

Additional nitrogen fertilization affects salt tolerance of lemon trees on different rootstocks

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ABSTRACT

Irrigation with saline water is one of the major problems in citrus crop in arid and semi-arid regions. Because rootstock and fertilization play an important role in citrus salt tolerance, we investigated the influence of the nitrogen fertilization and rootstock on salt tolerance of 2-year-old potted Fino 49 lemon trees. For that, trees grafted on *Citrus macrophylla* (M) or Sour orange (SO) rootstocks were watered for 12 weeks with complete nutrient solution containing either 0 mM NaCl (control, C), 50 mM NaCl (S), 50 mM NaCl with an additional 10 mM potassium nitrate (S + N), or 50 mM NaCl with a 1% KNO₃ (S + Nf) foliar spray application. Trees on M were more vigorous than trees on SO and saline treatments reduced leaf growth similarly in trees on both rootstocks. Trees on SO had a lower leaf Cl⁻ and Na⁺ concentration than those on M. Additional soil nitrogen (S + N) decreased leaf Cl⁻ concentration and increased leaf K⁺ concentration in salinized trees on both rootstocks. However, the salinity-induced reduction leaf growth was similar in S + N and S trees. This was due to osmotic effect, beside leaf Cl⁻ and Na⁺ toxicity, played an important role in the growth response of Fino 49 lemon to the salt stress. Additional foliar nitrogen in the S + Nf treatment also reduced leaf Cl⁻ concentration relative to the S treatment but trees from S + Nf treatment had the lowest leaf growth. Net assimilation of CO₂ (A_{CO₂}), stomatal conductance (g_s) and plant transpiration were reduced similarly in all three salt treatments, regardless rootstock. Salinity reduced leaf water and osmotic potential such that leaf turgor was increased. Thus, the salinity-induced A_{CO₂} reductions were not due to loss of turgor but rather due to high salt ion accumulation in leaves.

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1. Introduction

In the world ranking, Spain is the second-highest lemon fruit-producing country and the greatest exporter. About 80% of the Spanish production is located in the arid southeast where irrigation water often has high salinity from NaCl. Citrus trees have been classified as a salt-sensitive crop (Maas, 1993; Storey and Walker, 1999) as saline irrigation water reduces citrus tree growth and fruit yield (García-Sánchez et al., 2006; Grieve et al., 2007; Prior et al., 2007). Negative effects of saline irrigation water on citrus growth and physiological process (Cámara et al., 2003; Camara-Zapata et al., 2004) are generally due to Cl⁻ rather than to Na⁺ toxicity (Romero-Aranda et al., 1998), osmotic or salt-induced oxidative stress (Arbona et al., 2003). The osmotic adjustment in salinized citrus leaves is very effective because even when leaf water potential is reduced, the high leaf Cl⁻ and Na⁺ concentration reduces the osmotic potential such that leaf turgor is maintained or

even increased (García-Sánchez and Syvertsen, 2006). High concentrations of Cl⁻ and Na⁺ in leaves reduce net assimilation of CO₂ (A_{CO₂}) by a direct biochemical inhibition of photosynthetic capacity rather by decreases in stomatal conductance (Levy and Syvertsen, 2004; García-Sánchez and Syvertsen, 2006). Biochemical inhibition of A_{CO₂} in citrus can be linked to changes in leaf anatomy, interactions with leaf nutrients (Romero-Aranda et al., 1998) and/or reductions in the electron transport (Lopez-Climent et al., 2008).

Salt tolerance in citrus has been linked to the exclusion of toxic ions from the shoot (García-Sánchez 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 (Storey and Walker, 1999). For instance, in Fino lemon trees, Sour orange rootstock is considered a good Cl⁻ and Na⁺ excluder, whereas the *Citrus macrophylla* rootstock is a Cl⁻ and Na⁺ accumulator (Nieves et al., 1991). Historically, Sour orange (*Citrus aurantium* L.) has been the most commonly used rootstock in lemon trees, however, this rootstock is highly susceptible to the tristeza disease (Moreno et al., 2008) so this rootstock has been replaced mainly by the *C. macrophylla* rootstock in Spain. Citrus responses to salinity can also

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depend on the amount of irrigation, climate, soil type or fertilization (Levy and Syvertsen, 2004). Salinity may cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with nutrients such as K^+ , Ca^{2+} , Mg^{2+} and NO_3^- (Romero-Aranda et al., 1998) acting on biophysical and/or metabolic components of plant growth. However, an adequate fertilization and application of additional KNO_3 may enhance plant growth due to reductions in Cl^- toxicity (Camara-Zapata et al., 2004) and the maintenance of nutrient balances (Hu and Schmidhalter, 2005). In citrus, it has been reported that 10 mM KNO_3 is a much higher concentration which nitrate is reported to act as a chloride antagonist (Cerezo et al., 1999). At this concentration, potassium also can equalize the imbalances resulting from the excess of Na^+ without affecting the physiological responses (Bañuls et al., 1997; Moya et al., 1999). In Navelina orange, KNO_3 supplementation via root partially counteracted salt-stress reduced plant growth by increasing dry matter and new leaf area (Iglesias et al., 2004). However, high KNO_3 concentration in the nutrient solution could also have negative effects in the plant growth due mainly to increase the osmotic effect by increasing salt concentration in the root medium. In addition to osmotic effect, high K^+ concentration in a salinized nutrient solution increased the absorption of Cl^- citrus roots (Romero-Aranda et al., 1998). Thus, we hypothesized that a foliar application of 1% KNO_3 could avoid the negative osmotic effect and the high K^+ concentration in the nutrient solution. This would improve the mineral nutrition of N and K potentially decreasing leaf Cl^- concentration and increasing salt tolerance. The concentration of 1% KNO_3 was chosen because in citrus crops are normally used concentration between 1 and 3% for leaf spraying to control potassium deficiencies. Therefore, the objectives of this study were to evaluate the salt tolerance of Fino 49 lemon trees grafted on two contrasting rootstock, Sour orange or *C. macrophylla*, and if additional of KNO_3 in the irrigation water or via foliar application could improve the salt tolerance of Fino lemon trees.

2. Materials and methods

2.1. Plant material and growth condition

Two-year-old Fino lemon (*Citrus limon* Burm. f. cv. Fino 49) trees grafted on two rootstocks: the relatively salt tolerant Sour orange (*Citrus aurantium* L.; SO) and the more salt sensitive *C. macrophylla* (*C. macrophylla* wester; M) (Nieves et al., 1991). Trees were grown in 12-l pots filled with native clay-loam soil in a partially shaded greenhouse under maximum photosynthetically active radiation of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, day/night temperature of $35/18 \pm 3^\circ\text{C}$, a day/night relative humidity of $55/75 \pm 5\%$ and a 16-h photoperiod. Irrigation was carried out using a drip system at 41 h^{-1} per tree. The plants were irrigated with a complete nutrient solution of the following composition: 7.75 mM NO_3^- , 0.7 mM H_2PO_4^- , 4.05 mM K^+ , 2.20 mM Ca^{2+} , 0.5 mM Mg^{2+} , 0.5 mM SO_4^{2-} and 0.6 mM Fe, and containing either 0 mM NaCl (control treatment; $\text{EC} = 1.3 \text{ dS m}^{-1}$), 50 mM NaCl (S; $\text{EC} = 6.3 \text{ dS m}^{-1}$), 50 mM NaCl supplemented with 10 mM KNO_3 (S+N; $\text{EC} = 7.9 \text{ dS m}^{-1}$), or S plus a foliar spray application one time per week of 1% KNO_3 , equivalent to 98 mM KNO_3 (S+Nf; $\text{EC} = 6.3 \text{ dS m}^{-1}$). The plants were well watered and well nourished by watering every 2 days in a sufficient volume to leach from the bottom of all pots. To avoid an osmotic shock in the salt treatments, salinity was increased in increments of 10 mM NaCl per day until 50 mM NaCl was achieved. The experimental design was a 2×4 factorial of two rootstocks (Sour orange and *C. macrophylla*) by four irrigation treatments (C, S, S+N and S+Nf) with six replicate trees in each treatment. Treatments were continued for 12 weeks.

2.2. Plant water relations

All leaf measurements were done using a single mature leaf in the mid-stem region of each of the six replicate trees. Pre-dawn leaf water potential (Ψ_w) was measured at the end of the experiment using a Scholander-type pressure chamber (PMS instrument, Corvallis, OR; (Scholander et al., 1965). After Ψ_w was measured, the leaves were immediately wrapped tightly in aluminum foil, frozen by immersing in liquid nitrogen and subsequently stored in airtight plastic bags at -18°C . After thawing, Ψ_π of the expressed sap was measured at $25 \pm 1^\circ\text{C}$ with an osmometer (Digital Osmometer, Wescor, Logan, UT). Turgor potential was (Ψ_p) calculated as the differences between Ψ_w and Ψ_π . The midday relative water content (RWC) was measured using adjacent leaves, which were immediately weighed to obtain a leaf fresh mass. Leaves were placed in a beaker with the petioles submerged in water overnight in the dark, so leaves could become fully hydrated. Leaves were reweighed to obtain turgid mass and dried at 80°C for 24 h to obtain dry mass. RWC was calculated as $[(M_f - M_d)(M_t - M_d)^{-1}] \times 100$ according to Morgan (1984), where M_f is the leaf fresh mass; M_t is the turgid mass; and M_d is the dry mass. Leaf osmotic potential at full turgor (Ψ_π^{100}) was also measured on one leaf per plant after full hydration overnight as above. Fully turgid leaves were then frozen in liquid nitrogen, and Ψ_π^{100} was measured as above.

2.3. Leaf gas exchange parameters

Net assimilation of CO_2 (A_{CO_2}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), leaf transpiration (E) and instantaneous leaf water use efficiency ($\text{WUE} = A_{\text{CO}_2}/E$) were measured at the end of the experiment using a portable photosynthesis system (model LCA-4, ADC Bioscientific Ltd., Hoddesdon, UK) and a leaf chamber PLC-4N (11.35 cm^2), configured to an open system. All measurements were made in the morning from 08:00 to 10:00 h to avoid high temperatures and low humidity in the afternoon. During all measurements, leaf temperature was $30 \pm 2^\circ\text{C}$ and leaf-to-air vapor pressure difference was $2.4 \pm 0.4 \text{ kPa}$ within the cuvette.

2.4. Proline and quaternary ammonium compounds (QACs)

At the end of the experiment, proline was extracted from fresh leaf and root tissues with sulphosalicylic acid (3%) and quantified according to the protocol described by Bates et al. (1973). QAC was also extracted from fresh tissues with 1 M H_2SO_4 and quantified using methods in Grieve and Grattan (1983). Proline and QAC concentrations were expressed in units of $\text{mmol kg}^{-1} \text{ dw}$.

2.5. Growth and leaf nutrient concentration

At the end of the experiment, plants were harvested and separated into leaves, stems, and roots. Tissues were briefly rinsed with deionised water, oven-dried at 60°C for at least 48 h, weighed and ground to a fine powder. Subsamples of leaf and root tissues were extracted with deionised water, and tissue chloride concentration was measured with a Corning 926 chloridometer (Sherwood, UK). Tissue N concentration was measured using a Thermo-Finnigan 1112 EA elemental analyzer (Thermo-Finnigan, Milan, Italy). Tissue Na^+ , K^+ , Mg^{2+} , Ca^{2+} and P concentrations were determined by inductively coupled plasma emission optical spectrometry (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA) after previous acid digestion in $\text{HNO}_3:\text{H}_2\text{O}_2$ (5:3, v/v) in a microwave reaching 200°C in 20 min and holding at this temperature during 2 h (CEM Mars Xpress, North Carolina, USA).

2.6. Statistical analysis

Data were subjected to analysis of variance using a two-way ANOVA (SPSS statistical package, Chicago, IL) with two rootstocks \times four nitrogen treatments and six replicate plants or leaves per treatment. When interaction term were significant ($P < 0.05$), treatment means were separated using Duncan's multiple range test. When interaction term was not significant and, main factors (nitrate supplementation and/or rootstock) were significant, Duncan's multiple range test was run to the main factor regardless each other (Little and Hills, 1987).

3. Results

3.1. Growth

After 12 weeks of treatments, Fino 49 lemon trees grafted on M had higher leaf dry weight but similar stem dry weight than those on SO (Table 1). All salinized treatments reduced leaf and stem growth similarly in trees on both SO and M. The highest reduction of leaf growth occurred in the salinized S + Nf treatment, while there were no significant differences between S and S + N treatments. The growth reduction in stem dw was similar for all three salinized S, S + N and S + Nf treatments. Root growth was reduced similarly for all three salinized treatments in trees on SO but it was not affected by salinized treatment in trees on M. Shoot to root dw ratio was higher in trees on M than those on SO, but was not affected by the salt treatment in either M or SO.

3.2. Concentration of Cl and Na in leaves, stem and root

All salt treatments increased the Cl^- and Na^+ concentration in leaves, stems and roots of both SO and M trees as compared to control treatment (Fig. 1). The highest leaf Cl^- and Na^+ concentration occurred in Fino 49 leaves on M in the S treatment as both nitrate supplement treatments reduced salt ions similarly in leaves. However, in trees on SO, only S + N treatment decreased

leaf Cl^- concentration with respect to S treatment. Stem Cl^- concentration was similar in all three salinized treatments regardless of rootstock. Root Cl^- concentration was also similar in all three salinized treatments, but trees on SO had a higher concentration than those on M. There were no significant differences in the leaf Na^+ concentration in salinized trees on SO regardless of the nitrate supplement treatments. The S + N treatment of trees on M, and the S + N and S + Nf treatments in trees on SO decreased the concentration of Na^+ in stems as compared to their respective S treatment. Root Na^+ concentration was similar in the three salinized treatments in trees on M. However, in trees on SO, S + N treatment decreased root Na^+ concentration as compared to S and S + Nf treatments.

3.3. Mineral nutrient concentrations

In the non-salinized control treatment, trees on M had higher leaf K^+ concentration and lower leaf Mg^{2+} concentration than those on SO, while leaf Ca^{2+} , P and N concentration was similar in trees on both M and SO (Table 2). Salinized trees on M had lower leaf Ca^{2+} concentrations than the control treatment regardless of nitrate treatment. In trees on SO, soil applied KNO_3 lowered leaf Ca^{2+} more than foliar applied KNO_3 . The highest leaf K^+ concentration occurred in the S + N treatment for trees on both M and SO. In tree on M, foliar applied KNO_3 did not increase leaf K^+ concentration while in trees on SO, foliar applied KNO_3 increased leaf K^+ above the control but not as high as the soil applied KNO_3 treatment. The salinized S and S + N treatments both increased the concentration of leaf K^+ in trees on SO as compared to control treatment. Salinized treatments had no effect on leaf Mg^{2+} compared to the control treatment for trees on M. However, in trees on SO, all salinized treatments decreased leaf Mg^{2+} concentration where the lowest concentration occurred in for S + N trees. The highest leaf N concentration occurred in trees from S + Nf treatment, whereas there were no significant differences among the rest of treatments regardless of rootstock. In the non-salinized control treatment, trees on M had lower root Ca^{2+} concentration and higher root N concentration than those on SO (Table 3). Root K^+ , Mg^{2+} and P concentration was similar in trees on both M and SO. All salt treatments decreased root Ca^{2+} and N concentration, and salinized S + N and S + Nf treatments decreased root P concentration regardless rootstock. Leaf K^+ and Mg^{2+} concentrations were decreased in trees on M from all salt treatments. In trees on SO, the root K^+ concentration was decreased by the S treatment and the leaf Mg^{2+} concentration was higher for trees from S + Nf than for those from S and S + N treatments.

3.4. Water relation and osmotic adjustment

Salt treatments reduced both Ψ_w and Ψ_π such that Ψ_p was not affected by any treatment in trees on both M and SO (Fig. 2). However, in trees on M, Ψ_w and Ψ_π reductions were similar in trees from S + N and S + Nf but the NO_3 treatments lowered both values below those from C and S treatments.

In the non-salinized control treatment, trees on M had significantly lower ψ_π^{100} , and higher leaf RWC, proline and QAC's concentration than those on SO (Table 4). The ψ_π^{100} was significantly decreased by all salts treatments and leaves from S + N and S + Nf treatment had similarly lower values than those from S treatment regardless rootstock. All salinized treatments on both rootstocks had lower leaf RWC than controls. Leaf proline concentration was increased significantly by salinized S + N and S + Nf treatment regardless rootstock and leaves from S + N treatment had highest concentrations. Leaf QAC's concentrations were not affected by salt treatments.

Table 1

Effect of rootstock, 50 mM NaCl (S) and nitrate supplement treatment (Trt) on mean ($n = 6$) leaf, stem and root dry weight, and shoot to root dw ratio of Fino 49 lemon trees grafted on *C. macrophylla* (M) or Sour orange (SO) rootstocks.

Rootstock	Trt	Leaf (g dw)	Stem (g dw)	Root (g dw)	Shoot:root
M	C	262 Aa ^a	246 a	103 b	4.86 A
	S	143 b	171 b	77 b	4.13
	S + N	136 b	137 b	86 b	3.27
	S + Nf	113 c	151 b	86 b	3.19
SO	C	211 Ba	263 a	181 a	2.70 B
	S	100 b	141 b	103 b	2.59
	S + N	84 b	152 b	102 b	2.41
	S + Nf	61 c	158 b	82 b	2.74
		F-test			
		Leaf	Stem	Root	Shoot:root
Rootstock		***	ns	***	***
N treatment		***	**	***	ns
Rootstock \times N treatment		ns	ns	**	ns
cv (%)		47.3	34.4	37.8	32.6

ns indicates non-significant difference.

^a Significant differences ($P < 0.05$) between treatments are denoted with different lower case letters. Differences between rootstocks are indicated by different upper case letters, regardless nitrate supplementation treatment.

** Indicates significant difference at $P < 0.01$ for the two way interaction rootstock \times nitrate supplement treatments.

*** Indicates significant difference at $P < 0.001$ for the two way interaction rootstock \times nitrate supplement treatments.

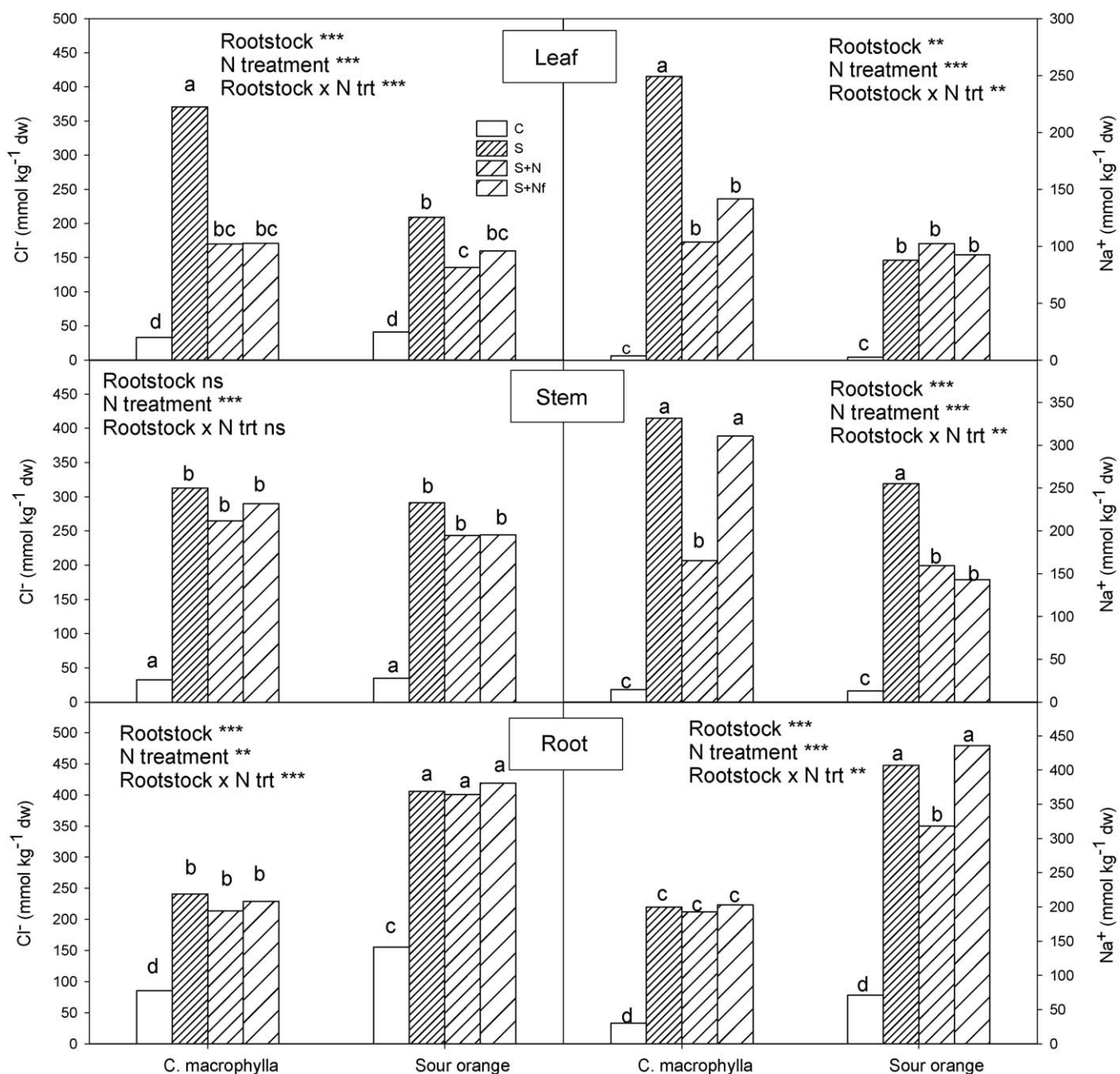


Fig. 1. Effect of rootstock and supplemental nitrate on mean ($n = 6$) leaf, stem and root Cl^- and Na^+ concentration (mmol kg^{-1} dry weight) in Fino 49 lemon trees grafted on *C. macrophylla* (M) and Sour orange (SO) watered with complete nutrient solution containing either 0 mM NaCl (control, C), 50 mM NaCl (S), 50 mM NaCl plus 10 mM KNO_3 (S + N), or 50 mM NaCl with 1% foliar KNO_3 (S + Nf). Ns, ** and *** indicate non-significant or significant differences at $P < 0.01$, or 0.001, respectively, for the two way interaction rootstock \times nitrate supplement treatments. Significant differences ($P < 0.05$) between treatments are denoted with different lower case letters.

3.5. Leaf gas exchange parameters

Leaves from trees on M had similar A_{CO_2} and g_s than those on SO (Fig. 3). Salinized treatments reduced A_{CO_2} , g_s , WUE as compared to the control treatment, whereas there were no significant differences among S, S + N and S + Nf treatments regardless rootstock.

4. Discussion

In citrus trees, there are important effects of the rootstock on leaf nutrient levels and growth parameters of the scion (Georgiou, 2002). In our experiment, non-salinized trees on M had higher N concentration, and lower leaf Ca^{2+} and Mg^{2+} than those on SO.

But overall, our data shows that, under non-saline or saline condition, Fino 49 lemon trees on M were more vigorous, based on greater leaf dw and it had higher shoot to root ratio, than those on SO. In Verna lemon, however, it has been previously observed that salinized trees grafted on SO had less reduced growth, measured as trunk circumference, than those on M (Cerdá et al., 1990). Thus, salinity effects in different varieties of lemon citrus trees may depend on scion \times rootstock combination and salinity tolerance can also be influenced by the scion (Cooper et al., 1952) In addition to salinity-induced reductions in leaf dry weight, root growth was particularly sensitive to salt treatment in trees on SO but not on M whereas root growth was not reduced.

This experiment confirmed that SO rootstock is a Cl^- and Na^+ excluder whereas M is a Cl^- and Na^+ rootstock accumulator

Table 2

Effect of rootstock and nitrate supplementation on mean ($n = 6$) leaf Ca^{2+} , K^+ , Mg^{2+} , P and N (expressed as mmol kg^{-1} dry weight) on Fino 49 lemon trees grafted on *C. macrophylla* (M) and Sour orange (SO) watered with 1/2 Hoagland solution containing 0 mM NaCl (C), 50 mM NaCl (S), 50 mM NaCl and supplemented with 10 mM KNO_3 via root (S + N), or 50 mM NaCl and supplemented with 1% KNO_3 via foliar (S + Nf).

Rootstock	Trt	K^+	Ca^{2+}	Mg^{2+}	P	N
M	C	831 d ^a	477 ab	74 cd	71	1964 b
	S	926 cd	359 c	66 de	74	2129 b
	S + N	1077 b	304 c	62 de	71	2136 b
	S + Nf	916 cd	342 c	58 de	65	2250 a
SO	C	701 e	559 a	119 a	71	1829 b
	S	888 cd	546 a	99 b	68	1743 b
	S + N	1315 a	299 c	66 e	74	1814 b
	S + Nf	1013 bc	454 b	86 bc	77	2064 a
<i>F</i> -test						
		K^+	Ca^{2+}	Mg^{2+}	P	N
Rootstock		ns	***	***	ns	***
N treatment		***	***	***	ns	**
Rootstock \times N treatment		**	*	***	ns	ns
cv (%)		20.2	28.5	27.9	9.0	11.5

ns indicates non-significant difference.

^a Significant differences ($P < 0.05$) between treatments are denoted with different lower case letters. Differences between rootstocks are indicated by different upper case letters, regardless nitrate supplementation treatment.

* Indicates significant difference at $P < 0.05$ for the two way interaction rootstock \times nitrate supplement treatments.

** Indicates significant difference at $P < 0.01$ for the two way interaction rootstock \times nitrate supplement treatments.

*** Indicates significant difference at $P < 0.001$ for the two way interaction rootstock \times nitrate supplement treatments.

(García-Sánchez et al., 2005). In our previous studies with citrus rootstock seedlings or citrus trees under saline stress, low leaf Cl^- concentration could be linked to low leaf transpiration, high shoot to root ratio (Syvertsen et al., 2008) and/or the ability of roots to retain a high Cl^- concentration (García-Sánchez and Syvertsen, 2006; Prior et al., 2007). In this experiment, leaf transpiration was not affected by rootstock and shoot to root was higher in trees on M than those on SO. Thus, the lowest leaf Cl^- and Na^+ concentration observed in leaves on trees on SO could be due to the SO roots accumulating a high Cl^- and Na^+ concentration and limiting their transport to the shoot. On the other hand, these differences in the leaf Cl^- and Na^+ concentrations between trees on M and SO did not produce significant differences in leaf growth.

The salt-induced growth reduction in trees on SO could have been due to an osmotic effect. Although leaf water potential was decreased by the salinity treatment, the leaves from trees on M had Ψ_w and Ψ_π that were both less negative than on those on SO. This occurred in despite that trees on M had a higher leaf Cl^- and Na^+ which could have contributed to decrease the Ψ_π (García-Sánchez and Syvertsen, 2006). Thus, to decrease leaf osmotic potential under saline condition without a high contribution from Cl^- and Na^+ , trees on SO could have increased the synthesis of organic solutes in part to amino acids, rather sugars as Arbona et al. (2005) showed a decrease in carbohydrates in citrus plants exposed to salt stress, which require a additional energetic cost that could have caused a reduction in the growth. In an experiment with SO and M seedlings, growth response to different salt treatments with similar osmotic pressure (isotonic solutions) was mainly affected by an osmotic effect in SO seedlings and by an ion toxicity in M seedlings (Ruiz et al., 1999).

Additional KNO_3 added to the nutrient solution had beneficial effects in the S + N treatment which had lower leaf Cl^- concentrations than those from S treatment. This leaf Cl^- reduction was higher in trees on M which is not considered a Cl^- excluding

Table 3

Effect of rootstock and nitrate supplementation on mean ($n = 6$) root Ca^{2+} , K^+ , Mg^{2+} , P and N (expressed as mmol kg^{-1} dry weight) on Fino 49 lemon trees grafted on *C. macrophylla* (M) and Sour orange (SO) watered with 1/2 Hoagland solution containing 0 mM NaCl (C), 50 mM NaCl (S), 50 mM NaCl and supplemented with 10 mM KNO_3 via root (S + N), or 50 mM NaCl and supplemented with 1% KNO_3 via foliar (S + Nf).

Rootstock	Trt	K^+	Ca^{2+}	Mg^{2+}	P	N
M	C	540 a ^a	796 Ba	95 a	129 a	2321 Aa
	S	340 c	472 b	70 b	81 ab	1921 b
	S + N	404 bc	432 b	58 b	65 b	2229 b
	S + Nf	345 c	444 b	66 b	65 b	2014 b
SO	C	514 a	1038 Aa	74 ab	94 a	1893 Ba
	S	420 bc	871 b	70 b	74 ab	1921 b
	S + N	530 a	851 b	66 b	52 b	2229 b
	S + Nf	463 ab	971 b	95 a	48 b	2014 b
<i>F</i> -test						
		K^+	Ca^{2+}	Mg^{2+}	P	N
Rootstock		**	***	ns	ns	***
N treatment		***	**	ns	***	***
Rootstock \times N treatment		*	ns	*	ns	ns
cv (%)		21.4	36.7	26.7	48.8	18.7

ns indicates non-significant difference.

^a Significant differences ($P < 0.05$) between treatments are denoted with different lower case letters. Differences between rootstocks are indicated by different upper case letters, regardless nitrate supplementation treatment.

* Indicates significant difference at $P < 0.05$ for the two way interaction rootstock \times nitrate supplement treatments.

** Indicates significant difference at $P < 0.01$ for the two way interaction rootstock \times nitrate supplement treatments.

*** Indicates significant difference at $P < 0.001$ for the two way interaction rootstock \times nitrate supplement treatments.

rootstock. The leaf Cl^- reduction in the nitrate-supplemented S + N trees was likely due to an antagonism between Cl^- and NO_3^- uptake by fibrous roots (Lea-Cox and Syvertsen, 1993; Tyerman and Skerrett, 1998; Cerezo et al., 1999) rather leaf transpiration or shoot to root ratio which were similar in the S and S + N treatments. In addition, the added KNO_3 in the nutrient solution also caused a reduction of leaf Na^+ concentration in trees on M, a Na^+ accumulator, but not in SO, a Na^+ excluder. A higher K^+/Na^+ ratio in the nutrient solution could have inhibited the Na^+ uptake by M due to the antagonism between these ions. Another beneficial effect of increasing the KNO_3 was the increase in the leaf K^+ concentration in salinized trees from S + N treatment. It has been well stabilized that high leaf K^+ concentration may alleviate salt stress effects by minimizing oxidative stress and/or contributing to osmotic adjustment (Cakmak, 2005). However, despite these beneficial effects, trees watered with additional KNO_3 in the basic nutrient solution had similar salt tolerance than those in the S treatment as the leaf growth reduction by salinity was similar in trees in the S and S + N treatments. Despite differences in leaf Cl^- concentration, this could have been due to the increased osmotic potential of the nutrient solution as a consequence of adding extra 10 mM KNO_3 (1.6 dS m^{-1} extra). In fact, trees on both M and SO from S + N treatment had a lower ψ_π^{100} than those from S treatment. This suggested that the leaf Cl^- and Na^+ concentration reduction in the S + N treatment decreased the contribution of these ions to ψ_π^{100} , and thus, the osmotic adjustment process had to be carried out using other ions or by synthesis of organic solutes as supported by the increased leaf proline concentration. The use of organic solutes instead of osmotically active ions for osmotic adjustment to balance tissue water relations may be a disadvantage since is an energy-consuming process at cellular level what could limit plant growth (Yeo, 1983).

Additional KNO_3 via foliar application also had beneficial effects in salinized trees on M where leaf Cl^- and Na^+ concentrations were

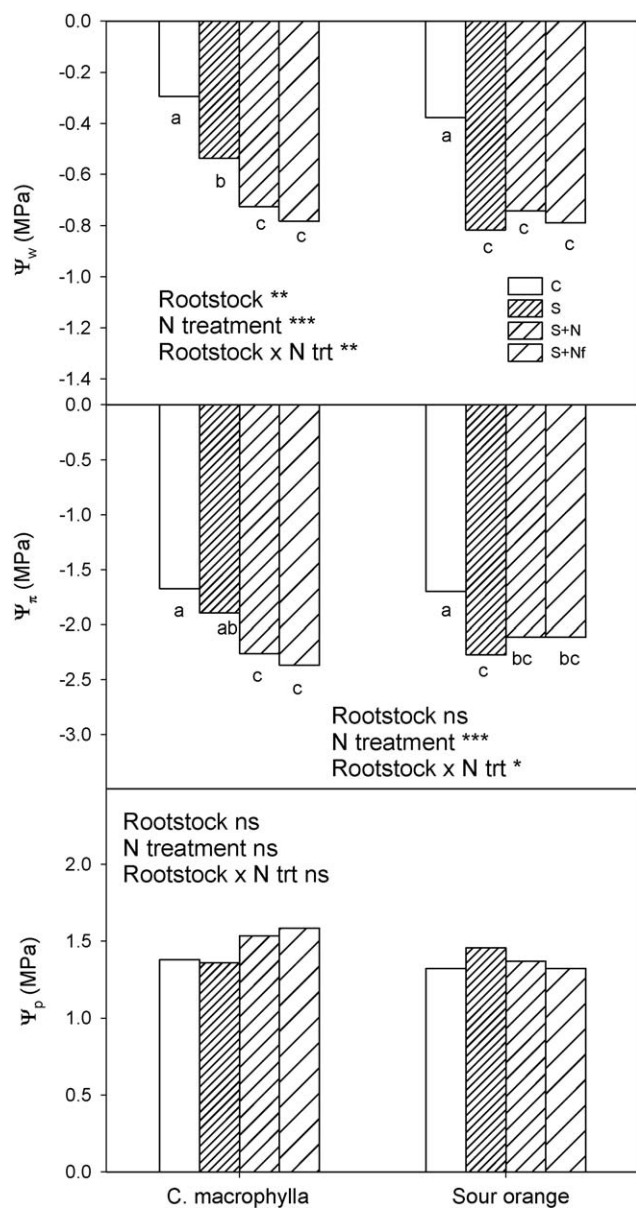


Fig. 2. Effect of rootstock and nitrate supplementation on mean pre-dawn leaf water potential (Ψ_w), osmotic potential (Ψ_π) and turgor potential (Ψ_p) of leaves on Fino 49 lemon trees grafted on *C. macrophylla* (M) and Sour orange (SO) watered with complete nutrient solution containing either 0 mM NaCl (control, C), 50 mM NaCl (S), 50 mM NaCl plus 10 mM KNO_3 (S + N), or 50 mM NaCl with 1% foliar KNO_3 (S + Nf). Ns, *, **, and *** indicate non-significant or significant differences at $P < 0.05$, 0.01, or 0.001, respectively, for the two way interaction rootstock \times nitrate supplement treatments. Significant differences ($P < 0.05$) between treatments are denoted with different lower case letters.

decreased on S + Nf trees relative to the S treatment. However, trees on M and SO from the S + Nf treatment had the highest leaf growth reduction relative to non-salinized control treatment. In the S + Nf treatment, the lower ψ_π^{100} in trees on M and SO, and lower Ψ_w in trees on M relative to S treatment, implied that trees from S + Nf treatment may have suffered a strong osmotic effect from salts deposited in the leaf surface. A white salt residue was visible 2 h after foliar application. Potassium nitrate tends to penetrate in the cuticular membrane only when humidity is close to 100%, so at lower humidities when penetration ceased, KNO_3 on the leaf surface can cause possible phytotoxicity effects (Schonherr and Luber, 2001).

Although salinity altered leaf water relations of trees on both SO and M rootstocks, turgor potential in salt-stressed plants was

Table 4

Effect of rootstock and nitrate supplementation on mean ($n = 6$) on leaf osmotic potential at full turgor (ψ_π^{100}), midday relative water content (RWC), leaf proline concentration, and leaf quaternary ammonium compounds concentration (QAC) on Fino 49 lemon trees grafted on *C. macrophylla* (M) and Sour orange (SO) watered with 1/2 Hoagland solution containing 0 mM NaCl (C), 50 mM NaCl (S), 50 mM NaCl and supplemented with 10 mM KNO_3 via root (S + N), or 50 mM NaCl and supplemented with 1% KNO_3 via foliar (S + Nf).

Rootstock	Trt	ψ_π^{100} (MPa)	RWC (%)	Proline (mmol kg^{-1} dw)	QAC (mmol kg^{-1} dw)
M	C	-2.19 Ba ^a	94.7 a	285.8 Ac	152.0 A
	S	-2.47 b	92.0 cd	340.5 bc	137.6
	S + N	-2.74 c	93.2 abc	431.7 a	168.8
	S + Nf	-2.89 c	92.1 cd	380.4 b	144.2
	SO	C	-1.65 Aa	93.8 b	282.3 Bc
	S	-2.26 b	91.2 de	316.2 bc	123.4
	S + N	-2.61 c	90.0 e	377.0 a	121.4
	S + Nf	-2.59 c	92.9 bc	296.2 b	125.6
F-test					
		ψ_π^{100}	RWC	Proline	QAC
Rootstock		**	**	*	**
N treatment		***	***	ns	ns
Rootstock \times N treatment		ns	**	ns	ns
cv (%)		19.0	1.8	20.0	16.0

ns indicates non-significant difference.

^a Significant differences ($P < 0.05$) between treatments are denoted with different lower case letters. Differences between rootstocks are indicated by different upper case letters, regardless nitrate supplementation treatment.

* Indicates significant difference at $P < 0.05$ for the two way interaction rootstock \times nitrate supplement treatments.

** Indicates significant difference at $P < 0.001$ for the two way interaction rootstock \times nitrate supplement treatments.

*** Indicates significant difference at $P < 0.001$ for the two way interaction rootstock \times nitrate supplement treatments.

similar to non-salinized plants. Thus, leaf Cl^- toxicity rather leaf water relations were responsible for reduction in A_{CO_2} and g_s by salinized treatments (Garcia-Sanchez et al., 2006). In addition, in this experiment, leaf Cl^- concentration from salinized treatments ranged between 0.6 and 1.2% dw, however, A_{CO_2} and g_s were similar among salinized trees from S, S + N and S + Nf treatments. Thus, A_{CO_2} of Fino 49 lemon trees were very sensitive to the Cl^- toxicity whenever leaf Cl^- concentrations exceeded 0.6%. In previous studies using 'Sunburst' mandarin grafted on different rootstocks, A_{CO_2} and g_s progressively declined in parallel with an increase in the leaf Cl^- concentration (Garcia-Sanchez et al., 2002).

Mineral nutrient concentration in leaves and roots were also altered depending on the rootstock and salt treatments. The concentration of Ca^{2+} decreased in leaves and roots in trees on M for all three salinized treatments. Root Ca^{2+} uptake probably was inhibited by the salt treatments as observed in other citrus scion \times rootstock combinations (Cámara et al., 2003). However, in trees on SO, leaf Ca^{2+} concentration was decreased only when treated with the additional KNO_3 , the S + N and S + Nf treatments. Additional KNO_3 in the nutrient solution could have decreased leaf Ca^{2+} concentration for antagonism between K^+ and Ca^{2+} ions (Kent and Lächli, 1985; Hansen and Munns, 1988). In addition, the reduction in K^+ uptake could have been caused by high Na^+ , a well-known competitive process in soil and plant roots (Hu and Schmidhalter, 2005). However, leaf K^+ concentration increased with the salinized S and S + Nf treatments in trees on SO but not on M. Thus, high leaf K^+ concentration in trees on SO could be the mechanism by which low Na^+ concentration was maintained. We found similar responses in salinized 'Sunburst' mandarin trees grafted onto Carrizo citrange rootstock, also considered Na^+ excluder (Garcia-Sanchez et al., 2002). In citrus, high concentration of Cl^- in nutrient solution can inhibit the low affinity nitrate transport system (Cerezo et al., 1997) but in our experiment, there

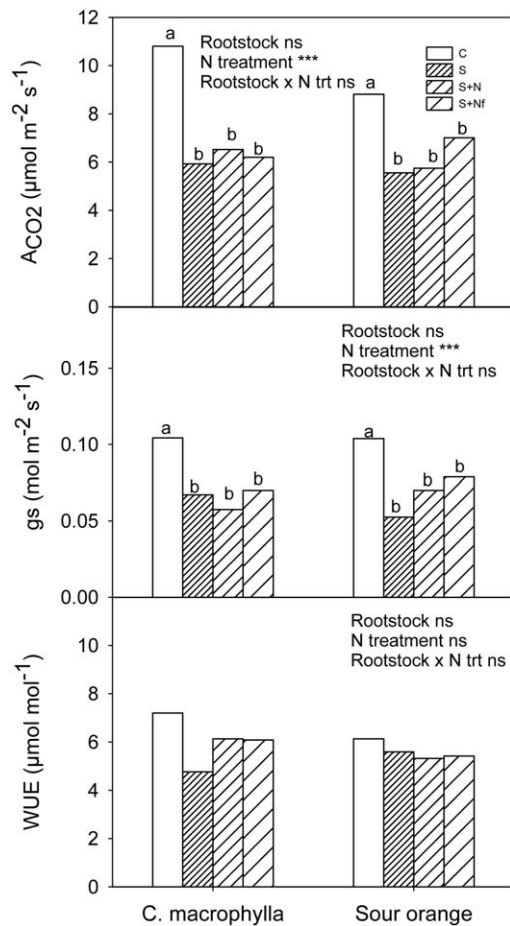


Fig. 3. Effect of rootstock and nitrate supplementation on net assimilation of CO_2 (A_{CO_2} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) and water use efficiency (A_{CO_2}/E , $\mu\text{mol mol}^{-1}$) of leaves on Fino 49 lemon trees grafted on *C. macrophylla* (M) and Sour orange (SO) watered with complete nutrient solution containing either 0 mM NaCl (control, C), 50 mM NaCl (S), 50 mM NaCl plus 10 mM KNO_3 (S + N), or 50 mM NaCl with 1% foliar KNO_3 (S + Nf). ns or *** indicate non-significant or significant differences at $P < 0.001$, respectively, for the two way interaction rootstock \times nitrate supplement treatments. Significant differences ($P < 0.05$) between treatments are denoted with different lower case letters.

was no significant change in leaf Total-N by salinity without additional nitrate. The reductions of shoot biomass near 50% may have offset the decreases in nitrate absorption.

5. Conclusion

In conclusion, *C. macrophylla* was a good rootstock for Fino 49 lemon trees since trees on M were more vigorous than trees on Sour orange regardless of salt treatment. However, Sour orange was a good Cl^- and Na^+ excluder since their roots retained high salt concentrations. Additional nitrogen fertilization via the soil solution had beneficial effects in salinized trees since leaf Cl^- and Na^+ concentration was reduced and leaf K^+ and N concentration was increased for trees from S + N and S + Nf treatment, respectively. Although salt tolerance, based on leaf growth reduction relative to the control treatment, was similar for trees from S + N and S treatment, additional 10 mM potassium nitrate could be an interesting treatment to alleviate the negative effects of high leaf Cl^- in salt treatments.

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References

- Arbona, V., Marco, A.J., Iglesias, D.J., Lopez-Climent, M.F., Talon, M., Gomez-Cadenas, A., 2005. Carbohydrate depletion in roots and leaves of salt-stressed potted *Citrus clementina* L. *Plant Growth Regul.* 46, 153–160.
- Arbona, V., Flors, V., Jacas, J., Garcia-Agustin, P., Gomez-Cadenas, A., 2003. 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.
- Bañuls, J., Serna, M.D., Legaz, F., Talon, M., Primo-Millo, E., 1997. Growth and gas exchange parameters of Citrus plants stressed with different salts. *J. Plant Physiol.* 150, 194–199.
- Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 391, 205–207.
- Cakmak, I., 2005. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *J. Plant Nutr. Soil Sci.* 168, 521–530.
- Cámara, J.M., García-Sánchez, F., Nieves, M., Cerdá, A., 2003. Effect of interstock ('Salustiano' orange) on growth, leaf mineral composition and water relations of one year old citrus under saline conditions. *J. Hortic. Sci. Biotechnol.* 78, 161–167.
- Camara-Zapata, J.M., García-Sánchez, F., Martínez, V., Nieves, M., Cerdá, A., 2004. Effect of NaCl on citrus cultivars. *Agronomie* 24, 155–160.
- Cerdá, A., Nieves, M., Guillen, M.G., 1990. Salt tolerance of lemon trees as affected by rootstock. *Irrigation Sci.* 11, 245–249.
- Cerezo, M., Garcia-Agustin, P., Primo-Millo, E., 1999. Influence of chloride and transpiration on net 15NO_3^- uptake rate by citrus roots. *Ann. Bot.* 84, 117–120.
- Cerezo, M., García-Agustín, P., Serna, M., Primo-Millo, E., 1997. Kinetics of nitrate uptake by Citrus seedlings and inhibitory effects of salinity. *Plant Sci.* 126, 105–112.
- Cooper, W.C., Gorton, B.S., Olson, E.O., 1952. Ion accumulation in citrus as influenced by rootstock and scion and concentration of salts and boron in the substrate. *Plant Physiol.* 27, 191–203.
- García-Sánchez, F., Botia, P., Fernandez-Ballester, G., Cerdá, A., Lopez, V.M., 2005. Uptake, transport, and concentration of chloride and sodium in three citrus rootstock seedlings. *J. Plant Nutr.* 28, 1933–1945.
- García-Sánchez, F., Jifon, J.L., Carvajal, M., Syvertsen, J.P., 2002. Gas exchange, chlorophyll and nutrient contents in relation to Na^+ and Cl^- accumulation in 'Sunburst' mandarin grafted on different rootstocks. *Plant Sci.* 162, 705–712.
- García-Sánchez, F., Perez-Perez, J.G., Botia, P., Martínez, V., 2006. The response of young mandarin trees grown under saline conditions depends on the rootstock. *Eur. J. Agron.* 24, 129–139.
- García-Sánchez, F., Syvertsen, J.P., 2006. Salinity tolerance of Cleopatra mandarin and Carrizo citrange rootstock seedlings is affected by CO_2 enrichment during growth. *J. Am. Soc. Hortic. Sci.* 131, 24–31.
- Georgiou, A., 2002. Evaluation of rootstocks for 'Clementine' mandarin in Cyprus. *Sci. Hortic.* 93, 29–38.
- Grieve, A.M., Prior, L.D., Bevington, K.B., 2007. Long-term effects of saline irrigation water on growth, yield, and fruit quality of Valencia orange trees. *Aust. J. Agric. Res.* 58, 342–348.
- Grieve, C.M., Grattan, S.R., 1983. Rapid assay for determination of water-soluble quaternary ammonium-compounds. *Plant Soil* 70, 303–307.
- Hansen, E.H., Munns, D.N., 1988. Effects of CaSO_4 and NaCl on growth and nitrogen fixation of *Leucaena leucocephala*. *Plant Soil* 107, 95–99.
- Hu, Y.C., Schmidhalter, U., 2005. Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J. Plant Nutr. Soil Sci.* 168, 541–549.
- Iglesias, D.J., Levy, Y., Gomez-Cadenas, A., Tadeo, F.R., Primo-Millo, E., Talon, M., 2004. Nitrate improves growth in salt-stressed citrus seedlings through effects on photosynthetic activity and chloride accumulation. *Tree Physiol.* 24, 1027–1034.
- Kent, L.M., Läuchli, A., 1985. Germination and seedling growth of cotton: salinity-calcium interactions. *Plant Cell Environ.* 8, 155–159.
- Lea-Cox, J.D., Syvertsen, J.P., 1993. Salinity reduces water use and nitrate-N-use efficiency of citrus. *Ann. Bot.* 72, 47–54.
- Levy, Y., Syvertsen, J.P., 2004. Irrigation water quality and salinity effects in citrus trees. *Hort. Rev.* 30, 37–82.
- Little, T.M., Hills, F.J., 1987. Métodos estadísticos para la investigación en la agricultura. Trillas, México, p. 270.
- Lopez-Climent, M.F., Arbona, V., Perez-Clemente, R.M., Gomez-Cadenas, A., 2008. Relationship between salt tolerance and photosynthetic machinery performance in citrus. *Environ. Exp. Bot.* 62, 176–184.
- Maas, E.V., 1993. Salinity and citriculture. *Tree Physiol.* 12, 195–216.
- Moreno, P., Ambros, S., Albiach, M., Guerri, J., Peña, L., 2008. Citrus tristeza virus: a pathogen that changed the course of the citrus industry. *Mol. Plant Pathol.* 9, 251–268.
- Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. *Annu. Rev. Plant Physiol.* 35, 299–319.
- Moya, J.L., Primo-Millo, E., Talon, M., 1999. 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.
- Nieves, M., Cerdá, A., Botella, M., 1991. Salt tolerance of two lemon scions measured by leaf chloride and sodium accumulation. *J. Plant Nutr.* 14, 623–636.

- Prior, L.D., Grieve, A.M., Bevington, K.B., Slavich, P.G., 2007. Long-term effects of saline irrigation water on Valencia orange trees: relationships between growth and yield, and salt levels in soil and leaves. *Aust. J. Agric. Res.* 58, 349–358.
- Romero-Aranda, R., Moya, J.L., Tadeo, F.R., Legaz, F., Primo-Millo, E., Talon, M., 1998. 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.
- Ruiz, D., Martínez, V., Cerdá, A., 1999. Demarcating specific ion (NaCl, Cl⁻, Na⁺) and osmotic effects in the response of two citrus rootstocks to salinity. *Sci. Hortic.* 80, 213–224.
- Scholander, P., Hammel, H., Bradstreet, E.Y., Hemmingsen, E., 1965. Sap Pressure in vascular plants. *Science* 37, 247–274.
- Schonherr, J., Luber, M., 2001. Cuticular penetration of potassium salts: effects of humidity, anions, and temperature. *Plant Soil* 236, 117–122.
- Storey, R., Walker, R.R., 1999. Citrus and salinity. *Sci. Hortic.* 78, 39–81.
- Syvertsen, J.P., Melgar J.C., García-Sánchez, F., 2008. On the relationships between salinity tolerance, growth and water use in citrus. *Proc. ISC*, in press.
- Tyerman, S.D., Skerrett, I.M., 1998. Root ion channels and salinity. *Sci. Hortic.* 78, 175–235.
- Yeo, A.R., 1983. Salinity resistance—physiologies and prices. *Physiol. Plant* 58, 214–222.