Potassium and calcium enrichment alleviate salinity-induced stress in hydroponically grown endives

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Abstract

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Salinity either of soil or of irrigation water causes disturbance in plant growth and nutrient balance and reduces crop yields. The effects of NaCl salinity and/or calcium or potassium level on the plant growth and severity of gray mold (*Botrytis cinerea* [De Bary] Whetzel) were investigated in endive (*Cichorium endivia* L., cv. Green Curled) grown with the nutrient film technique under greenhouse conditions during early spring. Plants were supplied with nutrient solutions containing 40 mmol/l of sodium chloride (NaCl) and/or 10 mmol/l potassium sulphate (K_2SO_4). Additionally, plants treated with foliar spray of 15 mmol/l calcium nitrate [$(CaNO_3)_2$] or distilled water. Salinity or K- and Ca-enrichment mainly affected the upper part of endive plants and reduced leaf area. However, when salinity combined with either K- or Ca-enrichment, the negative impact of salinity on plant growth was reversed. Salinized and/or K- and Ca-enriched, plants did not differ in plant biomass, leaf/root ratio, leaf fresh weight, leaf number, and root length. Salinity did not have any impacts on photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration. Indeed, photosynthetic rate and stomatal conductance increased with Ca foliar application and decreased with K while the opposite effects were observed for the intercellular CO_2 concentration. Total nutrient uptake was reduced 2-fold in salt-treated plants compared to controls. No symptoms of tip-burn or blackheart were recorded throughout the experimental study. Endive grown in the nutrient film technique had tolerance to NaCl salinity, and this method could be used to exploit saline water in soilless culture. These findings also suggest that a proper management of the salt concentration of the nutrient solution plus external elemental enrichment may provide an efficient tool to improve the quality of leafy vegetables with little effect on yield.

Keywords: calcium foliar spay; *Cichorium endivia*; growth; nutrient film technique, potassium; salinity; soilless culture

The use of low quality water causes an increase of soil salinity, which may have negative effects on growth and yield of crops. Soil and water management, irrigation and drainage technologies and developments in plant breeding and selection enhance and facilitate the use of low quality water for irrigation with minimum adverse impact on soil productivity and the environment (Chartzoulakis, Klapaki 2000). In many areas the availability of good quality water is limited. Hence, the increasing demands for irrigation water forces the growers to utilize semi-saline underground water, which causes a gradual build-up of Na and Cl in the root zone to levels (Sonneveld 2000). An efficient way to overcome salinity problems is the introduction of salt tolerance to crops. Exposure to NaCl salinity affects the water and ion transport processes in plant, which may change the nutritional status and ion balance (Lauchli, Epstein 1990) as well as many physiological processes (Munns, Termaat 1986). Several studies reported the tolerance and the effects of salt on greenhouse grown vegetables, including hydroponically grown crops (Sonneveld 1988; Chartzoulakis, Loupassaki 1997; Pardossi et al. 1999; Chartzoulakis, Klapaki 2000; Tzortzakis 2009), but gaps in the knowledge exist.

One approach to minimize effects of salinity is use of nutrient foliar application or nutrient enrichment into soil or soilless culture to increase tolerance to plant salinity by alleviating Na^+ and Cl^- injury to plants (Alpaslan et al. 1999; Pardossi et al. 1999; Tzortzakis 2009). Previous work indicated that nutrient foliar application alleviated harmful effects of NaCl salinity and corrected nutrient status causing increases in salt tolerance of cereals, leguminous and tomato (*Lycopersicon esculentum* Mill.) plants (El-Fouly et al. 2002). An alternative solution to the salinity problem is to select plant species capable of utilizing sufficiently high concentrations of NaCl, edible for humans, and featuring high productivity, with glasswort (*Salicornia europaea* L.) being one of the most promising candidates to be included in the human diet (Ushakova et al. 2005). Employing glycinebetaine or proline provided cell membrane protection under salinity stress in onion (*Allium cepa* L.) bulbs (Mansour 1998). Endive (*Cichorium endivia* L.) is categorized as being moderately salt tolerant (De Pascale, Barbieri 1995).

In soilless culture, one problem may be accumulation of salts (e.g. Na^+, Cl^-) in the recirculating nutrient solution in irrigation water that are not efficiently absorbed by plants. Use of poor-quality water accelerates salinity build-up in the substrate and may produce negative effects on salt-sensitive crops (Incrocci et al. 2006). The extent to which salinity stress affects plant growth/development, is dependent on various factors, including plant species, cultivar, phenological stage, soluble salt composition, stress intensity and duration, and edaphoclimatic conditions (Cramer et al. 1995). Each plant has a salinity tolerance limit above which yield is reduced in a linear fashion, which is called the salinity threshold (ST). Thus, salination (50 mmol/l NaCl) of the nutrient solution of greenhouse tomatoes markedly decreased K content of the leaves, fruit-set rate, number of flowers, dry weights of the plants, fruit sizes, and stem heights (Achilea 2002). Salt-treated (1% NaCl) endive and fennel had decreased marketable yield by about 60%, but for lettuce (*Lactuca sativa* L.) yield was reduced about 15% (lettuce and endive appeared to be more sensitive to tip-burn and necrotic symptoms occurring in the crop under saline-sodic conditions). In addition, the gas exchange rates, stomatal conductance and product quality were reduced by salinity (De Pascale, Barbieri 1995). These symptoms may be attributed to low uptake of calcium, decreased xylem transport of this element, or to disturbed partitioning of cations in plant tissues at high concentration of sodium ions in the soil solution (Sonneveld 1988).

The use of enhanced potassic and nitric plant nutrition is an efficient method of preventing Na^+- or Cl^--induced stress in many crops, respectively. Previous studies in tomato, lettuce, Chinese cabbage (*Brassica rapa* L. var. *chinensis*), sweet corn (*Zea mays* L. var. *rugosa* Bonaf.), and grape fruit (*Citrus* × *paradise* Macht.) indicated that the application of Multi-K (potassium nitrate) was a very efficient method of combating the aforementioned stresses and enhancing crops performance under saline conditions, and it increased yields (Achilea 2002). Other papers (as reviewed by Achilea 2002) have shown that K alleviates Na deleterious effects under sodic conditions.

This study was undertaken to investigate to what extent salinity reduced biomass and affected plant growth and incidence of blackheart and tip-burn in young endive leaves. The influence of potassium and/or calcium enrichment on response of endive to salinity and disease severity were also addressed.

MATERIAL AND METHODS

Experimental design

The experiment was carried out in an unheated glasshouse with a North-South orientation at the Institute for Olive Tree and Subtropical Plants of Chania, Crete, Greece. Endive, cv. Green Curled, plants were grown under natural light from February to May (in 2008). Average minimum and maximum air temperatures during this period were 13°C and 27°C, respectively. Seedlings were produced in plastic seedling trays filled with expanded clay. Four-week old seedlings (grown during late winter time) were transplanted in nutrient film technique (NFT) channels in late March. Two replicate plots per salinity and/or potassium treatments were used and each plot consisted of a separated (twin trough with a 3% slope) NFT system contained in total 80 plants (40 plants sprayed with calcium nitrate and 40 plants sprayed with distilled water) per treatment. Rows were 0.3 m apart and plants were separated by 0.2 m in rows. The soilless culture system was closed with the excess nutrient solution drained away to approximately 97 l capacity catchment tanks and then recirculated. A nutrient solution (1:100 v/v) in water containing: NO_3-N = 14.29, K = 10.23, PO_4-P = 0.97, Ca = 3.74, Mg = 2.88, SO_4-S = 1.56 and Na = 1.31 mmol/l; and B = 18.52, Fe = 71.56, Mn = 18.21, Cu = 4.72, Zn = 1.53, and Mo = 0.52 µmol/l, respectively, was used in the NFT unit at a continuous flow rate of 3 l/min. Actual pH values of the nutrient solution collected in catchment tanks fluctuated between 6.1 and 7.2 while electrical conductivity (EC) was between 2.18–2.93 dS/m.

Sodium chloride, potassium sulphate and calcium nitrate treatments

Plants were grown for 7 weeks with a basic nutrient solution. Plants were subjected to salinity with sodium chloride (NaCl; 40 mmol/l) and/or potassium sulphate (K_2SO_4; 10 mmol/l) added to the basic solution (starting on the second week after transplanting). The saline solution was prepared with tap water containing $\simeq$ 5 mmol/l NaCl. To avoid osmotic shock NaCl was applied in daily increments of approximately 10 mmol/l until the final level was reached with constant EC (3.0 dS/m). The K_2SO_4 applied at once in the second week. In order to prevent undesired changes of the ion concentration, solutions were adjusted for mineral concentration daily and completely renewed every 2–3 weeks (NaCl and K_2SO_4 applied in each nutrient solution renewal).

Foliar applications of calcium nitrate were started 2 weeks after transplanting. Treated plants were sprayed (30–40 ml per plant) with a 15 mmol/l solution twice a week until 5 days before harvest; approximately 6 mmol of calcium nitrate were supplied to each plant over the whole growing period. Distilled water (0 mmol/l calcium nitrate) was used on control plants.

Gas exchange measurements

Leaf photosynthesis (P_n), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured using a portable infra-red gas analyser (model LI-6200, Li-Cor, Inc., Lincoln, NE, USA). Measurements were carried out between 9:00 to 11:30 a.m. at air temperatures from 28–30°C, vapor pressure deficit (VPD) lower than 20 mbar and photosynthetic photon flux density of 800 to 1,000 mmol/ms at the ambient CO_2 concentration. Measurements were replicated on five plants in each treatment and on two fully expanded, healthy, sun-exposed leaves per plant.

Vegetative growth and yield

At harvest, five plants per treatment were sampled. Plants were harvested, divided into above-ground parts and roots. Fresh weight (g/plant), longest root length (cm/plant), and length of the longest leaf (cm) were determined. Dry matter content of leaves and roots was calculated as percent of fresh weight (following drying for 48 h at 80°C). Leaf number, leaf/root ratio and total leaf area (dm^2/plant) were determined from the same plants for each treatment.

Incidence of tip-burn, or blackheart, was assessed at harvest and the severity of blackheart was estimated by rating young and/or older leaves for symptoms as follows: none – 1; slight – 2; mild – 3; severe – 4; very severe – 5. Blackheart symptoms recorded when interior leaves became necrotic at tips, and eventually signs of infection, mycelia attributable to a secondary invader (*Botrytis cinerea* [De Bary] Whetzel) progressed to the internal head.

Elemental analysis of nutrient solution

Samples (100 ml) of nutrient solution outflow were analysed weekly for K, Ca, and Na content by a flame photometer (JENWAY, PEP-7 Jenway, Dunmow, UK) and N/NO_3 content determined spectrophotometrically at 210 nm and 275 nm (Pye Unicam Hitachi U-1100, Tokyo, Japan). The apparent mineral uptake/plant was observed by using differences of concentration values for two subsequent measurements and summed up by the concentration of the corresponding element in the stock solution added for the referred week. The additional amounts of Ca and NO_3 through the water and nitric acid supply were included, as well as the additional K amounts for K-enriched treatments. There were unaccountable losses from the solution in the drainage system

Table 1. Influence of salinity (40 mmol/l NaCl), potassium sulphate (10 mmol/l K_2SO_4), and calcium nitrate [15 mmol/l $Ca(NO_3)_2$] on plant biomass (g/plant), leaf fresh weight (g/plant), leaf number, leaf length (cm), leaf dry weight (g/plant), and leaf area (dm^2/plant) of endive (cv. Green Curled) plants grown hydroponically in nutrient film technique

NaCl (mmol/l)	K-sulphate (mmol/l)	Ca-nitrate (mmol/l)	Plant biomass (g/plant)	Leaf fresh weight (g/plant)	Leaf number	Leaf length (cm)	Leaf dry weight (g/plant)	Leaf area (dm^2/plant)
0	0	0	$317.1^{ab\ Y}$	265.0^{ab}	28.5^{ab}	40.7^{ab}	5.2^{bc}	44.9^{f}
	0	15	314.7^{ab}	254.5^{ab}	31.2^{ab}	38.1^{ab}	6.3^{a}	38.2^{g}
	10	0	462.0^{a}	387.7^{a}	39.5^{a}	42.7^{ab}	5.1^{bc}	119.7^{a}
	10	15	262.7^{b}	218.0^{b}	28.2^{ab}	37.0^{b}	5.7^{ab}	52.1^{e}
40	0	0	294.2^{ab}	246.0^{ab}	30.0^{ab}	43.0^{ab}	4.9^{c}	74.5^{c}
	0	15	336.2^{ab}	275.3^{ab}	30.7^{ab}	42.0^{ab}	5.7^{ab}	90.8^{b}
	10	0	285.7^{ab}	247.5^{ab}	31.2^{ab}	44.2^{a}	5.1^{bc}	61.6^{d}
	10	15	278.2^{ab}	224.7^{b}	25.7^{b}	43.5^{a}	6.1^{a}	36.8^{g}

[Y]values in columns followed by the same letter are not significantly different, $P \leq 0.05$

along the plant row due to precipitation and acquisition by algae present in the system.

Statistical analysis

Data were tested for normality, and then subjected to analysis of variance (ANOVA). Sources of variation were NaCl, potassium, and calcium levels. Significant differences between mean values were determined using Duncan's multiple range test ($P \leq 0.05$) following one- or two-way ANOVA. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, USA) and graphs produced using Prism v.2.0 (Graph Pad Inc., San Diego, USA).

RESULTS

Gas exchange

The photosynthetic rate (P_n) increased (up to 29%) with Ca foliar application and decreased (up to 16%) with K enrichment but when Ca and K enrichment combined, an intermediate impact was observed for hydroponically grown endives (Fig. 1). The P_n did not differ among salinity treatments. Similar observations were recorded for stomatal conductance (g_s) regarding Ca and K enrichment but salinity reduced (or had no effects) the g_s among the treatments. The opposite effects were observed for the intercellular CO_2 concentration (C_i), resulting in decreased C_i following Ca foliar application and increased C_i following K enrichment. Salinity did not have marked impacts on C_i. Not strong correlation, but still significant, was observed among P_n and C_i ($y = -2.304x + 6.22$; $r^2 = 0.55$; $P < 0.001$) as well as among g_s and C_i ($y = 8.299x + 8.67$; $r^2 = 0.41$; $P < 0.05$) for endive.

Plant growth

ANOVA indicated that: root dry weight, leaf length and leaf area were affected by NaCl concentration; leaf dry weight and leaf area were affected by calcium nitrate $Ca(NO_3)_2$ concentration; leaf area were affected by potassium sulphate (K_2SO_4) concentration. From examination of the interaction of the above parameters, it resulted that leaf dry weight and leaf area were affected by the interaction of the NaCl and K_2SO_4 concentration; leaf area was affected by the interactions of the NaCl and $Ca(NO_3)_2$ concentration, $Ca(NO_3)_2$ and K_2SO_4 concentration and NaCl, $Ca(NO_3)_2$ and K_2SO_4 concentration. Other measures were not affected by the treatment, i.e., plant biomass averaged 318.8 g/plant, leaf/root ratio averaged 5.3, leaf fresh weight averaged 264.8 g per plant, leaf number averaged 31 leaves/plant and root length averaged 41.7 cm. No plants showed symptoms of ion toxicity. The greatest plant biomass (462 g/plant) and leaf number (averaged 39.5) were observed in K-enrichment and the lowest plant bio-

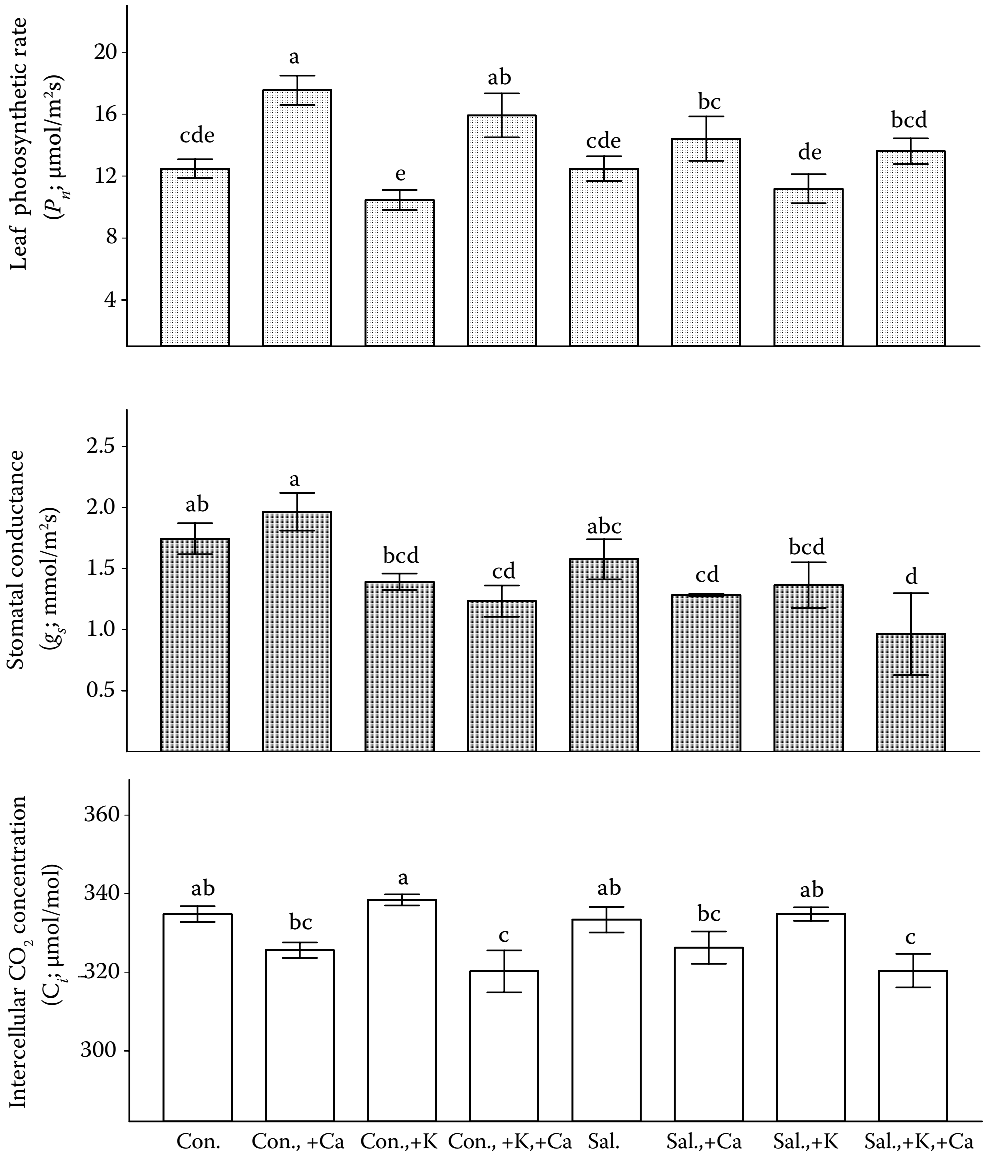

Fig. 1. Leaf photosynthetic rate (P_n, µmol/m^{2}s), stomata conductance (g_s, mmol/m^{2}s) and intercellular CO_2 concentration (C_i, µmol/mol) of fully expanded leaves of endive (cv. Green Curled) 5 weeks after treatment with NaCl

Conditions: CO_2 – 360 mbar; O_2 – 21% (v/v); PFD – 800–1,000 µmol/m^{2}s; air temperature – 29 ± 2°C. Values are means ± SE. Con. = control; Sal. = salinity (40 mmol/l of NaCl); +K = with potassium sulphate (10 mmol/l); +Ca = with calcium nitrate (15 mmol/l)

mass (262.7 g/plant) was observed when Ca foliar application combined with K-enrichment, including salinity for the lowest leaf number (averaged 25.7) (Table 1). Salinity increased leaf length and the effects were significant when salinity was combined with K- and/or Ca-enrichment. Potassium enrichment accelerated (up to 62%) leaf area produced by endive plants while Ca application reduced leaf area up to 14% compared with control plants. Indeed, salinity combined with K-enrichment reversed the impacts of the single K-enrichment itself.

Incidence of tip burn

There were no symptoms of tip-burn or blackheart severity in 40 mmol/l NaCl and/or Ca- or K-treated endive (data not presented).

Water and nutrient uptake

Plants treated with NaCl did not wilt. Total nutrient uptake was reduced 2-fold (21 ml of stock solu-

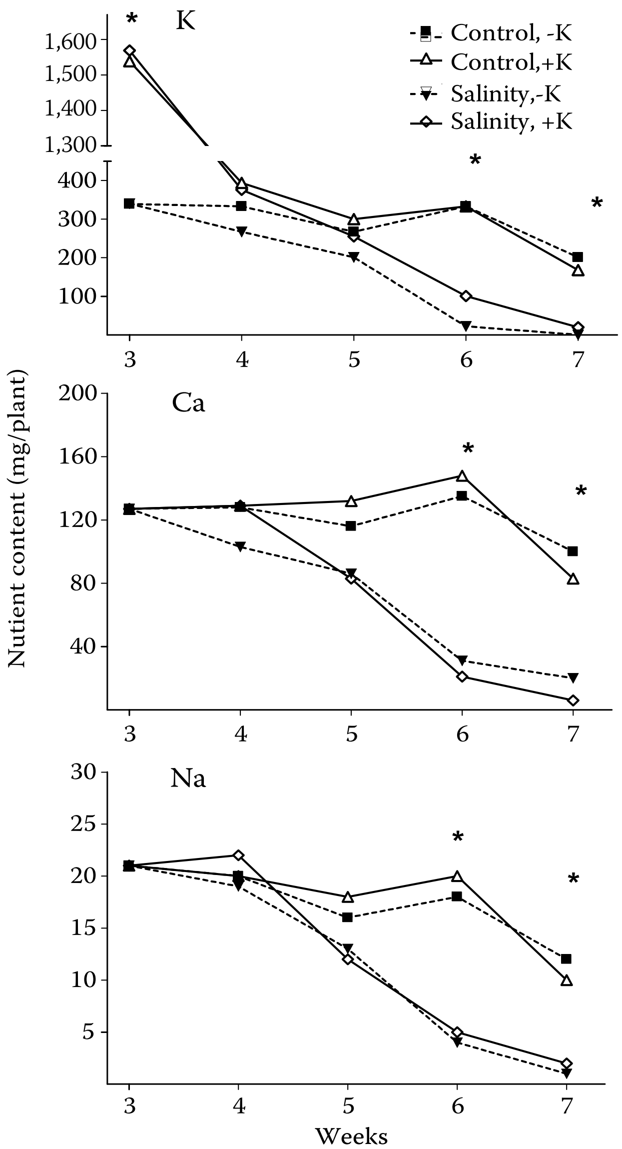

Fig. 2. Influence of NaCl salinity (40 mmol/l) and potassium sulphate (10 mmol/l K_2SO_4) on nutrient uptake of endive (cv. Green Curled) plants grown hydroponically in nutrient film technique. Stars symbolize statistical differences (at $P = 0.05$)

tion/plant) in plants treated with 40 mmol/l of NaCl compared to controls (46 ml of stock solution per plant); however, no differences were observed on water uptake among the treatments (data not presented). Salinity reduced significantly the elemental (K, Ca, and Na) uptake after the 5th week and became minimal following the 6th week (Fig. 2). Regression analysis revealed a linear relationship between time (weeks) of endive cultivation and K nutrient uptake for the treatment of salinity without K-enrichment ($y = -92.49x + 38.44$; $r^2 = 0.95$, $P < 0.005$). Moreover, a linear relationship observed between time (weeks) and salinity for Ca uptake (salinity without K; $y = -28.61x + 11.22$; $r^2 = 0.96$, $P < 0.005$, and salinity with K; $y = -35.01x + 19.10$; $r^2 = 0.92$, $P < 0.05$). However, K-enrichment had no impacts on the nutrient uptake rather than the increased initial value for K. No differences were observed on NO_3/N uptake by endive plants (data not presented).

DISCUSSION

Salinity is one of the major abiotic stresses affecting plant productivity. The response of plant growth and yield to salinity is the result of various salt effects, including reduced carbon fixation due to specific ion toxicity (Niu et al. 1995), restriction of photosynthesis due to partial stomata closure, waste of energy in the processes of osmotic adaptation and ion exclusion (Pasternak 1987) and growth limitations originating from nutritional imbalances (Grattan, Grieve 1999).

Vegetable crops are widely grown in regions where irrigation with saline water may have long-term negative effects on soil-water-plant relationships (Graifenberg et al. 1996) as well as plant grown (De Pascale, Barbieri 1995; Chartzoulakis, Klapaki 2000). In the present study, salinity (40 mmol/l NaCl) did not affect root development in hydroponically grown endives which contrasts with previous studies (Pardossi et al. 1999; Andriolo et al. 2005). Thus, this effect may be attributed to the salinity concentration and/or plant species employed. Indeed, salinity and/or elemental (K or Ca) enrichment affected mainly the upper part of the endive plants. The combination of salinity with enrichment of both elements reduced leaf dry matter and leaf area produced, while when either K or Ca was combined with salinity, no major differences were observed on plant growth. Calcium foliar sprays on salt-treated lettuce and endive revealed neutral or negative impacts on plant growth but exhibited protection on blackheart and tip-burn symptoms on lettuce plants (Tzortzakis 2009). However, the specific leaf weight increased with salinity (thicker leaves) on tomato, bean (*Phaseolus vulgaris* L.) and eggplant (*Solanum melongena* L.); this may related to plant species and/or salt concentration used (reviewed by De Pascale, Barbieri 1995). High NaCl levels inhibited leaf expansion, largely due to an inhibition of cell division rather than to cell expansion (Chartzoulakis, Klapaki 2000).

The reduction in photosynthetic rates in plants under stress is mainly due to reduction in water potential. The photosynthetic rates in the present study did not differ among salinity treatments but accelerated or depressed with Ca or K-enrichment, respectively. The opposite effects were marked for the intercellular CO_2 concentration (C_i). Previous studies on salt-treated peppers (*Capsicum annuum* L.) indicated that there was partial closure of stomata, as evidenced by the decline in stomatal conductance at those salinity levels that could have caused the reduction in photosynthesis. Inhibition of vegetative growth and yield in pepper at high salinity levels is associated with marked inhibition of photosynthesis (Chartzoulakis, Klapaki 2000) because it reduced assimilation of photosynthate, possibly as a consequence of reduced uridine diphosphoglucose (UDPG), uridine triphosphate (UTP), and adenosine triphosphate (ATP) pools in growing leaves (Nieman et al. 1988).

Taking into consideration nutrient concentration, the results revealed that decreasing the uptake values of plants exposed to NaCl levels was mainly due to the depressive effect of salinity on plant dry weight. Similar findings were found by Elgala et al. (1989) and El-Fouly et al. (2002). It was reported that macronutrients (N, P, K, Mg, and Ca) uptake were negatively affected with NaCl increase in the growth root medium whereas Na, Fe, and Zn were stimulated under saline conditions (El-Fouly et al. 2002). Reduction in macronutrients uptake in tomato may be attributed to the lack of energy that catalyzes and enhances translocation, as well as the sodium ions that cause severe membrane depolarization (Cramer et al. 1995) and due to the antagonism between Na and K, Ca, Mg, and P (Yahya 1998). Another possible reason was increasing HCO_3 level in the root growth medium due to NaCl salinity, which in turn was found to depress plant Fe concentration as well as total Fe uptake (Romheld, Marschner 1986), and this may also have contributed to increased leaf thickness (Feigin 1985).

Increased salinity generally reduces the yield of vegetables, but it may improve their quality, as observed in plants grown both in soil and soilless culture (Pardossi et al. 1999). It was suggested that salinity protected celery (*Apium graveolens* L.) and lettuce plants from the disorder (blackheart) by improving Ca nutrition of rapidly-growing tissues (Pardossi et al. 1999; Tzortzakis 2009). In hydroponically-grown tomato plants, increasing salinity of the nutrient solution increased concentration of dry matter, sugars and organic acids in fruit and provided the basis for better taste and higher firmness (Petersen et al. 1998). A positive effect of salinity on fruit quality was also found in pepper and cucumber (*Cucumis sativus* L.) grown in soilless culture (Sonneveld, Van der Burg 1991). In contrast, raising salinity level in the root zone was reported to increase occurrence of Ca-related physiological disorders, such as tip-burn in lettuce (Sonneveld 1988), blossom-end rot in tomato and pepper (Sonneveld 1988; Sonneveld, Van der Burg 1991), and internal fruit rot in eggplant (Savvas, Lenz 1994), which may be prevented by foliar application of calcium nitrate (Cox, Dearman 1978; Tzortzakis 2009).

In the foreseeable future production area under irrigation is expected to increase with large quantities of fresh water required for agriculture (Hamdi et al. 1995). To overcome water shortages, and to satisfy the increasing water demand for agricultural development, alternative use of marginal quality water (brackish, reclaimed, drainage) will become necessary in many countries (Chartzoulakis et al. 2002).

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