Europ.J.Hort.Sci., **75** (1). S. 33–41, 2010, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

The Importance of K^+ in Ameliorating the Negative Effects of Salt Stress on the Growth of Pepper Plants

J. S. Rubio[1)], F. García-Sánchez[1)], F. Rubio[1)], A. L. García[2)] and V. Martínez[1)]
([1)]Departamento de Nutrición Vegetal, Centro de Edafología y Biología Aplicada del Segura, CSIC. Campus Universitario de Espinardo, Espinardo, Murcia, Spain, and [2)]Departamento de Química Agrícola, Universidad de Murcia, Murcia, Spain.)

Summary

An experiment was conduced to study the response of pepper plants under saline conditions (30 mM NaCl) on growth, cation uptake, and water and ion relations. The physiological role of K^+ under the saline conditions was studied. Pepper plants were cultivated with five different nutrient solutions that included four K^+ treatments (0.2, 2.0, 7.0 and 14.0 mM K^+) with 30 mM NaCl and an additional treatment with 7 mM K^+ without salt. Plants were grown in hydroponics in a controlled-environment greenhouse and the experiment was carried out until the start of the fruiting phase. Salinity reduced reproductive organs dry weight and shoot dry weight but not root dry weight. Salinity did not affect water relations at dawn but affected ion relations in leaf sap and decreased relative water content at midday. The concentration in leaf sap of Cl^- and the organic solutes fructose, glucose and *myo*-inositol increased in salt-stressed plants. In addition, salinity produced nutrient unbalance in both leaf and root by increasing the Cl^-/NO_3^- ratio. On the other hand, increasing K^+ concentration in the nutrient solution increased salt tolerance as indicated by the increases in shoot and root dry weights. This effect was mainly due to the fact that plants under high K^+ concentrations are able to keep adequate water and ion relations with a high K/Na ratio while plants under low K^+ decreased the osmotic potential and showed dehydration leaf signs. Leaf sap oxalic acid concentration increased in parallel to leaf sap K^+ concentration and leaf sap fructose and glucose decreased with the increase of the K^+ level in the nutrient solution.

Key words. *Capsicum annuum* L. – growth – ion relations – salt stress – pepper – potassium – uptake – water

Introduction

Pepper is considered a salt sensitive species (Maas and Hoffman 2006) with a salinity threshold about 2.8 dS m^{-1} in soilless culture (Sonneveld and Vanderburg 1991). The adverse effects of salinity in the vegetative growth of pepper plants are well documented (Chartzoulakis and Klapaki 2000; De Pascale et al. 2003; Navarro et al. 2003; Lycoskoufis et al. 2005). Stomatal conductance and photosynthesis rate of pepper plants decrease due to the accumulation of Na^+ and/or Cl^- in the leaf laminas (Bethke and Drew 1992). In addition to the toxic effect, the osmotic effect contributes to the growth reduction. It has been observed in pepper plants subjected to high NaCl or Na_2SO_4 concentrations that growth reduction could have been due to leaf turgor potential decrease (Navarro et al. 2003), suggesting that pepper plants could not adjust osmotically under severe saline conditions. On the other hand, growth reduction at high Na^+ concentrations in the external medium may be due to a decrease in the K^+ concentrations and the Na/K ratio in leaves and roots as it has been previously reported in maize (Botella et al. 1997) and in wheat (Hu and Schmidhalter 2005).

Potassium is an important macronutrient in plants, which carries out vital functions in metabolism, growth and stress adaptation (Marschner 1995). Whereas, most of K^+ is located within the central vacuole of plant cells, where it functions as an osmoticum (Leigh and Jones 1984), in the cytosol the requirement for K^+ appears to be related to its role as an activator of biochemical processes, particularly protein synthesis, photosynthesis and oxidative metabolism (Maathuis 2006). As a major inorganic osmolyte, the role of K^+ in cell osmoregulation and turgor maintenance is crucial in processes such as stomatal aperture (Graham and Ulrich 1972), cell expansion and turgor-driven movements (Maathuis and Amtmann 1999). K^+ is not replaceable in its cytoplasmic functions and the plant maintains the cytoplasmic concentration of K^+ in the range of 100 to 200 mM (Leigh and Jones 1984). In fact, most of the plants can cope (i. e. show no symptoms of either deficiency or toxicity) with external K^+ concentrations ranging from low μM to tens of mM (Asher and Ozanne 1967). However, the optimum physiological K^+ concentrations narrow in the presence of increasing amounts of Na^+ (Maathuis and Amtmann 1999). While the beneficial effects that the extra supply of K^+ may play on growth and yield of pepper growth under saline stress

have been documented (Kaya et al. 2001; Kaya and Higgs 2003), the physiological basis for these effects is not completely clear. In a recent study carried out with pepper seedlings under saline conditions (10 mM NaCl) it has been observed that Na^+ net uptake is inhibited when the K^+ concentration is increased in the nutrient solution (Rubio et al. 2003).

Plant subjected to water stress (drought or salinity) accumulate osmolytes to avoid leaf dehydration and to maintain leaf turgor. Plant species can differ in the contribution of the different osmolytes to osmotic adjustment (Morgan 1984). In pepper leaves, it has been observed that K^+ is the major osmolyte (Martinez-Ballesta et al. 2004). The use of ionic osmolytes may be advantageous in terms of metabolic energy because the use of ions to balance tissue water potential has a lower energy cost than the use of carbohydrates or amino acids (Yeo 1983). Therefore, we hypothesized that K^+ fertilization in pepper plants subjected to salt stress might improve the salt tolerance during the vegetative phase, because of the beneficial effects on water and ion relations. The objectives of the present study were to determine the effects of moderate saline stress (30 mM NaCl) on pepper plant growth, the effect of different K^+ concentrations in the growth solution on salt tolerance and to establish the extent to which K^+ in the nutrient solution can alleviate the negative effects of salt stress. Thus, we studied in pepper plants growing in hydroponics culture under 30 mM NaCl the effects of four different concentrations of K^+ (0.2, 2.0, 7.0 and 14.0 mM) on growth parameters, cation uptake, tissue mineral concentration, water and ion leaf relations.

Materials and Methods

Plant culture, treatments and experimental conditions

Sweet pepper (*Capsicum annuum* L. cv. 'Somontano') seedlings coming from commercial nursery were transferred to 120 L continuously aerated-containers in a greenhouse. After 15 d with Hoagland solution, plants were submitted to different nutrient solutions that contained four K^+ treatments (0.2, 2.0, 7.0 and 14.0 mM). The salt treatment was started at the same time as the K^+ treatments by adding an additional 10 mM NaCl for 3 d to give a final NaCl concentration of 30 mM. In addition, plants from the 7.0 mM K^+ treatment were maintained without NaCl and employed as the control treatment. In total there were five treatments, 4 (K^+ + NaCl) treatments and a non-salinized control treatment with a 7.0 mM K^+ concentration (Table 1). Salt and K^+ treatments continued for 94 d.

During the experimental period the temperature was maintained between 25 and 32 °C day and between 15 and 18 °C night, and the relative humidity between 55 and 70 % day and between 80 and 90 % night. Deionised water was used to prepare all nutrient solutions. Nutrient solutions were sampled weekly and analysed to supply the nutrients depleted and the pH was maintained in the range of 5.5–6.2 using H_2SO_4 or NaOH. In addition, the nutrient solution from the 0.2 mM K^+ treatment was sampled every 2 d and K^+ was added because K^+ was depleted in this solution to 0 in 4–5 d. Water was added when needed to maintain the ± 5 % the initial volume. The experiment was carried out in the greenhouse under conditions of ambient light during winter and early spring. The experimental designs consisted of a randomized complete block, with 5 blocks and 2 replicates per block (ten plants per treatment).

Growth parameters and mineral analysis

Five plants per treatment were harvested at 74 (late vegetative phase) and 94 (starting fruiting phase) days after transplanting (DAT). Plants were separated in young and old leaves, stem, root, little fruits (only in the second harvest) and flowers, and the fresh and dry weights of each tissue were measured. Tissue dry weight was measured after drying the material in an air forced oven at 70 °C for 72 h. Dried plant tissue was digested in a concentrated HNO_3-$HClO_4$ (2/1, v/v) solution and K^+, Ca^{2+}, Mg^{2+} and Na^+ concentrations were determined by inductively coupled plasma spectrometry (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA). Anions were extracted from dry tissue with deionised water and

Table 1. Electrical conductivity (EC) and concentration of nutrient source (mmol L^{-1}) used for the nutrient solutions in this experiment.

Nutrient Source	Non-salt plus K^+ (in mM)	Salt (30.0 mM NaCl) plus K^+ (in mM)			
	7.0	0.2	2.0	7.0	14.0
EC (dS m^{-1})	1.9	4.9	4.9	4.9	5.8
KNO_3	6.0	0.2	2.0	6.0	6.0
$Ca(NO_3)_2 \cdot 4(H_2O)$	4.0	4.0	4.0	4.0	4.0
$NaNO_3$	0.0	5.8	4.0	0.0	0.0
K_2SO_4	0.0	0.0	0.0	0.0	3.5
$MgSO_4 \cdot 7(H_2O)$	1.0	1.0	1.0	1.0	1.0
$NaH_2PO_4 \cdot 2(H_2O)$	0.0	1.0	1.0	0.0	0.0
KH_2PO_4	1.0	0.0	0.0	1.0	1.0
KCl	0.05	0.0	0.0	0.0	0.0
$CaCl_2 \cdot 6(H_2O)$	0.0	0.0	0.0	0.0	0.0
NaCl	0.0	30.0	30.0	30.0	30.0

Cl^-, NO_3^-, $H_2PO_4^-$ and SO_4^{2-} were determined by ion chromatography, using conductivity detection (Dionex DX-100), an Ionpac AG14 (4 × 50) guard column, an Ionpac AS14 (10 × 32) analytical column and a Dionex ASRS-I4- mm self regenerating suppression system.

Leaf expansion rate in single leaves was studied during 12 d of leaf growth (from 60 to 72 DAT) in 10 leaves from the 16, 17 or 18 nodes per treatment. The average initial leaf area of sampled leaves for each treatment was 15.6 cm^2. Absolute rate of leaf area expansion were determined from leaf length (L) and maximum width (W) measurements and the following equation;

leaf area (LA) in cm^2 = 0.0062 * L * W + 1.1475,

$P < 0.0001$, $r^2 = 0.988$, where L and W are in mm. This equation is the result of linear regression analysis among the known length, width and leaf area (Licor-3100 Area meter) of 30 leaf samples. Absolute rate of leaf expansion (cm^2 $leaf^{-1}$ day^{-1}) was determined as follows; ($LA_{60\ DAT}$ − $LA_{72\ DAT}$)/12.

Cation uptake rate

To determinate the cation uptake rate of K^+, Ca^{2+}, Mg^{2+} and Na^+, plants (vegetative and reproductive tissue) harvested at 74 and 94 DAT were used. Specific absorption rate was calculated as the rate of mineral accumulation in the tissue per unit of root dry weight and defined as:

$$\text{Uptake} = [(M2 - M_1)\ (t_2 - t_1)^{-1}]\ [(\ln RDW_2 - \ln RDW_1)\ (RDW_2 - RDW_1)^{-1}],$$

where M_1 and M_2 are the whole plant content of K^+, Ca^{2+}, Mg^{2+} or Na^+ at harvest 1 and 2 respectively and RDW_1 and RDW_2 are the root dry weights at each harvest.

Leaf water and ion relations

Leaf water relations were measured at predawn at the end of the experiment (94 DAT) in 5 plants per treatment. Four fully-expanded leaves per plant were used for leaf water potential (Ψ_w), leaf osmotic potential (Ψ_π), leaf osmotic potential at full turgor (Ψ_p) and relative water content (RWC). Ψ_w was measured using a pressure chamber technique (Turner 1988). For Ψ_π leaves were frozen in liquid nitrogen *in situ* after removed of the plant. Leaf sap was extracted by spinning in perforated Eppendorf tubes during 5 min at 2000 rpm at 25 °C. Sap osmolarity was measured at 25 °C after 30 min after thawing by using a freezing-point depression osmometer (Digital Osmometer, Roebling, Berlin). Sap Ψ_π was then calculated at 25 °C, based on the Van't Hoff equation (Nobel 1991). Ψ_p was calculated as the difference between Ψ_w and Ψ_π. Measurement of the osmotic potential at full turgor (Ψ_π^{100}) was performed on one leaf per plant using the same procedure described above after incubation of leaves in water for 24 h at room temperature to regain full turgor and freezing them in liquid nitrogen. Relative water content was calculated according to the equation:

$$RWC = 100 \times (FW - DW) / (TW - DW),$$

where FW is the leaf fresh weight, DW is the leaf dry weight and TW is the leaf turgid weight.

Two fully-expanding leaves per plant were removed at midday (1200 a.m) for determination of RWC and ion relations in leaf sap at the end of the experimental period. Ions relations were determined in leaves frozen in liquid nitrogen immediately after harvest and stored at −80 °C. Leaf saps were collected as described above and Ψ_π, inorganic ions, oxalic acid, *myo*-inositol, fructose, glucose and sucrose concentration were measured. Oxalic acid and anions were determined by ion chromatography (Diones DX-100) and cations by atomic absorption spectrometry (Perkin-Elmer Inc., Wellesley, MA, USA, model 5500). Soluble sugars determination was performed immediately after sap extraction using a Dionex DX-600 chromatograph equipped with an ED50A conductivity detector. A Dionex IonPac AG11-HC (4 × 50) guard column and a Dionex CarboPac PA10 4 × 250 were used.

Statistical analysis

Statistical analyses included analysis of variance, regression analysis, and orthogonal comparisons where appropriate. Statistical Analysis Systems Software for PC (SPSS v.13.0) was used for all data analyses. Levels of significance are represented as follows: *P < 0.05; ** P < 0.01; *** P < 0.001; NS, not significant.

Results

Growth parameters

Salinity reduced shoot and reproductive dry weights, whereas root dry weight was not affected. The shoot-to-root dry weight ratio (S/R) and the absolute rate of leaf area expansion were also significantly decreased by salinity. In the growth parameters, shoot, root and ALAE were significantly affected by the K^+ treatments in the nutrient solution. Increasing external K^+ concentration from 0.2 to 14 mM linearly increased shoot, root and ALAE, being the shoot dry weight increased by 33 %, the root dry weight by 32 % and the ALAE by 40 % (Table 2).

Cations uptake

Calcium and Na^+ uptake were significantly affected by salinity (Fig. 1) as Ca^{2+} uptake was inhibited by 25 % and Na^+ uptake was increased by 270 % compared to the control treatment. No significant differences were found on K^+ and Mg^{2+} uptake between non-salinized and salinized-K7 plants. The increase of external K^+ concentration from 0.2 to 14 mM linearly decreased Ca^{2+}, Mg^{2+} and Na^+ uptake, and increased K^+ uptake.

Leaf and root mineral concentration

The salt treatment significantly increased the leaf Na^+ and Cl^- concentrations, being the leaf Cl^- concentration 16-fold higher than the leaf Na^+ concentration (Table 3). The salt treatment decreased the leaf NO_3^- concentration. Under saline conditions, the K^+ treatments significantly affected the leaf Ca^{2+}, K^+, Mg^{2+}, NO_3^- and $H_2PO_4^-$ concentrations. Increasing K^+ concentrations in the nutrient solution decreased leaf Ca^{2+}, Mg^{2+} and $H_2PO_4^-$ concentrations and increased leaf K^+ concentrations. K^+ con-

Table 2. Shoot, root and reproductive (fruit and flowers) dry weight per plant, shoot/root ratio (S/R) and absolute rate of leaf expansion (ALAE) in pepper plants grown under different salt and K^+ treatments at the end of the experimental period.

Treatment	K^+ (mM)	Dry weight (g)			S/R	ALAE (cm^{-2} $leaf^{-1}$ day^{-1})
		Shoot	Root	Reproductive		
Non-salt	7.0	45.1	18.1	18.2	2.48	3.99
Salt (30 mM NaCl)	0.2	26.8	12.8	9.4	2.09	2.73
	2.0	29.6	16.2	10.5	1.83	3.25
	7.0	29.5	16.6	9.8	1.79	3.30
	14.0	35.7	16.9	12.1	2.13	3.84
Non-salt *vs*. Salt		**	Ns	**	**	*
K^+ effects	Linear	*	*	Ns	Ns	*
	Quadratic	Ns	Ns	Ns	Ns	Ns

Non-salt *vs*. Salt (each with 7.0 mM K^+); * and ** indicate significant differences between means at 0.05 and 0.01 level of probability, respectively. NS indicate non-significant. Values are means of 5 plants.

Table 3. Concentration of anions and kations in leafs and roots of pepper plants grown under different salt and K^+ treatments at the end of the experimental period.

Treatment	K^+ (mM)	Concentration of kations and anions (mg g^{-1} dry weight)							
		Ca^{2+}	K^+	Mg^{2+}	Na^+	Cl^-	NO_3^-	$H_2PO_4^-$	SO_4^{2-}
					Leaf				
Non-salt	7.0	45.7	55.2	8.2	0.4	1.5	26.7	8.4	12.5
Salt (30 mM NaCl)	0.2	56.8	28.0	10.4	1.0	15.1	15.9	20.2	14.3
	2.0	52.5	41.4	9.3	1.1	20.0	20.6	13.4	9.1
	7.0	42.8	51.8	7.8	0.7	16.5	18.4	10.2	10.8
	14.0	36.4	60.0	7.3	0.5	15.7	15.4	8.1	10.1
Non-salt *vs*. Salt		Ns	Ns	Ns	**	***	*	Ns	Ns
K^+ effects	Linear	**	***	*	Ns	Ns	Ns	***	Ns
	Quadratics	**	***	*	Ns	Ns	Ns	**	Ns
					Root				
Non-salt	7.0	7.6	53.6	4.6	1.0	3.9	83.5	49.1	9.4
Salt (30 mM NaCl)	0.2	7.2	16.6	8.1	13.2	17.9	43.7	55.0	10.3
	2.0	5.8	39.2	3.8	9.4	19.0	55.5	51.5	10.7
	7.0	6.6	40.1	4.1	7.4	15.9	57.5	46.2	8.5
	14.0	5.0	51.0	3.6	8.1	20.6	66.3	43.7	9.9
Non-salt *vs*. Salt		Ns	*	Ns	***	***	**	Ns	Ns
K^+ effects	Linear	Ns	***	*	**	Ns	**	Ns	Ns
	Quadratic	Ns	***	**	**	Ns	*	Ns	Ns

Non-salt *vs*. Salt (each with 7.0 mM K^+); *, ** and *** indicate significant differences between means at 0.05, 0.01 and 0.001 level of probability, respectively. NS indicate non-significant. Values are mean of 5 plants.

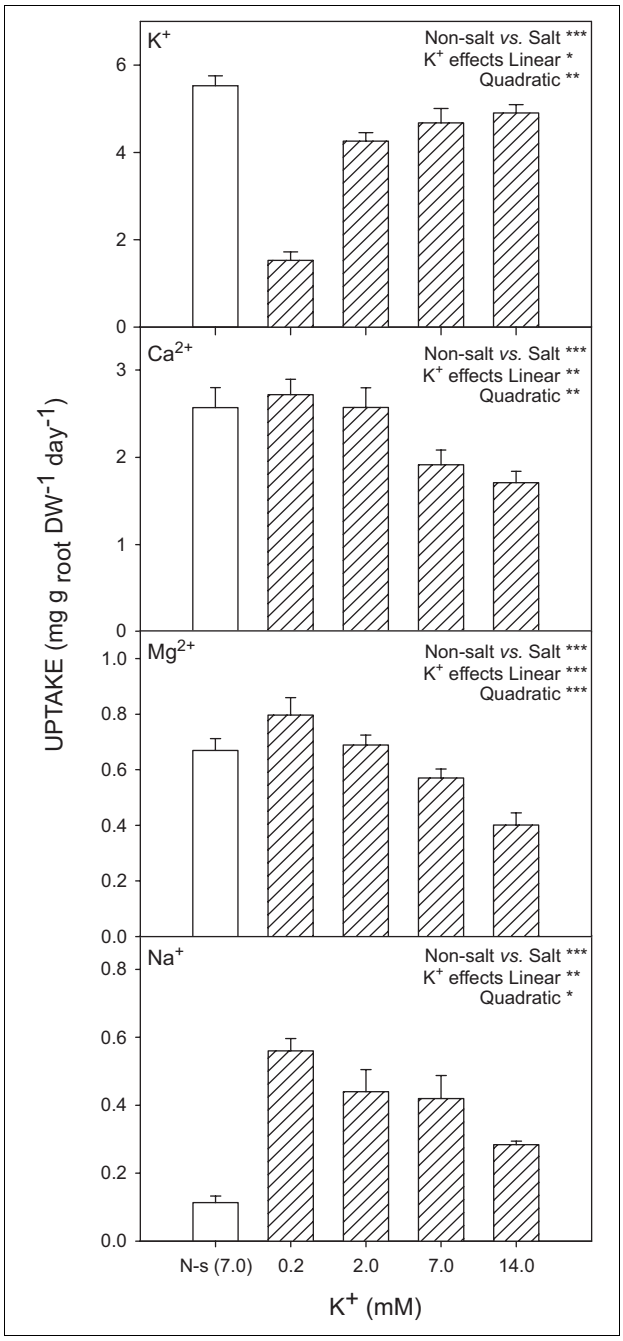


Fig. 1. Salt and K^+ treatments effects on K^+, Ca^{2+}, Mg^{2+} and Na^+ uptake. The uptake of the different cations (mg in the whole plant per g of root dry weight per day) was calculated from the cation contents 74 and 94 d after transplanting. Five replica per treatment were performed and the averages and standard errors (S.E.) (error bars) are reported besides the statistical analysis.

centrations in the nutrient solution had no significant effect on leaf Na^+, Cl^- and SO_4^{2-} concentrations.

In roots, salinity significantly increased the concentration of Cl^- and Na^+ (Table 3). Salinity also significantly decreased root K^+ and NO_3^- concentrations, while no significant differences in the rest of the nutrients were observed. Under saline conditions, increasing the K^+ concentration in the nutrient solution significantly increased the root K^+ and NO_3^- concentrations and decreased the Mg^{2+} and Na^+ concentrations.

Leaf water and ion relations

The salt treatment had no significant effect on the leaf water relations parameters at dawn (Table 4). However, leaf osmotic potential (Ψ_π) and osmotic potential at full turgor (Ψ_π^{100}) decreased, and leaf turgor potential (Ψ_p) and RWC increased with increasing K^+ concentration in the nutrient solution. The quadratic regression was significant but the linear regression was not. No significant differences were found among K^+ treatments regarding leaf water potential (Ψ_w).

At midday RWC of salinized plants decreased relative to control plants (Table 5). The osmotic potential showed no significant differences between control and salt-treated plants and among the plants subjected to the K^+ treatments. However, significant differences in the ionic and organic composition of the leaf sap were observed (Table 5 and 6). Salinity significantly increased Na^+, Cl^-, *myo*-inositol, fructose and glucose, and decreased NO_3^-, $H_2PO_4^-$, SO_4^{2-}. The major contributor to osmotic potential under salt conditions was Cl^-. On the other hand, increasing K^+ concentration in the nutrient solution increased RWC, K^+ and oxalic acid and, decreased Ca^{2+}, Mg^{2+}, Na^+, $H_2PO_4^-$, and SO_4^{2-} in leaf sap. A positive correlation ($r^2 = 0.69$, $P < 0.001$) was found between K^+ and oxalic acid in leaf sap. In addition, increasing K^+ concentration in the nutrient solution lead to an increase in the total cation content and a decrease in the total anion content (data not show). Decreasing K^+ concentration in the external solution decreased fructose and glucose in leaf sap, whereas *myo*-inositol and sucrose concentrations were unaffected by the K^+ level in the nutrient solution.

Discussion

Effects of salinity on pepper plants

As expected, in our experiment, growth of pepper plants decreased after 94 d under saline condition (Table 2). This indicates that, as it has been shown by others authors, pepper plant behaves as salt sensitive species (Sonneveld and Vanderburg 1991). In plants, growth reduction due to saline condition may be due to several causes as incapacity of plants for osmotic adjustment, unbalanced essential nutrients and, Cl^- and/or Na^+ toxic effects (Grattan and Grieve 1999; Munns 2002). The results of the present study show that the main factors that lead to a reduction in growth of pepper plants exposed to 30 mM NaCl are related to the high leaf Cl^- concentration, the low leaf NO_3^-/Cl^- ratio and the incapacity of maintaining an adequate leaf water status at midday as showed low the RWC values. The low leaf Na^+ concentrations exclude the possibility that Na^+ toxicity is negatively affecting plant growth (Table 3). High leaf Cl^- concentrations may cause a reduction in photosynthesis rates, possibly by non-stomatal factors as damage of chloroplasts (Bethke and Drew 1992), root hydraulic conductance reduction and reduction in aquaporin functionality (Martinez-Ballesta et al. 2004). On the other hand, salinity decreased leaf NO_3^- concentrations but did not

Table 4. Leaf water potential (Ψ_w), leaf osmotic potential (Ψ_π), leaf turgor potential (Ψ_p), osmotic potential at full turgor (Ψ_π^{100}) and relative water content (RWC) measured at dawn in pepper plants grown under different salt and K^+ treatments at the end of the experimental period.

Treatment	K^+ (mM)	Ψ_w (MPa)	Ψ_π (MPa)	Ψ_p (MPa)	Ψ_π^{100} (MPa)	RWC (%)
Non-salt	7.0	−0.17	−1.18	1.02	−1.09	98.6
Salt (30 mM NaCl)	0.2	−0.27	−1.03	0.83	−0.99	96.7
	2.0	−0.20	−1.20	0.97	−1.08	97.8
	7.0	−0.19	−1.20	1.01	−1.15	98.1
	14.0	−0.16	−1.25	1.09	−1.20	98.1
Non-salt *vs.* Salt		Ns	Ns	Ns	Ns	Ns
K^+ effects	Linear	Ns	Ns	Ns	Ns	Ns
	Quadratic	Ns	*	*	*	*

Non-salt *vs*. Salt (each with 7.0 mM K^+); * indicate significant differences between means at 0.05 level of probability. NS indicate non-significant. Values are means of 5 plants.

Table 5. Relative water content (RWC), leaf osmotic potential (Ψ_π), and leaf sap concentration measured at midday in pepper plants grown under different K^+ and salt treatments at the end of the experimental period.

Treatment	K^+ (mM)	RWC (%)	Ψ_π (MPa)	Leaf sap concentration (mEq L^{-1})								
				Ca^{2+}	K^+	Mg^{2+}	Na^+	Cl^-	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	Oxalic acid
Non-salt	7.0	96.5	−1.21	0.47	199	52	0.24	5	122	58	71	17
Salt (30 mM NaCl)	0.2	93.2	−1.18	0.71	175	49	1.43	45	92	74	62	9
	2.0	94.1	−1.18	0.48	199	56	1.31	38	94	52	55	17
	7.0	95.2	−1.20	0.47	212	49	1.37	46	86	44	48	18
	14.0	96.0	−1.22	0.19	231	41	0.55	46	79	47	48	26
Non-salt *vs.* Salt		**	Ns	Ns	Ns	Ns	***	***	***	**	***	Ns
K^+ effects	Linear	**	Ns	***	***	**	*	Ns	Ns	*	*	***
	Quadratic	*	Ns	**	***	**	*	Ns	Ns	*	**	***

Non-salt *vs.* Salt (each with 7.0 mM K^+); *, ** and *** indicate significant differences between means at 0.05, 0.01 and 0.001 level of probability, respectively. NS indicate non-significant. Values are means of 5 plants.

produce total-N deficit (data not shown). However, in plants, it has been observed that a low ratio of NO_3^-/Cl^- may cause a reduction in growth (Grattan and Grieve 1999). On the other hand, although leaf Na^+ and Cl^- concentrations increased by salinity, Cl^- concentrations were 16-fold the Na^+ concentrations in leaf. These data indicate that pepper plants can restrict high accumulation of Na^+ in leaves. A certain capacity to restrict Na^+ transport to leaves by accumulating it in pith cells of the stem and the root has been observed in pepper plants (Blom-Zandstra et al. 1998), although the mechanism by which this takes places remains unclear. Ours results show that pepper plants did not promote an adequate biomass allocation for increasing the salt tolerance since shoot to root ratio was decreased by salinity (Table 2). Plant S/R ratios have also been shown to be affected by salinity and salinity tolerant plants show an increase in S/R ratios while salinity intolerant plants show a decrease in S/R because a decrease in shoot growth (Cheeseman and Wickens 1986; Bayuelo-Jimenez et al. 2003).

Although leaf osmotic potential was similar in control and salt-treated plants at midday, the leaf water content and leaf sap ionic concentration were significantly affected by the salt treatment (Table 5 and 6). The maintenance of a sufficiently-high leaf water content has been pointed out as an essential factor in plant water relations under salinity conditions to allow proper functioning of the processes involved in growth (Munns et al. 2000). The increase in the Cl^- concentration in the leaf sap in plants subjected to the salt treatment was concomitant

Table 6. Leaf sap concentration (mEq L^{-1}) of *myo*-ionsitol and soluble carbohydrates measured at midday in pepper plants grown under different K^+ and salt treatments at the end of the experimental period.

Treatment	K^+	Leaf sap concentration (mEq L^{-1})			
	(mM)	*myo*-Inositol	Fructose	Glucose	Sucrose
Non-salt	7.0	1.08	0.65	0.31	0.56
Salt (30 mM NaCl)	0.2	1.50	1.44	0.77	0.61
	2.0	1.63	1.31	0.71	0.59
	7.0	1.67	1.29	0.71	0.60
	14.0	1.66	1.21	0.64	0.66
Non-salt *vs.* Salt		**	***	***	Ns
K^+ effects	Linear	Ns	*	*	Ns
	Quadratic	Ns	*	Ns	Ns

Non-salt *vs.* Salt (each with 7.0 mM K^+); *, ** and *** indicate significant differences between means at 0.05, 0.01 and 0.001 level of probability, respectively. NS indicate non-significant. Values are means of 5 plants.

with a decrease in the anions concentration (NO_3^-, $H_2PO_4^-$ and SO_4^{2-}). This could be explained by a reduction in anion uptake and transport when high Cl^- concentrations were applied to the root medium. Antagonism between Cl^- and NO_3^- has been previously observed in other plant species (Abdelgadir et al. 2005). Other important effect of salinity in the leaf ion relations was the increase in the *myo*-inositol and the soluble carbohydrates fructose and glucose (Table 6). *myo*-Inositol was the most abundant polyol found in salt-adapted pepper leaves, constituting 40 % of the major soluble carbohydrates. It is one of the most abundant polyol in *Solanaceae* (Lauchli and Epstein 1990) and it is implicated in the adaptation to salt of tomato (Sacher and Staples 1985) and kiwifruit (Klages et al. 1999) plants. *myo*-Inositol may act as a cytoplasmatic osmoticum, it may be involved in scavenging of oxygen radicals, and it may act as photosynthetic product storage which is not utilized under stress conditions (Nelson et al. 1998). The increase in soluble hexoses fructose and glucose by salinity is other possible adaptive response of pepper plants to salt stress. The accumulation of soluble carbohydrates in plants has been showed as a response to salinity, despite that both photosynthesis and growth were negatively correlated with sugar accumulation (Balibrea et al. 2000). The utilization of soluble hexoses in the fully-expanding leaves for osmotic functions instead of use it for new tissue formation could have had an important influence in the reduction of shoot growth and ALAE (Table 2). The regulation of carbon allocation and partitioning in plants subjected to salinity affect more growth rate and yield than carbohydrate availability (Munns 1993).

Effects of external K^+ concentration on pepper plants under saline conditions

Potassium plays a key role in the response of plants to salinity (Maathuis 2006). In our experiment, decreasing the K^+ concentration in the nutrient solution decreased salt tolerance of pepper plants as observed by the reduction in shoot and root growth (Table 2). This was mainly due to the fact that plants under the low K^+ treatments did not show an adequate leaf water status and had low K/Na ratios. Decreasing K^+ concentrations in the nutrient solution decreased the Ψp (Table 4) and leaf tissue dehydration was observed as indicated by the low content of water in leaves at dawn and at midday. In the leaf sap, our data show that the major ion contributing to osmotic potential was K^+, therefore, a decrease in K^+ concentration caused an increase in leaf osmotic potential and a decrease in leaf turgor potential. Similar conclusions were observed in bluegrass subjected to different K^+ concentrations in the nutrient solution (Carroll et al. 1994). It was found that plants under low K^+ had a higher leaf osmotic, and a lower turgor potential and a relative low dry weight in comparison with plants under high K^+ (Carroll et al. 1994). In addition, in other plant species, it has been observed that other organic solutes can replace K^+ in the vacuole to keep leaf turgor potential (Leigh and Jones 1984). However, in our experiment, K^+ and leaf osmotic potential decreased in parallel suggesting that organic solutes did not contribute to restore the low turgor potential caused by the low K^+ treatment. On the other hand, the observed beneficial effect of external K^+ on growth under moderate salinity stress could be also due to an increase in the K/Na ratio in plant tissue as indicated above. It has been pointed out (e.g. (Yeo 1998; Maathuis 2006) that one of the key factors in salinity tolerance is the capacity to maintain a high cytosolic K/Na ratio. In our experiment, the K/Na ratio increased in fully expanded leaves from 28 to 120 by increasing K^+ concentration in the root system (data not shown) which could be explained by a reduction of Na^+ uptake and by an increase of K^+ uptake. A clear inhibition of Na^+ uptake by K^+ has been observed in pepper plants (Rubio et al. 2003), indicating that a portion of the influx of both cations may take place through the same transporters.

Despite that leaf K^+ concentration decreased with decreasing K^+ concentration in the nutrient solution, K^+ deficit was not the cause of the decrease in growth in the

low K^+ treatments. The lowest plant tissue concentration of K^+ on a tissue water basis was 134.5 mM and K^+ deficiency is observed at a concentration below 100 mM (LEIGH and JONES 1984). Therefore, our results show that in pepper plants, K^+ uptake, transport and distribution is a well regulated process in the range of 0.2 to 14 mM K^+. The fact that 0.2 mM K^+ in the nutrient solution did not cause K^+ deficiency could be due to the fact that pepper plants induce a high affinity K^+ uptake system when the root K^+ concentration is lower than 30 mg $g_{root}DW^{-1}$ (MARTINEZ-CORDERO et al. 2004). In our experiments, the concentrations of K^+ in the roots of plants grown at 0.2 mM K^+ were 31.1 mg g^{-1} at 74 DAT and 16.6 mg g^{-1} at 94 DAT which could explain the induction of the high-affinity K^+ uptake system. On the other hand, Ca^{2+} and Mg^{+2} uptakes were decreased with increasing the K^+ concentration in the nutrient solution. This reduction in Ca^{2+} uptake and transport to shoot by K^+ could be due to competition at the site of absorption as it has been previously reported by (ROBSON and PITMAN 1983).

Interestingly, the oxalic acid concentration in the leaf sap increased with the increase in K^+ level in the nutrient solution. Oxalic acid occurs in many plants as soluble salts of K^+, and could play a major role in ion relations and osmoregulation (LIBERT and FRANCESCHI 1987). On the other hand, water-soluble carbohydrates in leaves, glucose and fructose, increased with decreasing K^+ concentration in the nutrient solution (Table 6). Although not specifically documented in pepper, a deficiency in K^+ often produces an increase in the concentration of soluble carbohydrates (WANG and GALLETTA 1998; PETTIGREW 1999). It is not clear if this accumulation is due to the reduced rate of carbohydrate export or to other process. The accumulation of the reducing sugars, fructose and glucose, in K-deficient leaves is consistent with the results reported by HUBER (1985) and the accumulation could be explained by the decrease of the activities of K-dependent enzymes, such as pyruvate kinase (EVANS and SORGER 1966).

In conclusion, in this work we have shown that growth reduction of pepper plants grown under conditions of moderate saline could be due to the Cl^- toxicity and/or to the high Cl^-/NO_3^- ratios and an incapacity for maintaining a proper leaf water status at midday. The partitioning of soluble carbohydrates for osmotic adjustment could be also implicated in the growth reduction. On the other hand, increasing K^+ concentrations in the nutrient solution increased the salt tolerance as observed by the increase in shoot and root growth. This was mainly due to the fact that plants under high K^+ concentrations are able to keep adequate water and ion relations and a high K/Na ratio. However, high K^+ concentrations in the nutrient solution decreased Ca^{2+} and Mg^{2+} uptake and their concentration in plant tissue compared with low K^+ concentrations in the nutrient solution. Oxalic acid, Ca^{2+} and Mg^{2+} concentrations in plants has been related with blossom-end rot incidence (ALONI et al. 1994) and fruit quality (DILMAGHANI et al. 2004). Further work should be undertaken to study the capacity of osmotic adjustment in pepper plants subjected to moderate saline conditions and to study if the effect of K^+ on oxalic acid, Ca^{2+} and Mg^{2+} contents could affect fruit quality and commercial yield of pepper plants. This type of studies could allow establishing the optimum K^+ concentrations in the root system for high fruit yield under saline conditions.

Acknowledgement

J.S. Rubio was a recipient of a FPU fellowship from Ministerio de Educación y Ciencia (Spain). This work was funded by the project AGR/18/FS/02 (Fundacion Séneca, CARM). The authors thank Brian Hua for the correction of the English in the manuscript.

References

ABDELGADIR, E.M., M. OKA and H. FUJIYAMA 2005: Nitrogen nutrition of rice plants under salinity. Biol. Plant. **49**, 99–104.
ALONI, B., L. KARNI, I. RYLSKI and Z. ZAIDMAN 1994: The effect of nitrogen-fertilization and shading on the incidence of color spots in Sweet-Pepper (*Capsicum annuum*) fruit. J. Hort. Sci. **69**, 767–773.
ASHER, C.J. and P.G. OZANNE 1967: Growth and potassium content of plants in solution cultures maintained at constant potassium concentrations. Soil Sci. **103**, 155–161.
BALIBREA, M.E., J. DELL'AMICO, M.C. BOLARIN and F. PEREZ-ALFOCEA 2000: Carbon partitioning and sucrose metabolism in tomato plants growing under salinity. Physiol. Plant. **110**, 503–511.
BAYUELO-JIMENEZ, J.S., D.G. DEBOUCK and J.P. LYNCH 2003: Growth, gas exchange, water relations, and ion composition of *Phaseolus* species grown under saline conditions. Field Crops Res. **80**, 207–222.
BETHKE, P.C. and M.C. DREW 1992: Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. Plant Physiol. **99**, 219–226.
BLOM-ZANDSTRA, M., S.A. VOGELZANG and B.W. VEEN 1998: Sodium fluxes in sweet pepper exposed to varying sodium concentrations. J. Exp. Bot. **49**, 1863–1868.
BOTELLA, M.A., V. MARTINEZ, J. PARDINES and A. CERDA 1997: Salinity induced potassium deficiency in maize plants. J. Plant Physiol. **150**, 200–205.
CARROLL, M.J., L.H. SLAUGHTER and J.M. KROUSE 1994: Turgor potential and osmotic constituents of Kentucky Bluegrass leaves supplied with 4 Levels of Potassium. Agron. J. **86**, 1079–1083.
CHARTZOULAKIS, K. and G. KLAPAKI 2000: Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci. Hort. **86**, 247–260.
CHEESEMAN, J.M. and L.K. WICKENS 1986: Control of Na^+ and K^+-Transport in *Spergularia marina*. 2. Effects of plant size, tissue ion contents and root-shoot ratio at moderate salinity. Physiol. Plant. **67**, 7–14.
DE PASCALE, S., C. RUGGIERO, G. BARBIERI and A. MAGGIO 2003: Physiological responses of pepper to salinity and drought. J. Am. Soc. Hort. Sci. **128**, 48–54.
DILMAGHANI, M.R., M.J. MALAKOUTI, G.H. NEILSEN and E. FALLAHI 2004: Interactive effects of potassium and calcium on K/Ca ratio and its consequences on apple fruit quality in calcareous soils of Iran. J. Plant Nutr. **27**, 1149–1162.
EVANS, H.J. and G.J. SORGER 1966: Role of mineral elements with emphasis on univalent cations. Ann. Rev. Plant Physiol. **17**, 47–76.
GRAHAM, R.D. and A. ULRICH 1972: Potassium deficiency-induced changes in stomatal behavior, leaf water potentials, and root system permeability in *Beta-Vulgaris* L. Plant Physiol. **49**, 105–109.
GRATTAN, S.R. and C.M. GRIEVE 1999: Salinity mineral nutrient relations in horticultural crops. Sci. Hort. **78**, 127–157.
HU, Y.C. and U. SCHMIDHALTER 2005: Drought and salinity: A comparison of their effects on mineral nutrition of plants. J. Plant Nutr. Soil Sci. **168**, 541–549.
HUBER, S.C. 1985: Role of potassium in photosynthesis and respiration. In: MUNSON, R.D. (ed.), Potassium in Agriculture. ASA, CSSA, and SSSA, Madison, WI, pp 369–395.
KAYA, C., H. KIRNAK and D. HIGGS 2001: Effects of supplementary potassium and phosphorus on physiological development and mineral nutrition of cucumber and pepper cultivars grown at high salinity (NaCl). J. Plant Nutr. **24**, 1457–1471.
KAYA, C. and D. HIGGS 2003: Supplementary potassium nitrate improves salt tolerance in bell pepper plants. J. Plant Nutr. **26**, 1367–1382.
KLAGES, K., H. BOLDINGH and G.S. SMITH 1999: Accumulation of *myo*-inositol in *Actinidia* seedlings subjected to salt stress. Ann. Bot. **84**, 521–527.

Lauchli, A. and E. Epstein 1990: Plant responses to saline and sodic conditions. In: Tangi, K.K. (ed.): Agricultural salinity assessment and management. Am. Soc. Civil Eng., New York, pp. 113–137.
Leigh, R.A. and R.G.W. Jones 1984: A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant-cell. New Phytol. **97**, 1–13.
Libert, B. and V.R. Franceschi 1987: Oxalate in crop plants. J. Agric. Food Chem. **35**, 926–938.
Lycoskoufis, I.H., D. Savvas and G. Mavrogianopoulos 2005: Growth, gas exchange, and nutrient status in pepper (*Capsicum annuum* L.) grown in recirculating nutrient solution as affected by salinity imposed to half of the root system. Sci. Hort. **106**, 147–161.
Maas, E.V. and G.J. Hoffman 2006: Crop salt tolerance. J. Irrig. and Drain. Div. **103**, 115–134.
Maathuis, F.J.M. and A. Amtmann 1999: K^+ nutrition and Na^+ toxicity: The basis of cellular K^+/Na^+ ratios. Ann. Bot. **84**, 123–133.
Maathuis, F.J.M. 2006: The role of monovalent cation transporters in plant responses to salinity. J. Exp. Bot. **57**, 1137–1147.
Marschner, H. 1995: Mineral Nutrition of Higher plants. London, pp. 299–312.
Martinez-Ballesta, M.C., V. Martinez and M. Carvajal 2004: Osmotic adjustment, water relations and gas exchange in pepper plants grown under NaCl or KCl. Environ. Exp. Bot. **52**, 161–174.
Martinez-Cordero, M.A., V. Martinez and F. Rubio 2004: Cloning and functional characterization of the high-affinity K^+ transporter HAK1 of pepper. Plant Mol. Biol. **56**, 413–421.
Morgan, J.M. 1984: Osmoregulation and water-stress in higher-plants. Ann. Rev. Plant Physiol. Mol. Biol. **35**, 299–319.
Munns, R. 1993: Physiological processes limiting plant-growth in saline soils – some dogmas and hypotheses. Plant Cell Environ. **16**, 15–24.
Munns, R. 2002: Comparative physiology of salt and water stress. Plant Cell Environ. **25**, 239–250.
Munns, R., J.B. Passioura, J.M. Guo, O. Chazen and G.R. Cramer 2000: Water relations and leaf expansion: importance of time scale. J. Exp. Bot. **51**, 1495–1504.
Navarro, J.M., C. Garrido, V. Martinez and M. Carvajal 2003: Water relations and xylem transport of nutrients in pepper plants grown under two different salts stress regimes. Plant Growth Reg. **41**, 237–245.
Nelson, D.E., G. Rammesmayer and H.J. Bohnert 1998: Regulation of cell-specific inositol metabolism and transport in plant salinity tolerance. Plant Cell. **10**, 753–764.
Nobel, P.S. 1991: Physiochemical and Environmental Plant Physiology. San Diego, CA. p. 635.
Pettigrew, W.T. 1999: Potassium deficiency increases specific leaf weights and leaf glucose levels in field-grown cotton. Agron. J. **91**, 962–968.
Robson, A.D. and M.G. Pitman 1983: Interactions between nutrients in higher plants. In: Lauchli, A. and R.L. Bieleski, (ed.): Encyclopedia of plant physiology. Springer Verlag, Berlin, Germany, pp 147–180.
Rubio, F., P. Flores, J.M. Navarro and V. Martinez 2003: Effects of Ca^{2+}, K^+ and cGMP on Na^+ uptake in pepper plants. Plant Sci. **165**, 1043–1049.
Sacher, R.F. and R.C. Staples 1985: Inositol and sugars in adaptation of tomato to salt. Plant Physiol. **77**, 206–210.
Sonneveld, C. and A.M.M. Vanderburg 1991: Sodium-Chloride salinity in fruit vegetable crops in soilless culture. Netherl. J. Agri. Sci. **39**, 115–122.
Turner, N.C. 1988: Measurement of plant water status by the pressure chamber technique. Irrigation Sci. **9**, 289–308.
Wang, S.Y. and G.J. Galletta 1998: Foliar application of potassium silicate induces metabolic changes in strawberry plants. J. Plant Nutr. **21**, 157–167.
Yeo, A.R. 1983: Salinity resistance – physiologies and prices. Physiol. Plant. **58**, 214–222.
Yeo, A. 1998: Molecular biology of salt tolerance in the context of whole-plant physiology. J. Exp. Bot. **49**, 915–929.

Received December 17, 2008 / Accepted August 06, 2009

Addresses of authors: Jose Salvador Rubio, Francisco García-Sánchez (corresponding author), Francisco Rubio, and V. Martínez, Departamento de Nutrición Vegetal, Centro de Edafología y Biología Aplicada del Segura, CSIC, Campus Universitario de Espinardo, Espinardo, 30100, Murcia, Spain and Antonio Lino García, Departamento de Química Agrícola, Universidad de Murcia, P.O. Box 4021, 30071, Murcia, Spain, e-mail: fgs@cebas.csic.es.