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Ammonium, bicarbonate and calcium effects on tomato plants grown under saline conditions

Josefa M. Navarro, Vicente Martínez, Micaela Carvajal *

Departament Fisiología y Nutrición Vegetal, Centro de Edafología y Biología Aplicada del Segura, CSIC. P.O. Box 4195, 30080 Murcia, Spain

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Abstract

Tomato plants (70 days old) were grown in hydroponic culture into a greenhouse, where supply of inorganic carbon, ammonium and calcium to saline nutrient solution, was investigated in order to reduce the negative effect of salinity. After 70 days, an ameliorating effect upon the decrease in growth observed under salinity was only observed with the treatments $NaCl + Ca^{2+}$ and $NaCl + HCO_3^- + NH_4^+ + Ca^{2+}$. A large reduction of hydraulic conductance (L_0) and stomatal conductance (G_s) was observed with all treatments, compared with the control. However, the reductions were less when NaCl and Ca^{2+} were added together. Organic acids (mainly malic acid) in the xylem were decreased with all treatments except with $NaCl + NH_4^+$ and with all single treatments added together ($NaCl + HCO_3^- + NH_4^+ + Ca^{2+}$). Amino acid concentrations in the xylem (mainly asparagine and glutamine) decreased when plants were treated with NaCl and $NaCl + Ca^{2+}$, but there was a large increase in the plants treated with $NaCl + NH_4^+$ or with all treatments together. As HCO_3^- is an important source of carbon for NH_4^+ assimilation, the increase in the concentration of amino acids and organic acids caused by the treatments that contained NH_4^+, support the idea that fixation of dissolved inorganic carbon was occurring and that the products were transported via the xylem to the shoot. The ameliorating effect of Ca^{2+} on root hydraulic conductivity plus the increase of NH_4^+ incorporation into the amino acid synthesis pathway possibly due to dissolved inorganic carbon fixation, could reduce the negative effect of salinity on tomato plants.

1. Introduction

Saline waters for irrigation appear in many semi-arid regions of the world where they inhibit the growth and yields of crops [1]. The physiology of the plant responses to salinity and their relation to salinity resistance have been much researched and frequently reviewed [2,3]. However, one problem is that there is still no clear consensus concerning the physiological traits that are primarily responsible for growth inhibition by salinity [4]. When the saline ions reach the apoplast, the aqueous and ionic thermodynamic equilibria are altered, which results in hyperosmotic stress, ionic imbalance and toxicity. Thus, it becomes vital for exposed plants to re-establish cellular ion homeostasis for proper metabolic functioning and growth. In other words, they have to adapt to the saline environment. Osmotic adjustment helps plant cells to withstand salt stress and water deficits by maintaining sufficient turgor for growth [5]. It involves the transport, accumulation, and compartmentation of inorganic ions and organic solutes [6–8], but even so, a decrease in the hydraulic conductivity of root membranes has been observed [9,5].

Calcium has been shown to ameliorate the adverse effects of salinity on plants [10]. Calcium ions are well known to have regulatory roles in metabolism [11], and sodium ions may compete

Abbreviations: G_s, stomatal conductance; L_0, hydraulic conductance; PEPc, phosphoenol pyruvate carboxylase; OAA, oxaloacetic acid.

* Corresponding author. Tel.: +34-968-396310; fax: +34-968-396213.

E-mail address: mcarvaja@natura.cebas.csic.es (M. Carvajal).

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with calcium ions for membrane-binding sites. Therefore, it has been hypothesised that high calcium levels can protect the cell membrane from the adverse effects of salinity [12]. Probably as a consequence, calcium plays an important role in the water transport of plants growing under salt stress as its concentration in the nutrient solution can determine the restoration of root hydraulic conductivity [9].

It has been reported that HCO_3^- is an important source of carbon for NH_4^+ assimilation in several species [13–15]. This is a consequence of the increase in phosphoenol pyruvate carboxylase (PEPc) activity associated with HCO_3^- uptake [16], which are also increased with NH_4^+ nutrition. Both HCO_3^- and PEP yield oxaloacetic acid (OAA) which is used as an auxiliary carbon skeleton for the amides fixation products.

As salinity has been observed to decrease NO_3^- assimilation, the enhancement of HCO_3^- in the growth medium relieved the limitation of carbon supply for root nitrogen assimilation, thus ameliorating the influence of salinity on hydroponically grown tomato plants [14]. Therefore, in the present investigation, we grew tomato plants under greenhouse conditions for testing whether increases of NH_4^+ and Ca^{2+} concentrations and additions of HCO_3^- to the nutrient solution, could ameliorate the negative effect of salinity. For the study, root hydraulic conductivity and concentrations of amino acids, organic acids and sugars were determined and related to the different treatments.

2. Material and methods

2.1. Experimental design

Seeds of tomato (*Lycopersicon esculentum*, Mill.) cv. Daniella were germinated in peat trays. On 14 May 1999, after removing all peat from the root system, one seedling was transplanted into each of the 36 continuously-aerated 120 l capacity containers with the following macronutrients (mM): 6 KNO_3, 4 $Ca(NO_3)_2$, 1 KH_2PO_4, 1 $MgSO_4$ and the micronutrients (μM) were applied in the form of 20 Fe-EDTA, 25 H_3BO_3, 2 $MnSO_4$, 2 $ZnSO_4$, 0.5 $CuSO_4$ and 0.5 H_2MoO_4. The solutions were prepared using deionized water. During the experiment, the volume of the nutrient solutions was maintained by adding deionized water daily. All solutions were analyzed weekly and readjusted to initial concentrations when necessary. The pH was kept within the range of 6.0–6.5 by adding H_2SO_4 or KOH except for the treatments containing HCO_3^- which gave a higher pH (7).

The experimental design consisted of one saline level, made by the addition of 60 mM NaCl to all treated plants, the substitution of 3 mM NO_3^- by 3 mM NH_4^+, added as 2 mM $NH_4Cl + 1$ mM $NH_4H_2PO_4$ to give a NO_3^-:NH_4^+ ratio of 11:3 (K^+ was then added as KCl), and 6 mM extra Ca^{2+}, added as 6 mM $CaCl_2$, or 2 mM HCO_3^-, added as $NaHCO_3$. Another treatment consisted of all treatments together. Therefore, they was a control and five treatments (NaCl, $NaCl + HCO_3^-$, $NaCl + NH_4^+$, $NaCl + Ca^{2+}$, $NaCl + HCO_3^- + NH_4^+ + Ca^{2+}$). Each treatment had six replicates. Calcium, ammonium and bicarbonate treatments were added on the day of transplantation. The solutions were brought to their correct salinity levels by adding 30 mM NaCl on days 10 and 12 after transplanting to give the 60 mM NaCl.

On 23 June, one day before the harvest, root hydraulic conductance and stomatal conductance were measured. Young fully expanded leaves were frozen and kept for sap extraction.

2.2. Root hydraulic conductance

The method was based on volume flow through detached root systems. The aerial parts of the plant were removed, leaving the base of the stem, which was sealed with silicone grease into a tapered plastic tube. After 2 h, the exuded xylem sap was collected using a Pasteur pipette and transferred to an assay tube. The amount of sap was determined by weight. The roots were removed and weighed. Sap-flow (Jv) was expressed in mg g^{-1} root fresh weight h^{-1}. The samples of sap (50 μl) were placed in Eppendorf tubes and the osmotic potential of the sap and the root bathing medium were measured using an osmometer (Digital Osmometer, Roebling, Berlin), which was calibrated using a standard solution of KNO_3. The osmotic pressure-difference between the xylem sap and the external solution, $\Delta\Psi_\pi$, was calculated from the osmolarity values. The driving force, $\Delta\Psi_\pi$, used to estimate root hydraulic conductance, L_0, which has the units mg g^{-1} root fresh weight h^{-1} MPa^{-1} was:

$$L_0 = \frac{Jv}{\Delta \Psi_\pi}$$

The solute flux in the xylem, *Js*, was estimated as the product of osmolarity and *Jv*.

2.3. Stomatal conductance

Adaxial stomatal conductance to water vapour exchange (G_s) was measured in one leaf per plant with a LI-COR model 1600 diffusion porometer between 10:00 and 12:00 h on 22 June.

2.4. Leaf sap extraction

On 23 June, before the plants were harvested, one leaf from each plant was harvested and put into plastic bags and rapidly frozen with liquid nitrogen. They were subsequently thawed and pressed to extract the cell sap.

The osmotic potential (Ψ_π) of the leaf sap was calculated after measuring sap osmolarity using an osmometer.

2.5. Sugars analysis

The concentrations of glucose and fructose in leaf sap were determined directly by HPLC using a LiCrospher 100 NH_2 5 μm column coupled with a differential refractrometer detector. The mobile phase was acetonitrile:water (85:15) with a flow rate of 1.5 ml min^{-1}.

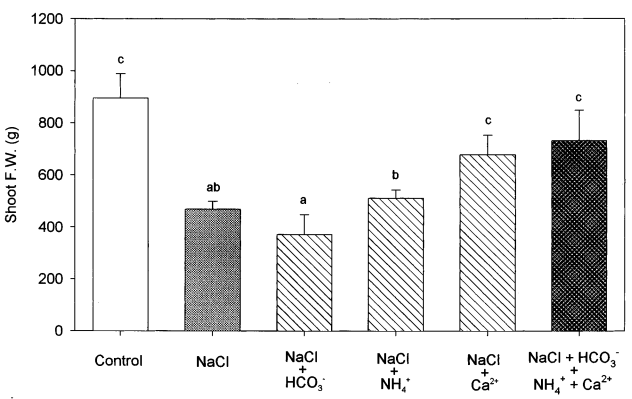

Fig. 1. Shoot fresh weight of control tomato plants and plants grown with NaCl (60 mM), bicarbonate (2 mM), ammonium (3 mM) and/or calcium (10 mM). Each value is mean ± SE ($n = 6$). Bars with the same letter are not significantly different by LSD at 0.05 level.

2.6. Analysis of amino acids and organic acids

Aliquots of xylem and leaf sap were lyophilized in derivatization vials for GLS analysis [17]. The lyophilized extracts were sonicated for 1 h in a bath sonicator with a 1:1 mixture of anhydrous acetonitrile and MTBSTFA (*N*-methyl-*N*-(tert-butyldimethylsisilyl)trifluoroacetamide), and allowed to stand overnight at room temperature. The derivatized mixtures were injected directly in a GC fitted with a capillary TRB-5 column (Tracer), 30 m length, 0.25 mm i.d. The column temperature was programmed from 60 to 150°C at 20°C min^{-1} and from 150 to 300°C at 6°C min^{-1}. GLC flame ionization detection signals were assigned by comparing retention times with those of pure standards run under the same conditions. Compounds were quantified by normalising the total ion response of the identified compounds in the extract against those of the standards.

3. Results

The fresh weight of the shoot (Fig. 1) decreased, compared to the control, when NaCl was applied alone and when plants were treated with $NaCl + NH_4^+$. The greatest decrease was obtained when NaCl was applied with HCO_3^-. However, for the $NaCl + Ca^{2+}$ treatment and when all treatments were applied together, only slight differences were obtained with respect to the control. Similar results were observed for dry weight (data not shown).

The hydraulic conductance (L_0) of the roots (Fig. 2a) was measured from 11 to 13 h when transpiration was maximal [18]. A high reduction of L_0 was observed with all treatments compared with the control. However, the reduction was less when NaCl (60 mM) and Ca^{2+} (10 mM) were added together. The stomatal conductance of the most recent fully expanded leaves (Fig. 2b) was also reduced in all treated plants compared with controls. This reduction was higher in the plants treated only with NaCl and smaller when NaCl was added with Ca^{2+}.

The flux of solutes into the xylem (Fig. 3a) was estimated. A reduction occurred in plants treated only with NaCl, and this was greater when HCO_3^- was added and for all treatments together. However, $NaCl + NH_4^+$ and $NaCl + Ca^{2+}$ treatments

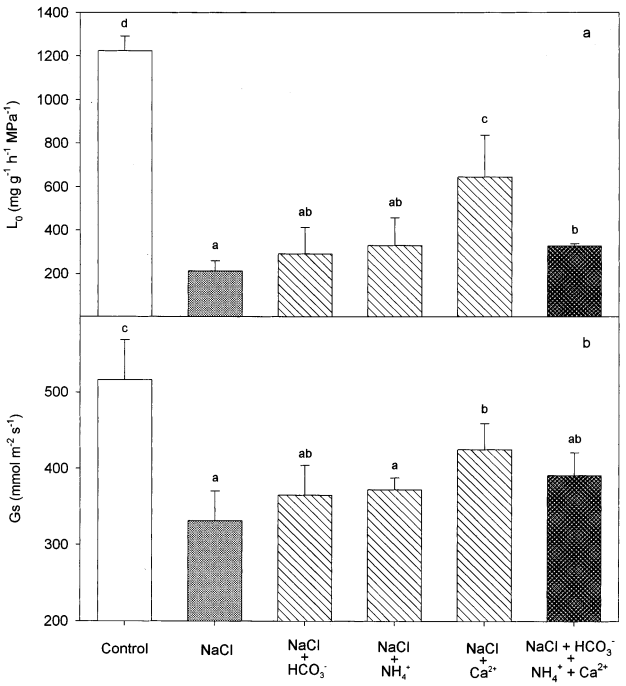

Fig. 2. Root hydraulic conductivity (a) and stomatal conductance (b) of control tomato plants and plants grown with NaCl (60 mM), bicarbonate (2 mM), ammonium (3 mM) and/or calcium (10 mM). Each value is mean ± SE ($n = 6$). Bars with the same letter are not significantly different by LSD at 0.05 level.

produced no significant differences from the control. Organic acids (malic, oxaloacetic, lactic, fumaric, pyruvic, citric) were analyzed in the xylem sap (Fig. 3b). A slight decrease was observed with all treatments except when NaCl and HCO_3^- were combined and when all treatments were added together ($NaCl + HCO_3^- + NH_4^+ + Ca^{2+}$). Amino acids were also analysed in the extracted xylem sap (Fig. 3c). A decrease with respect to the control was observed in plants treated with NaCl and $NaCl + Ca^{2+}$, no differences in those treated with $NaCl + HCO_3^-$ and a high increase in the plants treated with $NaCl + NH_4^+$ or with all treatments together.

When individual amino acids and organic acids were studied, it was observed that only the abundance of some of them change as a consequence of the treatments (Table 1). Asparagine concentration was significantly increased by the treatments $NaCl + HCO_3^-$ and $NaCl + NH_4^+$ but the increase was higher when all treatments were applied together ($NaCl + HCO_3^- + NH_4^+ + Ca^{2+}$). Glutamine was significantly increased in the xylem sap of the plants treated with $NaCl + NH_4^+$ but, in the case of asparagine, the increase was more pronounced when all treatments were applied together. Arginine concentrations were reduced to non-detected levels in the xylem sap of plants treated with $NaCl + HCO_3^-$ and $NaCl + Ca^{2+}$. For all the organic acids determined, only malic acid levels were found to change with the treatments. A great increase was observed in the xylem sap of NaCl treated plants, when HCO_3^- was also added and when all treatments were applied together.

Analysis of leaf sap revealed that the osmotic potential (Fig. 4a) was significantly increased with all treatments compared with control. However, there were no significant differences between treatments. The total organic acid concentration (Fig.

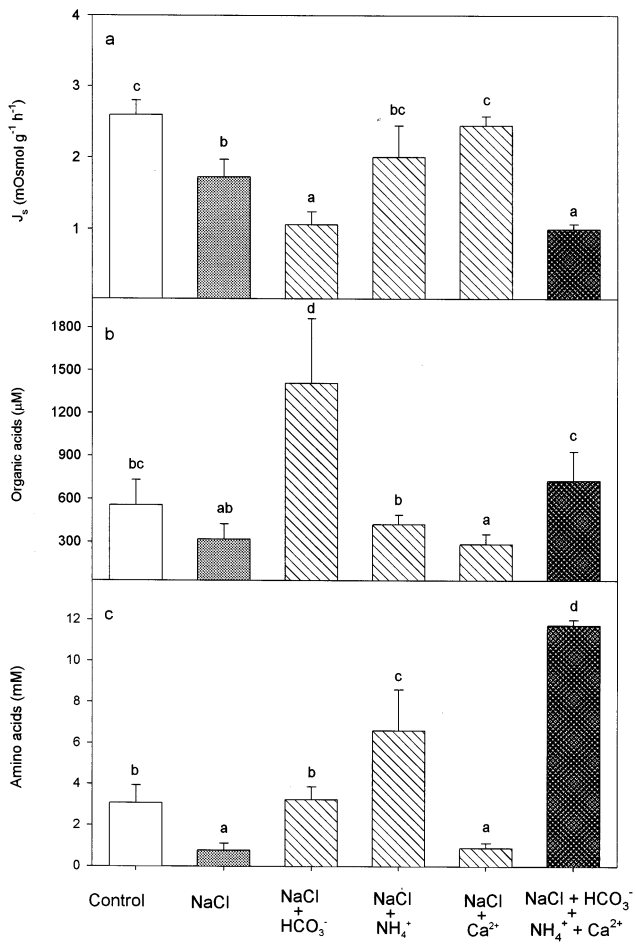

Fig. 3. Flux of solutes into the xylem (a), concentrations of organic acids (b) and amino acids (c) in the xylem sap of control tomato plants and plants grown with NaCl (60 mM), bicarbonate (2 mM), ammonium (3 mM) and/or calcium (10 mM). Xylem was obtained from adult plants under natural exudation. Each value is mean ± SE ($n = 6$). Bars with the same letter are not significantly different by LSD at 0.05 level.

Table 1
Concentrations of amino acids and malic acid in the xylem sap of control tomato plants and plants grown with NaCl (60 mM), bicarbonate (2 mM), ammonium (3 mM) and/or calcium (10 mM)[a]

	Asparagine (μM)	Glutamine (μM)	Arginine (μM)	Malic acid (μM)
Control	∠1	97.4 ± 68.8	2048.2 ± 550.0	∠1
NaCl	∠1	129.6 ± 74.8	1371.9 ± 370.1	139.7 ± 32.9
$NaCl+HCO_3^-$	243.9 ± 86.5	43.2 ± 15.3	∠1	175.8 ± 38.1
$NaCl+NH_4^+$	245.8 ± 70.6	899.1 ± 151.8	3761.9 ± 1046.2	∠1
$NaCl+Ca^{2+}$	∠1	28.5 ± 20.2	∠1	∠1
$NaCl+HCO_3^-+NH_4^++Ca^{2+}$	340.4 ± 33.3	2287.8 ± 616	2260.9 ± 305.5	98.8 ± 28.5

[a] Xylem was obtained from adult plants under natural exudation. Each value is mean ± SE ($n = 6$).

4b) was slightly increased with all treatments, compared with the control but the differences were not significantly different. The total amino acid concentration (Fig. 4c) was increased significantly with all treatments except when NaCl was added alone.

Glucose and fructose were also determined in the sap of the leaves (Fig. 5). Glucose concentration strongly decreased in plants treated with NaCl, $NaCl + NH_4^+$ and $NaCl + Ca^{2+}$. However, when NaCl was added with HCO_3^- and when all treatments were applied together, no significant differences were found with respect to control values. The fructose concentration was slightly increased when NaCl and $NaCl + NH_4^+$ treatments were applied.

4. Discussion

Fresh and dry weights of all salinised plants were significantly reduced in comparison to the control, but the addition of HCO_3^- and NaCl together produced a more negative effect than did salinity alone. Cramer and Lips [13] showed that dissolved inorganic carbon resulted in greater biomass accumulation in leaves and roots of salinised plants. However, they treated the tomato plants with 100 mM NaCl, which is much higher than the concentration used in our experiments (60 mM). In the same way, they applied CO_2 as a carbon supply to the nutrient solution. The reduction in biomass production that is shown in this paper could be due to the high pH (7) caused by the dissolved HCO_3^-, which could affect nutrient uptake. It has been reported that alkalinity by HCO_3^- limited plants growth [19,20] showing that HCO_3^- interfered with nutrient accumulation, but even at the highest level (6 mM HCO_3^-), nutrient deficiencies did not appear. In our experiments, the other treatment containing HCO_3^- ($NaCl + HCO_3^- + NH_4^+ + Ca^{2+}$), the opposing effects of

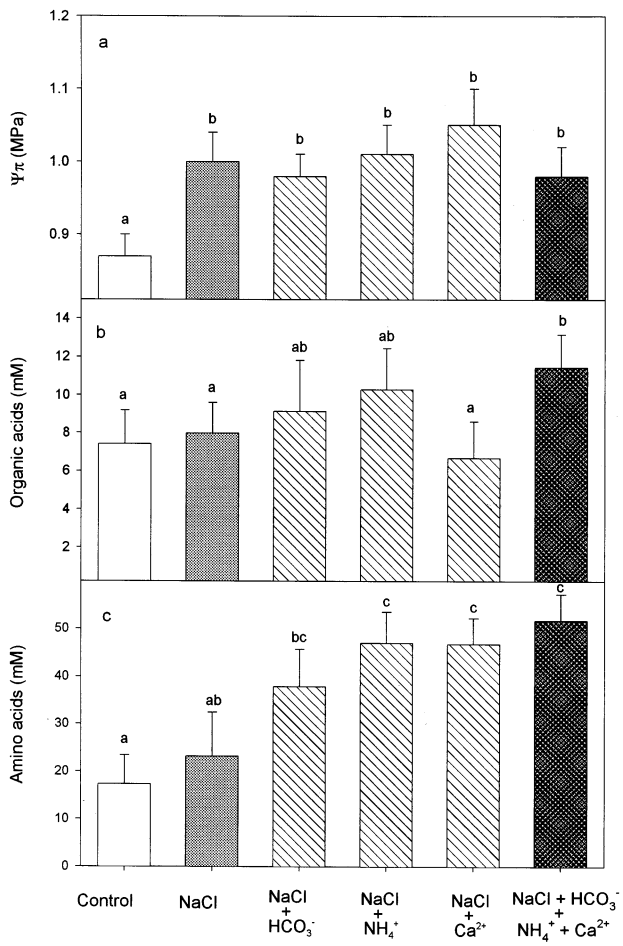

Fig. 4. Osmotic potential (a), concentrations of organic acids (b) and amino acids (c) in the leaf sap of control tomato plants and plants grown with NaCl (60 mM), bicarbonate (2 mM), ammonium (3 mM) and/or calcium (10 mM). Each value is mean ± SE ($n = 6$). Bars with the same letter are not significantly different by LSD at 0.05 level.

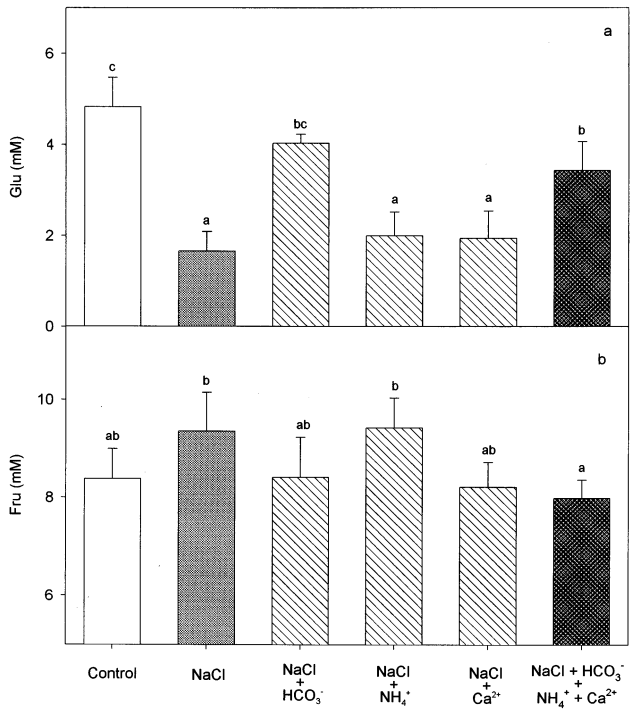

Fig. 5. Concentrations of glucose (a) and fructose (b) in the leaf sap of control tomato plants and plants grown with NaCl (60 mM), bicarbonate (2 mM), ammonium (3 mM) and/or calcium (10 mM). Each value is mean ± SE ($n = 6$). Bars with the same letter are not significantly different by LSD at 0.05 level.

NH_4^+ and HCO_3^- made the pH stable, at around 6.0. In that case, although the HCO_3^- concentration was lowered to 0.5 mM by the decrease in pH, from the nutrient analysis of the xylem sap it could be observed that the nutrient availability was not affected. Therefore, addition of all treatments together had a positive effect on growth compared with the application of NaCl alone, and produced only a small difference in growth with respect to the control.

In the experiments described in this paper, a strong reduction of L_0 in roots of tomato plants after NaCl supply has been observed. Reductions in root hydraulic conductivity, *Lp*, of salinised plants have been shown in several reports [21–23] suggesting that the osmotic concentration had negative effects on *Lp*. However, in our previous work [5] it was reported that NaCl produced a decrease in the L_0 of pepper roots whereas high concentrations of macronutrients had no effect. Therefore, it was concluded that there must have been a toxic effect of NaCl, rather than an osmotic effect on L_0. In the experiments of this paper, Ca^{+2} was able to partially compensate for the toxic effect of NaCl on tomato root hydraulic conductance (Fig. 2a). It also has been reported that short-term experiments performed by adding supplemental calcium to saline growth media only had an ameliorative effect on *Lp* under NaCl stress [9,24]. The beneficial effect of calcium on the development of plants grown under saline conditions reported previously [11,25,26] occurred in our experiments, since only slight shoot weigh reductions compared with the control were found (Fig. 1). Our results also indicate that the addition of extra calcium to salinised plants could restore the flux of solutes into the xylem to a level similar to that of control plants (Fig. 3a).

Transpiration rates generally tend to decline with increasing rhizospheric salinity in non-halophytes. This may be due to lowered water potentials in the roots, and the transfer of abscisic acid from root to shoot as a signal [27], but at high concentration, this reduction in transpiration rate has been attributed to salt damage to the photosynthetic tissue, to stomatal effects with the consequent restriction of the CO_2 availability for carboxylation or to the acceleration of senescence [28]. There is another potential action of NaCl on transpiration, occurring as a result of the direct inhibition of stomatal opening by apoplastic Na^+ concentration [29]. In our plants, the reduction observed in G_s after all saline treatments could be related to the reduction of root L_0, since the smallest reduction was obtained when Ca^{2+} was added with NaCl. Therefore, as all treatments containing NaCl produced similar Na^+ concentrations in the leaves (data not shown), the signal to promote stomatal closure could have been generated in the roots rather than in the leaves, as a consequence of the changes of root L_0.

Analyses of exuded xylem sap can provide valuable information about absorption of water and mineral nutrients by roots. However, there are several factors to be considered in their interpretation. Xylem sap collected from decapitated plants (exudate) represents only that fraction of water flow driven by the root pressure. For the evaluation of the transpirational component, exudates have to be collected, either under vacuum from the cut stump, or by increasing the external pressure in the root zone (pressure chamber). With both methods, xylem volume flow is increased and mineral nutrient concentration usually decreases. However, with these methods, calculated transport

rates to the shoot might be quite different from the results found in intact plants [30,31]. In an earlier study on changes in hydraulic conductance of roots due to nutrient deficiencies, sap-flow data, acquired from the de-topped root, was interpreted as being a result of changes in water-channel function [18]. Therefore, the effect of salinity could decreased the passage of water through the membrane and roots by reducing the activity of Hg-sensitive water-channels [5].

The capacity of the plant to provide carbon skeletons through dark fixation of dissolved inorganic carbon by roots may determine the capacity of the plant to assimilate NH_4^+. However, the increase of the concentration of amino acids caused by the treatment that only contained NH_4^+, in this paper (Fig. 3c) suggested that NH_4^+ assimilation was occurring although to a lesser extent than when HCO_3^- was present ($NaCl + HCO_3^- + NH_4^+ + Ca^{2+}$) and the products transported via the xylem to the shoot. It has been reported [32] that dark fixation of inorganic carbon is unlikely to be related to passive uptake of inorganic carbon (^{14}C) via transpiration, because the label was associated with organic compounds. Therefore, the higher concentrations of amino acids and organic acids in the xylem must be a consequence of the incorporation of carbon into the carbon skeletons necessary for conversion of NH_4^+ into amino acids.

It has been reported that NO_3^- uptake is reduced by salinity [33]. Although it has been suggested that the inhibitory effect of salinity on tomato growth may be partially overcome by increased nitrogen supply [34], other authors [35] showed that the influence of salinity on NO_3^- uptake was associated with anionic interference with NO_3^- translocation across the root plasma membrane. In most plants only part of the nitrate-*N* is reduced to ammonia in the roots while the great part of nitrate-*N* is translocated to the shoot to be assimilated [36]. Hence the assimilation of inorganic *N* in roots is promoted more through ammonium nutrition than through nitrate nutrition [15]. In many species, NH_4^+ nutrition increases the activity of PEPc in the roots, which has been linked to the requirement for carbon, amino acids and organic acid synthesis [37,38]. Oxaloacetate derived from PEP carboxylation may be converted to malate by malate dehydrogenase, be transaminated by aspartate transaminase to form aspartate or may enter the tricarboxylic acid cycle to form 2-oxoglutarate, which may in turn enter the glutamine synthetase/glutamate synthetase pathway for synthesis of glutamine and glutamate. In our plants treated with NH_4^+, a great increase of total amino acids and organic acids was found in the xylem. However, for the treatment consisting of the combination of all ($NaCl + HCO_3^- + NH_4^+ + Ca^{2+}$), the increase of amino acids was much higher than in the other treatments, suggesting that HCO_3^- increased the supply of carbon skeletons necessary for NH_4^+ incorporation into amino acids that were then transported to the shoot. The fact that only the amides asparagine and glutamine were increased to a much higher extent in treatments with NH_4^+ than in the other treatments confirms that NH_4^+ is mainly incorporated into amides. This effect could be the consequence of the higher growth obtained with that combination of treatments. In addition to that, the increase of glucose in the leaf sap of the plants treated with HCO_3^- and with the combination of all treatments suggests that HCO_3^- promoted the synthesis of carbon skeletons, which probably demanded more glucose formation.

In conclusion, in salinised tomato plants, dissolved inorganic carbon could increase root inorganic carbon uptake, providing carbon skeletons for NH_4^+ incorporation into amino acid synthesis. In addition to that, the ameliorating effect of Ca^{2+} on the root hydraulic conductivity reduces the negative effect of salinity on water uptake. Therefore, further research should be carried out in order to investigate the effects of all these factors on fruit yield and quality.

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References

[1] H. Greenway, R. Munns, Mechanism of salt tolerance in nonhalophytes, Annu. Rev. Plant Physiol. 31 (1980) 149–190.
[2] J.M. Cheeseman, Mechanisms of salinity tolerance in plants, Plant Physiol. 8 (1988) 547–550.

[3] R. Munns, Physiological processes limiting plant growth in saline soil: some dogmas and hypotheses, Plant Cell Environ. 16 (1993) 15–24.
[4] P. Neumann, Salinity resistance and plant growth revisited, Plant Cell Environ. 20 (1997) 1193–1198.
[5] M. Carvajal, V. Martínez, C.F. Alcaraz, Physiological function of water-channels, as affected by salinity in roots of paprika pepper, Physiol. Plant. 105 (1999) 95–101.
[6] D.T. Clarkson, Calcium transport between tissues and its distribution in the plant, Plant Cell Environ. 7 (1984) 449–456.
[7] J. Cuartero, R. Fernandez-Muñoz, Tomato and salinity, Sci. Hortic. 78 (1999) 83–125.
[8] N. Dali, D. Michaud, S. Yelle, Evidence for the involvement of sucrose phosphate synthase in the pathway of sugar accumulation in sucrose-accumulating tomato fruits, Plant Physiol. 99 (1992) 434–438.
[9] H. Azaizeh, E. Steudle, Effects of salinity on water transport of excised maize (*Zea mays* L.) roots, Plant Physiol. 97 (1991) 1136–1145.
[10] D.L. Ehret, R.E. Remann, B.L. Harvey, A. Cipywnyk, Salinity-induced calcium deficiencies in wheat and barley, Plant Soil 128 (1990) 143–151.
[11] G.R. Cramer, A. Läuchli, E. Epstein, Effects of NaCl and $CaCl_2$ on ion activities in complex nutrient solutions and root growth of cotton, Plant Physiol. 81 (1986) 792–797.
[12] D.S. Bush, Calcium regulation in plant cells and his role in signalling, Ann. Rev. Plant Physiol. 46 (1995) 95–122.
[13] G.R. Cramer, S.H. Lips, Enriched rhizosphere CO_2 concentrations can ameliorate the influence of salinity on hydroponically grown tomato plants, Physiol. Plant. 94 (1995) 425–432.
[14] M.D. Cramer, A. Schierholt, Y.Z. Wang, S.H. Lips, The influence of salinity on the utilization of root anaplerotic carbon and nitrogen metabolism in tomato seedlings, J. Exp. Bot. 46 (1995) 1569–1577.
[15] M. Ikeda, K. Mizoguchi, T. Yamakawa, Stimulation of dark carbon fixation in rice and tomato roots by application of ammonium nitrogen, Soil Sci. Plant Nutr. 38 (1992) 315–322.
[16] M. Popp, R.E. Summons, Phosphoenolpyruvate carboxylase and amino acid metabolism in roots, Physiol. Veg. 21 (1983) 1083–1089.
[17] A. Ortiz, V. Martínez, A. Cerdá, Effect of osmotic shock and calcium on growth and solute composition of *Phaseolus vulgaris*, Physiol. Plant. 91 (1994) 468–476.
[18] M. Carvajal, D.T. Cooke, D.T. Clarkson, Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function, Planta 199 (1996) 372–381.
[19] R.C. Pearce, Y. Li, L.P. Bush, Calcium and bicarbonate effects on the growth and nutrient uptake of burley tobacco seedlings: float system, J. Plant Nutr. 22 (1999) 1079–1090.
[20] R.C. Pearce, Y. Li, L.P. Bush, Calcium and bicarbonate effects on the growth and nutrient uptake of burley tobacco seedlings: hydroponic culture, J. Plant Nutr. 22 (1999) 1079–1090.
[21] D. Evlagon, Y. Ravina, P.M. Neumann, Interactive effects of salinity and calcium on hydraulic conductivity, osmotic adjustment and growth in primary roots of maize seedlings, Isr. J. Bot. 39 (1990) 239–247.
[22] R. Munns, J.B. Passioura, Hydraulic resistance of plants. III.: effects of NaCl in barley and lupin, Aust. J. Plant Physiol. 11 (1984) 351–359.
[23] J. Shalhevet, E.V. Mass, G.J. Hoffman, G. Ogata, Salinity and the hydraulic conductance of roots, Physiol. Plant. 38 (1976) 224–232.
[24] H. Azaizeh, B. Gunse, E. Steudle, Effects of NaCl and $CaCl_2$ on water transport across root cells of maize (*Zea mays* L.) seedlings, Plant Physiol. 99 (1992) 886–894.
[25] G.R. Cramer, A. Läuchli, V.S. Polito, Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells, Plant Physiol. 79 (1985) 207–211.
[26] G.R. Cramer, E. Epstein, A. Läuchli, Kinetics of root elongation of maize in response to short-term exposure to NaCl and elevated calcium concentration, J. Exp. Bot. 39 (1988) 1513–1522.
[27] J. Zhang, W.J. Davies, Antitranspirant activity in xylem sap of maize plants, J. Exp. Bot. 42 (1991) 317–321.
[28] M. Pessarakli, Handbook of Plant and Crop Stress, Marcel Dekker, New York, 1994.
[29] L.K.R.R. Parera, T.A. Mansfield, A.J.C. Malloch, Stomatal responses to sodium ions in *Aster tripolium*: a new hypothesis to explain salinity regulation in above-ground tissues, Plant Cell Environ. 17 (1994) 335–340.
[30] M. Salim, M.G. Pitman, Pressure-induced water and solute flow through plant roots, J. Exp. Bot. 35 (1984) 869–881.
[31] S. Allen, J.A. Raven, J.I. Sprent, The role of long-distance transport in intracellular pH regulation in *Phaseolus vulgaris* grown with ammonium or nitrate as nitrogen source, or nodulated, J. Exp. Bot. 39 (1988) 513–528.
[32] G.R. Cramer, O.M.A. Lewis, S.H. Lips, Inorganic carbon fixation and metabolism in maize roots as affected by nitrate and ammonium nutrition, Physiol. Plant. 89 (1993) 632–639.
[33] M.A. Botella, A. Cerdá, S.H. Lips, Kinetics of NO_3^- and NH_4^+ uptake by wheat seedlings: effect of salinity and nitrogen source, J. Plant Physiol. 144 (1994) 53–57.
[34] A.P. Papadopoulos, V.V. Rendig, Interactive effect of salinity and nitrogen on growth and yield of tomato plants, Plant Soil 73 (1983) 47–57.
[35] M. Aslam, R.C. Huffaker, D.W. Rains, Early effects of salinity on nitrate assimilation in barley seedlings, Plant Physiol. 76 (1984) 321–325.
[36] Y. Oji, G. Izawa, Quantitative changes of free amino acids and amides in barley plants during ammonia and nitrate assimilation, Plant Cell Physiol. 13 (1972) 249–259.
[37] P. Schweizer, K.H. Erismann, Effect of nitrate and ammonium nutrition of non-nodulated *Phaseolus vulgaris* L. on phoshoenolpyruvate carboxylase and pyruvate kinase activity, Plant Physiol. 78 (1985) 455–458.
[38] P.A. Arzonis, J.A. Niemans, G.R. Findenegg, Phosphoenolpyruvate carboxylase activity in plants grown with either NO_3^- or NH_4^+ as inorganic nitrogen source, J. Plant Physiol. 132 (1988) 23–27.