DOI: 10.1007/s11099-014-0068-z PHOTOSYNTHETICA 52 (4): 589-596, 2014

Effects of supplementary potassium nitrate on growth and gas-exchange characteristics of salt-stressed citrus seedlings

D. KHOSHBAKHT[*, +], A. GHORBANI[**], B. BANINASAB[*], L.A. NASERI[***], and M. MIRZAEI[#]

Department of Horticultural Science, College of Agriculture, Isfahan University of Technology,
Isfahan PO Box 8415683111, Isfahan, Iran[*]
Department of Horticultural Science, College of Agriculture, Azad University, Makoo, Iran[**]
Department of Horticultural Science, College of Agriculture, University of Urmia, West Azarbaijan, Iran[***]
Department of Horticultural Science, College of Agriculture, Azad University, Garmsar, Iran[#]

Abstract

We investigated the effects of supplementary KNO_3 and NaCl on one-year-old, potted Valencia orange (*Citrus sinensis*) scions grafted on Iranian mandarin Bakraii [*Citrus reticulata* × *Citrus limetta*] (Valencia/Bakraii) and Carrizo citrange [*C. sinensis* × *Poncirus trifoliata*] (Valencia/Carrizo) rootstocks. After watering plants for 60 days with 50 mM NaCl, the lowest reduction in dry mass, stomatal conductance, and chlorophyll (Chl) content was found in Valencia/Bakraii. Bakraii accumulated more Cl^- and Na^+ in roots and transferred less to Valencia leaves compared with Carrizo rootstock. Moreover, higher net photosynthetic rate was found in Valencia/Bakraii than those on Carrizo rootstock. NaCl caused a decrease in the maximal efficiency of PSII photochemistry (F_v/F_m) and effective quantum yield (Φ_{PSII}) but elevated coefficient of nonphotochemical quenching. Salinity reduced Ca^{2+}, Mg^{2+}, and total N contents, and increased Na^+/K^+ ratio in leaves and roots of both grafting combinations. Salinity increased K^+ and proline content in leaves and decreased K^+ concentrations in roots of both grafting combinations. In salinized plants, nitrate supplementation (10 mM KNO_3) reduced leaf abscission, Cl^-, Na^+, Na^+/K^+, and Ca^{2+} concentrations in leaves and roots of both combinations. K^+ and N concentrations and proline increased in leaves of the nitrate-supplemented salinized plants. Supplementary nitrate increased leaf number and area, stem elongation, Chl content, F_v/F_m, and Φ_{PSII} and stimulated photosynthetic activity. Thus, nitrate ameliorated the deleterious effects of NaCl stress and stimulated the plant metabolism and growth. It can be used as a vital treatment under such condition.

Additional key words: chlorophyll fluorescence; mineral nutrition; net gas exchange; nitrogen; rootstocks; salinity.

Introduction

Salinity is amongst the most significant, environmental factors responsible for substantial losses in agricultural production worldwide. It is also one of the most serious problems confronting sustainable agriculture in irrigated production systems in arid and semiarid regions (Ravindran *et al.* 2007). Nearly 20% of the world cultivated area and about half of the world irrigated lands are affected by this stress (Munns and Tester 2008). Salinity causes various injuries in plants, such as tissue burning, yield reduction, and finally plant death (Romero-Aranda *et al.* 1998), reduces water uptake and tree growth (Syvertsen and Yelenosky 1988, Maas 1993), causes leaf senescence, reduction of Chl content (Chen *et al.* 1991) and PSII activities (Nishihara *et al.* 2003), decreasing stomatal conductance (g_s) and net photosynthetic rate (P_N) (Garcia-Sanchez *et al.* 2002), enhancing membrane permeability (Dhindsa *et al.* 1981), nutritional imbalances, and toxicity (Grattan and Grieve 1992).

Received 19 September 2013, *accepted* 18 April 2014.

[+] Corresponding author; tel: +98 311 3913447, fax: +98 311 3913356, e-mail: davod.khoshbakht@gmail.com

Abbreviations: C_a – atmospheric CO_2 concentration; Chl – chlorophyll; DM – dry mass; FM – fresh mass; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence of the light-adapted state; F_s – steady-state fluorescence; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; S – NaCl salinity stress; SN – NaCl + KNO_3; Φ_{PSII} – effective quantum yield of PSII photochemistry.

Acknowledgements: We would like to thank to the Department of Horticulture, College of Agriculture, Isfahan University of Technology, for financial support of the research.

Citrus are one of the most globally important horticultural crops considered as salt-sensitive (Al-Yassin 2005). Salt tolerance in citrus has been linked to the exclusion of toxic ions from shoots (Garcia-Sanchez *et al.* 2002). Thus, citrus rootstocks have a great influence on the amount of Cl^- and/or Na^+ accumulated in the foliage of grafted trees (Gimeno *et al.* 2009). Adaptation of plants to salinity is associated with osmoregulation adjustment and proline has been known to serve as a compatible osmolyte, protectant of macromolecules, and also as a scavenger of reactive oxygen species under stressful conditions (Ashraf and Foolad 2007). Chl fluorescence can be used for analyzing photosynthetic performance under field or laboratory conditions (Maxwell and Johnson 2000). It has been reported that nitrogenous compounds enhance salinity tolerance by reducing Cl^- uptake (Tyerman and Skerrett 1999, Iglesias *et al.* 2004) and/or maintenance of nutrient balance (Hu and Schmidhalter 2005).

We hypothesized that supplemental KNO_3 could improve growth and development of Valencia orange variety grafted on Bakraii (*C. reticulata* × *C. limetta*) and/or Carrizo citrange (*C. sinensis* × *P. trifoliata*) rootstocks under salinity stress.

Materials and methods

Plants and growth conditions: One-year old, potted plants of Valencia orange scion (*C. sinensis*) was grafted on two rootstocks: Iranian mandarin Bakraii (*C. reticulata* × *C. limetta*) and/or Carrizo citrange (*C. sinensis* × *P. trifoliata*). Plants were grown under greenhouse conditions, located at Department of Horticultural Science, College of Agriculture, Isfahan University of Technology, Iran. Similar scions were selected and used for T-budding. Both grafting combinations (scion/rootstock) were further referred as followed: Valencia/Bakraii and Valencia/Carrizo. They were transplanted into 30 cm wide plastic pots containing fine sand, then placed in a greenhouse at 25–28/18°C (day/night), 900 μmol m^{-2} s^{-1} of PAR, 50–60% relative humidity, and a 16-h photoperiod. The whole experiment lasted for 90 d; first, all plants were irrigated three times per week with half-strength Hoagland's solution for 30 d (Banuls *et al.* 1997). Treatments included 50 mM NaCl (S), 50 mM NaCl + supplementary 10 mM KNO_3 (SN), and control (C). In order to avoid osmotic shock, NaCl was applied in the increasing amount of 10 mmol per day, to reach the final NaCl concentration of 50 mmol. Treatments lasted for 60 d.

Growth measurements: Leaf abscission was recorded during the experiment. Moreover, the shoot length, number of leaves, and total leaf area per plant, fresh (FM) and dry mass (DM) of leaves, stems, and roots were measured at the end of the experiment. Total leaf area was measured with a leaf area meter (*Delta T Devices Ltd,* Cambridge, UK) and data were shown as % compared with control. For DM, tissues were separately oven-dried at 70°C for 3 d and then weighed.

Leaf and root tissue mineral analysis: At the end of experiment, leaves and roots of each plant were separated and washed with deionized water. Tissues were separately oven-dried at 70°C for 3 d and dried parts were milled to a powder. Ground samples were ashed in porcelain crucibles at 550°C for 6 h. Concentration of Na^+ and K^+ were determined by flame photometry (*Model PEP7*, *Jenway*, Dunmow, UK). Ca^{2+} and Mg^{2+} concentrations were measured by using an atomic absorption spectrophotometer (*Analyst Model 200, Perkin Elmer*, USA). Total N was determined by the method described by Gomez-Cadenas *et al.* (2000) and samples were extracted according to the semi-micro Kjeldahl method (Bremner 1965), using sulfate-selenium catalysis and steam distillation. Cl^- was extracted from the dried tissue with 500 mg of DM from leaf or root tissue with 0.1 N HNO_3 in 10% (v/v) glacial acetic acid; samples were incubated overnight at room temperature and then filtered. Finally, Cl^- concentration was determined by silver ion titration (Moya *et al.* 1999).

Proline: At the end of the experiment, proline content was extracted from fresh leaf tissue with sulphosalicylic acid (3%) and quantified according to the protocol described by Bates *et al.* (1973).

Leaf Chl content was determined according to Lichtenthaler (1987). After gas-exchange measurements, Chl was extracted from 500 mg of leaf discs which were chosen from the same leaf used for P_N measurements (avoiding major veins) using 80% aqueous acetone. Absorbance of centrifuged extracts (10,000 × *g*, 10 min, 4ºC) was measured at 645 and 663 nm using the spectrophotometer (*U-2000, Hitachi Instruments,* Tokyo, Japan).

Chl fluorescence: At the end of experiment, measurement of fluorescence parameters were performed between 9:00–11:00 h, using a *Plant Efficiency Analyzer* (*PEA, Hansatech Instrument Ltd,* King's Lynn, Norfolk, UK). The undamaged, mature leaves were darkened with a lightweight plastic leaf clips for 30 min before the measurement. Maximal PSII photochemical efficiency (F_v/F_m) was calculated automatically as: [$F_v/F_m = (F_m - F_0)/F_m$]; where F_m and F_0 are the maximum and basal fluorescence yields of dark-adapted leaves, respectively. The effective quantum yield (Φ_{PSII}) in the light was calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ after 45 s of illumination, when steady state was achieved. All calculations were performed according to Maxwell and Johnson (2000).

Gas-exchange parameters, net photosynthetic rate (P_N) and stomatal conductance (g_s), were measured between

9:00–11:00 h at the end of the experiment using a portable photosynthetic system (*LI-6200, LI-COR Inc.,* Lincoln, NE, USA). Top, fully expanded leaf was clamped to the leaf chamber and the observations were recorded when relative humidity and atmospheric CO_2 concentration (C_a) reached a stable value. PAR, air temperature, relative humidity, and CO_2 concentration inside the sensor head were set at 1,300 ± 100 μmol m^{-2} s^{-1}, 31 ± 2ºC, 60%, and 335–340 μmol mol^{-1}, respectively, when measuring P_N.

Statistics: Treatment effects were evaluated by analyses of variance, and comparisons of means were made by the Least Significant Differences (*LSD*) method ($P<0.05$). Statistical analysis was performed using *SAS* software, *version 9.1* (*SAS Inc.*, Cary, NC, USA).

Results

Growth: The DM of leaves, stems, and roots, leaf area and number, and plant height significantly decreased by the S treatment. The highest reduction of growth parameters were observed in Valencia/Carrizo compared with Valencia/Bakraii (Tables 1, 2). SN treatment increased DM of leaves (29.1 and 14.3% in Valencia/Carrizo and Valencia/Bakraii, respectively) and stems (32.1 and 16.7% in Valencia/Carrizo and Valencia/Bakraii, respectively. Although root DM was unaffected by SN treatment in Valencia/Bakraii, it increased in Valencia/Carrizo (Table 1). In comparison with S treatment, SN increased leaf area by 25.9% in Valencia/Carrizo and by 15.7% in Valencia/ Bakraii. The highest and lowest leaf number and stem elongation were observed in control and S plants, respectively, and SN treatment reduced leaf abscission by 17% in Valencia/Carrizo and by 11% in Valencia/Bakraii (Table 2).

Table 1. Effect of nitrate supplementation on mean (*n* = 6) leaf, stem, and root dry mass (DM) in Valencia orange seedlings grafted on Iranian mandarin Bakraii or Carrizo citrange treated for 60 days with 0 mM NaCl (C, control), 50 mM NaCl (S) or 50 mM NaCl + supplementary 10 mM KNO_3 (SN). DM – dry mass. For each rootstock, means within each column followed by *the same letter* do not differ significantly at $P\leq0.05$.

Rootstock	Treatment	Leaf DM [g]	Stem DM [g]	Root DM [g]
Bakraii	C	12.40^{a}	7.40^{a}	6.20^{a}
	S	8.30^{c}	5.50^{c}	5.40^{b}
	SN	9.70^{b}	6.60^{b}	5.30^{b}
Carrizo	C	10.40^{a}	6.80^{a}	5.40^{a}
	S	5.46^{c}	3.60^{c}	2.70^{c}
	SN	7.70^{b}	5.30^{b}	4.00^{b}

Mineral nutrients: The leaf and root Cl^- and Na^+ concentrations (Tables 3, 4) in both grafting combinations increased under salinity stress. Trees grafted on Bakraii rootstock accumulated less Cl^- and Na^+ in leaves than those on Carrizo rootstock (Table 3). By contrast, the concentrations of both ions were higher in roots of Bakraii compared with Carrizo (Table 4). Salinity increased K^+ of leaves and Na^+/K^+ ratio in leaves and roots, but decreased Ca^{2+}, Mg^{2+}, total N concentration of both parts, and K^+ in roots in both grafting combinations. Leaf and root Ca^{2+}, Mg^{2+}, and N concentrations were higher in Valencia/Bakraii than in Valencia/Carrizo. Supplementary nitrate significantly inhibited Cl^- and Na^+ accumulation in leaves and roots and also the leaf and root Na^+/K^+ ratios of both grafting combinations. SN treatment increased the concentration of leaf and root K+ of both grafting combinations, compared with control. Nitrate supplementation stimulated total N accumulation in leaves of Valencia/Bakraii and Valencia/Carrizo and in roots of both rootstocks. Although a similar pattern was observed in roots of Valencia/Bakraii, the differences were not statistically significant (Tables 3, 4).

Proline and leaf Chl: Proline content increased by S and SN treatments in Valencia/ Bakraii and Valencia/Carrizo (Table 5). Both Chl *a* and Chl *b* declined significantly by S treatment and supplementary nitrate improved their contents. Chl *a* was significantly affected by treatments,

Table 2. Effect of nitrate supplementation on mean leaf area. Leaf abscission, number of leaves, stem elongation in Valencia orange seedlings grafted on Iranian mandarin Bakraii or Carrizo citrange treated for 60 days with 0 mM NaCl (C – control), 50 mM NaCl (S) or 50 mM NaCl + supplementary 10 mM KNO_3 (SN). For each rootstock, means within each column followed by *the same letter* do not differ significantly at $P\leq0.05$. (*n* = 6).

Rootstock	Treatment	Leaf area [%]	Leaf abscission [%]	Number of leaves [%]	Stem elongation [%]
Bakraii	C	100^{a}	0^{c}	100^{a}	100^{a}
	S	75^{c}	24^{a}	76^{c}	67^{c}
	SN	89^{b}	13^{b}	88^{b}	79^{b}
Carrizo	C	100^{a}	0^{c}	100^{a}	100^{a}
	S	57^{c}	46^{a}	45^{c}	37^{c}
	SN	77^{b}	29^{b}	64^{b}	57^{b}

Table 3. Effect of nitrate supplementation on mean leaves Cl^-, Na^+, K^+, Na^+/K^+ ratio, Ca^{2+}, Mg^{2+} and N in leaves of Valencia orange seedlings grafted on Iranian mandarin Bakraii or Carrizo citrange treated for 60 days with 0 mM NaCl (C – control), 50 mM NaCl (S) or 50 mM NaCl + supplementary 10 mM KNO_3 (SN). For each rootstock, means within each column followed by *the same letter* do not differ significantly at $P \leq 0.05$. (n = 6).

Rootstock	Treatment	Cl^- [%]	Na^+ [%]	K^+ [%]	Na^+/K^+ ratio	Ca^{2+} [%]	Mg^{2+} [%]	N [%]
Bakraii	C	0.30^{c}	0.12^{c}	2.40^{c}	0.05^{c}	2.70^{a}	0.38^{a}	3.20^{a}
	S	2.50^{a}	2.80^{a}	2.58^{b}	1.07^{a}	2.40^{b}	0.32^{ab}	2.70^{c}
	SN	1.40^{b}	2.20^{b}	3.12^{a}	0.70^{b}	2.00^{c}	0.27^{b}	2.94^{b}
Carrizo	C	0.80^{c}	0.59^{c}	2.20^{b}	0.27^{c}	2.90^{a}	0.33^{a}	3.00^{a}
	S	3.80^{a}	3.80^{a}	2.60^{a}	1.46^{a}	2.00^{b}	0.23^{b}	2.42^{c}
	SN	2.20^{b}	2.60^{b}	2.79^{a}	0.95^{b}	1.60^{c}	0.19^{b}	2.73^{b}

Table 4. Effect of nitrate supplementation on mean roots Cl^-, Na^+, K^+, Na^+/K^+ ratio, Ca^{2+}, Mg^{2+} and total N in Valencia orange seedlings grafted on Iranian mandarin Bakraii or Carrizo citrange treated for 60 days with 0 mM NaCl (C – control), 50 mM NaCl (S) or 50 mM NaCl + supplementary 10 mM KNO_3 (SN). For each rootstock, means within each column followed by *the same letter* do not differ significantly at $P \leq 0.05$. (n = 6).

Rootstock	Treatment	Cl^- [%]	Na^+ [%]	K^+ [%]	Na^+/K^+ ratio	Ca^{2+} [%]	Mg^{2+} [%]	N [%]
Bakraii	C	0.34^{c}	0.24^{c}	2.20^{a}	0.11^{c}	1.60^{a}	0.28^{a}	2.84^{a}
	S	3.50^{a}	2.20^{a}	1.60^{b}	1.37^{a}	1.30^{b}	0.18^{b}	2.22^{b}
	SN	2.80^{b}	1.60^{b}	2.30^{a}	0.65^{b}	1.20^{b}	0.14^{b}	2.47^{b}
Carrizo	C	0.60^{c}	0.16^{c}	2.00^{a}	0.08^{c}	1.20^{a}	0.30^{a}	2.82^{a}
	S	3.20^{a}	1.80^{a}	1.30^{c}	1.48^{a}	0.80^{b}	0.12^{b}	2.12^{b}
	SN	2.60^{b}	1.40^{b}	1.70^{b}	0.88^{b}	0.60^{b}	0.07^{b}	2.56^{a}

Table 5. Effect of nitrate supplementation on mean proline, chlorophyll (Chl) *a*, Chl *b*, Chl (*a*+*b*) content, and Chl *a*/*b* ratio in Valencia orange seedlings grafted on Iranian mandarin Bakraii or Carrizo citrange treated for 60 days with 0 mM NaCl (C – control), 50 mM NaCl (S) or 50 mM NaCl + supplementary 10 mM KNO_3 (SN). Chl – chlorophyll. For each rootstock, means within each column followed by *the same letter* do not differ significantly at $P \leq 0.05$. (n = 6).

Rootstock	Treatment	Proline [μmol g^{-1}(FM)]	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Chl (*a*+*b*) [mg g^{-1}(FM)]	Chl *a*/*b* ratio
Bakraii	C	27.40^{c}	0.88^{a}	0.26^{a}	1.14^{a}	3.38^{a}
	S	72.20^{b}	0.58^{c}	0.18^{b}	0.76^{c}	3.22^{b}
	SN	79.30^{a}	0.66^{b}	0.20^{b}	0.86^{b}	3.30^{ab}
Carrizo	C	21.20^{c}	0.86^{a}	0.24^{a}	1.10^{a}	3.58^{a}
	S	50.30^{b}	0.41^{c}	0.12^{c}	0.53^{c}	3.33^{c}
	SN	62.00^{a}	0.59^{b}	0.17^{b}	0.76^{b}	3.47^{b}

and the lowest Chl *a* content was observed in Valencia/Carrizo under S treatment. Chl *a* concentration of leaves of salt-treated Valencia/Bakraii and Valencia/Carrizo always increased significantly in response to nitrate supplementation. Similarly to Chl *a* and Chl *b*, S treatment significantly reduced total Chl content and Chl *a*/*b* ratio; however, supplementary nitrate improved it. Moreover, similar to Chl *a* results, the lowest total Chl content was observed in Valencia/Carrizo under S treatment (Table 5).

Leaf gas exchange, F_v/F_m, Φ_{PSII}, and NPQ: Leaf gas-exchange parameters were reduced by S treatment in Valencia scions grafted on both rootstocks (Table 6). In comparison to control treatment and under NaCl salinity, P_N was reduced by 62.1% in plants grafted on Carrizo and by 41.1% in plants grafted on Bakraii. On the other hand, supplementary nitrate increased photosynthetic rates of salt-stressed Valencia/Carrizo by 38.5% and by 26.4% in Valencia/Bakraii. The F_v/F_m was reduced by salinity in Valencia scions grafted on both rootstocks. The S treatment showed more negative effect on this variable in Valencia/Carrizo (reduced by 27.2%) compared with this treatment in Valencia/Bakraii (reduced by 15%). Supplementary nitrate improved F_v/F_m in both grafting combinations, compared with S treatment, and Valencia/Bakraii showed the better response than another combination. S treatment significantly reduced Φ_{PSII} in both grafting combinations, however, Valencia/Carrizo showed higher reduction (by

Table 6. Effect of nitrate supplementation on mean (*n* = 6) P_N, g_s, F_v/F_m, Φ_{PSII}, and NPQ in Valencia orange seedlings grafted on Iranian mandarin Bakraii or Carrizo citrange treated for 60 days with 0 mM NaCl (C, control), 50 mM NaCl (S) or 50 mM NaCl + supplementary 10 mM KNO_3 (SN). P_N – net photosynthetic rate, g_s – stomatal conductance, F_v/F_m – maximum photochemical efficiency of PSII, Φ_{PSII} – effective quantum yield, NPQ – nonphotochemical quenching. For each rootstock, means within each column followed by *the same letter* do not differ significantly at $P \leq 0.05$. (*n* = 6).

Rootstock	Treatment	P_N [µmol(CO_2) m^{-2} s^{-1}]	g_s [mmol(H_2O) m^{-2} s^{-1}]	F_v/F_m	Φ_{PSII}	NPQ
Bakraii	C	9.00^a	0.15^a	0.80^a	0.72^a	0.81^c
	S	5.30^c	0.09^c	0.68^b	0.62^b	1.20^a
	SN	7.20^b	0.12^b	0.77^a	0.68^a	0.94^b
Carrizo	C	9.50^a	0.11^a	0.81^a	0.76^a	0.84^c
	S	3.60^c	0.04^c	0.59^c	0.53^c	1.42^a
	SN	5.85^b	0.06^b	0.68^b	0.63^b	1.10^b

30.3%) than other one (by 13.9%), compared with control treatment. In Valencia scions grafted on both rootstocks, NPQ in response to the S treatment increased and SN reduced Φ_{PSII}, compared with S treatment. NPQ in stressed plants of Valencia/Carrizo was higher (increased by 69% compared with control) than in Valencia/Bakraii (increased by 48.1% compared with control). SN reduced NPQ in Valencia/Carrizo and Valencia/Bakraii by 22.5 and 21.7%, respectively (Table 6).

Discussion

Salt stress led to growth parameter reduction of Valencia/Carrizo and Valencia/Bakraii combinations. In this experiment, the reduction in leaf, stem, and root DM were lower in Valencia/Bakraii than in Valencia/Carrizo. Plant growth and development is an important criterion to determine salinity tolerance in different citrus rootstocks. The reduction in growth parameters is obvious because of disturbances in physiological and biochemical activities under saline conditions (Craine 2005). It has been reported that the reduction in biomass in response to salinity is due to the reduction in leaf area and the number of leaves (Romero-Aranda *et al.* 1998). Supplementary nitrate increased leaf production of salt-treated plants. The reduction in leaf abscission in response to supplementary nitrate was related to leaf Cl^- concentration, inhibition of ethylene production *via* high nitrogen availability (Bar *et al.* 1998) and/or high carbohydrate concentrations (Gomez-Cadenas *et al.* 2000), which may result from increment of photosynthetic rates of salt-treated plants supplemented with KNO_3 (Iglesias *et al.* 2003).

Our data suggested that growth inhibition of both rootstocks under NaCl resulted from accumulation of Na^+ and Cl^- in leaves. Root accumulation and low transport of these ions are resistance mechanisms under NaCl salinity. Bakraii accumulated more Cl^- and Na^+ than Carrizo in root tissues, thus, lesser amount of these ions could be found in Valencia/Bakraii compared with Valencia/Carrizo leaves. It showed that Bakraii could partially exclude Cl^- and Na^+ from Valencia leaves by accumulating them in roots. This experiment confirmed that Bakraii rootstock is a Cl^- and Na^+ excluder, whereas Carrizo rootstock is a Cl^- and Na^+ accumulator. It was in agreement with Zekri and Parsons (1992) concerning Cleopatra mandarin as Na^+ accumulator in leaves. Supplementary nitrate had beneficial effects in the SN treatment which showed lower leaf Cl^- and Na^+ concentrations than those from S treatment. The leaf Cl^- reduction in the nitrate-supplemented plants was likely due to an antagonism between Cl^- and NO_3^- uptake by roots (Tyerman and Skerrett 1999). In addition, a higher K^+/Na^+ ratio in the nutrient solution could have inhibited the Na^+ uptake by rootstocks due to the antagonism between these ions (Gimeno *et al.* 2009). An imbalance of essential nutrients may also be a factor involved in the salt-induced decrease in gas-exchange parameters and consequently in growth reduction. Leaf K^+ concentration increased under salinity treatment and it was in agreement with Behboudian *et al.* (1986) and Khayyat *et al.* (2014). However, K^+ content in roots decreased. This implies that not only K^+ accumulation was reduced by competition with Na^+, but also its redistribution to the aerial tissues was affected in response to excess Cl^- accumulated in leaves. In this experiment, the root K^+ decreased with increasing salinity that was in agreement with Zekri and Parsons (1992) and Garcia-Sanchez *et al.* (2002). It could be attributed to the replacement of Na^+ with K^+ in the root tissue or replacement of Ca^{2+} with Na^+ ions in root cell membrane that induces K^+ leakage from root (Zekri and Parsons 1992). Since K^+ reduces osmotic potential participation in root tip (Marschner 1995), losses of K^+ in roots may lead to a reduction of nutrient transfer to the shoot. Another beneficial effect of KNO_3 was the increase in the leaf and root K^+ concentration in salinized trees during SN treatment. It has been well known that high leaf K^+ concentration may alleviate salt-stress effects by minimizing oxidative stress and/or contributing to osmotic adjustment (Cakmak 2005, Grieve and Walker 1983). The amount of Ca^{2+} in roots and leaves of both grafting combinations decreased by the S treatment that was in agreement with Zekri and Parsons (1992), Fernandez-Ballester *et al.* (2003), and Ruiz *et al.* (1997). It can be due to the competitive effects of other ions with Ca^{2+}, thus, it can disrupt the transfer of this element (Botella *et al.* 1997). Ca^{2+} is important in preserving membrance integrity (Rengel 1992), signaling in osmoregulation (Mansfield *et al.* 1990), and influencing K^+/Na^+ selectivity (Cramer *et al.* 1987). In both grafting combinations, leaf Ca^{2+} decreased when treated with SN because of antagonistic relationship between K^+ and Ca^{2+} ions (Hansen and Munns 1988). In both grafting combinations, Mg^{2+}concentration in leaves and roots decreased in response to salinity which was in agreement with Ruiz *et al.* (1997) and Garcia-Sanchez (2002), and it might be one of the reasons for the chlorosis observed in NaCl-treated plants. Mg^{2+} deficiency might reduce Chl and P_N, and could contribute to the reduction of DM. In Mg^{2+}-deficient leaves, formation of superoxide radicals and hydrogen peroxide is enhanced and therefore the leaves become highly photosensitive (Tozlu *et al.* 2000). Leaf and root N concentrations were reduced by salinity which might result from a competitive inhibition between Cl^- and NO_3^- uptake, proposed by Cerezo *et al.* (1997).

Leaf proline concentration increased significantly by S and SN treatments and leaves from SN treated plants showed the highest contents. In the S and SN treatments, trees on Bakraii had significantly higher leaf proline concentration than those on Carrizo rootstock. This suggested that the leaf Cl^- and Na^+ concentration were reduced after nitrate treatment; it decreased their contribution to leaf osmotic potential, and thus, the osmotic adjustment had to be carried out using other ions or by synthesis of organic solutes such as proline (Gimeno *et al.* 2009). In term of osmotic adjustment, it is known that accumulation of salts in the substrate triggers a transient water deficit that induces an increase in ABA accumulation and stomatal closure (Gomez-Cadenas *et al.* 2002). Then, plants accumulate more proline and ions in leaves to decrease water potential and to maintain a proper water flux and leaf turgor (Arbona *et al.* 2003), and also to affect the solubility of various proteins (Abraham *et al.* 2003) to protect them against denaturation under salinity (Tonon *et al.* 2004).

Chl, P_N, g_s, F_v/F_m, and Φ_{PSII} were reduced by salinity in both grafting combinations, however, the highest reduction of Chl was found in Valencia leaves on Carrizo rootstock that could be associated with accumulation of Na^+ and Cl^- in the leaves (Anjum 2007, Yang *et al.* 2008), and/or Mg^{2+} deficiency (Tozlu *et al.* 2000), and formation of proteolytic enzymes, such as chlorophyllase (Sabater and Rodriguez 1978). Anjum (2007) found that under salt stress, Chl contents decreased in Troyer citrange and Cleopatra mandarin and the seedlings of Troyer citrange were more affected than those of Cleopatra mandarin. It has been proposed that the reduction of leaf gas exchange in response to salinity is due to increasing leaf Na^+ concentration (Garcia-Legaz *et al.* 1993, Walker *et al.* 1993) and/or high concentration of Cl^- (Banuls *et al.* 1997). These results showed that salt stress exerts its deleterious effects, at least in part, through the reduction in F_v/F_m and Φ_{PSII} in both grafting combinations, indicating that electron transport through photosynthetic electron chain was reduced in response to salinity (Lopez-Climent *et al.* 2008). The F_v/F_m ratio is correlated with the efficiency of leaf photosynthesis and decline of this ratio provides an indicator of photoinhibitory damage caused by the incident photon flux density to plants under different environmental stresses (Björkman and Demmig 1987). The reduction in F_v/F_m and Φ_{PSII} were correlated with an increase in NPQ suggesting that salt treatment induced higher dissipation of damaging excessive energy. This energy could be dissipated at antennae through heat emission, although other mechanisms are also involved in energy dissipation, *e.g.* related to a transmembrane H^+ gradient generated by ATPase activity and uncoupling of electron transport, that my lead to oxidative stress (Maxwell and Johnson 2000). Under salinity, reduction of photosynthetic activity and Chl concentration are a consequence of leaf Cl^- accumulation (Heuer and Feigin 1993). In this experiment, potassium nitrate supplementation reduced leaf Cl^- and Na^+ concentration and increased Chl, g_s, F_v/F_m, and Φ_{PSII} that might keep a higher photosynthetic activity under this condition.

In conclusion, the Valencia/Bakraii combination introduced as a good grafting combination for saline conditions. The lower Cl^- and Na^+ concentration in trees on Bakraii than those on Carrizo, suggests that the salinity tolerance of Bakraii is associated with ion sequestration in roots with lesser transport to leaves. The data suggest that, in salinized citrus, the Cl^- and Na^+ overloading, low Mg^{2+}, stomatal closure, and Chl loss may contribute to reduction of P_N. Chl fluorescence parameters indicated severe impairments of photosynthetic activity under salinity stress. From our data, higher resistance to salinity could be associated to the ability to keep higher Chl, P_N, and better compartmentation of Cl^- and Na^+ in leaves and better maintenance of nutrient uptake even in saline condition. Additional potassium nitrate fertilization *via* the soil had beneficial effects on salinized trees since leaf Cl^- and Na^+ concentrations were reduced and leaf K^+ and N concentrations increased. Moreover, nitrate supplementation improved Chl content, Φ_{PSII}, and F_v/F_m, stimulated P_N and growth and reduced leaf abscission, resulted in Cl^- and Na^+ dilution, and led to a reduction in Cl^- and Na^+ concentration.

References

Abraham, E., Rigo, G., Szekely, G. *et al.*: Light dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteriod in *Arabidopsis*. – Plant Mol. Biol. **51**: 363-372, 2003.

Al-Yassin, A.: Review: adverse effects of salinity on citrus. – Int. J. Agric. Biol. **7**: 668-680, 2005.

Anjum, M.A.: Effect of NaCl concentration in irrigation water on growth and polyamine metabolism in two citrus rootstocks with different levels of salinity tolerance. – Acta Physiol. Plant. **30**: 43-52, 2007.

Arbona, V., Flors, V., Jacas, J. *et al.*: Enzymatic and non-enzymatic antioxidant responses of Carrizo citrange, a salt sensitive citrus rootstock, to different levels of salinity. – Plant Cell Physiol. **44**: 388-394, 2003.

Ashraf, M., Foolad, M.R.: Roles of glycine betaine and proline in improving plant abiotic stress resistance. – Environ. Exp. Bot. **59**: 206-216, 2007.

Banuls, J., Serna, M.D., Legaz, F. *et al.*: Growth and gas exchange parameters of citrus plants stressed with different salts. – J. Plant Physiol. **150**: 194-199, 1997.

Bar, Y., Apelbaum, A., Kafkafi, U. *et al.*: Ethylene association with chloride stress in citrus plants. – Sci. Hortic.-Amsterdam **73**:99-109, 1998.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. – Plant Soil **39**: 205-207, 1973.

Behboudian, M.H., Torokfalvy, E., Walker, R.R.: Effects of salinity on ionic content, water relations and gas exchange parameters in some citrus scion-rootstock combinations. – Sci. Hort.-Amsterdam **28**: 105-116, 1986.

Björkman, O., Demmig, B.: Photon yield of oxygen evolution and chlorophyll fluorescence characteristics at 77°K among vascular plants of diverse origin. – Planta **170**: 489-504, 1987.

Botella, M.A., Martinez, V., Pardines, J. *et al.*: Salinity induced potassium deficiency in maize plant. – J. Plant Physiol. **150**: 200-205, 1997.

Bremner, J.M.: Total nitrogen. – In: C.A. Black (ed.): Methods of Soil Analysis. Part 2. Pp. 1149-1178. American Society of Agronomy, Madison 1965.

Cakmak, I.: The role of potassium in alleviating detrimental effects of abiotic stresses in plants. – J. Plant Nutr. Soil Sc. **168**: 521-530, 2005.

Cerezo, M., Garcia-Agustin, P., Serna, M.D. *et al.*: Kinetics of nitrate uptake by Citrus seedlings and inhibitory effects of salinity. – Plant Sci. **126**: 105-112, 1997.

Chen, C.T., Li, C.C., Kao, C.H.: Senescence of rice leaves. Changes of chlorophyll, proteins and polyamine contents and ethylene production during senescence of a chlorophyll-deficient mutant. – J. Plant Growth Regul. **10**: 201-205, 1991.

Craine, J.M.: Reconciling plant strategy theories of Grime and Tilman. – J. Ecol. **93**: 1041-1052, 2005.

Cramer, G.R., Lynch, J., Lauchli, A. *et al.*: Influx of Na^+, K^+ , and Ca^{2+}, into roots of salt-stressed cotton seedlings. Effects of supplemental Ca^{2+}. – Plant Physiol. **83**: 510-516, 1987.

Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A.: Leaf senescence correlated with increased levels of membrane permeability and lipid per oxidation, and decreased levels of superoxide dismutase and catalase. – J. Exp. Bot. **32**: 93-101, 1981.

Fernandez-Ballester, G., Garcia-Sanchez, F., Cerda A. *et al.*: Tolerance of citrus rootstock seedlings to saline stress based on their ability to regulate ion uptake and transport. – Tree Physiol. **23**: 265-271, 2003.

Garcia-Sanchez, F., Jifon, J.L., Garrajal, M. *et al.:* Gas exchange, chlorophyll and nutrient content in relation to Na^+ and Cl^- accumulation in sunburst mandarin grafted on different rootstock. – Plant Sci. **162**: 705-712, 2002.

Garcia-Legaz, M.F., Ortiz, J.M., Garcia-Lidon, A.G. *et al.*: Effect of salinity on growth, ion content and CO_2 assimilation rate in lemon varieties on different rootstock. – Physiol. Plantarum **89**: 427-432, 1993.

Gimeno, V., Syvertsen, J.P., Nieves, M. *et al.*: Additional nitrogen fertilization affects salt tolerance of lemon trees on different rootstocks. – Sci. Hortic.-Amsterdam **121**: 298-305, 2009.

Gomez-Cadenas, A., Arbona, V., Jacas, J. *et al.*: Abscisic acid reduces leaf abscission and increases salt tolerance in citrus plants. – J. Plant Growth Regul. **21**: 234-240, 2002.

Gomez-Cadenas, A., Mehouachi, J., Tadeo, F.R. *et al.*: Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. – Planta **210**: 636-643, 2000

Grattan, S.R., Grieve, C.M.: Mineral element acquisition and growth response of plants grown in saline environments. – Agr. Ecosyst. Environ. **38**: 275-300, 1992.

Grieve, A.M., Walker, R.R.: Uptake and distribution of chloride, sodium and potassium ions in salt-treated citrus plants. – Aust. J. Agr. Res. **34**: 133-143, 1983.

Hansen, E.H., Munns, D.N.: Effects of $CaSO_4$ and NaCl on growth and nitrogen fixation of *Leucaena leucocephala*. – Plant Soil **107**: 95-99, 1988.

Heuer, B., Feigin, A.: Interactive effects of chloride and nitrate on photosynthesis and related growth parameters in tomatoes. Photosynthetica **28**: 549-554, 1993.

Hu, Y.C., Schmidhalter, U.: Drought and salinity: a comparison of their effects on mineral nutrition of plants. – J. Plant Nutr. Soil Sc. **168**: 541-549, 2005.

Iglesias, D.J., Levy, Y., Gomez-Cadenas, A. *et al.*: Nitrate improves growth in salt-stressed citrus seedlings through effects on photosynthetic activity and chloride accumulation. – Tree Physiol. **24**: 1027-1034, 2004.

Iglesias, D.J., Tadeo, F.R., Primo-Millo, E. *et al.*: Fruit set dependence on carbohydrate availability in citrus trees. – Tree Physiol. **23**:199-204, 2003.

Khayyat, M., Tehranifar, A., Davarynejad, G.H. *et al.*: Vegetative growth, compatible solute accumulation, ion partitioning and chlorophyll fluorescence performance of 'Malas-e-Saveh' and 'Shishe-Kab' pomegranates in response to salinity stress induced by NaCl under field condition. – Photosynthetica **52**: 301-312, 2014.

Lichtenthaler, R.K.: Chlorophylls and carotenoids-pigments of photosynthetic biomembranes. – In: Colowick, S. P., Kaplan, N. O (ed.): Methods in Enzymology. Vol. 148. Pp. 350-382. Academic Press, San Diego, New York, Berkeley, Boston, London, Sydney, Tokyo, Toronto 1987.

Lopez-Climent, M.F., Arbona, V., Perez-Clemente, R.M. *et al.*: Relationship between salt tolerance and photosynthetic machinery per formation in citrus. – Environ. Exp. Bot. **62**: 176-184, 2008.

Maas, E.V.: Salinity and citriculture. – Tree Physiol. **12**: 195-216, 1993.

Mansfield, T.A., Hetherington, A.M., Atkinson, C.J.: Some aspects of stomatal physiology. – Annu. Rev. Plant Phys. **41**: 55-75, 1990.

Marschner, H.: Mineral Nutrition of Higher Plants. Pp. 889. Academic Press, San Diego 1995.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Moya, J.L., Primo-Millo, E., Talon, M.: Morphological factors determining salt tolerance in citrus seedlings: the shoot to root ratio modulates passive root uptake of chloride ions and their accumulation in leaves. – Plant Cell Environ. **22**: 1425-1433, 1999.

Munns, R., Tester, M.: Mechanism of salinity tolerance. – Annu. Rev. Plant Biol. **59**: 651-681, 2008.

Nishihara, E., Kondo, K., Masud Parvez, M. *et al.*: Role of 5-aminolevulinic acid (ALA) on active oxygen-scavenging system in NaCl-treated spinach (*Spinacia oleracea*). – J. Plant Physiol. **160**: 1085-1091, 2003.

Ravindran, K.C., Venkatesan, K., Balakrishnan, V. *et al.*: Restoration of saline land by halophytes for Indian soils. – Soil Biol. Biochem. **39**: 2661-2664, 2007.

Rengel, Z.: The role of calcium in salt toxicity. – Plant Cell Environ. **15**: 625-632, 1992.

Romero-Aranda, R., Moya, J.L., Tadeo, F.R. *et al.*: Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. – Plant Cell. Environ. **21**: 1243-1253, 1998.

Ruiz, D., Martinez, V., Cerada, A.: Citrus response to salinity: growth and nutrient uptake. – Tree Physiol. **17**: 141-150, 1997.

Sabater, B., Rodriguez, M.T.: Control of chlorophyll degradation in detached leaves of barley and oat through effect of kinetin on chlorophyllase levels. – Physiol. Plantarum **43**: 274-276, 1978.

Syvertsen, J.P., Yelenosky, G.: Salinity can enhance freeze tolerance of citrus rootstock seedlings by modifying growth, water relations and mineral nutrition. – J. Am. Soc. Hortic. Sci. **113**: 889-893, 1988.

Tonon, G., Kevers, C., Faivre-Rampant, O. *et al.*: Effect of NaCl and mannitol iso-osmotic stresses on proline and free polyamine levels in embryogenic *Fraxinus angustifolia* callus. – J. Plant Physiol. **161**: 701-708, 2004.

Tozlu, I., Moore, G.A., Guy, C.L.: Effect of increasing NaCl concentration on stem elongation, dry mass production, and macro- and micro- nutrient accumulation in *Poncirus trifoliate*. – Aust. J. Plant Physiol. **27**: 35-42, 2000.

Tyerman, S.D., Skerrett, I.M.: Root ion channels and salinity. – Sci. Hortic.-Amsterdam **78**:175-235, 1999.

Walker, R.R., Blackmore, D.H., Qing, S.: Carbon dioxide assimilation and foliar ion concentration in leaves of lemon (*Citrus limon* L.) trees irrigated with NaCl or Na_2SO_4. – Aust. J. Plant Physiol. **20**: 173-185, 1993.

Yang, C.W., Wang, P., Li, C.Y. *et al.*: Comparison of effects of salt and alkali stresses on the growth and photosynthesis of wheat. – Photosynthetica **46**: 107-114, 2008.

Zekri, M., Parsons, L.P.: Salinity tolerance in citrus rootstock: Effect of salt on root and leaf mineral concentrations. – Plant Soil **147**: 171-181, 1992.