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Effects of sodium chloride stress on gas exchange, chlorophyll content and nutrient concentrations of nine citrus rootstocks

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Abstract

We investigated the influence of salinity (0, 25, 50, or 75 mM NaCl) on gas exchange and physiological characteristics of nine citrus rootstocks (Cleopatra mandarin, Carrizo citrange, Macrophylla, Iranian mandarin Bakraii, Rangpur lime, Rough lemon, Sour orange, Swingle citrumelo, and Trifoliate orange) in a greenhouse experiment. Total plant dry mass, total chlorophyll (Chl) content, and gas-exchange variables, such as net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration, were negatively affected by salinity. In addition, ion concentrations of Cl^- and Na^+ increased by salinity treatments. Salinity also increased Mg^{2+} content in roots and reduced Ca^{2+} and Mg^{2+} concentrations in leaves. The K^+ concentration in leaves was enhanced at low salinity (25 mM NaCl), whereas it decreased with increasing salinity stress. Salinity caused a decline in K^+ contents in roots. The rootstocks showed major differences in the extent of Cl^- and Na^+ accumulation in leaves and in their ability to maintain the internal concentrations of essential nutrients in response to different salinity. Therefore, in addition to inhibitory effects of high concentrations of Cl^- and Na^+, an imbalance of essential nutrients may also contribute to the reduction in gas exchange under saline conditions. Higher tolerance of rootstocks to salinity could be associated with the reduction of Cl^- and Na^+ uptake and transport to leaves, ability to keep higher Chl, g_s, P_N, and better maintenance of nutrient uptake even under high salinity. We found that Sour orange and Cleopatra mandarin were the rootstocks most tolerant to salinity of all nine studied. In addition, Trifoliate orange, Carrizo citrange, and Swingle citrumelo were the rootstocks most sensitive to salt stress followed by the Rough lemon and Macrophylla that showed a low-to-moderate tolerance, and Rangpur lime and Bakraii, with a moderate-to-high tolerance to high salinity.

Additional key words: growth analysis; mineral nutrition; net photosynthetic rate; salinity.

Introduction

Efficient use of the limited water resources in arid and semiarid regions is becoming more and more vital because of the rapid expansion of irrigated agriculture (Al-Yassin 2004). Salt stress is a major stress problem in arid and semiarid regions and irrigated areas. Almost 7% of the world land area, 20% of the cultivated land, and nearly half of the irrigated land is affected by high salt concentrations (Sudhir and Murthy 2004).

High amount of salt in water can cause shoot dehydration (Kaya *et al.* 2002), nutrient imbalances (Qadar 1998), and particular toxicity that is a result of increasing amount of Na^+ and Cl^- in the cytoplasm (Ashraf and Harris 2013). The leaf senescence is an important negative effect of salt stress (Agrawal *et al.* 2013). Leaf senescence goes together with the reduction of chlorophyll content (Juan *et al.* 2005) and increase of membrane permeability at high NaCl concentrations (Liu and Baird 2004). High concentration of Na^+ and Cl^- in leaves also causes substantial reduction in g_s and P_N in citrus (García-Sánchez *et al.* 2002). The effects of salinity on photosynthesis range from the restriction on CO_2 diffusion into the chloroplast, *via* limitations on stomatal opening mediated by shoot- and root-generated hormones, and on the mesophyll transport of CO_2, to alterations in leaf photochemistry and carbon metabolism. These effects vary according to the intensity and duration of stress and the plant species (Chaves *et al*. 2009).

Citrus is grown commercially in more than 50 countries

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Abbreviations: C_a – atmospheric CO_2 concentration; C_i – intercellular CO_2 concentration; Chl – chlorophyll; DM – dry mass; g_s – stomatal conductance; P_N – net photosynthetic rate.
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and ranks as the top fruit crop in world production (García-Sánchez *et al*. 2002). Citrus plants are sensitive to salts (Al-Yassin 2005) because of the specific toxicity of Cl^- and/or Na^+ and to the osmotic effect caused by the high concentration of salts (García-Sánchez 2000). As judged by the ability to inhibit the accumulation of Cl^- and/or Na^+ in leaves of the scion, rootstocks differ in their salinity tolerance. Grafting scions onto salt-tolerant rootstocks is one of the ways to improve the citrus salt tolerance (Storey and Walker 1999).

In an attempt to better understand the relative effects of salinity on growth, gas-exchange parameters, Chl, and nutrient contents, the present study compared the responses of nine citrus rootstocks to salinity stress.

Materials and methods

Plant material and growth conditions: Seeds of nine citrus rootstocks were obtained from Citrus Research Station, Ramsar, Iran.

Plants were grown in a greenhouse located at the Department of Horticultural Science, College of Agriculture, Isfahan University of Technology, Iran. Seeds were sown in pots filled with a mixture of sterile sand and perlite. Two months after emergence, uniform seedlings of rootstock cultivars were transplanted to 30 cm wide plastic pots containing fine sand. Prior to the onset of treatment, plants were allowed to acclimate in the greenhouse for three months under maximum photosynthetically active radiation (PAR) of 800 μmol m^{-2} s^{-1}, day/night temperature of 25/17°C, a day/night relative humidity of 60/70 ± 5%, and a 16-h photoperiod. Plants were irrigated in 2-d intervals with 500 ml of water and fertilized with a commercial water-soluble fertilizer containing macro- and micronutrients (*Floral Mixed fertilizer*, *IFO*, Italy).

Rootstock		Abbreviation
Sour orange	*Citrus aurantium*	SO
Bakraii	*Citrus reticulata* × *Citrus limetta*	Bak
Cleopatra mandarin	*Citrus reshni*	CM
Rangpur lime	*Citrus limonia*	RaLi
Rough lemon	*Citrus jambhiri*	RLe
Macrophylla	*Citrus macrophylla*	Mac
Swingle citrumelo	*Poncirus trifoliata* × *Citrus paradisi*	SwC
Carrizo citrange	*Poncirus trifoliata* × *Citrus sinensis*	CaCi
Trifoliate orange	*Poncirus trifoliata*	TO

Stress imposition: After the period of acclimation (five months for the seedlings), each seedling was exposed to one of several salt-stress treatments. The salt treatments (0, 25, 50, or 75 mM NaCl) were applied to the pots at 2-d intervals in 500 ml of irrigation water. To avoid osmotic shock, the NaCl concentrations were increased gradually by adding increments of 25 mM NaCl every 2 d until the desired concentration was reached. The various analyses were performed 60 d after the start of each salt treatment.

Gas-exchange parameters: Measurements of P_N, g_s, and intercellular CO_2 concentration (C_i) were made between 9:00–14:00 h on a sunny day 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 RH and atmospheric CO_2 concentration (C_a) reached a stable value. During measuring P_N, PAR was set at 1,300 ± 100 μmol m^{-2} s^{-1}, air temperature at 31 ± 2ºC, relative humidity at 60%, and CO_2 concentration inside the sensor head at 335–340 μmol m^{-1}.

Leaf Chl content: Chl content was determined according to Lichtenthaler (1987). After gas-exchange measurements, Chl was extracted from 500 mg of leaf tissue that were chosen from the same leaf used for P_N measurements (avoiding major veins) using 80% aqueous acetone. After filtering, absorbance of centrifuged extracts was measured at 645 and 663 nm using a spectrophotometer (*U-2000, Hitachi Instruments,* Tokoyo, Japan).

Analysis of ion concentration: At the end of experiment (60 d after the start of salt treatment), the plants were carefully uprooted; leaves and roots were separated and washed in deionized water. Tissues were oven-dried at 70°C for 3 d, then grounded to pass through a 30-mesh screen. The ground material was ashed in a muffle furnace at 550°C for 6 h, and the ash was then dissolved in 2 ml of hot HCl and made up to 100 ml with distilled water. Contents of Na^+ and K^+ were determined using a flame photometer (*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). Cl^- was estimated according to Gilliam (1971). Cl^- was extracted from 500 mg of dry mass (DM) of 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. Cl^- concentration was

determined by silver ion titration (Moya *et al.* 1999). Ion concentrations were expressed as a percentage of DM.

DM: At the end of experiment, the seedlings were harvested, carefully washed, and leaves, stems, and roots were separated. Tissues were separately oven dried at 70°C for 3 d and then weighed. For standardizing data, percentages were calculated on a DM basis relative to the control plants of each rootstock.

Experimental design: The layout was a 4 × 9 factorial experiment in a completely randomized design, with five replications and one plant (*i.e.,* pot) per replication. Data were analyzed for significant differences using a factorial analysis of variance, with NaCl concentration and rootstock as the main factors. Statistical analysis was performed using *SAS* software, *Version 9.1* (*SAS Inc.,* Cary, NC, USA) and means were compared by using least significant difference (LSD) test at $P<0.05$. Interaction effects were compared by *MSTATC* software (*Michigan State University*, USA). Regression analyses were made using *SPSS* statistical package (*SPSS*, Chicago).

Results

Total DM: Salt treatment decreased total DM in all rootstocks, although the effect varied with rootstock species (Table 1). In 25 mM NaCl, DM was significantly reduced compared with the control. At this salinity level, SO (21% reduction) and CM (22% reduction) showed the highest and TO (50% reduction) and SwC (49% reduction) showed the lowest DM. The percentage reduction in DM was greater at 50 and 75 mM NaCl and the highest and the lowest DM were obtained for the SO (49% reduction) and TO (80% reduction), respectively at 75 mM NaCl (Table 1).

Mineral nutrient concentrations: Tissue concentrations of Cl^- and Na^+ increased significantly in response to the salt treatments (Table 2). The nine tested rootstocks accumulated more Cl^- and Na^+ ions in the leaves than in the roots. For all rootstocks, leaf and root Cl^- concentrations were higher than Na^+ concentrations (Table 2). Leaves of RLe and CM accumulated higher amount of Na^+ under 25 mM NaCl. At higher salinity (50 and 75 mM), Bak, RaLi, and SO accumulated the least amounts of Na^+ in leaves (Table 2). Leaves of CM accumulated lower Cl^- under 25 mM NaCl. At 50 and 75 mM NaCl, CM and RaLi showed the lower Cl^- in leaves but higher Cl^- was observed in leaves of TO compared with other rootstocks (Table 2). Rootstocks of SO, RaLi, and Bak excluded Na^+ from the leaves *via* accumulating the highest concentration of Na^+ in the roots (Table 2). The ability to limit the transfer of Na^+ to leaves under 25 mM NaCl was observed in TO, CaCi, and SwC, but this ability was not observed at the two higher concentrations (50 and 75 mM NaCl) (Table 2). Salinity induced changes in the concentrations of the other elements analyzed that varied with a plant organ and element (Tables 3, 4). The concentrations of K^+ in leaves significantly increased at 25 mM NaCl compared with the control plants (0 mM NaCl) except for RLe and CM, but it was decreased in leaves at 50 and 75 mM NaCl (Table 3). Salinity lowered K^+ concentrations in roots of all rootstocks (Table 3). In all rootstocks, salinity decreased Ca^{2+} and Mg^{2+} concentrations in leaves and Ca^{2+} concentrations in roots; whereas Mg^{2+} concentrations increased in the roots (Tables 3, 4).

Table 1. Effects of rootstock and NaCl (0, 25, 50, or 75 mM NaCl) on total seedling dry mass of nine citrus rootstocks after 60 days of applying salinity treatments. DM – dry mass. Values are means ($n = 5$). * – Within each column, means followed by *the same letters* are not significantly different at $P\leq0.05$. ** – Percentages were calculated on a DM basis relative to the control plants of each rootstock. SO – Sour orange; CM – Cleopatra mandarin; RaLi – Rangpur lime; Bak – Bakraii; RLe – Rough lemon; Mac – Macrophylla; SwC – Swingle citrumelo; CaCi – Carrizo citrange; TO – Trifoliate orange.

Rootstock	NaCl [mM]							
	0		25		50		75	
	DM [g]	DM [%]	DM [g]	DM [%]	DM [g]	DM [%]	DM [g]	DM [%]
SO	16.44^{c*}	100	13.01^{d}	79.0^{**}	10.79^{fg}	65.5	08.39^{i}	51.0
CM	12.78^{d}	100	09.97^{gh}	78.0	08.05^{i}	63.0	05.95^{kl}	46.6
RaLi	16.74^{bc}	100	11.72^{e}	70.0	10.55^{g}	63.0	07.97^{i}	47.6
Bak	19.51^{a}	100	12.80^{d}	65.6	10.60^{g}	54.0	07.02^{j}	36.0
RLe	17.54^{b}	100	10.31^{gh}	57.7	08.58^{i}	48.0	05.43^{lmn}	30.4
Mac	13.24^{d}	100	08.47^{i}	64.0	05.78^{lm}	43.7	03.39^{op}	25.6
SwC	09.71^{h}	100	04.95^{mn}	51.0	03.88^{o}	40.0	02.65^{pq}	27.3
CaCi	11.57^{ef}	100	06.73^{jk}	56.6	04.76^{n}	40.0	02.85^{p}	24.0
TO	09.96^{gh}	100	04.99^{mn}	50.2	03.39^{op}	34.0	01.99^{q}	20.0

Table 2. Sodium (Na^+) and chloride (Cl^-) composition of leaf and root tissue of nine citrus rootstocks after 60 days of applying salinity treatments (0, 25, 50, or 75 mM NaCl). Values are means (n = 5). * – Within each column, means followed by *the same letters* are not significantly different at $P \leq 0.05$. SO – Sour orange; CM – Cleopatra mandarin; RaLi – Rangpur lime; Bak – Bakraii; RLe – Rough lemon; Mac – Macrophylla; SwC – Swingle citrumelo; CaCi – Carrizo citrange; TO – Trifoliate orange.

Rootstock	NaCl [mM]							
	0	25	50	75	0	25	50	75
	Leaf Na^+ [%]				Root Na^+ [%]			
SO	0.14^{p*}	1.30^{klm}	1.60^{ij}	2.10^{efg}	0.18^{lm*}	0.62^{gh}	0.99^{b}	1.40^{a}
CM	0.15^{p}	1.70^{hi}	2.10^{efg}	2.40^{d}	0.12^{m}	0.38^{j}	0.60^{h}	0.80^{de}
RaLi	0.14^{p}	1.22^{lmn}	1.50^{ijk}	2.00^{fg}	0.29^{k}	0.50^{i}	0.96^{bc}	1.34^{a}
Bak	0.13^{p}	1.17^{lmn}	1.70^{hi}	1.90^{gh}	0.18^{lm}	0.64^{gh}	0.94^{bc}	1.40^{a}
RLe	0.15^{p}	1.90^{gh}	2.40^{d}	3.40^{a}	0.29^{k}	0.30^{jk}	0.50^{i}	0.73^{ef}
Mac	0.15^{p}	1.38^{jkl}	1.90^{gh}	3.30^{ab}	0.23^{kl}	0.60^{h}	0.74^{ef}	0.94^{bc}
SwC	0.11^{p}	1.10^{mno}	2.30^{de}	2.98^{c}	0.15^{lm}	0.70^{fg}	0.88^{cd}	0.94^{bc}
CaCi	0.12^{p}	1.00^{no}	2.15^{ef}	3.12^{bc}	0.14^{m}	0.64^{gh}	0.7^{fg}	0.84^{d}
TO	0.11^{p}	0.90^{o}	2.26^{de}	3.30^{ab}	0.12^{m}	0.68^{fgh}	0.74^{ef}	0.8^{de}
	Leaf Cl^- [%]				Root Cl^- [%]			
SO	0.70^{qrs*}	2.73^{kl}	3.80^{h}	4.50^{e}	0.54^{l*}	1.30^{jk}	1.70^{ghi}	2.00^{efg}
CM	0.45^{s}	2.00^{n}	2.80^{jk}	3.80^{h}	0.53^{l}	1.10^{k}	1.40^{ijk}	1.90^{fg}
RaLi	0.53^{rs}	2.40^{m}	3.00^{j}	4.00^{gh}	0.53^{l}	1.30^{jk}	1.40^{ijk}	1.80^{fgh}
Bak	0.88^{pq}	2.80^{jk}	3.40^{i}	4.40^{ef}	0.63^{l}	1.30^{jk}	1.50^{hij}	1.90^{fg}
RLe	0.79^{qr}	2.99^{jk}	4.80^{cd}	5.89^{a}	0.67^{l}	1.70^{ghi}	2.00^{efg}	2.40^{cd}
Mac	0.53^{rs}	2.50^{lm}	3.40^{i}	4.80^{cd}	0.58^{l}	1.45^{ij}	1.70^{ghi}	2.00^{efg}
SwC	1.23^{o}	3.40^{i}	4.20^{fg}	5.30^{b}	0.73^{l}	2.00^{ef}	2.40^{cd}	2.70^{abc}
CaCi	0.90^{pq}	3.52^{i}	4.60^{de}	5.46^{b}	0.77^{l}	1.70^{ghi}	2.66^{bc}	3.00^{a}
TO	1.06^{op}	3.80^{h}	4.90^{c}	5.82^{a}	0.74^{l}	1.80^{fgh}	2.30^{de}	2.90^{ab}

Table 3. Potassium (K^+) and calcium (Ca^{2+}) composition of leaf and root tissue of nine citrus rootstocks after 60 days of applying salinity treatments (0, 25, 50, or 75 mM NaCl). Values are means (n = 5). * – Within each column, means followed by *the same letters* are not significantly different at $P \leq 0.05$. SO – Sour orange; CM – Cleopatra mandarin; RaLi – Rangpur lime; Bak – Bakraii; RLe – Rough lemon; Mac – Macrophylla; SwC – Swingle citrumelo; CaCi – Carrizo citrange; TO – Trifoliate orange.

Rootstock	NaCl [mM]							
	0	25	50	75	0	25	50	75
	Leaf K^+ [%]				Root K^+ [%]			
SO	2.10^{ghij*}	2.70^{bcd}	2.40^{defg}	2.20^{fghi}	0.96^{ef*}	0.89^{g}	0.81^{h}	0.75^{hijk}
CM	2.20^{fghi}	2.00^{hijk}	1.80^{jk}	1.80^{jk}	1.18^{abcd}	0.96^{ef}	0.90^{fg}	0.80^{hi}
RaLi	2.00^{hijj}	3.00^{ab}	2.60^{cde}	2.40^{defg}	1.13^{cd}	1.02^{e}	0.80^{hi}	0.74^{ijk}
Bak	2.30^{efgh}	3.20^{a}	2.80^{bc}	2.50^{cdef}	1.12^{d}	0.78^{hij}	0.72^{jk}	0.62^{m}
RLe	2.00^{hijk}	1.80^{jk}	1.40^{lm}	1.10^{m}	1.23^{a}	0.71^{k}	0.61^{m}	0.50^{n}
Mac	2.10^{ghij}	2.60^{cde}	2.00^{hijk}	1.30^{m}	1.21^{ab}	0.70^{kl}	0.64^{lm}	0.60^{m}
SwC	1.96^{hijk}	2.50^{cdef}	1.90^{ijk}	1.40^{lm}	1.19^{abc}	0.72^{jk}	0.60^{m}	0.40^{o}
CaCi	1.69^{kl}	2.30^{efgh}	1.70^{kl}	1.10^{m}	1.16^{bcd}	0.62^{m}	0.50^{n}	0.30^{p}
TO	1.80^{jk}	2.60^{cde}	1.90^{ijk}	1.20^{m}	1.20^{ab}	0.70^{kl}	0.60^{m}	0.30^{p}
	Leaf Ca^{2+} [%]				Root Ca^{2+} [%]			
SO	2.6^{abcd*}	2.6^{abcd}	2.4^{cdef}	2.0^{ghi}	2.70^{ab*}	2.50^{abc}	2.20^{cdef}	2.20^{cdef}
CM	2.9^{a}	2.8^{ab}	2.6^{abcd}	2.2^{efg}	2.00^{efgh}	2.40^{bcd}	1.90^{fghi}	1.60^{ij}
RaLi	2.7^{abc}	2.7^{abc}	2.5^{bcde}	2.1^{fgh}	2.20^{cdef}	2.00^{efgh}	1.90^{fghi}	1.70^{hij}
Bak	2.4^{cdef}	2.0^{ghi}	1.8^{hij}	1.6^{j}	2.30^{cde}	1.85^{ghi}	1.70^{hij}	1.20^{klm}
RLe	2.6^{abcd}	2.0^{ghi}	2.0^{ghi}	1.6^{j}	2.00^{efgh}	2.30^{cde}	1.40^{jk}	1.20^{klm}
Mac	2.7^{abc}	2.0^{ghi}	1.8^{hij}	1.7^{ij}	2.80^{a}	2.40^{bcd}	1.20^{kl}	1.00^{lmn}
SwC	2.8^{ab}	2.1^{fgh}	1.8^{hij}	1.5^{jk}	1.80^{ghi}	1.60^{ij}	1.00^{lmn}	0.90^{mn}
CaCi	2.9^{a}	2.3^{defg}	2.0^{ghi}	1.7^{ij}	2.10^{defg}	2.00^{efgh}	0.85^{n}	0.70^{n}
TO	2.4^{cde}	2.0^{ghi}	1.6^{j}	1.2^{k}	1.90^{fghi}	2.20^{cdef}	1.40^{jk}	1.00^{lmn}

Table 4. Magnesium (Mg^{2+}) composition of leaf and root tissue of nine citrus rootstocks after 60 days of applying salinity treatments (0, 25, 50, or 75 mM NaCl). Values are means (n = 5). * – Within each column, means followed by *the same letters* are not significantly different at $P \leq 0.05$. SO – Sour orange; CM – Cleopatra mandarin; RaLi – Rangpur lime; Bak – Bakraii; RLe – Rough lemon; Mac – Macrophylla; SwC – Swingle citrumelo; CaCi – Carrizo citrange; TO – Trifoliate orange.

Rootstock	NaCl [mM] 0	25	50	75	0	25	50	75
	Leaf Mg^{2+} [%]				Root Mg^{2+}[%]			
SO	0.39^{a*}	0.37^{abc}	0.34^{cd}	0.28^{ghi}	0.34^{ijkl}	0.41^{defg}	0.44^{bcd}	0.48^{a}
CM	0.37^{abc}	0.33^{de}	0.29^{fgh}	0.26^{hij}	0.46^{abc}	0.48^{a}	0.46^{abc}	0.46^{abc}
RaLi	0.35^{bcd}	0.32^{def}	0.29^{fgh}	0.27^{ghij}	0.31^{lmn}	0.38^{ghi}	0.40^{efh}	0.43^{cde}
Bak	0.39^{a}	0.34^{cd}	0.29^{fgh}	0.26^{hij}	0.36^{ijk}	0.36^{ijk}	0.38^{ghi}	0.40^{efgh}
RLe	0.35^{bcd}	0.30^{efg}	0.25^{ijk}	0.20^{lm}	0.40^{efgh}	0.42^{def}	0.42^{def}	0.46^{abc}
Mac	0.32^{def}	0.30^{efg}	0.26^{hij}	0.20^{lm}	0.37^{hij}	0.39^{fghi}	0.43^{cde}	0.47^{ab}
SwC	0.38^{ab}	0.34^{cd}	0.24^{jk}	0.16^{no}	0.26^{o}	0.33^{klm}	0.33^{klm}	0.36^{ijk}
CaCi	0.34^{cd}	0.32^{def}	0.22^{kl}	0.16^{no}	0.30^{mn}	0.36^{ijk}	0.40^{efgh}	0.40^{efgh}
TO	0.34^{cd}	0.30^{efg}	0.18^{mn}	0.14^{o}	0.28^{no}	0.32^{lm}	0.32^{lm}	0.36^{ijk}

Leaf Chl content: Total Chl contents were reduced by salinity in all rootstocks (Table 5). The lesser percentage reduction in total Chl content was recorded in SO (*i.e.*, 40.7% at 75 mM NaCl), CM (38% at 75 mM NaCl), and RaLi (with 37.4% at 75 mM NaCl). The percentage reduction in the total Chl content was greater at 50 and 75 mM NaCl and the highest reduction was obtained for SwC (77.9%), CaCi (79%), and TO (84%) at 75 mM NaCl (Table 5).

Leaf gas-exchange parameters: When plants were irrigated with water containing NaCl, P_N dropped in all rootstocks (Table 5). Comparison among the rootstocks at two lower concentrations (25 and 50 mM NaCl) indicated that CM (17.7% and 18.2% reduction, respectively) and SO seedlings (30.6% and 39.4% reduction, respectively) were less affected by the salt treatment. The percentage reduction of P_N was greater at 75 mM NaCl and the highest reduction was obtained for the TO (86.6%) and CaCi (84.4%) at 75 mM NaCl, followed by SwC with 80.3% (Table 5). High values of g_s were recorded in the controls (0 mM). The presence of NaCl in the root medium induced a significant decrease in g_s in leaves of all the rootstocks studied (Table 5). The g_s was lower as the salt concentration increased and reached the minimum values at 75 mM NaCl. Comparison among the rootstocks at 75 mM NaCl indicated lesser reduction in CM (43.3%), SO (46%), and RaLi (50%) than in SwC (68.2%), CaCi (73.6%), and TO (82%) (Table 5). C_i gradually decreased with an increase in the NaCl concentration. Except for CM, the other rootstocks showed a decrease of C_i at 25 mM NaCl. The reductions were more pronounced at 50 mM, especially for TO and CaCi, in which it attained 35.6 and 49% of the control, respectively. When NaCl concentration increased from 50 to 75 mM, the rootstocks except for CM showed an increase in C_i (Table 5).

Correlation coefficients: The statistical significance of correlations between Na^+ and Cl^- contents in roots and leaves and concentrations of these ions in the external solution was determined using linear regression analyses (Table 6). In all rootstocks, Cl^- and Na^+ uptake and transport to leaves increased linearly with the increase of the external salt concentration (regressions were significant at $P \leq 0.001$) (Table 6). Regression equations of P_N, DM, Chl, and g_s with foliar Na^+ and Cl^- contents were calculated to evaluate the relative importance of foliar ion contents and their effects on these parameters. Results indicated that, in citrus rootstocks, many physiological disturbances and nutritional imbalances caused by salinity were linked to leaf Na^+ and Cl^- accumulation. The percentage of Na^+ and Cl^- in leaves of all rootstocks negatively correlated with the DM (Table 7). In all rootstocks, Na^+ and Cl^- also negatively correlated with the photosynthetic variables and with the Chl content (Table 7). A strong correlation was found between P_N and DM, Chl, g_s (Table 8). The correlation coefficients indicated that salinity reduced P_N *via* reduction in Chl, g_s, and nutritional disturbance (Table 8).

Discussion

Salt stress led to a decrease in total plant DM in all rootstocks after 60 days of treatments. If we define salt tolerance as growth reduction, data presented herein indicate that SO and CM were the most tolerant, while TO, CaCi, and SwC were the most sensitive to salt stress. Rootstocks of RLe and Mac showed a low-to-moderate tolerance to salt stress, whereas RaLi and Bak exhibited a moderate-to-high tolerance. Reduction in DM might occurr due to the increased osmotic pressure in the root zone after the enhanced salt concentration in the soil solution, the accumulation of ions (especially Na^+ and Cl^-) in the plant tissues to the toxic concentrations, and the

Table 5. Effects of rootstock and NaCl (0, 25, 50, or 75 mM NaCl) on total chlorophyll (Chl), net photosynthetic rate (P_N), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) in leaves of of nine citrus rootstocks after 60 days of applying salinity treatments. FM – fresh mass. Values are means ($n = 5$). * – Within each column, means followed by *the same letters* are not significantly different at $P \leq 0.05$. ** – Reduction in comparison to the control [**%**]. SO – Sour orange; CM – Cleopatra mandarin; RaLi – Rangpur lime; Bak – Bakraii; RLe – Rough lemon; Mac – Macrophylla; SwC – Swingle citrumelo; CaCi – Carrizo citrange; TO – Trifoliate orange.

Rootstock	NaCl [mM] 0	25		50		75	
	Chl [mg g^{-1}(FM)]						
SO	1.08^{ab*}	0.92^{cd}	14.8^{**}	0.77^{ef}	28.7^{**}	0.64^{ghi}	40.7^{**}
CM	1.07^{ab}	0.95^{cd}	11.2	0.74^{fg}	30.8	0.66^{gh}	38.0
RaLi	0.99^{bc}	0.85^{de}	14.1	0.72^{fgh}	27.3	0.62^{hi}	37.4
Bak	0.98^{bc}	0.73^{fg}	25.5	0.55^{ij}	43.9	0.42^{klm}	57.0
RLe	0.95^{cd}	0.70^{fgh}	35.7	0.46^{jkl}	52.0	0.35^{mno}	63.0
Mac	0.99^{bc}	0.78^{ef}	33.3	0.38^{lmn}	61.6	0.30^{nop}	69.7
SwC	0.95^{cd}	0.55^{ij}	42.0	0.25^{opq}	73.7	0.21^{pq}	77.9
CaCi	1.00^{bc}	0.50^{jk}	50.0	0.24^{pq}	76.0	0.21^{pq}	79.0
TO	1.13^{a}	0.41^{klmn}	62.6	0.18^{q}	84.0	0.18^{q}	84.0
	P_N [μmol(CO_2) m^{-2} s^{-1}]						
SO	6.6^{a*}	5.4^{bc}	18.2^{**}	4.0^{efg}	39.4^{**}	3.1^{hij}	50.0^{**}
CM	6.2^{a}	5.1^{cd}	17.7	4.3^{e}	30.6	3.3^{ghi}	46.8
RaLi	6.6^{a}	4.4^{de}	33.3	3.5^{fgh}	46.9	2.7^{ijkl}	59.0
Bak	6.0^{ab}	4.0^{efg}	33.3	3.0^{hijk}	50.0	2.4^{jklm}	60.0
RLe	6.4^{a}	3.5^{fgh}	45.3	2.2^{lmn}	65.6	1.8^{mno}	71.9
Mac	6.5^{a}	4.2^{ef}	35.8	2.3^{klm}	64.8	1.9^{mno}	70.9
SwC	6.1^{ab}	3.0^{hijk}	50.8	1.8^{mno}	70.5	1.2^{op}	80.3
CaCi	6.4^{a}	2.8^{hijkl}	56.2	1.5^{nop}	76.6	1.0^{p}	84.4
TO	6.0^{ab}	2.3^{klm}	61.6	1.0^{p}	83.3	0.8^{p}	86.6
	g_s [mmol(H_2O) m^{-2} s^{-1}]						
SO	0.150^{a*}	0.120^{bc}	20.0^{**}	0.098^{de}	34.7^{**}	0.085^{efg}	43.3^{**}
CM	0.130^{b}	0.110^{cd}	15.4	0.095^{def}	30.0	0.070^{ghij}	46.0
RaLi	0.150^{a}	0.120^{bc}	20.0	0.095^{def}	36.7	0.075^{gh}	50.0
Bak	0.130^{b}	0.098^{de}	26.4	0.078^{gh}	40.0	0.058^{ijkl}	55.4
RLe	0.120^{bc}	0.070^{ghij}	41.7	0.063^{hijk}	47.5	0.040^{mno}	66.7
Mac	0.130^{b}	0.080^{fg}	38.5	0.074^{ghi}	43.1	0.045^{lmno}	65.4
SwC	0.110^{cd}	0.055^{jklm}	50.0	0.050^{klmn}	54.5	0.035^{nop}	68.2
CaCi	0.110^{cd}	0.050^{klmn}	54.5	0.040^{mno}	63.9	0.029^{op}	73.6
TO	0.120^{bc}	0.040^{mno}	66.7	0.030^{op}	75.0	0.021^{p}	82.0
	C_i [μmol mol^{-1}]						
SO	292^{a*}	245^{bcd}		159^{opqr}		240^{bcde}	
CM	255^{bc}	228^{cdef}		191^{hijklm}		173^{klmnop}	
RaLi	263^{ab}	170^{lmnop}		160^{nopqr}		214^{efghi}	
Bak	247^{bcd}	189^{ijklmn}		200^{fghijk}		220^{defgh}	
RLe	228^{cdef}	163^{mnopqr}		146^{pqrs}		182^{jklmno}	
Mac	207^{fghij}	129^{st}		139^{qrs}		181^{jklmno}	
SwC	197^{ghijkl}	140^{qrs}		100^{tu}		146^{pqrs}	
CaCi	210^{fghij}	129^{st}		103^{t}		150^{pqrs}	
TO	221^{defg}	137^{rs}		72^{u}		167^{mnopq}	

excessive concentration of soluble ions that might have resulted in nutrient imbalance in the soil solution and plant tissues (Liu *et al*. 2005, Bhatt *et al.* 2008). Moreover, Can *et al*. (2003) reported that irrigation with saline water reduced the photosynthetic capacity per unit area as well as further depressed vegetative growth of the whole tree.

Ion toxic effects of salts are attributed to excessive accumulation of certain ions in plant tissues and to nutritional imbalances caused by such ions. Leaf and root Cl^- and Na^+ concentrations generally increased with increasing salt concentrations in the nutrient solution. Salt damage to citrus plants has been mainly attributed to excessive accumulation of Cl^- and Na^+ in the leaves (Moya *et al*. 2003, Anjum 2007). Root accumulation and low transport may be one of the mechanisms in determining salinity tolerance in citrus (Zekri and Parsons 1992).

Table 6. Correlation coefficient analysis between increasing salinity (0, 25, 50, or 75 mM NaCl) in the external solution and accumulation of Cl^- and Na^+ in leaves and roots of citrus rootstocks. Significant effects indicated by asterisks: *** – $P<0.001$. SO – Sour orange; CM – Cleopatra mandarin; RaLi – Rangpur lime; Bak – Bakraii; RLe – Rough lemon; Mac – Macrophylla; SwC – Swingle citrumelo; CaCi – Carrizo citrange; TO – Trifoliate orange.

Rootstock	Roots		Leaves	
	Cl^-	Na^+	Cl^-	Na^+
SO	0.95***	0.99***	0.94***	0.99***
CM	0.99***	0.99***	0.98***	0.99***
RaLi	0.90***	0.98***	0.95***	0.98***
Bak	0.95***	0.99***	0.94***	0.99***
RLe	0.92***	0.89***	0.98***	0.89***
Mac	0.91***	0.96***	0.98***	0.96***
SwC	0.88***	0.84***	0.95***	0.84***
CaCi	0.96***	0.84***	0.93***	0.84***
TO	0.97***	0.75***	0.93***	0.75***

Our data suggest that the mechanism of Na^+ exclusion depends on preferential accumulation in roots. For example, SO roots accumulated a higher concentration of Na^+ than CM roots, but SO leaves accumulated less Na^+ than CM leaves, showing that SO could partially exclude Na^+ from leaves by accumulating Na^+ in the roots. However, the root Cl^- concentrations were similar in SO and CM, while SO exhibited a higher leaf Cl^- concentration. These results suggest that the difference in capacity for Cl^- exclusion in leaves of the citrus rootstocks is based on the ability to restrict Cl^- transport from roots to shoots and not on the preferential accumulation of Cl^- in roots. Linear regressions showed that Na^+ and Cl^- accumulation significantly correlated with increasing concentrations of these ions in the external solution, and the increase was paralleled by reductions in DM and gas-exchange parameters. The rapid uptake and accumulation of Na^+ and Cl^- in leaves with increasing salinity may underline the inhibitory effects of salinity on growth in citrus rootstocks (Moya *et al*. 2003, Anjum 2007).

Table 7. Correlation coefficient analysis between leaf Cl^- and leaf Na^+ with dry mass (DM), net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), total chlorophyll (Chl), leaf Ca^{2+}, Mg^{2+} and K^+ in citrus rootstocks in response to increasing salinity (0, 25, 50, or 75 mM NaCl) in the external solution. Significant effects indicated by asterisks: *** – $P<0.001$; ** – $P<0.01$; * – $P<0.05$; ns – not significant. SO – Sour orange; CM – Cleopatra mandarin; RaLi – Rangpur lime; Bak – Bakraii; RLe – Rough lemon; Mac – Macrophylla; SwC – Swingle citrumelo; CaCi – Carrizo citrange; TO – Trifoliate orange.

Rootstock	DM	P_N	g_s	C_i	Chl	Ca^{2+}	Mg^{2+}	K^+
	Leaves Cl^-							
SO	–0.91***	–0.98***	–0.99***	–0.68**	0.98**	–0.79***	–0.88***	0.22ns
CM	–0.89***	–0.90***	–0.91***	–0.99***	–0.95**	–0.93***	–0.92***	–0.85***
RaLi	–0.99***	–0.99***	–0.98***	–0.58*	–0.98**	–0.82***	–0.98***	0.46 ns
Bak	–0.99***	–0.99***	–0.99***	–0.54*	–0.99**	–0.99***	–0.98***	0.29 ns
RLe	–0.97***	–0.97***	–0.96***	–0.67**	–0.99**	–0.94***	–0.99***	–0.97***
Mac	–0.99***	–0.97***	–0.99***	–0.34ns	–0.95**	–0.95***	–0.94***	–0.63**
SwC	–0.99***	–0.99***	–0.98***	–0.72**	–0.97**	–0.99***	–0.93***	–0.47ns
CaCi	–0.99***	–0.99***	–0.98***	–0.76**	–0.99**	–0.99***	–0.89***	–0.39ns
TO	–0.99***	–0.98***	–0.97***	–0.38ns	–0.97**	–0.96***	–0.92***	–0.32ns
	Leaves Na^+							
SO	–0.91***	– 0.96***	–0.98***	–0.62**	–0.97***	–0.78***	–0.88***	0.28ns
CM	–0.95***	–0.94***	–0.91***	–0.74**	–0.91***	–0.79***	–0.94***	–0.96***
RaLi	–0.99***	–0.99***	–0.98***	–0.61*	–0.97***	–0.79***	–0.97***	0.49*
Bak	–0.98***	–0.99***	–0.98***	–0.62*	–0.98***	–0.99***	–0.97***	0.34 ns
RLe	–0.99***	–0.97***	–0.99***	–0.68**	–0.97***	–0.99***	–0.97***	–0.94***
Mac	–0.97***	–0.92***	–0.97***	–0.23 ns	–0.92***	–0.91***	–0.97***	–0.70**
SwC	–0.94***	–0.95***	–0.91***	–0.69**	–0.97***	–0.98***	–0.98***	–0.63**
CaCi	–0.95***	–0.91***	–0.88***	–0.57*	–0.92***	–0.97***	–0.98***	–0.65**
TO	–0.92***	–0.87***	–0.82***	–0.44 ns	–0.85***	–0.99***	–0.99***	–0.62**

We found that the salt treatments altered mineral nutrient distribution and decreased absorption of all of the nutrients studied. K^+ concentration at low salinity in most rootstocks initially increased in leaves and then decreased at higher salinity. Increased accumulation of K^+ within cells could be a regulatory mechanism to maintain osmotic balance against the high concentrations of Cl^- under salinity stress (Grieve and Walker 1983). In this experiment, the concentration of K^+ ions was significantly reduced with increasing salinity in roots. In this regard, some authors reported that reduction in root K^+ could be attributed to a direct effect of Na^+ displacing K^+ from the root tissue or to replacement of Ca^{2+} with Na^+ ions in root membrane cells that induced leakage of K^+ from the roots (Zekri and

Table 8. Correlation coefficient analysis between net photosynthetic rate (P_N) and dry mass (DM), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), chlorophyll (Chl), leaves Ca^{2+}, Mg^{2+} and K^+ in citrus rootstocks in response to increasing salinity (0, 25, 50, or 75 mM NaCl) in the external solution. Significant effects indicated by asterisks: *** – $P<0.001$; ** – $P<0.01$; * – $P<0.05$; ns – not significant. SO – Sour orange; CM – Cleopatra mandarin; RaLi – Rangpur lime; Bak – Bakraii; RLe – Rough lemon; Mac – Macrophylla; SwC – Swingle citrumelo; CaCi – Carrizo citrange; TO – Trifoliate orange.

Rootstocks	P_N DM	g_s	C_i	Chl	Ca^{2+}	Mg^{2+}	K^+
SO	0.94^{**}	0.99^{***}	0.63^{**}	0.99^{***}	0.88^{***}	0.94^{***}	-0.2^{ns}
CM	0.99^{***}	0.99^{***}	0.91^{***}	0.98^{***}	0.95^{***}	0.99^{***}	0.94^{***}
RaLi	0.99^{***}	0.98^{***}	0.63^{**}	0.98^{***}	0.78^{***}	0.98^{***}	-0.48^{ns}
Bak	0.99^{***}	0.99^{***}	0.58^{*}	0.99^{***}	0.99^{***}	0.98^{***}	-0.31^{ns}
RLe	0.98^{***}	0.98^{***}	0.81^{***}	0.97^{***}	0.94^{***}	0.94^{***}	0.90^{***}
Mac	0.99^{***}	0.95^{***}	0.46^{ns}	0.98^{***}	0.97^{***}	0.88^{***}	0.54^{*}
SwC	0.99^{***}	0.99^{***}	0.82^{***}	0.99^{***}	0.99^{***}	0.88^{***}	0.37^{ns}
CaCi	0.99^{***}	0.99^{***}	0.83^{***}	0.99^{***}	0.98^{***}	0.84^{***}	0.29^{ns}
TO	0.99^{***}	0.99^{***}	0.52^{*}	0.99^{***}	0.90^{***}	0.87^{***}	0.17^{ns}

Parsons 1992, García-Sánchez *et al*. 2002). The decrease in foliar concentration of Ca^{2+} by salinity indicated that Ca^{2+} translocation was inhibited. Reduction in the amount of Ca^{2+} in the shoots under salt stress can be caused by the competitive effects of ions with Ca^{2+} in vessels of the plant, which disrupt the transfer of this element (Botella *et al*. 1997). Ca^{2+} is important in cell biology (especially membrane biology) during salt stress, *e.g.* in preserving membrance integrity (Rengel 1992), signaling in osmoregulation (Mansfield *et al*. 1990), and influencing K^+/Na^+ selectivity (Cramer *et al*. 1987). Foliar concentration of Mg^{2+} was reduced by salinity, but Mg^{2+} increased by salinity in roots. The foliar concentration of Mg^{2+} was highly correlated with P_N in all rootstocks. Very little is known about salinity and Mg^{2+} interactions in *Citrus*. However, besides its role in Chl structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is the export of photosynthates, which is impaired and leads to enhanced degradation of Chl in Mg^{2+}-deficient source leaves, resulting in increased oxygenase activity of Rubisco (Marschner and Cakmak 1989). In Mg^{2+}-deficient leaves, formation of superoxide radicals and H_2O_2 is enhanced and therefore the leaves become highly photosensitive (Tozlu *et al*. 2000). This suggests that Mg^{2+} deficiency may be one of the reasons for the chlorosis observed in salinity-treated leaves.

Total Chl content was markedly reduced by the salt treatment. A decrease in leaf Chl content has been described in citrus rootstocks irrigated with high NaCl concentration (García-Sánchez *et al*. 2002). Chl reduction could be due to salt stress–induced activity of chlorophyllase (Ashraf 2003), enhanced H_2O_2 production, and Chl photodamage (Hossain *et al*. 2011).

In the present experiment, P_N and g_s decreased with increasing salinity. The P_N and g_s were strongly negatively correlated with leaf Cl^- and Na^+. High correlation between leaf gas-exchange parameters and foliar concentration of Cl^- and Na^+ suggests that the toxic effect of the accumulated ions could be involved in reduction of P_N and g_s. P_N showed a highly significant correlation with g_s, which indicated that stomatal closure drove, at least in part, P_N reduction under salt stress. An imbalance of essential nutrients may also be a factor involved in the salt-induced decrease in gas-exchange parameters and consequently in rootstocks growth reduction. It has been reported that long-term exposure to salt decreased photosynthetic rate, which might be due to reduced g_s (Ouerghi *et al.* 2000). Leaf stomata closure due to toxic Na^+ and Cl^- ions decreases g_s that further decreases photosynthesis and reduces growth (Shahbaz and Zia 2011). The P_N and g_s were in agreement with C_i values. A decrease in C_i should occur with the decrease in P_N if stomatal limitations to CO_2 diffusion are a dominant limitation (Farquhar and Sharkey 1982). In citrus, salinity reduces P_N and carbohydrate accumulation (García-Sánchez and Syvertsen 2006), which is probably associated with the salt-induced reduction in CO_2 diffusion by stomata (Paranychianakis and Chartzoulakis 2005). The subsequent increase in C_i may indicate nonstomatal limitations (Moya *et al.* 2002). It can be concluded therefore that the decrease in the P_N reported herein was not due to biochemical limitations, but it could be attributed mainly to stomatal components.

Based on our results, we found that SO and CM were the rootstocks most tolerant to salinity of all nine studied. In addition, TO, CaCi, and SwC were the rootstocks most sensitive to salt stress. Mac and RLe showed a low-to-moderate tolerance to salt stress, while RLe and Bak exhibited the moderate-to-high tolerance to salinity. From our data, a higher resistance to salinity could be associated with the ability to keep the higher Chl content, P_N, to compartmentalize Cl^- and Na^+ in leaves better, and to maintain better nutrient uptake even in elevated concentrations of saline. Therefore, the data suggest that, in salinized citrus, an imbalance of essential nutrients, in combination with several metabolic components such as Na^+ and Cl^- overloading, low Mg^+, stomatal closure, and Chl loss, may contribute to reduction in P_N.

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