**CSIRO** PUBLISHING

*Functional Plant Biology*, 2013, **40**, 459–465
http://dx.doi.org/10.1071/FP12287

# Effects of NaCl addition to the growing medium on plant hydraulics and water relations of tomato

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**Abstract.** This work reports on experimental evidence for the role of ion-mediated changes of xylem hydraulic conductivity in the functional response of *Solanum lycopersicum* L. cv. Naomi to moderate salinity levels. Measurements were performed in fully developed 12-week-old plants grown in half-strength Hoagland solution (control, C-plants) or in the same solution added with 35 mM NaCl (NaCl-plants). NaCl-plants produced a significantly less but heavier leaves and fruits but had similar gas-exchange rates as control plants. Moreover, NaCl-plants showed higher vessel multiple fraction ($F_{VM}$) than control plants. Xylem sap potassium and sodium concentrations were significantly higher in NaCl-plants than in control plants. When stems were perfused with 10 mM NaCl or KCl, the hydraulic conductance of NaCl plants was nearly 1.5 times higher than in control plants. Accordingly, stem hydraulic conductance measured *in planta* was higher in NaCl- than in control plants. Our data suggest that tomato plants grown under moderate salinity upregulate xylem sap [$Na^+$] and [$K^+$], as well as sensitivity of xylem hydraulics to sap ionic content, thus, increasing water transport capacity.

**Additional keywords:** ionic effect, salt stress, xylem features, xylem hydraulic conductance, xylem sap.

Received 28 September 2012, accepted 7 January 2013, published online 14 February 2013

## Introduction

Soil salinity is a major limiting factor for crop productivity. About 6% of cultivated land is somehow affected by salinity (Munns and Tester 2008) and this percentage is predicted to increase over the next few decades. Salt stress can impact plant growth as a consequence of ion imbalances, toxic effects, osmotic stress, as well as production of reactive oxygen species (Hasegawa *et al*. 2000; Zhu 2001; Mühling and Läuchli 2002). However, several halophytes are known to thrive at high salt concentrations in the soil, as a likely result of their ability to limit $Na^+$ and $Cl^-$ uptake (Watson *et al*. 2001; Tester and Davenport 2003) or eliminate these ions at the leaf level, e.g. by accumulation in specialised salt glands (Fahn 1988). Other salt-tolerant plants are known to transport $Na^+$ and $Cl^-$ into root and leaf cell vacuoles (Blumwald 2000; James *et al*. 2006). Increases in [$Na^+$] and [$Cl^-$] in the vacuole are paralleled by an increase of [$K^+$] to subtoxic levels and production of compatible solutes (sugars, polyols, amino acids and their derivatives), which are accumulated in different subcellular compartments to balance the vacuole osmotic potential (Munns and Tester 2008).

Halophytes typically show optimal growth and productivity at salt concentrations that are strongly limiting or even lethal to most other plant species. Although molecular processes regulating $Na^+$ compartmentalisation in vacuoles have been thoroughly investigated, other essential processes involved in tissue tolerance of high $Na^+$ and $Cl^-$ concentrations remain relatively unknown (Munns and Tester 2008). As an example, little is known about long distance signalling of salt stress as well as regulation of sodium transport and partitioning between xylem and phloem. In particular, xylem transport might play an important role in salt tolerance mechanisms (Lopez-Portillo *et al*. 2005).

Recent studies have shown that changes in the cationic concentration of xylem sap are responsible for short-term changes in xylem hydraulic conductance ($K_{xyl}$, Zwieniecki *et al*. 2001; Nardini *et al*. 2011). This so-called 'ionic effect' is likely involved in the compensation for embolism-induced reduction of $K_{xyl}$ under drought stress (Trifilò *et al*. 2008, 2011) as well as in modulation of water delivery to branches exposed to different environmental conditions (Nardini *et al*. 2010; Sellin *et al*. 2010). Although species-specific differences in the magnitude of the ionic effect have been partially explained on the basis of variability of anatomical and biochemical xylem features (Zwieniecki *et al*. 2001; Boyce *et al*. 2004; Jansen *et al*. 2011), the exact mechanism underlying this phenomenon is still debated. It has been suggested that the ionic effect arises as a

consequence of the polyelectrolytic nature of pectins in intervessel pit membranes (Ryden *et al*. 2000). Cations in the xylem sap may induce shrinking of the pectic matrix in the pit membranes and consequent increase in the dimensions of their pores (Zwieniecki *et al*. 2001; Gortan *et al*. 2011). However, no direct evidence confirming this hypothesis has been provided so far. Nardini *et al*. (2007) reported that modification of homogalacturonan in transgenic lines of tobacco did not influence the ion-mediated hydraulic enhancement of $K_{xyl}$. Moreover, no or very little pectin has been found in the interconduit pit membranes of some Angiosperms (Plavcová and Hacke 2011). In a recent paper, van Doorn *et al*. (2011) proposed alternative hypotheses to explain the ionic effect, suggesting that the behaviour of other polymers (e.g. cellulose or lignin) in pit membranes might explain the phenomenon.

Because cations in the xylem sap can increase $K_{xyl}$, it can be hypothesised that increased $Na^+$ concentration in the xylem sap of plants exposed to soil salinity might improve their water transport capacity, as observed in some mangrove species (Lopez-Portillo *et al*. 2005). In the present study, we tested this hypothesis in plants of tomato, a moderately salt-tolerant crop (Cuartero and Fernandez-Munoz 1998) where $K_{xyl}$ has been reported to be sensitive to changes in sap cationic concentration (Zwieniecki *et al*. 2003).

## Materials and methods

### *Plant material and growth conditions*

Experiments were performed during summer 2010 on 60 plants of a moderately salt-tolerant cultivar of tomato (*Solanum lycopersicum* L. cv. Naomi), grown in a greenhouse at the Dipartimento di Scienze Biologiche e dell'Ambiente, University of Messina. Four-week-old seedlings provided by a local nursery (height = 18 ± 2 cm) were planted in 1 L pots after soil removal from the root system. Pots were filled with expanded clay and provided with half-strength Hoagland solution at pH = 6. The nutrient solution was replaced every 2 days. Three weeks after planting (plant height = 26 ± 5 cm), 35 randomly selected plants were maintained in the half-strength Hoagland solution (control, C-plants) and other 25 plants were additionally supplied with 35 mM $Na^+$ (NaCl-plants). This salt concentration was reached in a 3-week time interval, with weekly increments of 12 mM NaCl. The water potential of the nutrient solution ($\Psi_{soil}$), as estimated using an osmometer (One-ten Osmometer, Fiske Associates, Norwood, MA, USA), was –0.04 ± 0.01 MPa for control plants and –0.21 ± 0.04 MPa for NaCl plants ($n = 5$).

Measurements were performed 2 weeks after reaching the desired NaCl concentration. At this stage, plants were 12–14 weeks old and were bearing many green and some red mature fruits. During the whole experimental period, day/night air temperatures were maintained at 28 ± 3 and 20 ± 3°C respectively. RH and photosynthetic photon flux density (PPFD) were recorded at midday and were 56 ± 2% and 1600 ± 200 µmol $m^{-2}$ $s^{-1}$ respectively.

At the end of each experiment, plant height ($h$), stem diameter above the root collar (Ø), leaf surface area ($A_L$, measured using a portable leaf area meter LI-3000A, Li-Cor Inc., Lincoln, NE, USA), and the number of leaves and fruits per plant were recorded. Leaf and fruit water content and dry mass as well as degree of leaf succulence (maximum water content per unit leaf area) were also measured in eight randomly selected plants per group.

### *Xylem conduit anatomical analysis*

To check the possibility that the amount of grouped xylem conduits had an influence on the magnitude of ionic effect, three shoots from both control and NaCl-plants were collected after hydraulic measurements, cross-sectioned at their middle plane using fresh razor blades and observed under a microscope at a magnification of ×25 (Laborlux S, Leitz GmbH, Stuttgart, Germany) to estimate the vessel multiple fraction ($F_{VM}$, ratio of grouped vessels to total number of vessels, Jansen *et al.* 2011).

### *Xylem sap potassium and sodium concentrations*

To estimate xylem sap [$K^+$] and [$Na^+$] of both control and NaCl-plants, eight shoots were detached by cutting plants at their base between 1200 and 1300 hours. Xylem sap was immediately extracted using the vacuum chamber technique (Améglio *et al.* 2004). All leaves were removed before extraction. Shoot cut surface was repeatedly rinsed with deionised water to remove eventual phloem exudate and debris and then put into a 1.5 mL Eppendorf vial (Sigma-Aldrich, St Louis, MO, USA), which rested in a container filled with ice to minimise evaporation. Xylem sap was extracted under a pressure of –0.08 MPa. Shoots were cut sequentially into ~1.5 cm long segments to open vessels and allow for xylem sap outflow. Potassium and sodium concentrations were measured using $K^+$ and $Na^+$ selective electrodes (Horiba compact ion meter, Horiba Ltd, Kyoto, Japan).

### *Measurements of stem hydraulic conductance ($K_{stem}$)*

Preliminary anatomical measurements of the maximum xylem conduit length for both control and NaCl-plants were performed to select the length of stem samples for hydraulic measurements. Five stems per group were sampled, connected to the hydraulic apparatus (see below), and flushed at a pressure ($P$) of 0.2 MPa to remove eventual emboli. Samples were then injected with silicone (Rhodorsil RTV-141, Rhodia, Cranbury, NJ, USA) mixed with a blue pigment (Pentasol, Prochima, Pesaro, Italy) at $P = 0.5$ MPa for 3 h (Sperry *et al*. 2005). Stems were left in air for 12 h to complete silicone hardening and then cut into 2 cm long segments. Conduits filled with blue silicone were counted under a microscope on cross-sections and referred to the total number of conduits per section. The conduit length distribution was calculated according to Sperry *et al*. (2005). Maximum vessel length was <0.1 m for both control and NaCl-plants.

Hydraulic conductance measurements were performed on eight stems per group, which were detached under water and re-cut to a length of 0.3 m. This sample length maximised the number of intact conduits (see above) as well as the number of interconduit pits to be crossed by the perfused solution (Gascò *et al*. 2006). Stems were connected to the hydraulic apparatus (XYL'EM, xylem embolism meter, Bronkhorst, Montigny les Cormeilles, France), and flushed at $P = 0.2$ MPa for 15 min to remove eventual native emboli. Stem hydraulic conductance ($K_{stem}$) was recorded at $P = 7.5$ kPa for all the tested solutions. The reference solution was a commercial mineral water containing several ions (0.51 mM $Ca^{2+}$, 0.07 mM $Mg^{2+}$,

0.08 mM $Na^+$, 0.046 mM $K^+$, 0.03 mM, $NO_3^-$, 0.96 mM $HCO_3^-$, 0.15 mM $SO_4^{2-}$, 0.01 mM $F^-$), filtered to 0.2 µm. The effect of increasing cation concentrations on $K_{stem}$ with respect the reference solution ($\Delta K_{stem}$) was tested by perfusing stems with solutions obtained by adding to the reference solution either NaCl or KCl adjusted to 10 and 25 mM.

Three plants per treatment were used to check for changes in xylem sap [$K^+$] and [$Na^+$] in the outward perfusion fluid during hydraulic conductance measurements. Stems were connected to the hydraulic apparatus as described above. The cut surface was repeatedly rinsed with deionised water. The stems were flushed with the reference solution at $P = 0.2$ MPa, and sap samples were collected for ~10 s to get a total sap volume of ~15 µL. The outward solution was collected using a pipette and [$Na^+$] and [$K^+$] were measured with ion-selective electrodes. Sap samples were collected every 15 min during the first 90 min of the experiment and every 30 min for the subsequent 150 min.

### *Estimation of whole-plant hydraulic conductance*

Root, stem and leaf hydraulic conductance of eight control and eight NaCl-plants were estimated *in planta* using the evaporative flux method (EFM). The evening before measurements, two apical and two basal leaves were wrapped in plastic film and tin foil to equilibrate leaf water potential ($\Psi_L$) to the xylem pressure ($P_x$) of the adjacent stem. Leaf transpiration rate ($E_L$) was measured between 1130 and 1300 hours using a steady-state porometer (LI-1600, Li-Cor Inc. Lincoln, NE, USA). Immediately after $E_L$ measurements, leaf water potential of apical transpiring leaves ($\Psi_L$) as well as that of wrapped basal and apical leaves ($P_{x\ apical}$ and $P_{x\ basal}$) were measured using a pressure chamber equipped with a precision pressure test gauge (mod. A4A, Ashcroft Inc., Stratford, CT, USA), allowing readability of water potential measurements within a 0.03 MPa interval.

Root, stem and leaf hydraulic conductance ($K_{root}$, $K_{stem}$ and $K_{leaf}$ respectively) were calculated as:

$$K_{root} = E_L/(\Psi_{soil} - \Psi_{x\ basal}), \quad (1)$$

$$K_{stem} = E_L/(P_{x\ basal} - P_{x\ apical}), \quad (2)$$

$$K_{leaf} = E_L/(P_{x\ apical} - \Psi_L). \quad (3)$$

All $K$ values were corrected for water viscosity at a temperature of 20°C.

To check for eventual short-term effects of salt stress on plant hydraulic conductance, five control plants were irrigated with half-strength Hoagland solution enriched with 35 mM NaCl the evening before hydraulic measurements ($NaCl_{S2}$ plants). After hydraulic conductance measurements, shoots of control and $NaCl_{S2}$ plants were tested for xylem sap [$K^+$] and [$Na^+$].

## Results

Control and NaCl-treated plants had similar height, shoot diameter and leaf surface area. However, NaCl-plants produced ~25% less leaves and fruits than control plants (Table 1). Plants growing in salt solution showed an increase in leaf (~25%) and fruit (~35%) dry mass as well as in leaf succulence with respect to control plants (~55%, Table 2). Moreover, they showed statistically higher vessel multiple fraction than controls (0.68 ± 0.04 vs 0.52 ± 0.07; $P < 0.05$).

Xylem sap [$K^+$] and [$Na^+$] were significantly higher in NaCl-plants than in control plants. In particular, [$K^+$] and [$Na^+$] were 11.9 ± 2.4 mM and 0.2 ± 0.5 mM, respectively, in control plants, and 19.0 ± 4.8 mM and 30.0 ± 5.9 mM in NaCl-plants (Fig. 1).

Because no statistical difference was recorded in terms of $\Delta K_{stem}$ induced by 10 mM or 25 mM salt solutions, only the data corresponding to the 10 mM solutions are reported (Fig. 2). $\Delta K_{stem}$ was ~10% in control plants, for both KCl and NaCl

**Table 1. Plant height (*h*), shoot diameter (Ø), mean leaf surface area ($A_L$), number of leaves per plant and number of fruits per plant as measured in control (C-) and salt-treated (NaCl-) plants of *Solanum lycopersicum* cv. Naomi**

Means are given ± s.d. ($n = 8$ per treatment). *P*-values for *t*-test are reported

| | *h* (cm) | Ø (cm) | $A_L$ ($cm^2$) | Number of leaves | Number of fruits |
|---|---|---|---|---|---|
| C-plants | 71.2 ± 5.3 | 0.82 ± 0.10 | 45.8 ± 5.5 | 26.5 ± 6.0 | 11.5 ± 2.3 |
| NaCl-plants | 68.2 ± 5.2 | 0.81 ± 0.03 | 45.5 ± 4.8 | 20.0 ± 3.7 | 8.7 ± 1.6 |
| *P*-value | 0.272 | 0.79 | 0.909 | 0.021 | 0.013 |

**Table 2. Degree of succulence, leaf dry mass, fruit water content and fruit dry mass of control (C-) and salt-treated (NaCl-) plants of *Solanum lycopersicum* cv. Naomi**

Means are given ± s.d. ($n = 8$). *P*-values for *t*-test are reported

| | Degree of succulence ($g\ m^{-2}$) | Leaf dry mass (g) | Fruit water content (gr) | Fruit dry mass (g) |
|---|---|---|---|---|
| C-plants | 34.4 ± 2.2 | 0.22 ± 0.07 | 4.60 ± 1.16 | 0.43 ± 0.08 |
| NaCl-plants | 53.8 ± 4.1 | 0.30 ± 0.07 | 4.62 ± 1.24 | 0.54 ± 0.09 |
| *P*-value | <0.001 | 0.038 | 0.974 | 0.022 |

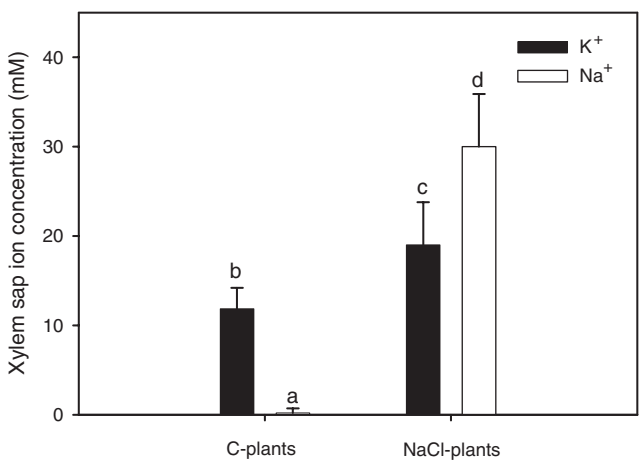


**Fig. 1.** Potassium (black column) and sodium (white column) xylem sap concentration as recorded in control (C-) and salt-treated (NaCl-) plants of *Solanum lycopersicum* cv. Naomi. Means are given ± s.d. ($n = 8$). Different letters indicate significant differences for Tukey pairwise comparisons ($P < 0.001$).

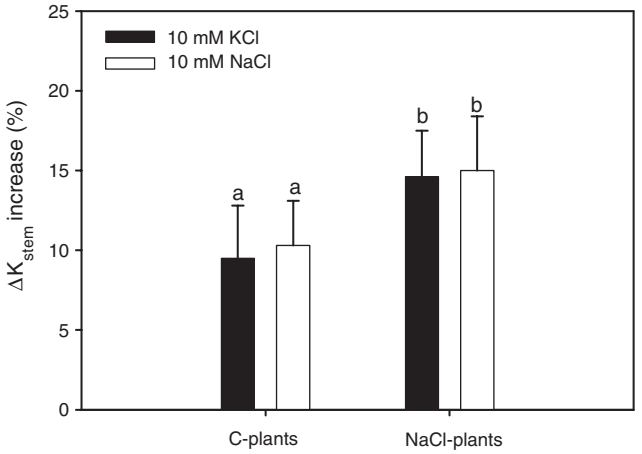


**Fig. 2.** Percentage increase of shoot hydraulic conductance ($\Delta K_{stem}$) as induced by 10 mM KCl (black column) or NaCl (white column) compared with the reference fluid and measured in control (C-) and salt-treated (NaCl-) plants of *Solanum lycopersicum* cv. Naomi. Means are given ± s.d. ($n = 8$). Different letters indicate significant differences for Tukey pairwise comparisons ($P < 0.05$).

solutions. In NaCl-plants, the effect of increasing cation concentrations on $K_{stem}$ was ~50% higher than that measured for control shoots.

Figure 3 reports changes of $[K^+]$ and $[Na^+]$ in the perfused solution collected after flowing through control and salt-treated stems. The first xylem sap samples extracted from control shoots had $[K^+]$ and $[Na^+]$ of $10.6 \pm 3.3$ mM and $0.2 \pm 0.02$ mM respectively. In salt-treated stems, $[K^+]$ was $38.7 \pm 3.4$ mM and $[Na^+]$ was $15.7 \pm 2.3$ mM. Both $[K^+]$ and $[Na^+]$ decreased during long-term perfusion. However, the concentrations of both cations were also significantly higher than that of the inlet reference solution (0.046 mM of $K^+$ and 0.08 mM of $Na^+$). Forty-five minutes after starting the experiments, $[K^+]$ and $[Na^+]$ were $2.39 \pm 0.25$ and $0.08 \pm 0.01$ mM, respectively, for control shoots but as high as $8.0 \pm 1.7$ mM and $12.5 \pm 5.5$ mM, respectively, for NaCl-plants, still higher than in the reference solution.

C- and NaCl-plants showed similar $g_L$ and $E_L$ but differed in terms of leaf water potential (Table 3). In particular, $g_L$ and $E_L$ were ~200 mmol $m^{-2}$ $s^{-1}$ and 3.8 mmol $m^{-2}$ $s^{-1}$, respectively, whereas $\Psi_L$ ranged from $-1.03 \pm 0.09$ MPa in control plants to $-1.25 \pm 0.16$ MPa in NaCl-plants.

Significant differences were also recorded between NaCl- and control plants in terms of $P_{x\ apical}$ and $P_{x\ basal}$ as well as in the pressure drop between stem base and apex ($P_{x\ basal} - P_{x\ apical}$). No differences were recorded in the pressure drop between the xylem at the stem apex and the adjacent transpiring leaves ($P_{x\ apical} - \Psi_L$) (Table 3). Hydraulic conductance values calculated on the basis of the transpiration rate and water potential drop revealed that, despite similar values of $K_{leaf}$ and $K_{root}$, the stem hydraulic conductance of salt-treated plants was 36% higher than that of the control plants ($50.9 \pm 13.0$ vs $37.2 \pm 7.2$ mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$, Fig. 4).

About 24 h after adding 35 mM NaCl to the growing medium, the xylem sap $[Na^+]$ of $NaCl_{S2}$ plants was nearly the same as the external one whereas $[K^+]$ was similar to that recorded in control

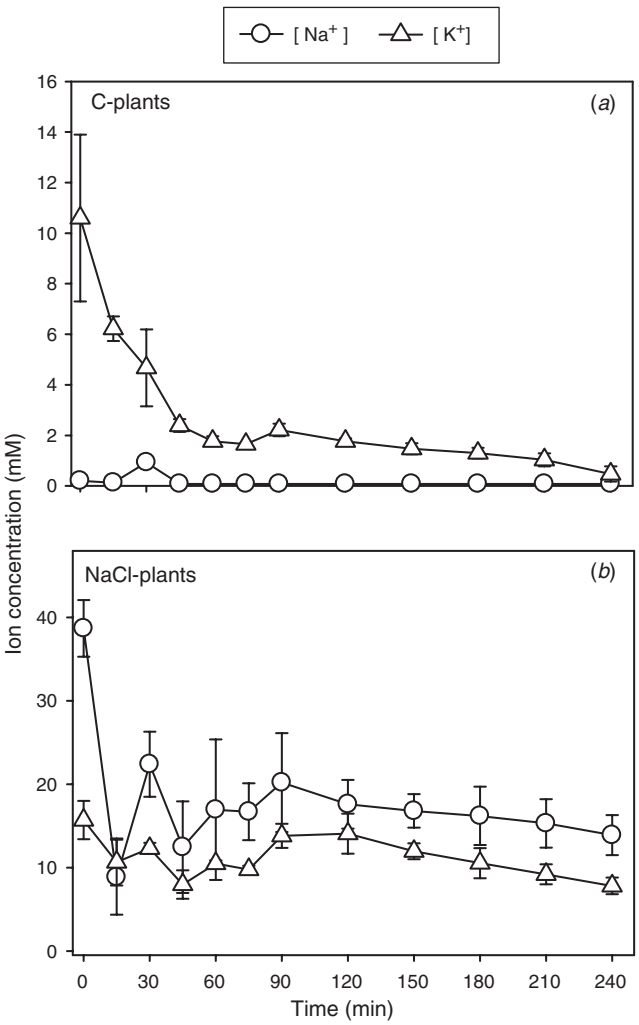


**Fig. 3.** Content of potassium (triangles) and sodium (circles) as recorded in the perfusion fluid collected after flowing through the shoots of control (C-) and salt-treated (NaCl-) plants of *Solanum lycopersicum* cv. Naomi. Means are given ± s.d. ($n = 3$).

**Table 3. Leaf conductance to water vapour ($g_L$), leaf transpiration rate ($E_L$), xylem pressure potential as measured at the stem apex ($P_{x\ apical}$) and base ($P_{x\ basal}$), leaf water potential ($\Psi_L$) and related pressure drops between stem base and apex ($P_{x\ basal} - P_{x\ apical}$) as well as between stem apex and leaves ($P_{x\ apical} - \Psi_L$)**

All variables were measured at midday in control and salt-treated (NaCl-) plants of *Solanum lycopersicum* cv. Naomi. Means are given ± s.d. ($n = 8$). *P*-values for *t*-test are reported

| | C-plants | NaCl-plants | *P*-value |
|---|---|---|---|
| $g_L$(mmol $m^{-2}$ $s^{-1}$) | 208 ± 64 | 190 ± 70 | 0.6 |
| $E_L$(mmol$m^{-2}$ $s^{-1}$) | 3.83 ± 0.53 | 3.87 ± 1.17 | 0.931 |
| $-P_{x\ basal}$ (MPa) | 0.83 ± 0.13 | 1.0 ± 0.07 | 0.006 |
| $-P_{x\ apical}$ (MPa) | 0.90 ± 0.11 | 1.06 ± 0.08 | 0.005 |
| $-\Psi_L$ (MPa) | 1.03 ± 0.09 | 1.25 ± 0.16 | 0.004 |
| ($P_{x\ basal} - P_{x\ apical}$) (MPa) | 0.086 ± 0.02 | 0.062 ± 0.02 | 0.031 |
| ($P_{x\ apical} - \Psi_L$) (MPa) | 0.12 ± 0.03 | 0.18 ± 0.09 | 0.095 |

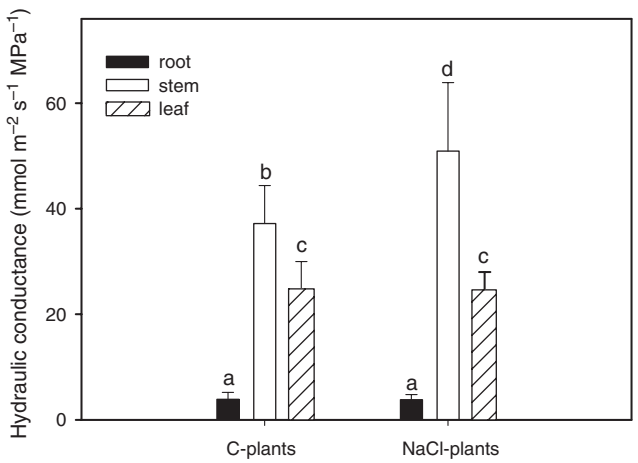


**Fig. 4.** Hydraulic conductance of root (black columns), stem (white columns) and leaves (striped columns) of control (C-) and salt-treated (NaCl-) plants as measured *in planta* using the evaporative flux method (EFM). Means are given ± s.d. ($n = 8$). Different letters indicate significant differences for Tukey pairwise comparisons ($P < 0.05$).

plants (~10 mM) (Table 4). No significant difference appeared in $K_{root}$ and $K_{stem}$ when comparing NaCl- and control plants. In contrast, a 50% reduction of $K_{leaf}$ was recorded in $NaCl_{S2}$ plants when compared with control plants (Table 4).

## Discussion

Moderate salt stress did not induce significant effects on plant size or leaf surface area in tomato plants, but NaCl-plants produced less leaves and fruits than control plants as well as higher vessel multiple fraction. Leaf gas-exchange rates of NaCl- and C-plants were comparable.

The invariance of gas-exchange rates in NaCl-plants with respect to control plants is somewhat surprising. Indeed, stomatal closure is known to be an early response to salt stress, as a likely consequence of osmotic effects at the soil and leaf level (e.g. Downton *et al*. 1985; James *et al*. 2002; Loreto *et al*. 2003). However, other studies reported gas exchanges or photosynthesis rates of salt-stressed tomato to be statistically similar (Gao *et al*. 1998) or enhanced (Xu *et al*. 1995) with respect to control plants. Relatively high gas-exchange rates under mild salt stress can be sustained only if plants are able to maintain adequate water supply to the leaves. Our data suggest that salt-stressed tomato plants might have improved water transport rates to the foliage through ion-mediated enhancement of xylem hydraulic conductance. Further, NaCl-plants showed substantially higher $K_{stem}$ sensitivity to changes in xylem sap cationic concentration, and higher xylem sap [$K^+$] and [$Na^+$], compared with C-plants. Accordingly, xylem hydraulic conductance, as measured *in planta* using the EFM, was higher in NaCl-plants than in control plants.

Ion-mediated increase of $K_{stem}$ was recorded in plants progressively acclimated to salinity. In contrast, tomato plants subjected abruptly to salt stress showed substantial reduction of leaf hydraulic conductance that resulted in strong reduction of gas-exchange rates (data not shown). This suggests that ion-mediated regulation of $K_{stem}$ in response to salt stress requires a period of acclimation. Ion-mediated changes of $K_{stem}$ have been interpreted on the basis of the presence of pectins or other components at the interconduit pit-membranes (Zwieniecki *et al*. 2001; Gortan *et al*. 2011; van Doorn *et al*. 2011). On the basis of our results, changes in vessels grouping patterns apparently played an important role in the acclimation of $K_{stem}$ responses to sap ionic content. Jansen *et al*. (2011) reported a positive correlation between the magnitude of the ionic effect and vessel grouping parameters across 20 angiosperm species. In accordance with our data, the vessel multiple fraction ($F_{VM}$) was higher in NaCl-plants (where higher ion-mediated changes of $K_{stem}$ were recorded) when compared with control plants. This finding suggests that intervessel connectivity plays a role in the magnitude of the ionic effect.

Modifications of xylem anatomy in response to salt stress have been previously reported in the literature, in terms of reduced vessel diameter (Reinhardt and Rost 1995; Baum *et al*. 2000) and increased vessel density (Schmitz *et al*. 2006). To the best of our knowledge, this is the first reported example of positive correlation between resistance to salt stress, xylem anatomical features and possible regulation of long distance transport as mediated by the ionic effect.

In a recent study, Oddo *et al*. (2011) reported clear relationships between short-term potassium fertilisation, potassium uptake and hydraulic conductance in young laurel plants. Because the xylem sap of NaCl-plants showed higher concentrations of both $K^+$ and $Na^+$ compared with C-plants, it is likely that both ions were involved in $K_{stem}$ modulation in tomato plants. In most previous studies, the attention has been focussed on the role of $K^+$ in the ion–mediated changes of $K_{stem}$, because it is the most abundant cation in the xylem sap in glycophytes and represents ~50% of total inorganic ions (Siebrecht *et al*. 2003). In the present study, similar $K_{stem}$ increase was recorded when using iso-osmotic solutions of $Na^+$ or $K^+$. Our data confirm the hypothesis that sodium can substitute for potassium in ion-mediated regulation of xylem hydraulic conductance in salt-resistant species, as also reported for mangroves by Lopez-Portillo *et al*. (2005). Higher concentrations of both $Na^+$ and $K^+$ were recorded in the xylem sap of NaCl-plants with respect to

**Table 4. Xylem sap [K+] and [$Na^+$], hydraulic conductance of roots ($K_{root}$), stems ($K_{stem}$) and leaves ($K_{leaf}$), as measured *in planta* using the evaporative flux method of control (C-) and short-term salt-treated ($NaCl_{S2}$) plants of *Solanum lycopersicum* cv. Naomi**

Means are given ± s.d. ($n = 5$). *P*-values for *t*-test are reported

| | [K+] (mM) | [$Na^+$] (mM) | $K_{root}$ (mmol m$^{-2}$ s$^{-1}$) | $K_{stem}$ (mmol m$^{-2}$ s$^{-1}$) | $K_{leaf}$ (mmol m$^{-2}$ s$^{-1}$) |
|---|---|---|---|---|---|
| C-plants | 10 ± 3 | 0.5 ± 0.2 | 3.8 ± 1.3 | 39 ± 5.4 | 22.2 ± 1.63 |
| $NaCl_{S2}$ plants | 11.4 ± 5.3 | 31 ± 8 | 3.3 ± 1.9 | 41 ± 5.3 | 11.6 ± 2.49 |
| *P*-value | 0.621 | <0.001 | 0.640 | 0.571 | <0.001 |

control plants. Even for plants adopting salt-exclusion strategies, xylem sap [$Na^+$] is a function of the concentration of the ion in the growing medium (Watson *et al*. 2001). Besides sodium, plants growing in saline conditions have higher xylem sap concentrations of other ions like potassium, phosphate and nitrate (Lohaus *et al*. 2000). The mechanism(s) underlying the uptake and accumulation of these ions are still unclear. Tomato plants growing under saline conditions might increase xylem sap [$K^+$] and [$Na^+$] through phloem-to-xylem re-circulation or via solute release by parenchyma cells. Exchanges of nutrients between xylem and phloem are well documented and have received much attention (Zwieniecki *et al*. 2004; Thompson 2006; Metzner *et al*. 2010; Lo Gullo *et al*. 2012). Re-circulation of $Na^+$ from the phloem to the xylem has been reported in different species and is apparently correlated with salt tolerance (Lohaus *et al*. 2000; Berthomieu *et al*. 2003; Tester and Davenport 2003; Davenport *et al*. 2007). However, the possibility that [$Na^+$] and [K+] increase in the xylem sap is due to an active pumping of these ions by nearby parenchyma cells cannot be excluded. Under salt stress, changes in the rate of ion transported across the tonoplast are recorded (e.g. Zhu 2001; Serrano and Rodriguez-Navarro 2001; Reisen *et al*. 2005). Overall, our data offer a possible mechanistic explanation for the positive effects exerted by enhanced xylem sap [$Na^+$] in plants' salt tolerance.

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