Europ.J.Hort.Sci., **78** (2). S. 86–95, 2013, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Growth, Gas Exchange and Water Relations in Pepper Grown in a Substrate Soilless Closed System as Affected by NaCl and Nutrient Salinity

F. Giuffrida
(Department of Agricultural and Food Science, Catania University, Catania, Italy)

Summary

In this experiment the effects of a moderate nutrient or NaCl salinity on growth, gas exchange and water relations in pepper grown in a substrate soilless closed system were studied. A control (2 dS m^{-1}) and two saline nutrient solutions (4 dS m^{-1}) differing in the salt sources (fertilizers or NaCl) were compared. Total plant dry mass was reduced more by NaCl salinity (−41 %) than nutrient salinity (−17 %) as well as total fruit yield (−48 and −33 %, respectively). In both salinity treatments, the yield decrease was due to a strong reduction in the mean fruit mass (−42 %), whereas NaCl-stressed plants also produced less fruits (−20 %). The results obtained on plant water relations, gas exchange and ion content in different plant organs demonstrated that the negative response of pepper to salt stress depends on both osmotic and ion specific effects. The detrimental effect on pepper response was more evident under NaCl stress. This could be mainly explained by the toxicity of Cl^-, although slight nutrient imbalances due to Na^+ and Cl^- antagonisms were observed.

Key words. dry mass – ion concentrations – leaf area – osmotic stress – photosynthesis – salt stress – water potential

Introduction

In many regions of the Mediterranean basin with intensive greenhouse cultivation, the use of irrigation water with high salt concentrations (mainly sodium chloride, sulphates and bicarbonates) and the application of excessive amounts of mineral fertilizer are the major cause of salt stress problems. Moreover, in these greenhouse areas, it is advisable to improve the water and nutrient use efficiency and to reduce the fertilizer pollution by using closed soilless cultivation systems. However, the recirculation of nutrient solutions prepared with saline water produces a progressive accumulation of unabsorbed ions, and also of nutrients when applied in higher amount than plants are able to take up (Giuffrida and Leonardi 2012). The consequent increase in electrical conductivity (EC) of the nutrient solution (NS) exposes the plants to osmotic stress.

Negative effects of salinity on plant growth and yield can be related to altered water status, metabolic ion toxicity and attenuated nutrient acquisition (Hasegawa et al. 2000). However, the precise contribution of these processes to plant growth inhibition is not clear (Munns and Tester 2008). As the first adaptive response to saline conditions, plants have to minimize water loss and maintain sufficient turgor for growth (Carvajal et al. 1996). To maintain the water uptake, a higher osmotic potential than the outside could be achieved by transport, accumulation and compartmentation of inorganic ions and organic solutes in the vacuole and/or cytoplasm (Jeschke et al. 1986; Blum et al. 1996). The use of inorganic salt for osmotic adjustment needs less energy and carbon than the adjustment by organic solutes (Wyn Jones 1981). Leigh and Storey (1993) supposed that the effectiveness of inorganic salt utilization for osmotic adjustment depends on the plant's ability to regulate internal Na^+ and Cl^- concentrations. Therefore, the type of salt involved is crucial for determining the responses of plants to salinity (Navarro et al. 2003). Among solanaceous species, tomato (Adams 1991) and eggplant (Savvas and Lenz 2000) respond similarly to different salinity sources (NaCl or nutrient) with no growth limitation but with a yield restriction compared to controls; thus the effects were osmotic and not salt specific.

Pepper (*Capsicum annuum* L.) is an important crop for greenhouse cultivation in the Mediterranean region due to the economic and nutritional value of fruits. Pepper is considered to be moderately sensitive to salinity (Ayers

and Westcot 1985) and its response to moderate and high NaCl stress has been well documented (Chartzoulakis and Klapaki 2000; Navarro et al. 2002; De Pascale et al. 2003; Martínez-Ballesta et al. 2004). The more negative influence of chloride salinity than sulphate on growth and yield of pepper (Navarro et al. 2002) indicates that different response mechanisms may be involved in plants under salt stress (Munns and Tester 2008). For pepper few investigations are available concerning the discrimination between the physiological effects related to water relations and those having ion-specific origin (Martínez-Ballesta et al. 2004; Eraslan et al. 2008; Silva et al. 2008). The use of nutrient and NaCl salinities to assess these effects on pepper was adopted by Silva et al. (2008) and Eraslan et al. (2008). Their experiments were carried out under severe salt stress conditions (30–60 mmol L^{-1} NaCl and iso-osmotic nutrient salinity) and for periods (7 and 60 d) not sufficiently large to acquire data on crop production.

The aim of this study was to evaluate the long term effects of moderate nutrient or NaCl salinity on growth, crop production, gas exchange and water relations of pepper grown in a substrate soilless closed system. The use of nutrient solutions with the same EC but prepared with different salinity sources could help to distinguish between osmotic and ion specific effects of salt stress on pepper plants.

Materials and Methods

Location, plant material, treatments and growth conditions

The experiment was conducted during spring and summer 2009 in an 800 m^2 unheated polyethylene greenhouse in southern Italy (37.3° N, 15.0° E; 20 m a.s.l.). Seedlings of pepper hybrid F_1 'Icaro' (Olter, Asti, Italy), 3/4 long type, were transplanted on April 8 at the third true-leaf stage, one for each pot (height 20 cm, diameter 19 cm) containing 5 liters of perlite (particle size 3–5 mm). Pots were placed in troughs with a distance of 33 cm between the pots and 100 cm between troughs, creating a plant density of 3 plants m^{-2}.

Three NS treatments were used: a control (2 dS m^{-1}) and two saline NS obtained by adding nutrients to the control maintaining the same ion ratio (nutrient salinity) or 18 mmol L^{-1} of NaCl (NaCl salinity). The NS of both salinity treatments had the same EC (4 dS m^{-1}) and almost the same molar concentrations (65.1 and 66.1 mmol L^{-1} in nutrient and NaCl salinity treatments, respectively). The control NS had the following composition (mmol L^{-1}): 11.0 NO_3^-, 1.3 $H_2PO_4^-$, 6.6 K^+, 0.9 SO_4^{2-}, 3.4 Ca^{2+}, 2.5 Mg^{2+}, 0.5 NH_4^+, 2.1 Na^+, 1.8 Cl^-. The nutrient salinity treatment had the following composition (mmol L^{-1}): 28.7 NO_3^-, 3.3 $H_2PO_4^-$, 16.1 K^+, 1.5 SO_4^{2-}, 6.2 Ca^{2+}, 4.2 Mg^{2+}, 1.2 NH_4^+, 2.1 Na^+, 1.8 Cl^-. The concentration of microelements (μmol L^{-1}) was: 15 Fe^{3+}, 10 Mn^{2+}, 0.75 Cu^{2+}, 5 Zn^{2+}, 30 B^{3+}, 0.5 Mo^{6+} for all treatments. The salinity treatments started a week after transplanting. Each experimental unit consisted of one trough containing 24 plants. The treatments were arranged in a randomized complete block design with four replicates per treatment.

A closed soilless cultivation system was adopted. Nutrient solutions were pumped from independent tanks with a drip irrigation system with one emitter per plant and a flow rate of 4 L h^{-1}. The drainage returned to the feeding tank where it was mixed with the new solution and added at each irrigation, to restore crop consumption. The pH of NS was continuously measured and adjusted to the predefined value (5.9) by adding sulphuric acid (96 %). The irrigation volume and frequency were determined according to the water retention curve of the substrate (measured by a Sandbox, Eijkelkamp, The Netherlands), the vapour pressure deficit inside the greenhouse and plant growth. Five to twenty irrigations were applied per day, while the volume per pot for each irrigation ranged from 50 to 180 cm^3 during the cultivation period. Recirculating NS was drained and replaced when the EC exceeded the initial value by 2 dS m^{-1}. The recirculating solution was changed 7, 30, and 22 times for control, NaCl salinity and nutrient salinity treatments, respectively. During the experimental period, the mean EC of the recirculating NS was 2.98 ± 0.06 dS m^{-1} for the control, 4.68 ± 0.09 dS m^{-1} and 4.68 ± 0.04 dS m^{-1} for NaCl and nutrient salinity, respectively.

Plants were grown under natural light conditions; windows were automatically opened when the air temperature exceeded 30 °C. The mean air temperature, the vapor pressure deficit and the global radiation inside the greenhouse during the experimental period were recorded on a data logger (CR10X, Campbell Scientific, Logan, Utah). The mean air temperature was 23.7 °C, the mean vapour pressure deficit 1.5 kPa and the mean global radiation 10.9 MJ m^{-2}. More specifically, the maximum and mean air temperatures were 26.1 and 18.7 °C in April, 31.0 and 23.1 °C in May, 32.6 and 25.4 °C in June, 35.5 and 27.7 °C in July. Harvest started in the first week of July and ended at 104 d after transplanting (end of experiment).

Data collection and analysis

The EC of recirculating NS was continuously measured by means of electrical conductivity probes connected to a datalogger (CR10X, Campbell Scientific, Logan, Utah).

At 49, 77 and 104 d after transplanting (DAT), 4 plants per replicate were divided into leaves, stems, fruits and roots and oven dried at 70 °C until they reached a steady weight for dry mass determination. Leaf area was measured using an area meter (WinDIAS 2; DELTA-T DEVICES Ltd, Cambridge, U.K.) calibrated to 0.01 cm^2.

The number of fruits, mean fruit mass and total yield were measured.

At 95 DAT, net CO_2 assimilation rate (ACO_2, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), chlorophyll content, leaf relative water content (RWC), leaf osmotic adjustment and leaf water potential were measured on the uppermost fully expanded leaves on 12 plants per treatment.

The gas exchange measurements were carried out using a portable photosynthesis system (LCi; ADC BioScientific Ltd., UK) with a 6.25 cm^2 leaf chamber. Leaf temperature was 34.4 ± 2 °C. Measurements were made between 11.00 and 13.00 hr.

For chlorophyll determination, the extraction was carried out according to Moran and Porath (1980) and quantified according to Porra et al. (1989) using an UV/VIS spectrophotometer (Lambda 3B; PERKIN-ELMER, Waltham, Massachusetts).

Leaf water potential was measured using a dewpoint potentiometer (WP4; Decagon Devices Inc., Pullman, WA). Total water potential (Ψ_w) was measured between 11.00 and 13.00 hr on one tissue disk (4 cm diameter) per plant. After the measurement of water potential, leaf disks were frozen at −80 °C. When thawed, osmotic potential (Ψ_π) was determined using the same instrument. Turgor potential (Ψ_p) was calculated as the difference between Ψ_w and Ψ_π (Hsiao 1973).

For leaf RWC, 30 disks of 10 mm diameter, for each replicate, were weighed to determine the fresh mass (FM). Turgid mass (TM) was obtained after floating leaf disks on distilled water at 20 °C in dark conditions for 24 h. Dry mass (DM) was determined after oven drying at 70 °C until weights were stable. RWC (%) was calculated according to the following equation: RWC = 100 × (FM−DM)/(TM−DM).

Leaf osmotic adjustment was determined as the difference: $\Psi_{\pi 0} \times V_0 - \Psi_\pi \times V$, where $\Psi_{\pi 0}$ and V_0 are respectively the osmotic potential and the osmotic volume of unstressed plants while Ψ_π and V are respectively the osmotic potential and the osmotic volume of salinized plants (Morgan 1984). The osmotic volume (V) was approximated by the corresponding RWC.

At 49, 77 and 104 DAT, dried plant organs (leaves, stems, fruits and roots) were ground separately and analyzed for Na^+ and Cl^- concentrations. The Na^+ and Cl^- concentrations of the whole plants were measured as the ratio between the sum of the ion amounts in each plant organ and the whole dry mass of the plants. In addition, the most recently matured leaves per each treatment were collected and analyzed for evaluating the K^+ and Ca^{2+} concentrations. The ion content was determined by means of ionic chromatography (Dionex IC 25 Ion Chromatograph, 40 EG Eluent Generator) using an Ion Pac AS11-HC separation column for Cl^- and an Ion Pac CS12A for Na^+, K^+ and Ca^{2+}.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine the effects of salinity. When significant differences were found, a Student-Newman-Keuls post hoc multiple comparison procedure was used to assess significant differences between individual means. Analyses were conducted on non-transformed data. An α level of 0.05 was selected for the ANOVA and Student-Newman-Keuls post hoc tests. Statistical analyses were performed with CoStat release 6.311 (CoHort Software, 2001).

Results

Plant growth, dry mass partitioning and yield

Dry mass production was significantly affected by salinity during the experiment (Table 1). At the first measurement (49 DAT) NaCl salinity reduced the plant dry mass (−29 %) compared to the mean of the control and nutrient salinity combined, and the reduction was greater at 77 DAT (−31 %) (Table 1). At the end of the experiment (104 DAT) nutrient salinity also negatively influenced (−17 %) the mass production of the plant compared to the control but to a lesser extent than NaCl salinity (−41 %) (Table 1). The dry matter partitioning showed that NaCl salinity affected the allocation of assimilates to stems and leaves more than to roots (Table 1). At 77 DAT the reduction of root dry mass of NaCl stressed plants was about 18 % compared to the control, whereas this decrease was of about 32 % for stems, leaves and fruits. At the end of the experiment (104 DAT), the negative effect of salinity on root dry mass was greater (−30 %) and was also evident for plants stressed by nutrient salinity (−30 %). At the later stages of the experiment (i.e. from 77 DAT and thereafter), the root dry mass limitation in salt-stressed compared to control plants was particularly evident. The highest reduction was observed for fruit dry mass under NaCl salinity, which was 44 % lower than that of the control, whereas a diminution of about 22 % was obtained with nutrient salinity (Table 1). For stems and leaves the responses were similar to those observed at 49 and 77 DAT.

Dry matter content of fruits for the salinity treatments was about 12 and 25 % higher than controls at 77 and 104 DAT respectively, whereas no differences were found in the other plant organs (data not shown).

Yield was significantly affected by salinity (Table 2). The total yield of nutrient and NaCl salinity treatments was reduced by 33 and 48 %, respectively, compared to the control. For all salt stressed plants this decrease was related to a strong reduction of mean fruit mass (−42 %), although the number of fruits was not different compared to the control. When NaCl and nutrient salinities were compared, the lower yield of NaCl treatment was accompanied by a significant decrease in fruit number (−20 %).

The effect of NaCl salinity on total leaf area was already manifested at the first measurement (49 DAT) when a reduction of about 35 % compared to the control was observed (Fig. 1A). A similar trend was observed at

Table 1. Dry mass production and partitioning (% in brackets, total dry mass = 100 %) of pepper as affected by nutrient and NaCl salinity.

Treatments	Dry mass (g plant^{-1})					Root/Shoot
	Roots	Stems	Leaves	Fruits	Total	
49 DAT						
Control	11.1 a$^+$ (29.8)	8.4 a (22.5)	17.8 a (47.7)	–	37.3 a	0.42 b
Nutrient salinity	12.1 a (33.9)	7.6 a (21.3)	16.0 a (44.8)	–	35.7 a	0.51 ab
NaCl salinity	9.7 b (37.6)	5.2 b (20.2)	10.9 b (42.2)	–	25.8 b	0.60 a
Significance	*	*	*	–	*	*
77 DAT						
Control	17.4 a (11.3)	19.3 a (12.5)	35.6 a (23.2)	81.4 a (53.0)	153.7 a	0.13 ab
Nutrient salinity	15.8 ab (10.3)	21.9 a (14.3)	37.3 a (24.5)	77.8 a (50.9)	152.8 a	0.11 b
NaCl salinity	14.2 b (13.4)	13.0 b (12.2)	24.0 b (22.6)	55.1 b (51.8)	106.3 b	0.15 a
Significance	*	***	**	*	**	*
104 DAT						
Control	22.9 a (7.9)	39.5 a (13.6)	54.5 a (18.8)	173.4 a (59.7)	290.3 a	0.09 ab
Nutrient salinity	16.7 b (7.0)	38.9 a (16.2)	49.9 a (20.8)	134.6 b (56.0)	240.1 b	0.07 b
NaCl salinity	15.7 b (9.1)	25.3 b (14.7)	32.8 b (19.2)	98.0 c (57.0)	171.8 c	0.10 a
Significance	*	**	*	***	**	*

DAT: days after transplanting
*, **, ***: Significant at $0.01 < P \leq 0.05$, $0.001 < P \leq 0.01$, $P \leq 0.001$, respectively.
$^+$ Values followed by a common letter in a column are not significantly different at P = 0.05 (Student-Newman-Keuls test).

Table 2. Yield, number and mean mass of pepper fruits as influenced by nutrient and NaCl salinity (104 DAT).

Treatments	Yield (kg plant^{-1})	Number of fruits (n plant^{-1})	Mean mass (g fruit^{-1})
Control	2.04 a$^+$	12.9 ab	159.4 a
Nutrient salinity	1.37 b	14.5 a	94.5 b
NaCl salinity	1.07 c	11.7 b	91.4 b
Significance	***	*	**

DAT: days after transplanting
*, **, ***: Significant at $0.01 < P \leq 0.05$, $0.001 < P \leq 0.01$, $P \leq 0.001$, respectively.
$^+$ Values followed by a common letter in a column are not significantly different at P = 0.05 (Student-Newman-Keuls test).

77 DAT. At 104 DAT, a further reduction was determined by NaCl (−40 %) and nutrient salinity also negatively affected the leaf area (−14 %) compared to the control (Fig. 1A). No significant differences were observed between the treatments for specific leaf area (Fig. 1B).

Leaf photosynthetic characteristics and plant water relations

Gas exchange (net assimilation), stomatal conductance and transpiration were not significantly affected by salt treatments (Table 3). The concentration of chlorophyll was slightly higher in plants under nutrient salinity with a significant increase of about 6 % compared to the other treatments (Table 3).

More evident were the effects of both salinities on leaf water relations (Table 4). Total leaf water potential and osmotic potential decreased with all salinity treatments compared to the control (−45 and −11 % respectively, considering the mean of salinities). In spite of the decrease in osmotic potential, the turgor potential was lower (−14 %) in salinity-stressed plants compared to the control (Table 4). A significant reduction of RWC (−9 %) was also caused by both salinities. The osmotic adjustment (Table 4) of plant leaves differed significantly between the salinity treatments, with a significantly higher value observed for NaCl salinity (0.35 *vs.* 0.29 MPa).

Sodium and chloride plant content

The sodium and chloride concentrations of plants irrigated with NaCl nutrient solution were higher than control and

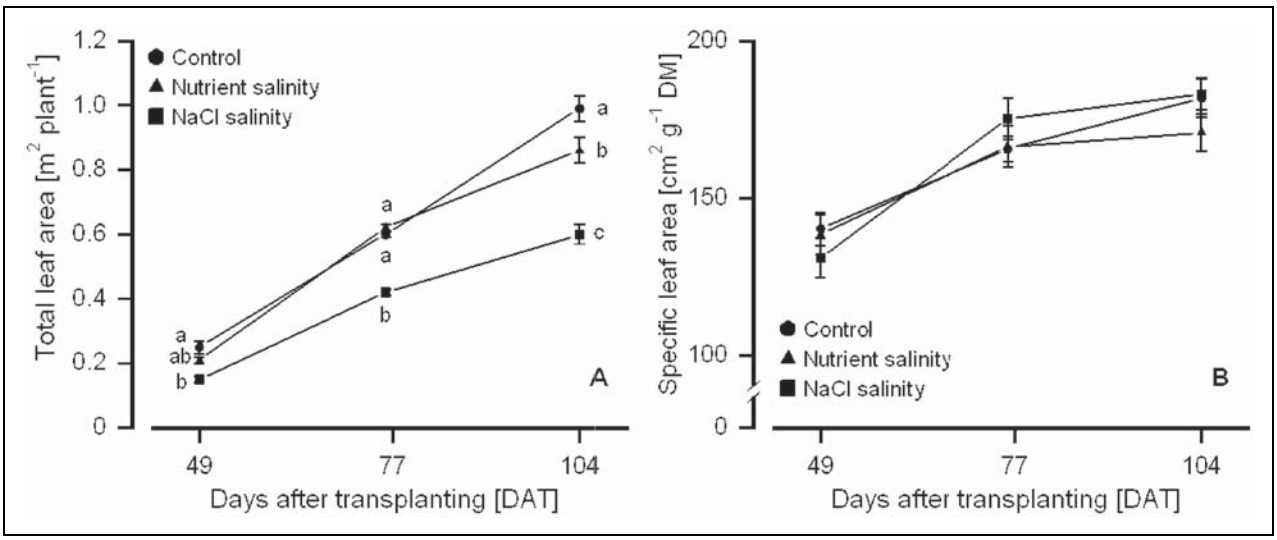


Fig. 1. Total (A) and specific (B) leaf area of pepper as affected by nutrient and NaCl salinity.
At each sampling date, different letters indicate significant differences at P = 0.05 (Student-Newman-Keuls). Vertical bars indicate ± SE of the mean (n = 3).

Table 3. Gas exchange and chlorophyll content of pepper as affected by nutrient and NaCl salinity (95 DAT).

Treatments	CO_2 net assimilation ($\mu mol\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)	Transpiration ($mmol\ m^{-2}\ s^{-1}$)	Chlorophyll ($\mu g\ cm^{-2}$)
Control	17.4	0.52	9.6	73.4 b[+]
Nutrient salinity	16.6	0.47	9.0	77.1 a
NaCl salinity	16.7	0.47	8.8	72.1 b
Significance	NS	NS	NS	*

DAT: days after transplanting
*, NS: Significant at $0.01 < P \leq 0.05$, or not significant, respectively.
[+] Values followed by a common letter in a column are not significantly different at P = 0.05 (Student-Newman-Keuls test).

Table 4. Water potential, osmotic potential, turgor potential, relative water content and osmotic adjustment of pepper as influenced by nutrient and NaCl salinity (95 DAT).

Treatments	Water potential (MPa)	Osmotic potential (MPa)	Turgor potential (MPa)	Relative water content (%)	Osmotic adjustment (MPa)
Control	−0.73 a[+]	−1.67 a	0.93 a	75.1 a	–
Nutrient salinity	−1.03 b	−1.83 b	0.80 b	67.6 b	0.29 b
NaCl salinity	−1.07 b	−1.87 b	0.80 b	69.2 b	0.35 a
Significance	**	*	*	*	*

DAT: days after transplanting
*, **: Significant at $0.01 < P \leq 0.05$, $0.001 < P \leq 0.01$, respectively.
[+] Values followed by a common letter in a column are not significantly different at P = 0.05 (Student-Newman-Keuls test).

nutrient salinity treatments in all of the measurements throughout the experiment (Fig. 2). At 77 and 104 DAT, the sodium concentrations of the nutrient salinity stress treatment were significantly lower compared to the control (−43 and −30 %, respectively) (Fig. 2A). A similar behavior was observed for Cl^- (Fig. 2B), but a significantly lower content of Cl^- in the plant tissues under nutrient salinity stress compared to unstressed plants was observed at 49 and 77 DAT.

Considering the distribution of Na^+ within the plant organs, it is evident that this ion is mainly accumulated in the roots, with considerable amounts also stored in the stems but only small amounts stored in the leaves and fruits (Table 5). The Na^+ content in all parts of the plant was higher under NaCl salinity than in other treatments, with a thirteen-fold increase in the leaves. A significant difference between nutrient salinity and control plants was found only for the Na^+ in the stems, which showed a lower content (about −60 %) in plants treated with nutrient salinity. The distribution of Cl^- showed a similar trend as Na^+ (Table 5).

K^+ and Ca^{2+} concentrations in the most recently matured leaves were significantly affected by NaCl salinity only at 104 DAT with a reduction, compared to other treatments, of about 16 and 20 %, respectively (Fig. 3).

Discussion

Increased salinity affected both growth and yield of pepper. The more detrimental effects determined by NaCl (18 mmol L^{-1}) than nutrient salinity showed that pepper

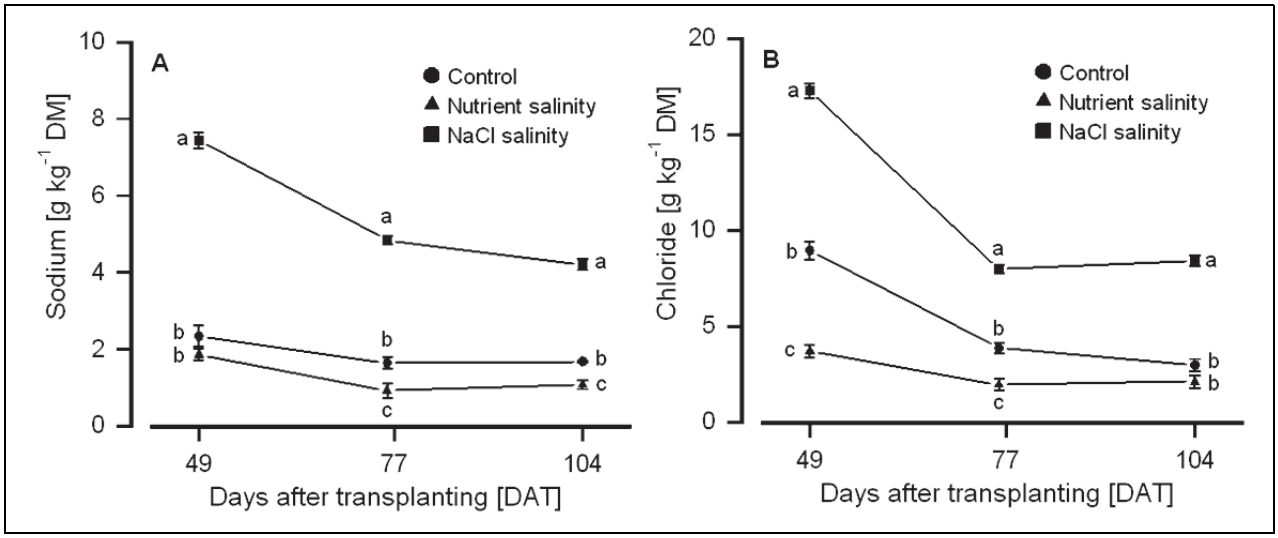


Fig. 2. Sodium (A) and chloride (B) concentrations in pepper plant as influenced by nutrient and NaCl salinity. At each sampling date, different letters indicate significant differences at P = 0.05 (Student-Newman-Keuls). Vertical bars indicate ± SE of the mean (n = 3).

Table 5. Sodium and chloride concentration (g kg^{-1} of DM) of leaves, stems, fruits and roots of pepper plants as influenced by nutrient and NaCl salinity (104 DAT).

Treatments	Na^+				Cl^-			
	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots
Control	0.1 b[+]	5.2 b	0.7 b	7.6 b	3.0 b	8.0 b	1.1 b	8.8 b
Nutrient salinity	0.1 b	2.0 c	0.9 b	5.1 b	1.8 b	4.4 c	1.0 b	6.7 b
NaCl salinity	1.3 a	9.8 a	1.3 a	19.3 a	13.1 a	18.9 a	2.2 a	21.5 a
Significance	***	***	*	***	*	***	**	***

DAT: days after transplanting

*, **, ***: Significant at $0.01 < P \leq 0.05$, $0.001 < P \leq 0.01$, $P \leq 0.001$, respectively.

[+] Values followed by a common letter in a column are not significantly different at P = 0.05 (Student-Newman-Keuls test).

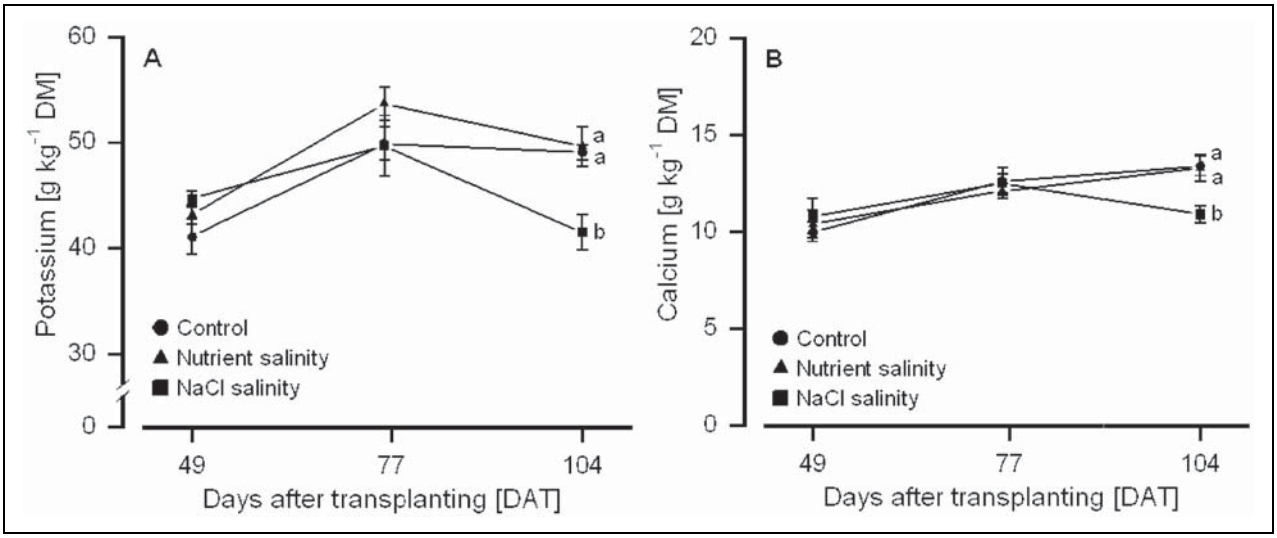


Fig. 3. Potassium (A) and calcium (B) concentrations in the most recently matured leaves as influenced by nutrient and NaCl salinity.
At each sampling date, different letters indicate significant differences at P = 0.05 (Student-Newman-Keuls). Vertical bars indicate ± SE of the mean (n = 3).

response to salt stress is both osmotic and ion specific. On the contrary, Silva et al. (2008) concluded, from the similar effects of 30 mmol L^{-1} NaCl and iso-osmotic nutrient salinity, that at the particular salt level the response could be attributed only to an osmotic effect. These different results could be explained by the short-term and long-term effects of salinity. In the study of Silva et al. (2008), the duration of the salinity treatments was shorter (7 d) than this experiment (104 d). Noteworthy, the negative effect of salinity increased during the cultivation period. The duration of salt stress, the plant age and size are key factors influencing the plants' response (De Pascale et al. 2003). In contrast to pepper, previous experiments on tomato and eggplant demonstrated that the response to salinity is of osmotic origin and, therefore, irrespective to salt composition from the nutrient solution in the root environment (Adams 1991; Savvas and Lenz 2000). Ion toxicity affected plant growth less than the osmotic effect, but at high salinity levels or in sensitive species ion toxicity may dominate the osmotic effect (Munns and Tester 2008). Pepper, tomato and eggplant are classified as moderately sensitive to salinity (Ayers and Westcot 1985; Maas 1993). Nevertheless, the rate of yield reduction of pepper to the increasing salinity was higher than that measured for tomato and eggplant (Maas 1993), indicating a more detrimental effect of salinity on pepper than tomato and eggplant.

The exposure of plants to salt stress and the consequent decrease of external water potential reduced the leaf water potential. Under these conditions, an accumulation of solutes in cells may occur in plants to lower the osmotic potential for maintaining the turgor pressure (Blum et al. 1996). In agreement with Martínez-Ballesta et al. (2004) and Silva et al. (2008), the reduction of osmotic potential did not compensate the decrease in water potential. Thus, the osmotic adjustment was not sufficient to maintain leaf turgor. The inability of pepper to adjust osmotically after few days of salt stress (8–10 d) (Martínez-Ballesta et al. 2004; Silva et al. 2008) and during a whole cultivation cycle (this experiment) highlights that this crop is unable to adapt to saline conditions. As a consequence of the water potential decrease, a reduction in leaf cell elongation and division can occur, which leads to a smaller final size of leaves (Munns and Tester 2008). This most likely explains the reduced plant leaf area observed in this experiment, given that the leaf number was not significantly different among the treatments (data not shown).

The lower leaf area of salt-stressed plants was the main cause for the decrease of dry mass production compared to the control. In our study, the photosynthetic rate did not change among the treatments. Although inhibition of vegetative growth and yield in pepper has been associated with marked reduction of photosynthesis under salinity stress conditions higher than that used in this experiment (Chartzoulakis and Klapaki 2000; De Pascale et al. 2003; Lycoskoufis et al. 2005). The further decrease in dry mass production of NaCl-stressed plants could be explained by the ion toxicity effect of NaCl salinity (Munns and Tester 2008), which reduced the leaf area more than nutrient salinity.

Results on dry mass partitioning showed that root growth was less affected by NaCl salinity than shoot growth, a result confirmed for pepper by De Pascale et al. (2003), for tomato by Cuartero and Fernández-Muñoz (1999), and also for cereals by Munns and Tester (2008).

At the end of the experiment, fruit growth was more impaired by salinity than stem and leave growth, as previously observed by Azuma et al. (2010). These authors suggested that the greatest reduction in fruit growth could be related to the higher concentration of Na^+ in fruits than in leaves, causing salt toxicity which induced a decrease in water influx and ionic imbalance in the fruit tissues. In the present investigation, however, no differences in Na^+ concentrations between leaves and fruits were found. It seems that the competitive capacity for attracting assimilates by various organs was modified by salt stress. This response differs between vegetable species: high salinity of the root medium increased the fraction of dry matter partitioned into the fruits of cucumber (Marcelis 1994), but had no effect in tomato (Ehret and Ho 1986). The higher reduction of fruit dry mass of NaCl-treated plants compared to nutrient stress is mainly related to the lower number of fruits that could be explained by the lower source strength as showed by leaf area and net assimilation rate. In pepper, it has previously been shown that when the source strength was decreased the rate of flower/fruit abortion increased (Marcelis et al. 2004).

The most detrimental effect of NaCl salinity on pepper growth could be explained by the toxicity of Na^+ and Cl^-, according their highest concentrations on the NaCl-stressed plants since the first measurement. However, severe toxic levels (> 100 mmol) (Ghanem et al. 2008) were observed for Cl^- (370 mmol kg^{-1} leaf DM at 104 DAT) but not for Na^+ (56 mmol kg^{-1} leaf DM at 104 DAT). Thus the main factor that lead to a reduction in growth of pepper plants exposed to NaCl salinity was related to Cl^- toxicity, as also observed by Rubio et al. (2010) on pepper plants exposed to 30 mmol L^{-1} NaCl. Nevertheless, the leaves of NaCl-stressed plants did not show toxicity symptoms despite the high concentration of Cl^-.

In this study, although the leaf sodium content of NaCl-stressed plants increased more than in the roots compared to the control (thirteen-fold and three-fold, respectively), Na^+ is mainly accumulated in roots. The ability of pepper to limit uptake and/or transport of Na^+ from the root zone to the leaves has also been shown by Chartzoulakis and Klapaki (2000) and by Blom-Zandstra et al. (1998), whereas Silva et al. (2008) found a higher content of Na^+ in shoots than in roots. However, pepper is unable to exclude Cl^- from the leaves, as previously observed by Chartzoulakis and Klapaki (2000). This may be due to its different capability for compartmentation of Na^+ and Cl^- ions in the root cells.

Our results showed that the effect of NaCl salinity on pepper is mainly related to Cl^- toxicity but ion imbalances also play a role. The most recently matured NaCl-treated pepper leaves were characterized by lower concentrations of K^+ and Ca^{2+} than non-stressed plants at the later stages of the experiment, although not to a level of deficiency. Na^+ competes with K^+ for binding sites essential for cellular function (Tester and Davenport 2003). More than 50 enzymes are activated by K^+ which cannot be substituted by Na^+ (Bhandal and Malik 1988). Moreover, protein synthesis requires high concentrations of K^+ and thus could be reduced by elevated Na^+ levels (Tester and Davenport 2003). In addition, high concentrations of Na^+ decrease Ca^{2+} availability of the plant as previously found by Silva et al. (2008), who reported lower K^+ and Ca^{2+} concentrations in pepper leaves specifically under NaCl stress compared to osmotic stress. As previously found by Rubio et al. (2010), the high content of Cl^- in the plants caused a significant reduction in NO_3^- in the leaves and stems, but did not affect total N accumulation compared to control (data not shown).

In the present research, both salinity treatments decreased total yield compared to the control by reducing the mean fruit mass but not fruit number. Similar results were found in pepper with Na^+ salinity by Navarro et al. (2002) and Rubio et al. (2009). The main effect of salinity on yield was related to a significant reduction of water accumulation in the fruits of both salinity treatments and also to a decrease of fruit dry mass. De Pascale et al. (2003) observed a reduction in the water accumulation of pepper fruits at the highest salinity level. According to Johnson et al. (1992) osmotic adjustment in salt-stressed plants is accompanied by a decrease in fruit water content. A lower water accumulation in the fruits, without significant effects on dry mass production, was found in eggplant (Savvas and Lenz 2000) and tomato (Ehret and Ho 1986; Adams 1991) exposed to salinity stress, irrespective of the salinity source.

In conclusion, the negative response of pepper to moderate salt stress depends on both osmotic and ion-specific effects. The osmotic adjustment of stressed plants was not sufficient to maintain leaf turgor. As a consequence, total leaf area decreased and a restriction of water accumulation in the fruits occurred. The reduction in plant leaf area resulted in a proportional decrease in dry mass production. The rate of photosynthesis was not affected by the present moderate salinity conditions. The detrimental effect on leaf growth was most evident under NaCl stress. This could be mainly explained by the toxicity of Cl^-, although slight nutrient imbalances due to Na^+ and Cl^- antagonisms were observed.

Acknowledgement

The author is very grateful to Ms. Hannah Wrighth for the English revision of the manuscript.

References

Adams, P. 1991: Effects of increasing the salinity of the nutrient solution with major nutrient or sodium chloride on the yield, quality and composition of tomatoes grown in rockwool. J. Hort. Sci. **66**, 201–207.

Ayers, R. and W. Westcot 1985: Water quality for agriculture. Irrigation and Drainage Paper No. 29. FAO, Roma, Italy.

Azuma, R., N. Ito, N. Nakayama, R. Suwa, N.T. Nguyen, J.A. Larrinaga-Mayoral, M. Esaka, H. Fujiyama and H. Saneoka 2010: Fruits are more sensitive to salinity than leaves and stems in pepper plants (*Capsicum annuum* L.). Sci. Hort. **125**, 171–178.

Bhandal, I.S. and C.P. Malik 1988: Potassium estimation, uptake, and its role in the physiology and metabolism of flowering plants. Inter. Rev. Cytol. **110,** 205–254.

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.

Blum, A., R. Munns, J.B. Passioura and N.C. Turner 1996: Genetically engineered plants resistant to soil dry and salt stress: How to interpret osmotic relations? Plant Physiol. **110,** 1050–1053.

Carvajal, M., D.T. Cooke and D.T. Clarkson 1996: Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function. Planta **199,** 372–381.

Chartzoulakis, K. and G. Klapaki 2000: Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci. Hort. **86**, 247–260.

Cuartero, J. and R. Fernández-Muñoz 1999: Tomato and salinity. Sci. Hort. **78**, 83–125.

De Pascale, S., C. Ruggiero and G. Barbieri 2003: Physiological responses of pepper to salinity and drought. J. Am. Soc. Hort. Sci. **128**, 48–54.

Ehret, D.L. and L.C. Ho 1986: The effects of salinity on dry matter partitioning and fruit growth in tomatoes grown in nutrient film culture. J. Hort. Sci. **61**, 361–367.

Eraslan, F., A. Güneş, A. İnal, N. Çiçek and M. Alpaslan 2008: Comparative physiological and growth responses of tomato and pepper plants to fertilizer induced salinity and salt stress under greenhouse conditions. Int. Meet. Soil Fert. Land Manag. Agroclimat. 687–696.

Ghanem, M.E., A. Albacete, C. Martínez-Andújar, M. Acosta, R. Romero-Aranda, I.C. Dodd, S. Lutts and F. Pérez-Alfocea 2008: Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). J. Exp. Bot. **59**, 3039–3050.

Giuffrida, F. and C. Leonardi 2012: Nutrient solution concentration on pepper grown in soilless closed system: yield, fruit quality, water and nutrient efficiency. Acta Agric. Scand. Section B – Soil Plant Sci. **62**, 1–6.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert 2000: Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Physiol. Plant Molec. Biol. **51**, 463–499.

Hsiao, T.C. 1973: Plant response to water stress. Ann. Rev. Plant Physiol. Plant Molec. Biol. **24**, 519–570.

Jeschke, W.D., Z. Aslam and H. Greenway 1986: Effect of NaCl on ion relations and carbohydrate status of roots and on osmotic regulation of roots and shoots of *Atriplex amnicola*. Plant Cell Environ. **9**, 559–569.

Johnson, R.W., M.A. Dixon and D.R. Lee 1992: Water relations of the tomato fruit during growth. Plant Cell Environ. **15**, 947–953.

Leigh, R.A. and R. Storey 1993: Intercellular compartmentation of ions in barley leaves in relation to potassium nutrition and salinity. J. Exp. Bot. **261**, 755–762.

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. 1993: Testing crops for salinity tolerance. In: Maranville, J.W., B.V. Baigar, R.R. Duncan and J.M. Yohe (eds.): Proceedings workshop on adaptation of plants to soil stresses. Intsormil, Lincoln, 234–247.

Marcelis, L.F.M. 1994: Fruit growth and dry matter partitioning in cucumber. Wageningen Agricultural University, Wageningen, PhD Diss.

Marcelis, L.F.M., E. Heuvelink, L.R. Baan Hofman-Eijer, J. Den Bakker and L.B. Xue 2004: Flower and fruit abortion in sweet pepper in relation to source and sink strength. J Exp. Bot. **55**, 2261–2268.

Martínez-Ballesta, M.C., V. Martínez 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.

Moran, R. and D. Porath 1980: Chlorophyll determination in intact tissues using *N,N*-dimethylformamide. Plant Physiol. **65**, 478–479.

Morgan, J.M. 1984: Osmoregulation and water stress in higher plants. Ann. Rev. Plant Physiol. **35**, 299–319.

Munns, R. and M. Tester 2008: Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. **59**, 651–681.

Navarro, J.M., C. Garrido, M. Carvajal and V. Martínez 2002: Yield and fruit quality of pepper plants under sulphate and chloride salinity. J. Hort. Sci. Biotech. **77**, 52–57.

Navarro, J.M., C. Garrido, V. Martínez and M. Carvajal 2003: Water relations and xylem transport of nutrients in pepper plants grown under two different salts stress regimes. Plant Growth Regul. **41**, 237–245.

Porra, R.J., W.A. Thompson and P.E. Kriedemann 1989: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Bioch. Bioph. Acta **975**, 384–394.

Rubio, J.S., F. García-Sánchez, F. Rubio and V. Martínez 2009: Yield, blossom-end rot incidence, and fruit quality in pepper plants under moderate salinity are affected by K^+ and Ca^{2+} fertilization. Sci. Hort. **119**, 79–87.

Rubio, J.S., F. García-Sánchez, F. Rubio, A.L. García and V. Martínez 2010: The importance of K^+ in ameliorating the negative effects of salt stress on the growth of pepper plants. Europ. J. Hort. Sci. **75**, 33–41.

Savvas, D. and F. Lenz 2000: Effects of NaCl or nutrient-induced salinity on growth, yield, and composition of eggplants grown in rockwool. Sci. Hort. **84**, 37–47.

Silva, C., V. Martínez and M. Carvajal 2008: Osmotic versus toxic effects of NaCl on pepper plants. Biol. Plant. **52**, 72–79.

Tester, M. and R.J. Davenport 2003: Na^+ transport and Na^+ tolerance in higher plants. Ann. Bot. **91**, 503–527.

Wyn Jones, R.J. 1981: Salt tolerance. In: Johnson, C.B. (ed.): Physiological processes limiting plant productivity, Butterworths, London, 271–292.

Received 07/30/2012 / Accepted 03/07/2013

Addresses of authors: Francesco Giuffrida, Department of Agricultural and Food Science, Via Valdisavoia 5, I-95123, Catania, Italy, e-mail: francesco.giuffrida@unict.it.