, being highest in plants treated with 80 mM NaCl. However, the concentration of Cl^- was higher obtained by the Scholander chamber, and the concentration of SO_4^{2-} was higher obtained by natural exudation. The NO_3^- concentrations were higher when the xylem sap was extracted by natural exudation but were significantly increased only by the treatment under pressure. There was no difference in the PO_4^{3-} concentration between control plants and those treated

with NaCl, when the sap was determined by both the natural exudation and Scholander chamber methods.

Effect of Salinity on Root Cell Anatomy

There were no significant effects of NaCl on the length of the roots ($P = 0.445$), with the mean root length being 54.364 ± 1.34 cm. Observations of the thin root sections with the microscope revealed a cell wall modification resembling phi thickenings in the cell walls of the internal cortical layer, proximal to the endodermis (fig. 6). These phi thickenings were present in all treatments, but there were more in plants treated with NaCl, and they were thicker in the presence of 80 mM NaCl (fig. 6C–6F). This thickening increased with time in NaCl-treated plants (fig. 6F). Thickenings were not formed continuously from the root tip to the base but were clearly evident in the portion 10 cm from the tip, the oldest part of the root.

Discussion

Salinity stress affects the water relations in plants (Zhu 2001), since it decreases the water potential in the environment surrounding the roots. This effect leads to an osmotic adjustment that involves the net accumulation of solutes in order to absorb water (Verslues et al. 2006). For this, plants have to decrease their internal water potential, which implies a decrease of the osmotic potential to maintain turgor and achieve osmotic adjustment (Blum et al. 1996). Therefore, the fact that in our plants a decrease in osmotic potential in leaves and roots was observed in relation to NaCl addition (fig. 1) could indicate a good osmotic adjustment. Also, the increase of the Na^+ and Cl^- concentrations detected in the sap flow after the NaCl addition (figs. 4, 5) could support the idea that the osmotic adjustment is produced in part by ion accumulation. These ions are involved in osmoregulation, although both are toxic at high concentrations (Munns 2002; Essah et al. 2003).

The root hydraulic conductance is known to change under high salinity (Carvajal et al. 1999). López-Berenguer et al. (2006) reported a decreased L_0 for broccoli plants 1 wk after applying different concentrations of NaCl. The effect of salinity on the L_0 in other species (Shalhevet et al. 1976; Munns and Passioura 1984; Eвлagon et al. 1990; Azaizeh and Steudle 1991) has been suggested to be due to the osmotic effect of Na^+ and Cl^- . However, Carvajal et al. (1999, 2000) and Martínez-Ballesta et al. (2000, 2004) suggested that the decrease of L_0

Table 1

Apoplastic Pathway Percentage, Measured by Apoplastic Tracer Light Green Dye, for Control and 40 and 80 mM NaCl Plants

Treatments	Scholander chamber	Natural exudation
Control	26.64 ± 3.10^a	$.92 \pm .03^a$
40 mM NaCl	$4.88 \pm .74^b$	$.77 \pm .08^{ab}$
80 mM NaCl	$4.56 \pm .61^b$	$.53 \pm .07^b$

Note. Data determined using a Scholander pressure chamber or by natural exudation 14 d after applying salinity stress ($n = 5 \pm SE$). Means with different letters in columns are statistically different ($P < 0.05$).

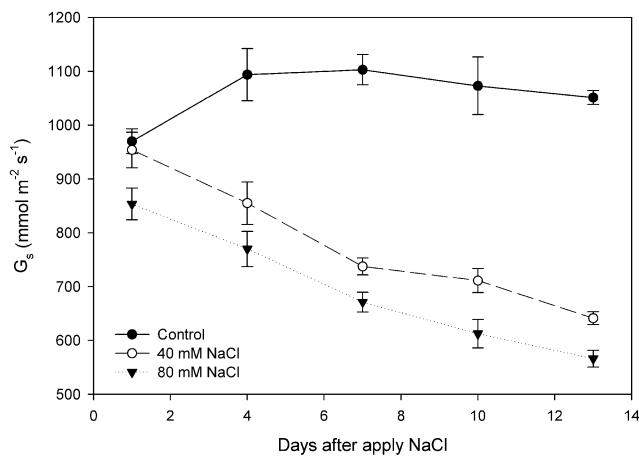


Fig. 3 Leaf stomatal conductance (G_s) of control and 40 and 80 mM NaCl plants ($n = 8 \pm \text{SE}$).

was due to a toxic effect of Na^+ and Cl^- , which mainly reduces the water transport through the plasma membrane. They also reported that a large reduction in root hydraulic conductance was related to the activity or concentration of aquaporins in the root plasma membrane. In our experiments, the higher

concentrations of Na^+ and Cl^- found in the sap and exudate of plants exposed to salinity (figs. 4, 5) could have had a toxic effect on aquaporin functionality. The treatments with NaCl produced a reduction in L_0 with respect to control plants (fig. 2). This decrease was 73% when determined using the Scholander chamber and 54% when determined by natural exudation. The fact that the flows were higher when measured using the Scholander chamber could be a consequence of pressurizing the roots. In this case, water movement occurs through the apoplast to a greater extent than when the measurements are carried out by natural exudation. Similar results were reported by Fernández-García et al. (2002) with tomato plants. This suggestion is confirmed by the data obtained from the experiment with the light green dye (table 1). However, the fact that the apoplastic pathway proportion, which was increased when plants were measured with the Scholander chamber, was not similar for all treatments (being much lower for NaCl-treated plants) suggests that the phi thickenings (fig. 6) could be a physical barrier to apoplastic water transport.

The significant decrease in G_s observed in plants treated with NaCl, with respect to control plants (fig. 3), was probably caused by closure of the stomata (Robinson et al. 1997) or decreased water uptake through the roots. Similar results were reported in pepper (Cabañero et al. 2004; Martínez-Ballesta

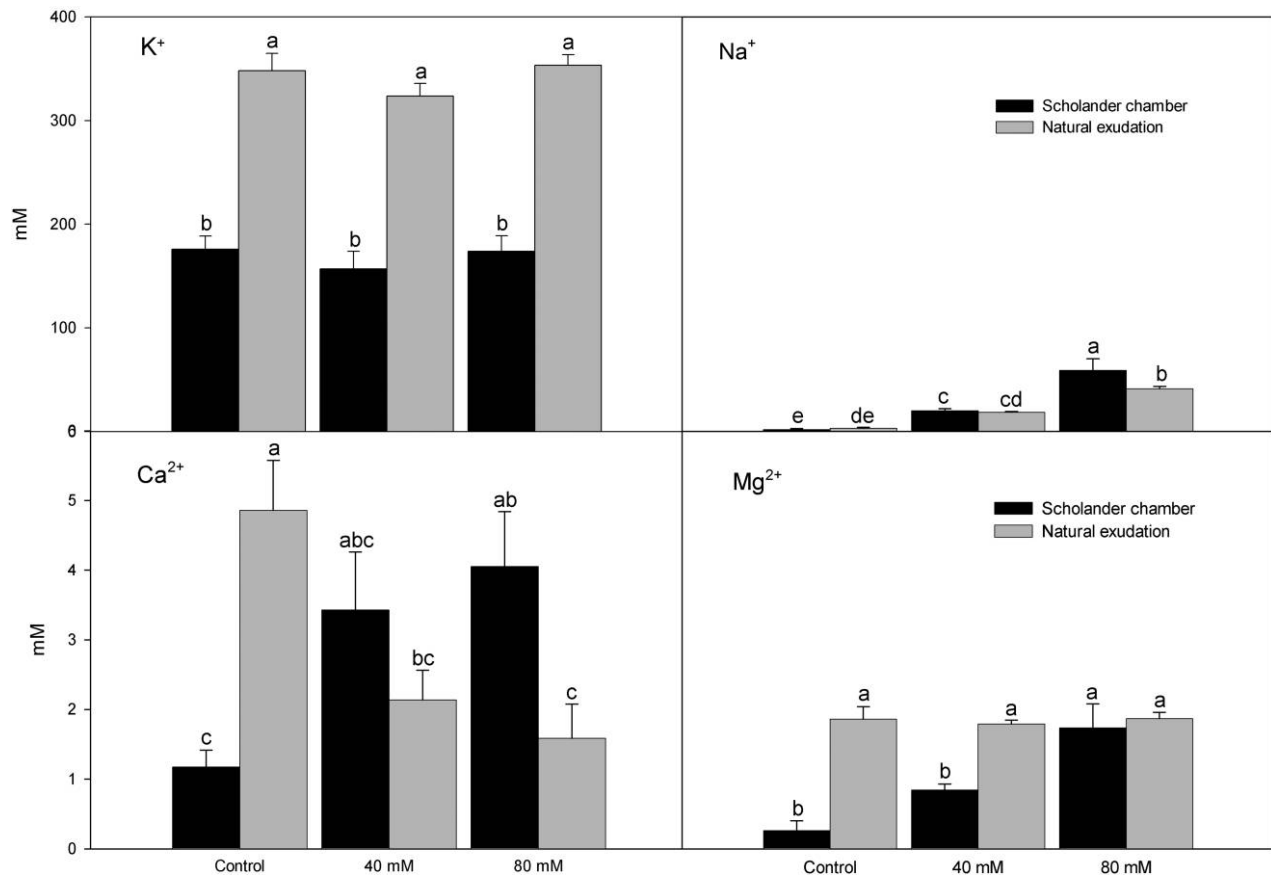


Fig. 4 Cation concentrations (K^+ , Na^+ , Ca^{2+} , and Mg^{2+}) in the xylem sap of control and 40 and 80 mM NaCl-treated broccoli plants, obtained by using natural exudation or a Scholander pressure chamber 14 d after applying salinity stress ($n = 5 \pm \text{SE}$). Columns with the same letters are not significantly different ($P < 0.05$, Tukey test).

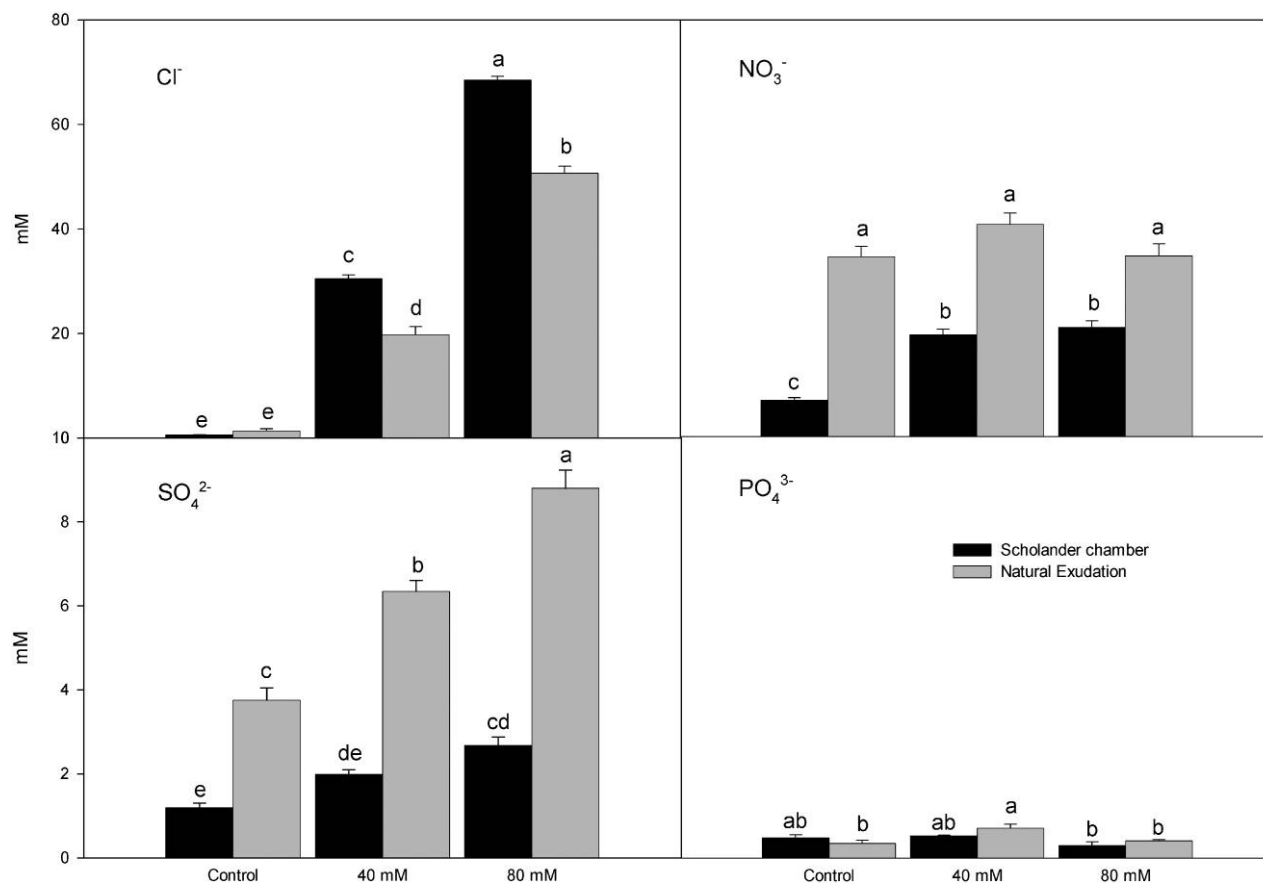


Fig. 5 Anion concentrations (Cl^- , NO_3^- , SO_4^{2-} , and PO_4^{3-}) in the xylem sap of control and 40 and 80 mM NaCl-treated broccoli plants, obtained by using natural exudation or a Scholander pressure chamber 14 d after applying salinity stress ($n = 5 \pm \text{SE}$). Columns with the same letters are not significantly different ($P < 0.05$, Tukey test).

et al. 2004), *Phaseolus* (Bayuelo-Jiménez et al. 2003), and broccoli (Ashraf 2001; López-Berenguer et al. 2006). A reduction in G_s could indicate a certain level of acclimation to salinity stress (Munns and Termaat 1986). The decreased G_s also could be due to higher concentrations of Na^+ and Cl^- in the leaf sap (figs. 4, 5). García-Legaz et al. (1993) and Walker et al. (1993) proposed that the reduction of leaf gas exchange in response to salinity is due to an increase in leaf Na^+ concentration. However, Bañuls et al. (1997) and García-Sánchez et al. (2002a, 2002b) associated reductions in stomatal conductance with high Cl^- concentrations.

The NaCl addition to the nutrient solution obviously caused a significant increase in Na^+ and Cl^- in the xylem sap (figs. 4, 5). The decreased SO_4^{2-} , NO_3^- , and Mg^{2+} concentrations in the xylem when sap was obtained by the Scholander chamber suggest that these ions were diluted as a consequence of pressurizing the roots that caused more water movement through the apoplast. Similar results were reported in tomato (Fernández-García et al. 2002). However, if the flux of solutes into the xylem is calculated for both methods, similar results are obtained (data not shown). The Cl^- concentration obtained by the Scholander chamber was found to be very similar to that of the outside nutrient solution, which could indicate that it is passively transported into the root system. The decrease in Ca^{2+} concentration obtained with salinity has been reported

previously (Savvas and Lenz 1994; Sánchez-Raya and Delgado 1996; Martínez-Ballesta et al. 2004). Under saline conditions, disorders may result from effects on Ca^{2+} availability, competitive uptake, transport, or partitioning within the plant (Grattan and Grieve 1999). Salinity has been shown to induce calcium deficiency in different species and affects water relations to a great extent (Kaya et al. 2002; Cabañero et al. 2004). Our results in previous experiments with pepper plants showed that salinity reduced the concentration of calcium in roots (intra- and extracellular) and that it was restored when Ca^{2+} was added to salinity-stressed plants (Cabañero et al. 2006). However, the fact that the Ca^{2+} concentration was increased with salinity treatments when it was obtained by the Scholander chamber needs to be assessed.

The broccoli plants grown with NaCl showed anatomical root cell changes. The most important modification in the anatomy of the root was phi thickening in some cases, with the typical shape of phi in the internal cortical layer. Two layers were detected (fig. 6D, 6E). It is the first time that the presence of cell wall modifications, like thickenings, has been reported in broccoli as a response to salinity. This thickening has been reported in other *Brassica* species such as *Brassica napus* (Enstone et al. 2003), but this was under flooding stress. Phi thickenings have been described already for other species (Haas et al. 1976; Mackenzie 1979; Peterson et al.

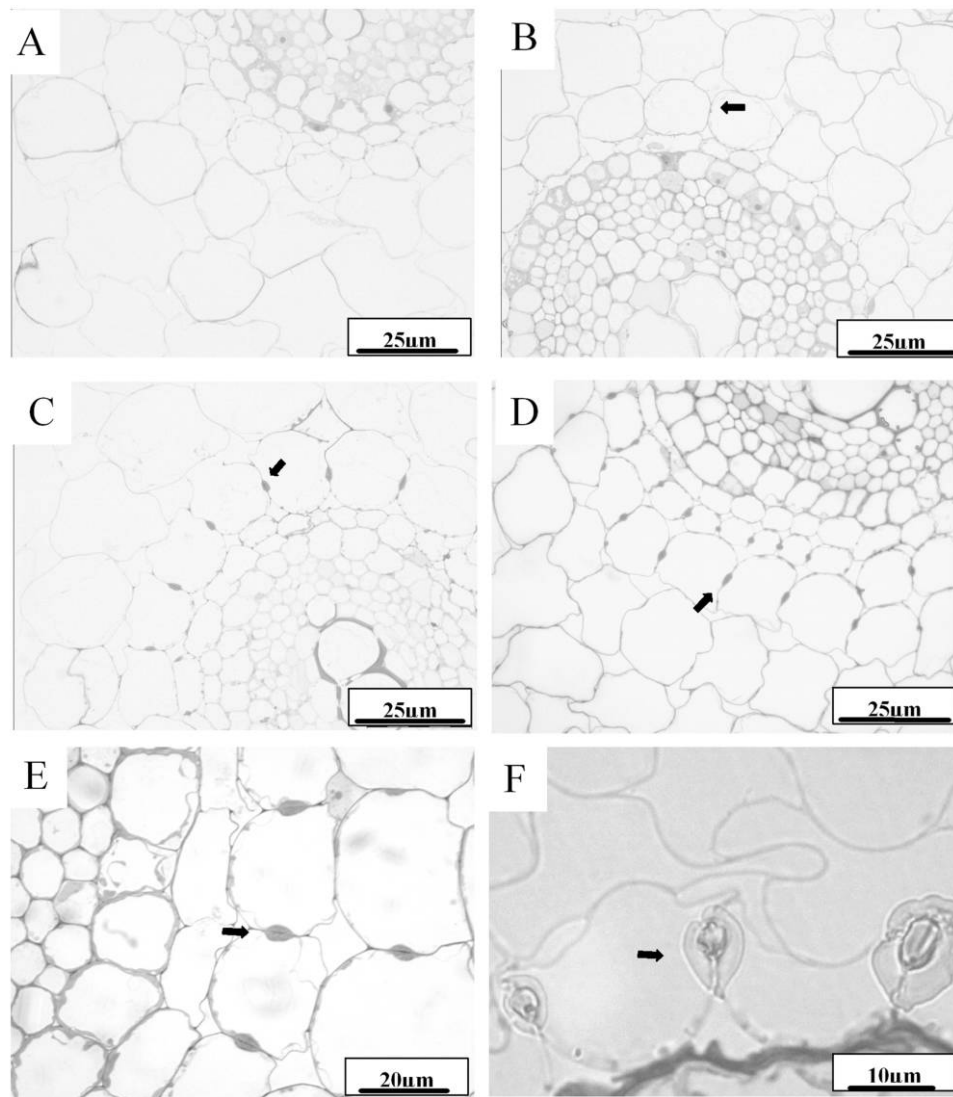


Fig. 6 Light microscopy showing phi thickenings in the cell walls of the cortical layer surrounding the endodermis (arrows) of control broccoli plants (A, B) and of 80 mM NaCl-treated plants (C–F). All images are at 10 cm from the tip and after 14 d of salinity treatment.

1981; Weerdenburg and Peterson 1983; Praktikakis et al. 1998; Degenhardt and Gimmler 2000; Gerrath et al. 2002, 2005; Soukup et al. 2004), but no special conditions were reported to be necessary to induce them. However, in *Cerato-*nia siliqua**, a phi layer appeared only when plants were grown in soil, not in perlite (Praktikakis et al. 1998). Also, in maize, phi thickenings were detected only in slag culture (Degenhardt and Gimmler 2000) but with no alteration under stress conditions. However, in our experiment, phi thickenings increased in salinity-stressed plants. In these plants, the apoplastic pathway was reduced by 60% with respect to control plants, which probably indicates that phi thickenings were affecting the apoplastic inflow of water, but not ions, to the stele. It has been shown that the functionality of aquaporins was greatly reduced in NaCl-treated broccoli plants in the short term (7 d) but to a lesser extent in the longer term (14 d) (López-Berenguer et al. 2006), which is directly re-

lated to water flow through the symplastic pathway. The fact that phi thickening developed after 14 d, when aquaporins functionality was partially restored, might provide an acclimation mechanism under salinity stress, in which plants can control water uptake.

The results of these experiments indicate that modifications in the cell walls of cortical cells could be an acclimation mechanism of broccoli plants to salinity stress. Such a mechanism certainly requires further investigation, but the fact that this layer could act as a physical barrier to the apoplastic pathway of this plant in saline conditions is a novel finding. Although, in the future, it will be a challenge to find out the physicochemical properties of the phi thickening and relate these to the permeability through both the symplastic and apoplastic pathways, the fact that these plants can regulate their water uptake under saline conditions implies a valuable physiological acclimation.

Acknowledgments

We thank Dr. D. Walker for correction of the English in the manuscript. L. López-Pérez was supported by a doctoral

fellowship from the Programa de Mejoramiento del Profesorado (Secretaría de Educación Pública), Mexico. This work was funded by the Centro de Investigación Científica y Tecnológica (AGL2005-00650/AGR).

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