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Different cation stresses affect specifically osmotic root hydraulic conductance, involving aquaporins, ATPase and xylem loading of ions in *Capsicum annuum*, L. plants

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Summary

In order to study the effect of nutrient stress on water uptake in pepper plants (*Capsicum annuum* L.), the excess or deficiency of the main cations involved in plant nutrition (K^+, Mg^{2+}, Ca^{2+}) and two different degrees of salinity were related to the activity of plasma membrane H^+-ATPase, the pH of the xylem sap, nutrient flux into the xylem (J_s) and to a number of parameters related to water relations, such as root hydraulic conductance (L_0), stomatal conductance (g_s) and aquaporin activity. Excess of K^+, Ca^+ and NaCl produced a toxic effect on L_0 while Mg^{2+} starvation produced a positive effect, which was in agreement with aquaporin functionality, but not with ATPase activity. The xylem pH was altered only by Ca treatments. The results obtained with each treatment could suggest that detection of the quality of the nutrient supply being received by roots can be related to aquaporins functionality, but also that each cation stress triggers specific responses that have to be assessed individually.

Introduction

An excess or absence of the main elements in plant nutrition can cause disorders with respect to nutrient availability, uptake, transport or partitioning within the plant. In addition, solubility and competition factors may result in deficiencies of

other nutrients, as well as a variety of physiological disorders (for example, related to water balance). Some of the parameters of water relations that can be affected include the hydraulic conductance of roots, stomatal conductance in the leaves, and, at the cellular level, the activity of aquaporins (Clarkson et al., 2000).

In plants, water transport across tissues is a fundamental process that must be adapted sufficiently to different environmental conditions in order to allow plants to survive. Similarly, nutrient uptake is a related function which, in most cases, depends on water movement. Under conditions of high transpiration, water moves passively across the roots at a high rate, whereas, under high humidity and in the absence of transpiration, the ascending water flow to the shoot is generated by root pressure with low flow rates (Steudle, 2000). Aquaporins, membrane channel proteins that facilitate and regulate the permeation of water across biological membranes (Baiges et al., 2002), have been found in the plasma membrane and tonoplast. Aquaporins make different contributions to water transport, depending on the environmental conditions. They are part of the mechanism of the transcellular pathway (Steudle, 2000), together with simple diffusion across the plasma membrane (Carvajal et al., 1998). In this way, water flow may be regulated by the opening and closing of aquaporins, which have been shown to be affected by various environmental factors, such as salinity (Cabañero et al., 2004), nutrient deprivation (Carvajal et al., 1996) and drought (North and Nobel, 2000). The molecular and cellular mechanisms that link hormonal, nutrient or stress stimuli to the activity of aquaporins in root cell membranes remain poorly understood.

The plasma membrane H^+-ATPase plays an essential role in the maintenance of plant cell turgor and extracellular pH. Using energy released by hydrolysis of ATP, the H^+-ATPase exports protons to create an electrochemical gradient across the plasma membrane, which is then used by the cell as the driving force for ion and metabolite transport. Regulation of this enzyme may play an important role in the response of plants to different stressing environments, in order to maintain control of ion transport. Also, previous studies have shown that aquaporins can be gated by protons (Gerbeau et al., 2002), indicating that H^+-ATPases may have an important role in water transport across plant membranes (Luu and Maurel, 2005).

The pH of the xylem sap is very relevant for loading/unloading processes, as it constitutes the driving force for antiport and symport activities and is the regulator of ion transporters or signalling molecules. Measurements on xylem sap collected from root exudates have shown a slightly acidic pH in the range of 5.5–6.5 (López-Millán et al., 2000). The xylem appears to exhibit a high capacity to maintain pH at a set value (Senden et al., 1992). However, in response to stress conditions, the xylem pH can fluctuate (Wilkinson and Davies, 1997), likely due to alterations in the H^+-ATPase or H^+-exchange systems in the plasma membrane of vessel-associated cells.

Potassium, calcium and magnesium are essential macronutrients and are normally the most abundant cations in plants. Potassium is essential for many metabolic processes and is a major contributor to cell turgor, whereas calcium plays a number of roles in stabilising cell walls and membranes and as a secondary messenger. Magnesium acts as a bridging element, forming complexes of different stabilities, which confers upon it a high capacity to interact with strongly nucleophilic ligands. In contrast, Na^+ is normally a non-essential element that is toxic to many plant species when present in high concentrations. Physiological interactions between these cations are well-documented (Carvajal et al., 2000a, b).

Often, an excess of certain nutrients in the external solution can be overcome by a controlled xylem-loading system, where the nutrients are introduced into the xylem stream and delivered to the whole plant. Since the fluxes and loading of Mg^{2+} and Ca^{2+} appear to be strongly related with the water stream, aquaporins and divalent-cation channels can mediate these processes. Sodium entry into the root symplast is most likely mediated by weakly-voltage-dependent, non-selective cation channels (Demidchik and Tester, 2002). Potassium loading into the root xylem may be a pH-sensitive process, the pH modulating the activity of K^+ channels responsible for loading K^+ into the xylem (Lacombe et al., 2000). Whereas the K^+ channel responsible for K^+ uptake into the root symplast is AKT1, xylem-loading of K^+ is regulated separately from K^+ uptake from the external solution (Engels and Marschner, 1992).

The aim of the present work was to examine the effects of an excess or deficiency of the main cations in plant nutrition (K^+, Mg^{2+}, Ca^{2+}), and of two different degrees of salinity with the water relations and xylem ion loading in pepper plants. Our hypothesis was that nutrient uptake disorders might influence plasma membrane H^+-ATPase activity, a parameter that is related closely to pH, nutrient flux into the xylem and to diverse parameters of water relations such as root hydraulic conductance, stomatal conductance and aquaporin activity.

Material and methods

Plant culture

Seeds of pepper (cv. California) were imbibed with deionised water for two days and placed in an incubator chamber at 30 °C, in darkness. The seeds were placed in trays with vermiculite as substrate. After 4–5 days, they were placed in 15-l containers (about 30 plants per container), containing a 50% modified-Hoagland nutrient solution (Epstein, 1972), and transferred to the definitive 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% (light period) and 80% (dark) and the photosynthetically active radiation (PAR) was 400 $\mu mol\, m^{-2}\, s^{-1}$, 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 approximately 15 days of growth, plants were transferred to new containers (with the same characteristics), where different treatments were carried out.

In order to study the influence of the nutritional state on the water relations of the pepper plants, we designed eight different treatments, plus the control (Hoagland nutrient solution at half-strength macronutrient concentrations). Treatments were modified in order to eliminate one cation or to add it in excess such that we obtained plants grown with deficit or excess of K^+, Mg^{2+} or Ca^{2+}, and with low and high salinity (10 and 100 mM NaCl,

respectively). The composition of each treatment was shown in Table 1.

Determinations were carried out during three consecutive days after treatment application. Also, in order to estimate the capacity of recovery from or the persistence in the changes which occurred, fresh nutrient solution was added again after the three days of stress application, and measurements were obtained after 24 h. Every 24 h, we used five plants to obtain different measurements. Therefore, each value is the average of five plants ±SE.

Root hydraulic conductance (L_0)

These measurements were obtained by assessing the natural exudation of detached roots. The aerial parts of the plant were removed, leaving a cylinder of leaf bases, which were sealed with silicone grease into tapered glass tubes. After 1 h (discarding the first 15 min), the exuded xylem sap was collected using Pasteur pipettes and transferred to Eppendorf tubes. The sap was weighed and the roots were removed and weighed. Sap flow was expressed in $mg\,(g\ root\ FW)^{-1}\,h^{-1}$. The osmotic potentials of the sap samples and the nutrient solution were measured using an osmometer (Digital osmometer, Roebling, Berlin, FRG). The osmotic potential difference between the xylem sap and the external solution, $\Delta\Psi_\pi$, was calculated from their osmolarity values. The hydraulic conductance, L_0, which has the units $mg\,(g\ root\ FW)^{-1}\,h^{-1}\,MPa^{-1}$, was

$$L_0 = J_v/\Delta\Psi_\pi,$$

Table 1. Composition (mM) and osmolalities (mOsm) of Hoagland modified nutrient solution for each treatment

	Control	$-K^+$	$+K^+$	$-Mg^{2+}$	$+Mg^{2+}$	$-Ca^{2+}$	$+Ca^{2+}$	$+Na^+$	$++Na^+$
KNO_3	3	—	3	3	3	3	3	3	3
$Ca(NO_3)_2$	2	3	2	2	2	—	20	2	2
KH_2PO_4	0.5	—	—	0.5	0.5	0.5	0.5	0.5	0.5
K_sHPO_4	—	—	13.5	—	—	—	—	—	—
$MgSO_4$	0.5	1	0.5	—	20	0.5	0.5	0.5	0.5
Micronutrients	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Fe-EDTA	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
$MgCl_2$	—	—	—	—	20	—	—	—	—
KCl	—	—	20	—	—	—	—	—	—
NaCl	—	—	—	—	—	—	—	10	100
$CaCl_2$	—	—	—	—	—	—	30	—	—
NaH_sPO_4	—	0.5	—	—	—	—	—	—	—
NH_4NO_3	—	1	—	—	—	—	—	—	—
$(NH_4)_2SO_4$	—	—	—	0.5	—	—	—	—	—
$NaNO_3$	—	—	—	—	—	2	—	—	—
$Mg(NO_3)_2$	—	—	—	—	10	1	—	—	—
Osmolality	11	10	73	15	99	13	124	32	181

[a]Normal values of Hoagland nutrient solution (Epstein, 1972).

where $J_v = $ mg xylem sap h^{-1} g $root^{-1}$ and $\Delta\Psi_\pi = \psi_0$ xylem sap$-\psi_0$ solution.

Anion and cation analysis

The xylem sap collected (25 μl) was filtered, diluted and injected into a Dionex-D-100 ion chromatograph with an Ionpac AS12A 4 mm (10–32) column and guard column. Chloride, NO_3^-, SO_4^{2-} and PO_4^{3-} were detected with a conductivity detector and quantified by comparing peak areas with those of known standards. The flow rate was 1.5 ml min^{-1} with an eluent of 2.7 mM Na_2CO_3/ 0.3 mM $NaHCO_3$.

The Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations in xylem sap were determined directly by atomic absorption spectrometry (Perkin-Elmer, 5500).

Stomatal conductance (g_s)

This was measured for intact leaves of living plants. Adaxial stomatal conductance to water vapour exchange (g_s) was measured in the first fully expanded leaf with a porometer (AP4 model, Delta-T Devices, Cambridge, UK), at the middle of the light period.

pH

The pH of the xylem sap obtained by natural exudation was measured directly in all samples after a 1:10 dilution with distilled water.

Aquaporin functionality

To examine whether aquaporins sensitive to Hg were present, 50 μM $HgCl_2$ was added to the nutrient solution of plants already used for measuring L_0. After 5 min, fresh nutrient solution without Hg was supplied and L_0 was again measured. Mercury was scavenged from roots with DTT (5 mM), and then L_0 was measured after this treatment.

Flow of solutes into the xylem (J_s)

The flow of different ions to the xylem, J_s, calculated as the product of sap flow and the ion concentration in the sap, was determined in every treatment for cations (K^+, Mg^{2+}, Ca^{2+} and Na^+) and anions (Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-}).

Plasma membrane isolation

Plasma membranes used in the ATPase assay were isolated using the two-phase aqueous polymer technique (Larsson et al., 1987). Approximately 20 g of fresh apical root material (3–5 cm from the tip; 16 4d-treated plants each sample, three samples per treatment) were finely chopped and vacuum-infiltrated with 40 ml of 50 mM Hepes plus 0.5 M sucrose, adjusted to pH 7.5 with NaOH, plus 1 mM DTT, 5 mM ascorbic acid and 0.6% insoluble PVP (w/v). The buffer-saturated material was homogenised using a pestle, mortar and sand, and filtered through a 240-μm-pore diameter nylon cloth. The filtrate was centrifuged for 15 min at 10,000g, at 4 °C (J2-21 centrifuge, Beckman, Palo Alto, CA, USA), and the supernatant further centrifuged at 100,000g for 35 min (L8-70 M Ultra-centrifugue, Beckman, Palo Alto, CA, USA) to yield a microsomal pellet, which was resuspended in 2 ml of 0.33 M sucrose in 5 mM phosphate buffer (pH 7.8). The suspension (2 ml) was added to 6 g of an aqueous two-phase mixture, producing 8 g of a two-phase system with a final composition of 6.2% (w/w) dextran T500 (Pharmacia), 6.2% (w/w) poly-ethyleneglycol (PEG) 3350 (Sigma), 3 mM KCl, 5 mM phosphate buffer pH 7.8 and 0.33 M sucrose. The phase system was centrifuged for 5 min at 4000g. The resulting plasma membranes (upper phase) were purified using a batch procedure (Larsson et al., 1987). The third upper phase was diluted with phosphate buffer pH 7.8 and centrifuged at 100,000g for 35 min; the resulting pellet was re-suspended in 0.9 ml of 5 mM MES-Tris plus 330 mM sucrose, pH 6.5, and stored at -20 °C. After membrane extraction, the protein content of samples was determined by the method of Bradford (1976), using the Bio-Rad reagent with BSA as standard.

ATPase activity

ATPase activity was measured in root plasma membrane vesicles (previously isolated as explained above) from 3d-treated plants by hydrolysis of ATP and the subsequent release of inorganic phosphate, using the method described by Coupland et al. (1991). To determine the "basal" activity, the assay medium contained 2 mM $MgCl_2$, 2 mM Na-ATP, 0.1 mM sodium molybdate, 0.1 mM sodium azide and 0.01% Triton X-100, made to a final volume of 0.5 ml with 40 mM Tris–MES, pH 6.5. To determine the K^+-stimulated activity, KCl (50 mM) was included in the above medium (data not shown). Values from different treatments

determined with or without K^+ followed the same pattern, so data of K^+-stimulated activity only are shown in results. The reaction was started by the addition of PM suspension (enough to contain 5–10 µg of protein) and incubated for 30 min at 37 °C. The latency, the ability of Triton X-100 to enhance the enzyme activity due to vesicle disruption, and the inhibition by sodium vanadate (Na_3VO_4) (22 mM) was assayed for the enzyme characterisation (Martínez-Ballesta et al., 2003b). The reaction was stopped by the addition of 1 ml of "stopping reagent" made from (A) Ammonium molybdate (4% w/v) plus 16 mM EDTA, and (B) PVP-40 (4% w/v), 87.5 mM sulphuric acid and 172 mM hydroxylamine monohydrochloride. A, B and water were combined in the ratio 3:2:1. Two minutes after adding the "stopping reagent", the colour was developed by adding 100 µl of a mixture of 6.47 M sodium hydroxide and 50 mM sodium carbonate. The absorbance was read at 720 nm.

Data analysis

Data were analysed statistically using the SPSS 7.5 software package, by Tukey's Multiple Range Test, to determine differences between means.

Results

The results for root hydraulic conductance are shown in Fig. 1. Control values did not change significantly during the days of measurements. Potassium, when not applied, produced values of L_0 similar to control values. However, when it was applied in excess, we could not obtain enough xylem sap to perform measurements. Values of L_0 were restored to control values when nutrient solution was re-established. Treatments with excess Mg^{2+} produced a slight but significant decrease of L_0. However, Mg^{2+}-starvation did not result in significant differences relative to the control after 24 or 48 h of treatment application. After 72 h, a significant increase was observed, that was restored to control values 24 h after adding fresh nutrient solution. Both an excess and absence of Ca^{2+} caused a decrease of L_0, but it was higher for the excess. In the case of salinity, 10 mM NaCl produced a significant decrease but again with 100 mM NaCl xylem sap was not obtained to perform measurements. The change to fresh Hoagland solution allowed full recovery of root hydraulic conductance in 24 h. The changes in L_0 were depending, in all treatments (except in NaCl), on the changes in J_v, since the driving force was not altered (data not shown).
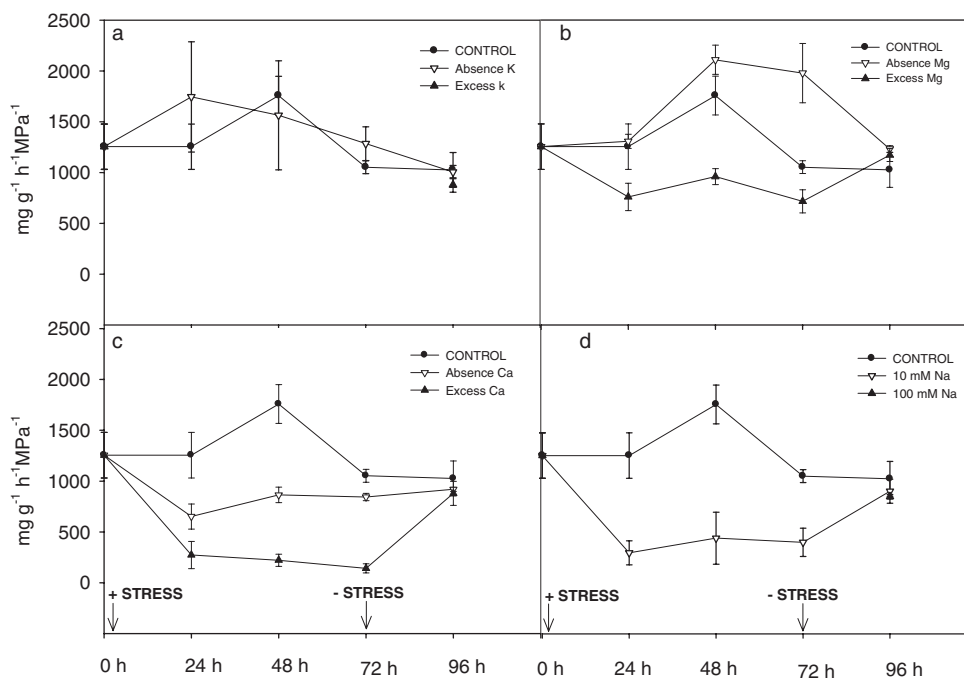

Figure 1. Root hydraulic conductance (L_0) of pepper plants grown in control nutrient solution or with absence/excess of (a) K^+, (b) Mg^{2+}, (c) Ca^{2+} or (d) low (10 mM NaCl) and high (100 mM NaCl) salinity. Each value is the mean of five samples ±SE.

Figure 2. Stomatal conductance (g_s) of pepper plants grown in control nutrient solution or with absence/excess of (a) K^+, (b) Mg^{2+}, (c) Ca^{2+} or (d) low (10 mM NaCl) and high (100 mM NaCl) salinity. Each value is the mean of five samples $\pm SE$.
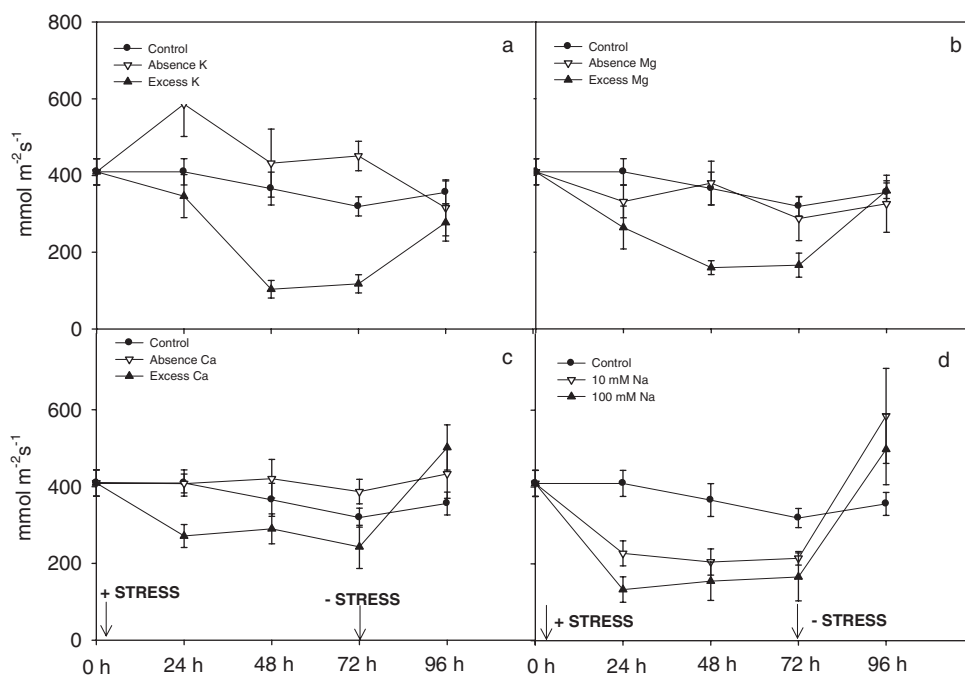
The stomatal conductance, g_s, (Fig. 2) of control plants did not vary during the time of the measurements. The excess of potassium caused a significant decrease of this parameter, corrected with 24 h in new, non-stressing solution. On the other hand, K^+-starvation produced a slight increase of g_s. Absence of Mg^{2+} did not produce any alteration with respect to the control. However, an excess of Mg^{2+} caused a decrease, but it was restored after removing the stress. Decreases of g_s were observed with excess calcium and they returned to control values when Ca^{2+} was back to normal values. Ca^{2+}-starvation did not produce differences with respect to the control. In both, 10 and 100 mM NaCl there was a decrease of g_s that was increased to above the control values after the change to fresh Hoagland solution.

The results for the measurements of pH are shown in Fig. 3. Treatments with K^+, Mg^{2+} or NaCl did not produce any significant alteration of pH, which was maintained at around 7.0. There were only significant changes of pH after both Ca^{2+} treatments. Excess Ca^{2+} produced a decrease of pH, while Ca^{2+}-starvation produced an increase. In both treatments, plants had recovered normal values 24 h after removing the stress.

The evaluation of aquaporin functionality, by the effect of $HgCl_2$ on L_0, is shown in Fig. 4. The figure shows percentage of inhibition of L_0 after Hg addition, for each treatment. The most-marked decreases in the percentage of L_0 inhibition were for K^+ or Ca^{2+}-starvation and the excess of Mg^{2+} and Ca^{+2}, being the lowest with excess Mg^{2+}. In the treatment with Mg^{2+}-starvation, the values of % inhibition were higher than control. Also, NaCl 10 mM decreases slightly the % inhibition. We used the DTT as scavenger for Hg^{2+} for checking the reversibility of the L_0 inhibition, and all treatments reverted to their original values (data not shown).

The evaluation of ATPase activity in plasma membrane vesicles isolated from roots is presented in Fig. 5. It can be observed that there are two treatments that showed a greater activity than the control: namely, the excesses of K^+ and Ca^{2+}. The absence of Mg^{2+} or Ca^{2+} gave values similar to the control. On the other hand, low values of ATPase activity were found in plants treated with absence of K^+, excess Mg^{2+}, 10 mM NaCl or 100 mM NaCl. In these last two treatments, the decrease of ATPase activity was strong, with no differences between the values obtained with the different concentrations of NaCl.

The statistical analysis of the flux of macronutrients into the xylem is shown in Table 2. The fluxes of K^+ and Ca^{2+} were reduced with all treatments except absence Mg^{2+}, for which
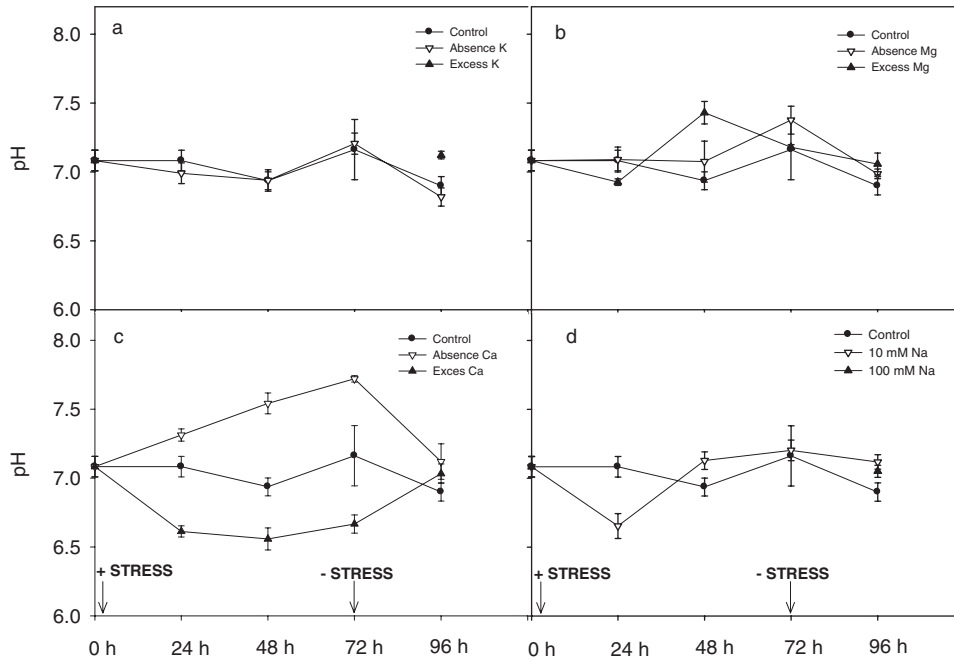
Figure 3. Measurement of the xylem sap pH in pepper plants grown in control nutrient solution or with absence/excess of (a) K^+, (b) Mg^{2+}, (c) Ca^{2+} or (d) low (10 mM NaCl) and high (100 mM NaCl) salinity. Each value is the mean of five samples $\pm$ SE.
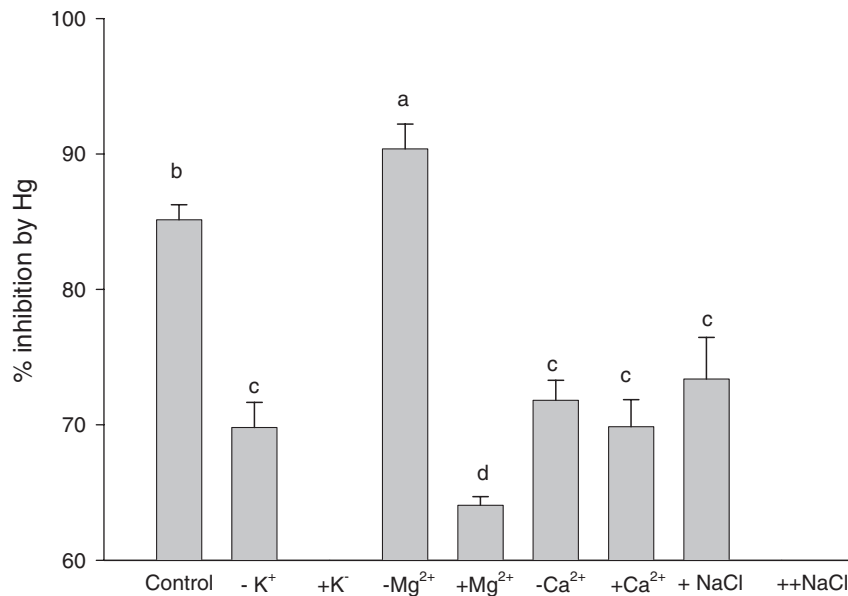

Figure 4. Percentage of root hydraulic conductance (L_0) inhibited by $HgCl_2$. in pepper plants grown in control nutrient solution or with absence/excess of (a) K^+, (b) Mg^{2+}, (c) Ca^{2+} or (d) low (10 mM NaCl) and high (100 mM NaCl) salinity. Figure show the level to which L_0 decreased after Hg addition, for each treatment. Each value is the mean of five samples $\pm$ SE.

there were no significant differences from the control. The flux of Mg^{2+} was increased significantly for excess Mg^{2+} and Ca^{2+}-starvation and decreased for excess Ca^{2+}. Salinity (10 mM NaCl) increased strongly only the flux of Na^+, but did not influence the flux of the other cations. However, the flux of Cl^- was increased by 10 mM NaCl or absence of Mg^{2+} and K^+. The flux of NO_3^- was reduced with all treatments (except the absence of Mg), strongly so with excess Ca^{2+}. The flux of PO_4^{3-} was significantly increased only by K^+-starvation and was significantly decreased by excess Ca^{2+}. There were
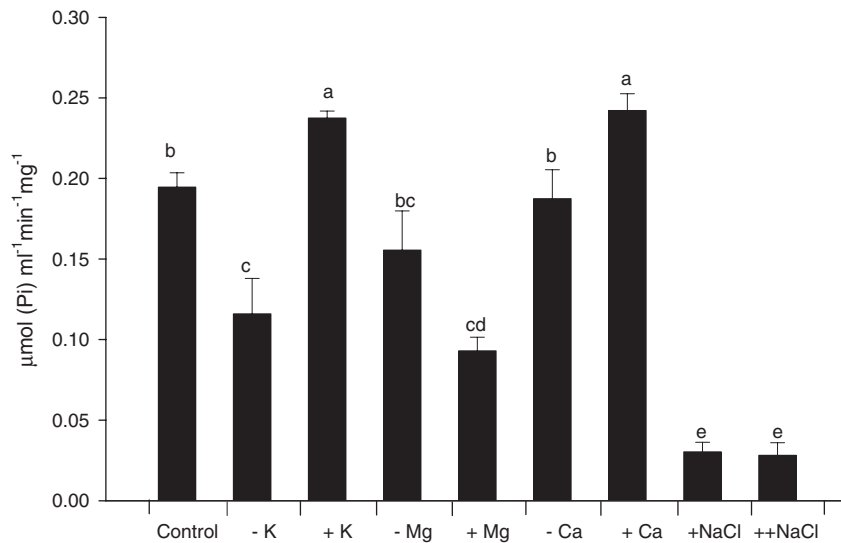
Figure 5. H^{+}-ATPase activity of isolated plasma membrane vesicles from pepper plants grown in control nutrient solution or with absence/excess of K^+, Mg^{2+}, Ca^{2+} or low (10 mM NaCl) and high (100 mM NaCl) salinity. Each value is the mean of five samples $\pm$ SE. Measurements were done in presence (black bars) or in absence (grey bars) of K (KCl 50 mM), for which statistical analysis was done separately.

Table 2. Statistical test (Tukey) of flux of solutes into the xylem (J_S) averages of the different treatments (T), absence of K^+, Mg^{2+}, Ca^{2+} ($-K$, $-Mg$, $-Ca$), excess of K^+, Mg^{2+}, Ca^{2+} ($+K$, $+Mg$, $+Ca$), 10 mM NaCl ($+Na$) and 100 mM NaCl ($++Na$), with regard to the control data

T	J_{K^+}				$J_{Mg^{2+}}$				$J_{Ca^{2+}}$				J_{Na^+}			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
−K	↓	↓	↓	=	=	=	=	=	↓	↓	↓	=	=	=	=	=
+K	ND	ND	ND	=	ND	ND	ND	=	ND	ND	ND	=	ND	ND	ND	=
−Mg	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
+Mg	↓	↓	↓	=	↑↑	↑	↑	=	↓	↓	↓	↓	=	=	=	=
−Ca	↓	↓	↓	=	=	↑	↑	↓	↓	↓	↓	=	=	=	=	=
+Ca	↓	↓	↓	=	↓	↓	↓	=	↓	↓	↓	=	=	=	=	=
+NaCl	↓	=	=	=	=	=	=	=	=	=	↓	=	↑	↑	↑	=
++NaCl	ND	ND	ND	=	ND	ND	ND	=	ND	ND	ND	=	ND	ND	ND	↑

T	J_{Cl^-}				$J_{NO_3^-}$				$J_{PO_4^{3-}}$				$J_{SO_4^{2-}}$			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
−K	=	=	=	=	=	↓	=	=	↑	↑	↑	=	=	=	=	=
+K	ND	ND	ND	=	ND	ND	ND	↓	ND	ND	ND	=	ND	ND	ND	↓
−Mg	=	↑	=	=	=	=	=	=	=	=	=	=	=	=	=	=
+Mg	↑	↑	=	=	=	↓	↓	=	=	=	=	=	=	=	↓	=
−Ca	=	=	=	=	=	↓	↓	↓	=	=	=	=	=	↓	↓	=
+Ca	=	=	=	=	↓	↓	↓	=	↓	↓	↓	=	↓	↓	↓	=
+NaCl	↑	↑	↑	=	=	↓	↓	=	=	=	=	=	=	↓	↓	=
++NaCl	ND	ND	ND	=	ND	ND	ND	↓	ND	ND	ND	=	ND	ND	ND	=

↑ increase, ↓ decrease with the level of significance: $P \leqslant 0.05$.

= : not significant differences; ND: no data.

significant decreases in $J_{SO_4^{2-}}$ with all treatments except K^+ and Mg^{2+}-starvation, which did not show differences from the control.

Discussion

The lack or excess of a nutrient due to changing environmental conditions is an important stress that can have consequences for multiple parameters of plant growth. These fundamental parameters include the water relations of the plant, in which aquaporins have an important role. Depriving plants of adequate supplies of the major nutrient ions has been shown to cause a reversible reduction of cell and root hydraulic conductivity (Carvajal et al., 1996). It has also been reported that, when a plant is subjected to nutrient deficiency, alterations in the aquaporins slow the movement of water throughout the plant (Clarkson et al., 2000; Shaw et al., 2002).

In our experiments, two of the treatments (excess potassium and strong salinity) directly inhibited the capacity of the root for water transport without the aerial part. Thus, although no sap was obtained with these treatments, we consider the natural exudation more suitable than pressurizing the roots for the experiments. The Schölander pressure chamber forces xylem sap out from decapitated plants. Although this method is also widely used, we previously observed that the values of L_0 were higher as a consequence of pressurizing the roots, since the water movement occurred through the apoplast to a greater extent than when the measurements are obtained by natural exudation (Fernandez-García et al., 2002). The interpretation of L_0 is complicated by the fact that solute and water fluxes interact to determine the value of the osmotic driving force between the xylem and the outside solution (Carvajal et al., 1996). Therefore, the most unequivocal evidence for a change in conductance is where there is a change in J_v without any change in the osmotic driving force as occurring in our experiments with the only exception of salinity treatment. In the latter treatment, the osmotic potential of the sap increased, but the osmotic driving force was lower than the control, producing a fall in L_0.

The effects of salinity on root hydraulic conductance and stomatal conductance havebeen reported widely (Cabañero et al., 2004; Martínez-Ballesta et al., 2004; Navarro et al., 2000), and are agreement with the results obtained in the present work. However, in previous work (Martínez-Ballesta

et al., 2004), an excess of K^+ produced a toxic effect on pepper plants, mainly by affecting the plant water relations. Potassium is the major osmoticum in plant cells (Marschner, 1995), and consequently essential, so cells maintain a constant level of K^+ in the cytosol (between 80 and 150 mM), while vacuolar K^+ concentrations vary dramatically, from extremely high (up to 0.6 M) to almost zero (Marschner, 1995; Shabala et al., 2003). Further, in our pepper plants, three days of the K^+ starvation treatments did not produce an effect on L_0 and g_s, most likely due to the high capacity of accumulation of this element in the vacuole. However, the decrease in aquaporin functionality observed, does not seem to fit in the whole picture. Therefore, further experiments should be undertaken to clarify this result.

Magnesium starvation seemed to cause an increase in L_0, but no changes, with respect to the control, for g_s. Some studies about deficiency of Mg^{2+} in *Beta vulgaris* have shown that the aerial biomass of plants decreased after 24 days of hydroponic culture in Mg^{2+}-free nutrient solution, whereas the root biomass was unaffected. In fact, shoot Mg^{2+} can fall to $3 \, mg \, g^{-1}$ DW without development of chlorosis and with no effect on photosynthetic parameters (Hermans et al., 2004). Our results appear to be in agreement, as only 3 days of this treatment was not sufficient to produce a deficiency, any visible symptom of chlorosis, or changes in the transpiration in pepper plants, whereas the effect on the root was the increase of root hydraulic conductance.

In our results, it can be observed that alterations in L_0 and g_s were reversible when control conditions were re-established. It has been reported that plants are able to reduce L_0, close its stomata and restrict leaf expansion with no detrimental effect on leaf water potential. When favourable conditions are re-established, the plant is able to return to its fully functional state (Clarkson et al., 2000; Shaw et al., 2002).

It was shown that high concentrations of NaCl, as well as Ca^{2+} and K^+-deprivation, lead to dramatic and divergent responses in aquaporin expression in *Arabidopsis thaliana* (Maathuis et al., 2003). Ca^{2+}-starvation generated a general down-regulation of aquaporin expression, but did not have a large impact on water relations at the whole-plant level (Maathuis et al., 2003). However, it is thought that aquaporin activity is post-translationally and negatively regulated by external Ca^{2+} and Mg^{2+} (Gerbeau et al., 2002), causing a decrease in osmotic water permeability. Taking into account our results regarding inhibition of aquaporin activity by $HgCl_2$, the absence of K^+ and Ca^{2+} and the excess of Mg^{2+}

and Ca^{2+} certainly led to a decrease of aquaporin activity. These results could be in agreement with those of Gerbeau et al. (2002). However, the fact that both the materials and the concentrations used are different, similarities must be interpreted with caution in this case. Further, in the case of pepper plants, it seems that the aquaporin activity present after 3 days of treatment tends to change in parallel with hydraulic conductance. It is necessary to bear in mind that the mercury is a general poison. In any case, as the majority of the aquaporins are known to be characteristically Hg-sensitive, it has been reported that $HgCl_2$ did not significantly reduce cells respiration during the short time of treatment in *Arabidopsis*, suggesting that the mercuric inhibition of root water flow was not due to metabolic inhibition (Martínez-Ballesta et al., 2003a).

It has been reported that abiotic stress may change the pH of the xylem sap. Under drought stress, the pH of the xylem sap from *Commelina communis* increased, and iron-stress conditions reduced the pH of sugar beet xylem sap (Wilkinson and Davies, 1997). In our results, the main variations were found with the Ca^{2+} absence or excess treatments. These results show that, while the lack of calcium produced an increase of pH, its excess caused an acidification of the xylem. However, this is not related to other parameter, which illustrates that the changes of pH were not a signal of these stresses.

Evidence that water transport across plant membranes (through aquaporins) can be regulated by intracellular pH has been reported during recent years (Gerbeau et al., 2002; Tournaire-Roux et al., 2003). Tournaire-Roux et al. (2003) demonstrated how decreases in cytosolic pH were associated with a decrease in root permeability in *Arabidopsis thaliana*. Altering the pH outside root cells did not have this effect, and no plant aquaporin has been shown to be regulated by extracellular protons so far (Luu and Maurel, 2005). Since ATPase activity is related to changes in nutrient uptake and the pH alteration, these affirmations could be in agreement with our results, where we could verify that a low activity of ATPases coincided with the treatments in which a low inhibition of L_0 by $HgCl_2$ appeared (treatments with excess of Mg^{2+} or K^+-starvation). However, in the case of Ca^{2+}-starvation, this relation does not appear.

With regard to the flow of solutes in the xylem sap, our results show that J_K^+ and $J_{NO_3^-}$ were the most affected by the treatments. At the same time, the data show how, in addition to the treatments of high salinity and excess K^+ that collapsed the xylem flow, the most aggressive treatments were the excess of Ca^{2+} and Mg^{2+} and Ca^{2+}-starvation. The flow of NO_3^- was affected by excess Ca^{2+}, Ca^{2+}-starvation, 10 mM NaCl and excess Mg^{2+}. This affirmation is important since deprivation of nitrogen has been shown to reduce the density of aquaporins and so the transport of water through the plant becomes more dependent on diffusion (Carvajal et al., 1998). In the case of PO_4^{3-}, previous studies have found that P nutrition of plants is an important factor in the uptake and translocation of Mg^{2+} and Ca^{2+}, and in increasing root osmotic hydraulic conductance and osmotically-driven xylem exudate flow (Reinbott and Blevins, 1999). Also, the aquaporin functionality could be affected by root P status, as suggested previously (Carvajal et al., 1996; Reinbott and Blevins, 1999).

The finding that nutrient stress caused by excess or depletion of specific cations (macronutrients) in the nutrient solution primarily affects water uptake and transport supports the idea that aquaporins are an interesting key factor. As has been suggested, aquaporins can play a central role in nutrient homeostasis, which is likely to comprise (i) support for ion fluxes through provision of accompanying water flow and (ii) active re-direction of apoplastic/symplastic water flow within tissues and the whole plant (Maathuis et al., 2003). Therefore, information about the quality of the nutrient supply being received by roots can be related to aquaporin functionality and ATPase activiy under certain circumstances, but not in general terms. It appears that each cation stress triggers specific responses, which probably depend on functional interactions with metabolism or other nutrients.

Aknowledgements

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