Journal of the American Pomological Society 63(2):72-80 2009

Performance of Forner-Alcaide 5 and Forner-Alcaide 13, hybrids of Cleopatra mandarin x *Poncirus trifoliata*, as Salinity-Tolerant Citrus Rootstocks

M. ANGELES FORNER-GINER[1], EDUARDO PRIMO-MILLO AND JUAN B. FORNER

Centro de Citricultura y Producción Vegetal. Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain

Abstract

Chloride and sodium concentrations and gas exchange parameters were measured on leaves of 'Valencia' orange scions grafted onto the rootstocks Cleopatra mandarin (CM), *Poncirus trifoliata* (PT) and their hybrids Forner-Alcaide 5 (FA-5) and Forner-Alcaide 13 (FA-13), grown in pots with different concentrations of NaCl in the external medium. Scions on CM accumulated less Cl^- in their leaves than scions on PT. However, Na^+ concentrations were lower in scions on PT than on CM. Leaf Cl^- exclusion appears to be transmitted to F1 hybrids FA-5 and FA-13. Also, the limited transport of Na^+ from rootstock to scions on PT was transmitted to FA-5. Leaf gas exchange parameters in salt-treated plants were reduced to different extents, depending on the rootstock. The decreases in net CO_2 assimilation and transpiration were more pronounced in CM, followed by PT and FA-13. The lowest reductions in both parameters occurred in scions on FA-5. The results indicate that, both in terms of saline ion exclusion and gas exchange parameters, FA-5 was the most tolerant genotype to salinity of the four studied, followed by FA-13.

Citrus is a salt-sensitive crop (21), which suffers physiological disturbances and growth reduction even at low to moderate salinities (2, 7, 23, 26). It is generally accepted that salinity effects are induced by factors such as salt-specific toxicity and ionic imbalance resulting from the accumulation of saline ions (7, 12, 24, 25, 27), as well as altered gas exchange parameters and adverse water relations due to the decrease in soil solution osmotic potential (5, 16, 17, 18, 19, 27) or combinations of these factors.

Rootstocks largely control the uptake and/or transport of salts to scions, and salt sensitivity is associated with the accumulation of excessive concentrations of chloride, and sometimes sodium, in leaves (7, 12). The main salts causing injury are chlorides, and the salt tolerance of some citrus rootstocks is determined by their capacity for chloride exclusion (1, 22, 25, 27).

In 1974, a program began at the Valencian Institute for Agricultural Research (IVIA) to breed citrus rootstocks by hybridizations, in which more than 500 hybrids were evaluated to determine their agronomic performances. Among them, two hybrids of Cleopatra mandarin (*Citrus reshni* Hort. *ex* Tan.) x *Poncirus trifoliata* (L.) Raf., specifically Forner-Alcaide nº5 (FA-5) and Forner- Alcaide nº13 (FA-13), were selected since both are semi-dwarfing, and induce high productivity and good fruit quality on the scion cultivar (8, 9). Moreover, these rootstocks are resistant to citrus tristeza virus (CTV) and display good resistance to *Phytophthora* spp. (8) In addition, FA-5 is resistant to the citrus nematode (*Tylenchulus semipenetrans* Cobb.) and appears to be tolerant to calcareous soils (8). FA-5 has been a commercial rootstock in the European Union since 2005 and FA-13 since 2008.

The aim of our study was to test the performance of FA-5 and FA-13 on exposure to salinity, studying chloride and sodium uptake, and leaf gas exchange parameters, compared with their parents, Cleopatra mandarin (CM) and *P. trifoliata* (PT).

---

[1] Corresponding author: telephone +34963.424.000, E-mail: forner_margin@gva.es, Fax +34963.424.001. Mailing address: Instituto Valenciano de Investigariones Agrarias, Apartado Oficial, 46113 Moncada (Valencia), Spain.

Materials and Methods

Plant material. Eighteen-month-old grafted plants of 'Valencia' orange (*C. sinensis* (L.) Osb.) scions on four rootstocks (CM, PT, FA-5 and FA-13) were used in this experiment. Plants were cultured under greenhouse conditions with supplementary light (<50 μmol $m^{-2}s^{-1}$, 400-700 nm) to extend the photoperiod to 16 h. Temperatures ranged between 16-18ºC at night and 24-28ºC by day. Relative humidity was maintained at approximately 80%. Plants were grown individually in 5 L pots filled with coarse sand. All plants were irrigated once weekly until the beginning of the experiment with the following nutrient solution: 3 mM $Ca(NO_3)_2$, 3 mM KNO_3, 2 mM $MgSO_4$, 2.3 mM H_3PO_4, 17.9 μM Fe-EDDHA and trace elements as prescribed by Hoagland and Arnon (14). Nutrient solution pH was adjusted to 6.0 with 1M KOH or 1M H_2SO_4. The water contained 3.80 mM Cl and 2.4 mM Na. When the experiment started, plants were given the above nutrient solution to which either 0 (control), 20, 40 or 60 mM NaCl was added. Salt treatments were maintained for 8 wk. The plants were irrigated twice weekly using 2 L of solution per pot at each watering event. Excess solution (approximately 1L) drained out of the pot, thereby avoiding salt accumulation in the sand. Plants growing as a single shoot were selected for uniformity of size at the beginning of the experimental treatments. Six plants were used for each treatment. The plants were randomized over the experimental area and analysed individually.

Gas exchange. Photosynthesis and transpiration of attached leaves were measured outdoors at the end of the experiment between 10:30 h and 14:30 h. Only fully expanded mature leaves were used for determinations of gas exchange parameters. Two leaves for each of the six plants per treatment and rootstock were used. Photosynthetically active radiation (PAR) at the leaf surface was adjusted to 1000 μmol m^{-2} s^{-1}, which exceeds the saturating value for citrus (15). A closed gas exchange Li-Cor LI-6400 portable Phosynthesis System (Li-Cor, Lincoln, Nebr. USA) was used for the measurements. Laminae of leaves were totally enclosed within a fan-stirred cuvette and maintained at 25 ±0.5 ºC, with a leaf-to-air vapour deficit of about 1.7 Pa. The air flow rate through the cuvette was 0.5-1.5 L min^{-1}. The CO_2 analyser was calibrated daily with a series of standard CO_2/air mixtures. Ten consecutive measurements were taken at 3 s intervals.

Harvesting and chemical analysis. After 8 wk salinization, plants were harvested, divided into root and aerial components, washed and weighed. The fractions were then dried in a forced-draft oven at 60ºC for 48 h and reweighed. For analysis of chloride and sodium, roots and aerial components were crushed in a hammer-mill and stored at room temperature. Sodium was determined by atomic absorption spectrophotometry with a Perkin-Elmer 4100 (Perkin-Elmer, Salem, Mass., USA) after extraction in nitric acid at 70ºC for 20 min. (6). Chloride was determined by silver ion titration with a Corning 926 chloridometer (Corning, Halstead, Essex, UK) (10).

Statistical analysis. The data presented correspond to the mean of at least six independent plants. Statistical analyses were performed with Statgraphics Plus version 5.1 (Statistical Graphics, Englewood Cliffs, NJ). Relationships between parameters were fitted to linear and nonlinear regressions. Main treatment effects were evaluated by analysis of variance (ANOVA).

Results

Chloride and sodium concentrations in leaves and roots of all scion-rootstock combinations increased when the NaCl treatment was increased from 0 to 60 mM (Fig. 1A). Leaves from scions grafted onto PT accumulated more Cl^- than leaves from scions on CM, FA-5 and FA-13 rootstocks, which showed much lower chloride concentrations. Comparison of the Cl^- contents of the roots of individual scion-rootstock combinations shows that FA-5 had the highest concentration of Cl^- followed by CM, whereas FA-13 and PT showed lower Cl^- levels (Fig. 1B). Total uptake of Cl^- per unit weight of whole plants

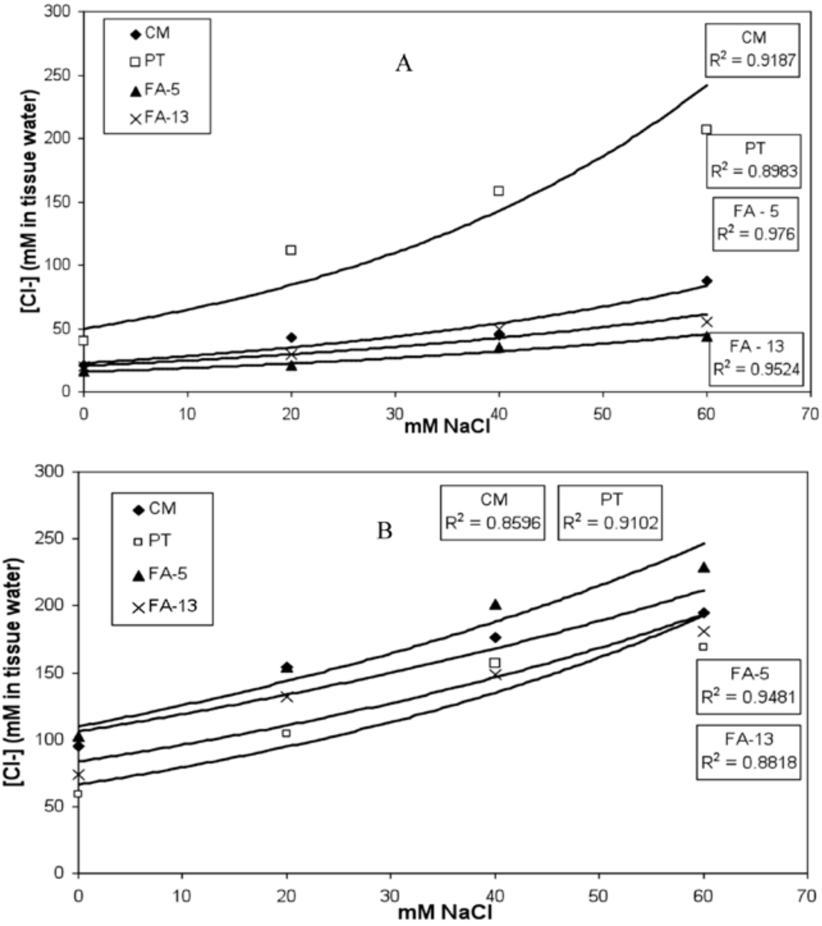

Figure 1. Effects of different NaCl treatments on chloride concentration in leaves (A) and roots (B) of plants grafted onto different rootstocks: Cleopatra mandarin (CM), *Poncirus trifoliata* (PT), Forner-Alcaide 5 (FA-5) and Forner-Alcaide 13 (FA-13).

was lower in plants grafted on CM, FA-5 and FA-13 than in plants on PT (Table 1).

Figure 2 represents the distribution of Cl^- between the different plant parts in all scion-rootstock combinations growing at 40 mM salt concentration, as the percentage contribution of the scion and the rootstock relative to the total ion content. There appears to be a marked influence of the rootstock on the relative distribution of the Cl^- between the different plant parts. Plants grafted on FA-5 accumulated relatively more Cl^- in the rootstock (73.08%) than on FA-13 and CM (47.62 and 48.15%, respectively), whereas plants grafted on PT showed the lowest amount (31.81%).

Comparison of the Na^+ contents of the leaves of individual scion-rootstock combinations (Fig. 3A) indicates that FA-5 and FA-13 accumulated by far the smallest concentrations. PT presents an intermediate concentration, although this effect could not be detected when external NaCl concentrations were higher than 40 mM. Leaves from scions on CM had the highest Na^+ concentrations. Roots

Table 1. Chloride uptake (in mg/g total plant dry weight) by plants grafted onto different rootstocks: Cleopatra mandarin (CM), *Poncirus trifoliata* (PT), Forner-Alcaide 5 (FA-5) and Forner-Alcaide 13 (FA-13). Data are means (n ≥ 6) ± std.dev.

NaCl treatment (mM)	**CM**	**PT**	**FA-5**	**FA-13**
0	2.6±0.7a^z	3.1±0.3a	2.9±0.7a	2.9±0.4a
20	3.3±0.4b	7.2±1.4a	4.3±0.4b	4.6±0.6b
40	4.3±0.6b	10.4±1.1a	5.7±0.6b	5.5±0.6b
60	5.1±0.8b	11.8±2.9a	6.2±0.5b	6.8±1.0b

^{z}Means within a row with the same letter are not significantly different ($P \geq 0.05$)

of FA-13 had the lowest Na^+ concentrations, whereas the highest root concentrations were recorded from PT (Fig. 3B).

All scion-rootstock combinations showed similar uptake of Na^+ per unit weight of whole plants (Table 2), but the relative distribution of Na^+ (the percentage contribution of each plant component relative to the total ion content) between the scion and the rootstock, appears to differ for each combination. Figure 4 shows the distribution of Na^+ between the different plant parts in all scion-rootstock combinations treated with 40 mM salt solution. Plants grafted on CM and FA-13 accumulated more Na^+ in scions (75% and 60%, respectively) than plants grafted on PT and FA-5 (50 % and 36.4%, respectively). Consistently, plants grafted on PT and FA-5 accumulated more Na^+ in rootstock (50% and 63.6%, respectively) than plants grafted on CM and FA-13 (25% and 40%, respectively).

Net CO_2 assimilation and leaf transpiration rates were reduced by NaCl during the 8 wk treatment period (Fig. 5). After this period, net CO_2 assimilation rates decreased between 29.5% (for plants on FA-5) and 47.6% (for plants on CM) in plants treated with 60 mM NaCl. Plants on FA-13 and PT rootstocks showed intermediate values (34.4% and 36.4%, respectively). Control plants on FA-5

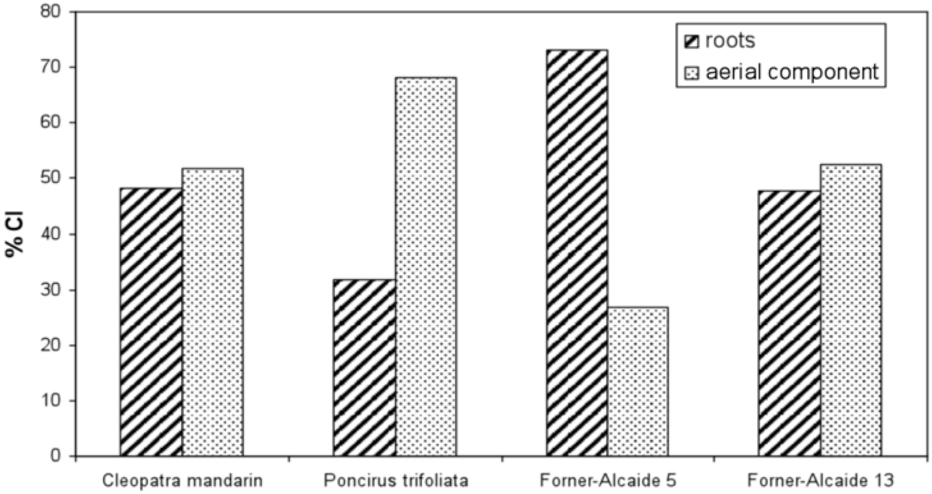

Figure 2. Fractional contribution of the rootstock and the scion to the total chloride content of plants grafted onto different rootstocks: Cleopatra mandarin (CM), *Poncirus trifoliata* (PT), Forner-Alcaide 5 (FA-5) and Forner-Alcaide 13 (FA-13). All plants were growing in 40 mM NaCl.

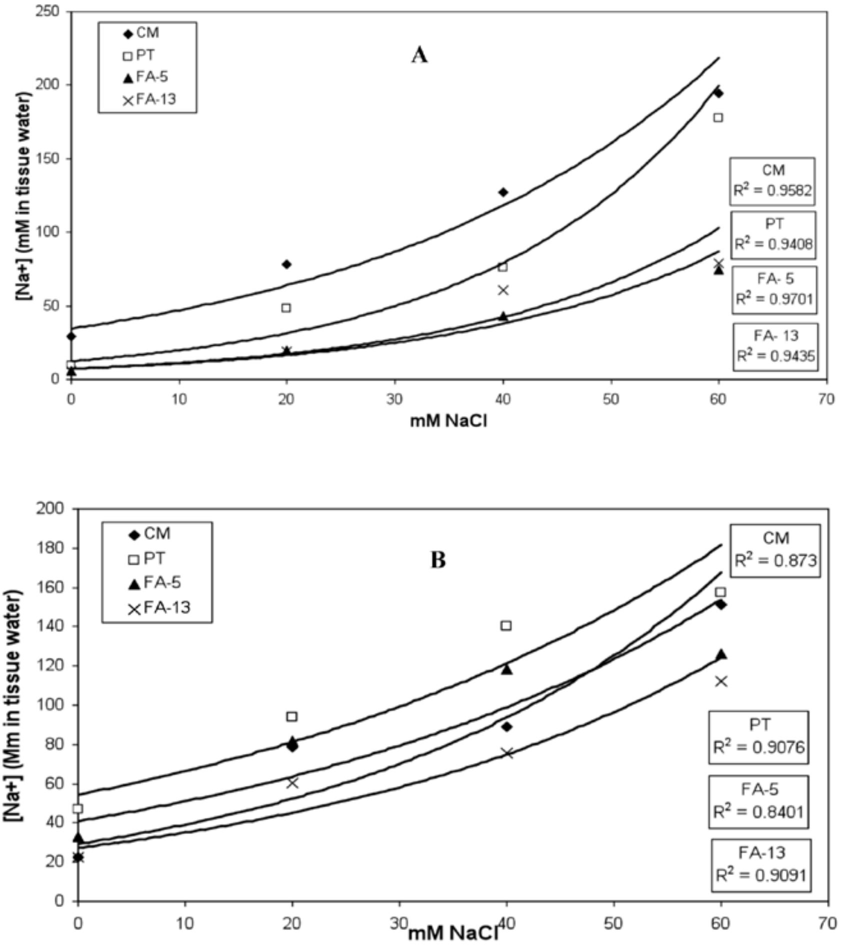

Figure 3. Effects of different NaCl treatments on sodium concentration in leaves (A) and roots (B) of plants grafted onto different rootstocks: Cleopatra mandarin (CM), *Poncirus trifoliata* (PT), Forner-Alcaide 5 (FA-5) and Forner-Alcaide 13 (FA-13).

and FA-13 showed higher rates of net CO_2 assimilation (6.11 and 7.57 μmol $m^{-2}s^{-1}$, respectively) than controls on CM and PT (3.49 and 4.18 μmol $m^{-2}s^{-1}$, respectively). Also, treatment with 60 mM NaCl over 8 wk resulted in reductions in leaf transpiration rates for scions on all tested rootstocks. The lowest decrease in leaf transpiration rate corresponded to FA-5 (44.3%), followed by FA-13 (58.3%), CM (66.9%) and PT (80.2%).

Discussion

Our work has confirmed that marked differences exist between CM and PT rootstocks in their ability to exclude saline ions, as observed previously (22, 27). Leaf chloride concentrations were lower in plants on CM than in plants on PT. This is the result of a reduced uptake of Cl^- by roots and a restricted ability to transport Cl^- from rootstock to scion in plants on CM (3, 25) Typically, salt tolerance of citrus has

Table 2. Sodium uptake (in mg/g total plant dry weight) by plants grafted onto different rootstocks: Cleopatra mandarin (CM), *Poncirus trifoliata* (PT), Forner-Alcaide 5 (FA-5) and Forner-Alcaide 13 (FA-13). Data are means (n ≥ 6) ± std.dev.

NaCl treatment (mM)	**CM**	**PT**	**FA-5**	**FA-13**
0	0.5±0.01c[z]	0.61±0.20c	0.54±0.050c	0.52±0.17c
20	2.42±0.39bc	2.77±0.69b	2.10±0.25bc	2.12±0.56bc
40	2.93±1.6b	3.26±0.4b	2.91±0.98b	2.71±1.10b
60	4.2±0.15a	6.82±2.60a	3.62±0.89ab	5.73±0.26a

[z] Means within a row with the same letter are not significantly different (P≥0.05)

been characterized by the absence of leaf toxic symptoms associated with a reduced accumulation of chloride ions in shoots and, therefore, CM is widely accepted as a salt-tolerant citrus rootstock.

On the other hand, plants on CM rootstocks showed higher concentrations of Na^+ in leaves, but lower concentrations in roots, compared to plants on PT. These data confirm CM as a relatively poor sodium excluder rootstock as previously reported (24, 27). There were no significant differences between plants on CM and PT in terms of total Na^+ uptake by the whole plant, indicating that the two rootstocks appear not to differ markedly in their ability to regulate Na^+ uptake at moderate salinities. However, the data on distribution of Na^+ between the scion and the rootstock suggests that in PT there was a limited transport of Na^+ from root to shoot, as is evidenced by the notable increase in Na^+ concentration in the roots. Therefore, PT can partially exclude Na^+ from leaves by accumulating Na^+ in the basal stem and roots (24).

The results clearly show that the accumulation of saline ions in leaves is mainly controlled by the rootstock, and suggest the existence of barriers in roots that restrict the entry of saline ions and also an ability to restrict root-to-shoot transport. Both mechanisms appear to have been transmitted to the F1 hybrids of Cleopatra mandarin x *P. trifoliata* studied here (FA-5 and FA-13). The restricted uptake of Cl^- ions by roots of CM appears in both hybrids

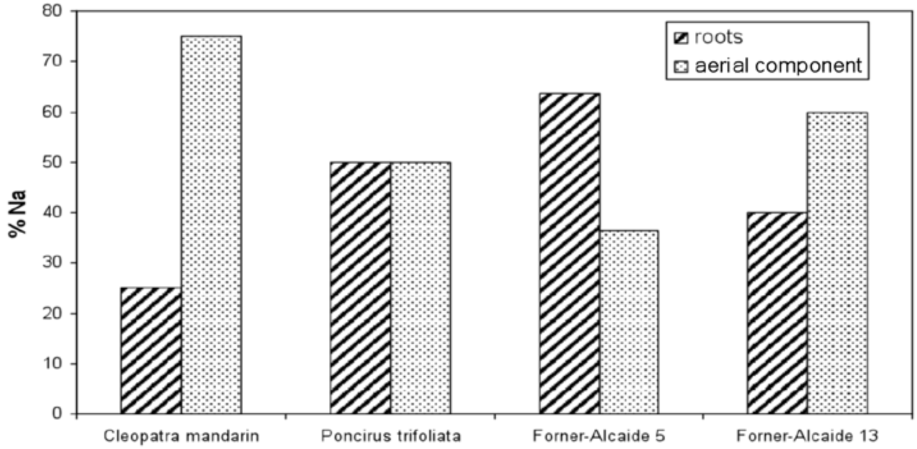

Figure 4. Fractional contribution of the rootstock and the scion to the total sodium content of plants grafted onto different rootstocks: Cleopatra mandarin (CM), *Poncirus trifoliata* (PT), Forner-Alcaide 5 (FA-5) and Forner-Alcaide 13 (FA-13). All plants were growing in 40 mM NaCl.

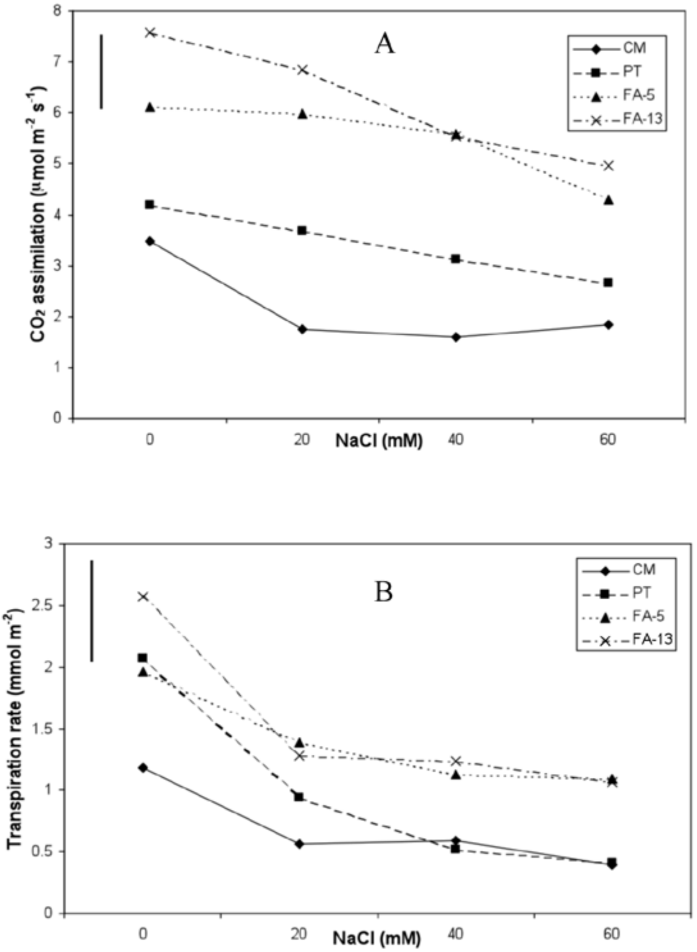

Figure 5. (A) CO_2 assimilation (umol $m^{-2}s^{-1}$) and (B) leaf transpiration rate (mmol m^{-2}) of plants grafted onto different rootstocks: Cleopatra mandarin (CM), *Poncirus trifoliata* (PT), Forner-Alcaide 5 (FA-5) and Forner-Alcaide 13 (FA-13). All plants were growing in 40 mM NaCl. Vertical bars show the maximal standard deviation.

and the reduced ability to transport Cl^- from roots to shoots of CM is also present in FA-13, and to a greater extent in FA-5. Furthermore, the limited transport of Na^+ from rootstock to scion of PT was transmitted to FA-5.

Leaf gas exchange of citrus plants on all four tested rootstocks was impaired by external NaCl. However, there were differences between rootstocks in the reductions of net CO_2 assimilation and transpiration observed in scion leaves of the salinized plants. The reductions in both parameters were less pronounced in plants on FA-5. The results presented here are in agreement with previous reports (3, 4, 27), which indicate that reductions in gas exchange parameters are not directly dependent on saline ion concentrations in leaves. It has been previously reported that, under salt conditions, stomatal conductance and net CO_2 assimilation shows a highly significant correlation, which indicates that stomatal closure drives, at least in part, gas exchange reductions in salinized plants (2, 20).

The different responses of citrus rootstocks to salinity indicates that, in these plants, the effects of salt on gas exchange parameters might be induced by a root-specific mechanism, probably modulated by a hormonal intermediate. It is generally accepted that abscisic acid (ABA) regulates stomatal conductance (13) and, in a previous work (11), it was reported that salt increases ABA levels in roots and leaves of citrus. It has also been shown that root to shoot transport of ABA increases under saline conditions, suggesting that ABA may function as a hormonal stress signal generated by the root system (11, 28, 29). Thus, ABA might act a modulator of the gas exchange responses to salt stress mostly through its involvement in stomatal processes. In conclusion, the data presented here indicate that, both in terms of saline ion exclusion from leaves and gas exchange parameter reductions, FA-5 was the most tolerant genotype to salinity of all four studied, followed by FA-13.

Acknowledgements

This work was funded by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and FEDER funds (RTA 2008-00060).

Literature Cited

1. Bañuls, J. and E. Primo-Millo. 1992. Effects of chloride and sodium on gas exchange parameters and water relations of Citrus plants. Physiol. Plant. 86:115-123.
2. Bañuls, J. and E. Primo-Millo. 1995. Effects of salinity on some Citrus scion-rootstock combinations. Ann. Bot. 76:97-102.
3. Bañuls, J., F. Legaz and E. Primo-Millo. 1990. Effect of salinity on uptake and distribution of chloride and sodium in some citrus scion-rootstock combinations. J. Hort. Sci. 65:715-724.
4. Bañuls, J., M.D. Serna, F. Legaz, M. Talon and E. Primo-Millo. 1997. Growth and gas exchange parameters of citrus plants stressed with different salts. J. Plant Physiol. 150:194-199.
5. Behboudian, M.H., E. Törökfalvy and R.R. Walker, 1986. Effects of salinity on ionic content, water relations and gas exchange parameters in some citrus scion-rootstocks combinations. Sci. Hortic. 28:105-116.
6. Chapman, H.D. and P.R. Pratt. 1961. Methods of analysis for soils, plants and waters. University of California Press, Riverside, Calif.
7. Cooper, W.C. and B.S. Gorton. 1952. Toxicity and accumulation of chloride salts in citrus various rootstocks. Proc. Amer. Soc. Hortic. Sci. 59:143-146.
8. Forner, J.B., M.A. Forner-Giner and A. Alcaide. 2003. Forner-Alcaide 5 and Forner-Alcaide 13: two new citrus rootstocks released in Spain. HortScience 38:629-630.
9. Forner-Giner, M.A., A. Alcaide, E. Primo-Millo and J.B. Forner. 2003. Performance of Navelina orange on 14 rootstocks in Northern Valencia (Spain). Scientia Hort. 98:223-232.
10. Gilliam, J.W. 1971. Rapid measurement of chloride in plant material. Proc. Am. Soil Sci. Soc. 35:512-513.
11. Gómez Cadenas, A., F. R. Tadeo, E. Primo-Millo and M. Talon. 1998. Involvement of abscisic acid and ethylene in the responses of citrus seedlings to salt shock. Physiol. Plant. 103:475-484.
12. Grieve, A.M. and R. R. Walker. 1983. Uptake and distribution of chloride, sodium and potassium ions in salt treated citrus plants. Aust. J. Agric. Res. 34:133-143.
13. Hartung, W. and W.J. Davies. 1991. Drought-inuced changes in physiology and ABA. Pp. 63-79. In: Abscisic acid. Physiology and biochemistry. W.J. Davies and H.G. Jones (Eds). BIOS Scientific, Oxford, UK.
14. Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circular 347:1-32.
15. Iglesias D.J., I. Lliso, F.R. Tadeo and M. Talon. 2002. Regulation of photosynthesis through source: sink imbalance in citrus is mediated by carbohydrate content in leaves. Physiol. Plant. 116:563-572.
16. Lloyd, A., P.E. Kriedemann and A. Aspinall. 1989. Comparative sensitivity of "Prior Lisbon" lemon and "Valencia" orange trees to foliar sodium and chloride concentrations. Plant Cell Environ. 12:529-540.
17. Lloyd, A., P.E. Kriedemann and A. Aspinall. 1990. Contrast between *Citrus* species in response to salinisation: an analysis of photosynthesis and water relations for different rootstock-scion combinations. Physiol. Plantarum 78:236-246.
18. Lloyd, A., P.E. Kriedemann and J.P. Syvertsen. 1987. Gas exchange, water relations and ion con-

centration of leaves on salt stressed "Valencia" orange, *Citrus sinensis* (L.) Osbeck. Aust. J. Plant Physiol. 14:387-396.

19. Lloyd, A., P.E. Kriedemann and J.P. Syvertsen. 1987. Salinity effects on leaf water relations and gas exchange of "Valencia" orange, *Citrus sinensis* (L.) Osbeck, on rootstocks with different salt exclusion characteristics. Aust. J. Plant Physiol. 14:605-617.
20. Lopez Climent, M.F., V. Arbona, R.M. Perez-Clemente and A. Gomez-Cadenas. 2008. Relationship between salt tolerance and photosynthetic machinery performance in citrus. Environ. Exper. Bot. 62:176-184.
21. Mass, E.V. and G.J. Hoffman. 1977. Crop salt tolerance current assesment. ASCE. J. Irrig. Drainage Div. 103:115-134.
22. Ream, C.L. and J.R. Furr. 1976. Salt tolerance of some *Citrus* species, relatives and hybrids tested as rootstocks. J. Amer. Soc. Hortic. Sci. 101:265-267.
23. Romero-Aranda, R., J.L. Moya, F.R. Tadeo, F. Legaz, E. Primo-Millo and M. Talon. 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.
24. Walker, R.R. 1986. Sodium exclusion and potassium-sodium selectivity in salt-treated trifoliate orange (*P. trifoliata*) and Cleopatra mandarin (*C. reticulata*) plants. Aust. J. Plant Physiol. 13:293-303.
25. Walker, R.R. and T.J. Douglas. 1983. Effect of salinity level on uptake and distribution of chloride, sodium and potassium ions in citrus plants. Aust. J. Agric. Res. 34:145-153.
26. Walker, R.R., E. Törökfalvy and W.J.S. Dowton. 1982. Photosynthetic responses of the citrus varieties Rangpur lime and Etrog citron to salt treatment. Aust. J. Plant Physiol. 9:783-790.
27. Walker, R.R., E. Törökfalvy, A.M. Grieve and L.D. Prior. 1983. Water relations and ion concentration of leaves on salt stressed citrus plants. Aust. J. Plant Physiol. 10:265-277.
28. Wolf, O., W. D. Jeschke and W. Hartung. 1990. Long distance transport of abscisic acid in NaCl-treated plants of *Lupinus albus*. J. Exp. Bot. 41:593-600.
29. Zhao, K., R. Munns and R. King. 1991. Ascisic acid synthesis in NaCl-treated barley, cotton and saltbush. Aust. J. Plant Physiol. 18:17-24.