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## Recovery of different *Citrus* rootstock seedlings previously irrigated with saline waters

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### Abstract

Potted plants of three citrus rootstocks, sour orange (*Citrus aurantium* (L.)), Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.) and *Citrus macrophylla* (*Citrus macrophylla* Wester) were irrigated with 5 (control), 25 and 50 mM of NaCl during a salinization period. Plants were then irrigated with fresh water (5 mM NaCl) during a desalinization period, and the levels of Cl<sup>-</sup>, Na<sup>+</sup> and K<sup>+</sup> were determined in leaves and roots. Rootstocks differences were evident at moderate and high levels of exogenous NaCl. Cleopatra mandarin seemed to be the most effective at excluding Cl<sup>-</sup>. During the desalinization period, Cleopatra mandarin irrigated at moderate salinity levels quickly recovered its foliar Cl<sup>-</sup> concentration, whereas sour orange and *C. macrophylla* needed two and three months, respectively; even after this time, *C. macrophylla* did not achieve the Cl<sup>-</sup> concentrations of the control plants. After three months of desalinization, Cl<sup>-</sup> levels in Cleopatra mandarin plants recovered but not Na<sup>+</sup> concentrations, whereas in sour orange basal Na<sup>+</sup> levels were quickly reached but not Cl<sup>-</sup>. In the case of *C. macrophylla*, Na<sup>+</sup> and Cl<sup>-</sup> concentrations remained high even after three months of desalinization. Root Cl<sup>-</sup> values increased in saline conditions in sour orange and *C. macrophylla* and during the desalinization period the Cl<sup>-</sup> concentration of sour orange reached those of the control roots. Root Na<sup>+</sup> concentration increased with salinity in all rootstocks and after desalinization all roots had similar Na<sup>+</sup> concentrations to control plants. The highest saline treatment reduced the dry weight of sour orange plants whereas plant dry weight in Cleopatra mandarin and *C. macrophylla* was not altered. The higher salt tolerance of Cleopatra mandarin with salinity could be related to its lower leaf Na<sup>+</sup> and Cl<sup>-</sup> levels compared with the other rootstocks.

### Introduction

Citrus is one of the most important horticultural crops in the world. Citrus trees are widely cultivated in south-eastern Spain, where the predominant climatic conditions are those typical of the semiarid zone, characterized by low rainfall and high evaporative demand, which frequently induce problems of drought. Lack of water is the major factor limiting the expansion of irrigated agriculture both in Murcia and in other arid regions of the world, as the scarcity of water resources forces growers to use low-quality water from aquifers containing excessive concentrations of soluble salts, mainly sodium chloride.

Citrus plants are classified as a salt-sensitive crop because relatively low salinity levels lead to physiological imbalances and reductions in both growth and fruit yield (3, 4, 16, 17). Cit-

rus is sensitive to the toxic effects of Cl<sup>-</sup> and/or Na<sup>+</sup> accumulation in the leaves (6, 14) and any tolerance to salinity in some genotypes has been related to the ability to restrict the uptake and/or transport of these ions from roots to shoots (9, 29, 31) and to the ability to maintain a high K<sup>+</sup>/Na<sup>+</sup> ratio in different plant tissues (23).

Rootstocks can influence the uptake and/or transport of salts to scions and each rootstock has a particular ability to exclude Cl<sup>-</sup> and/or Na<sup>+</sup> ions during both root absorption and during their translocation from shoots to roots (1, 7). For example, Rangpur lime (*Citrus limonia*) excludes Cl<sup>-</sup> and sequesters Na<sup>+</sup> in its basal parts while Cleopatra mandarin is considered among the rootstocks most capable of limiting Cl<sup>-</sup> uptake, although it has not been reported to be able to exclude Na<sup>+</sup> (6,

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14, 31). *Citrus macrophylla* seems to be able to exclude  $\text{Na}^+$  (10), while trifoliolate orange (*Poncirus trifoliata*) and its hybrids appear to be  $\text{Na}^+$  excluders but they transport  $\text{Cl}^-$  to leaves (31), and sour orange accumulates both  $\text{Na}^+$  and  $\text{Cl}^-$  (10, 29). This suggests the existence of apparently separate mechanisms that regulate the uptake and transport of  $\text{Cl}^-$  and  $\text{Na}^+$  ions in salt-stressed citrus (14, 29). Evidence for genotypic differences in the regulation of  $\text{Na}^+$  and  $\text{Cl}^-$  transport has also been found (8). Pathways of  $\text{Na}^+$  influx and efflux, such as channels and  $\text{Na}^+/\text{H}^+$  antiports, may vary in activity, depending on the rootstock, and the same may occur for  $\text{Cl}^-$  transporters, although these transport systems have not been described in citrus genotypes.

There are a number of publications dealing with the ability of citrus rootstocks to accumulate or restrict  $\text{Cl}^-$  and  $\text{Na}^+$  to the aerial parts of trees. However, there is comparatively little information about the processes that occur during a desalinization period. Under the climatic conditions in the southeast of Spain (high vapour pressure deficit in the summer period and occasional but heavy rainfall in winter (25)), the use of low-quality water for irrigation during the summer period (often reaching higher levels than 10 mM  $\text{Cl}^-$  (18) is likely to increase leaf  $\text{Cl}^-$  concentrations which may subsequently be reduced with some occasional rainfall during the winter period (i.e., during a desalinization period). In an attempt to better understand the behaviour of rootstocks irrigated under such conditions, we studied the ionic content of leaves and roots during salinization and desalinization periods. For this, we used three of the most commonly used rootstocks in lemon orchards, namely, sour orange (*Citrus aurantium* (L.)), Cleopatra mandarin (*Citrus reshni* Hort ex Tan) and *Citrus macrophylla* Wester, which are often irrigated with saline waters and which show different tolerance levels to salinity.

## Materials and Methods

**Plant material.** The three rootstocks selected are all commonly used with lemon

trees: sour orange, Cleopatra mandarin and *Citrus macrophylla*. Seeds were germinated into germination peat trays. Uniformly-sized, 6 month-old seedlings were selected and transplanted to 2 L pots filled with inert sand, and placed in a greenhouse providing average day/night temperatures of 30-32/13-15°C. Relative humidity was maintained at approximately 75%. The experiments were performed with 12 month-old potted plants.

Plants were irrigated three times per week with 2 L applied per pot at each application time, allowing drainage of any excess. The basic nutrient solutions used for irrigation had the following macronutrient composition (mM):  $\text{NO}_3^-$ , 12.2;  $\text{H}_2\text{PO}_4^-$ , 3.5;  $\text{SO}_4^{2-}$ , 3.7;  $\text{Ca}^{2+}$ , 5.3;  $\text{K}^+$ , 6.0;  $\text{Mg}^{2+}$ , 3.0. Three saline treatments were applied to the 12 month-old plants. These treatments were obtained by adding 5 (control), 25 and 50 mM NaCl to the above standard solution. After three months, each salinization treatment was substituted by a desalinization treatment and all the plants were irrigated with the control solution for three months during the summer period.

The experiment was a completely randomised design with a factorial arrangement ( $3 \times 3$ ): three levels of salinity (5, 25 and 50 mM NaCl) and three rootstocks (sour orange, Cleopatra mandarin and *C. macrophylla*). Plants were separated into two blocks of 54 plants, each comprising 3 salinization treatments  $\times$  3 rootstocks  $\times$  6 replicate plants.

**Harvest and chemical composition.** One group of 54 plants was harvested at the end of the salinization period and the other at the end of the desalinization period. Plant roots were separated carefully from the substrate and washed with distilled water and the dry weight of the roots was determined after oven-drying at 60°C for 5 days.

During the experiment, samples of leaves were taken on days 0, 22, 43, 64, 95 (end of salinization period), 125, 159 and 186 (end of desalinization period). The shoots were marked at the end of the salinization period so that new leaves were not harvested during the desalinization period. Leaf samples were

washed with distilled water, dried and ground.

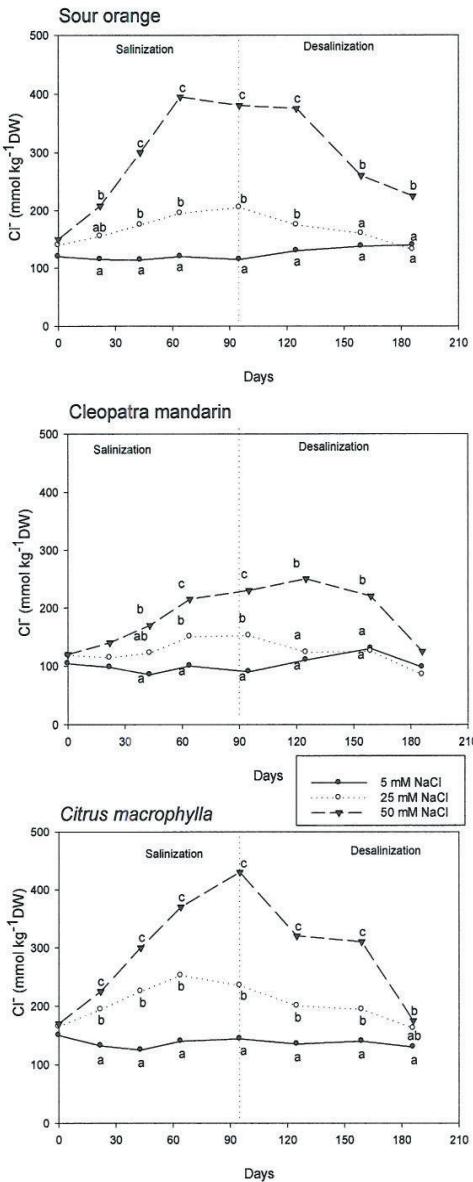
All roots and leaves were analyzed to determine  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  concentrations. Tissues were ashed and then dissolved in  $\text{HNO}_3$  and  $\text{Na}^+$  and  $\text{K}^+$  concentrations were then determined using atomic emission spectrometry (Thermo Elemental Solaar Waltham, Massachusetts, USA). Chloride was extracted by the dry tissue method (15) and determined by titration with an Ag electrode (Corning 926, Corning Ltd. Halstead, Essex, UK).

**Statistical analyses.** Data were analyzed using analysis of variance (ANOVA) procedures with the Statgraphics Plus software (Statistical Graphics Corporation, Englewood Cliffs, NJ) and means were separated by LSD at 95%.

## Results and Discussion

Chloride concentrations in the leaves of all of the rootstock genotypes increased with both 25 and 50 mM saline treatments during the salinization period (Fig. 1). Leaves of plants irrigated with 25 mM NaCl accumulated significantly more  $\text{Cl}^-$  than control plants but less than plants irrigated with 50 mM NaCl. Sour orange and *C. macrophylla* reached the highest foliar  $\text{Cl}^-$  concentrations at the end of the salinization period, whereas Cleopatra mandarin had the lowest concentrations, demonstrating its capacity to prevent the accumulation of leaf  $\text{Cl}^-$  and confirming its ability to exclude this ion (6, 27, 29). The regulation of leaf  $\text{Cl}^-$  concentration in citrus leaves has been linked to transpiration (19, 20, 25), so the rootstock's capacity to exclude  $\text{Cl}^-$  from the leaves has also been related to growth rate. Leaves on rapidly growing trees are exposed to relatively more  $\text{Cl}^-$  in the transpiration stream than leaves on slower-growing trees since rapidly growing trees use more water than slower-growing trees (24).

During desalinization the leaves showed declining chloride concentrations (Fig. 1). However, the response was different for each of the genotypes and saline treatments (Fig. 1). Chloride concentrations in leaves of Cleopatra mandarin previously irrigated with 25 mM NaCl quickly declined to control levels



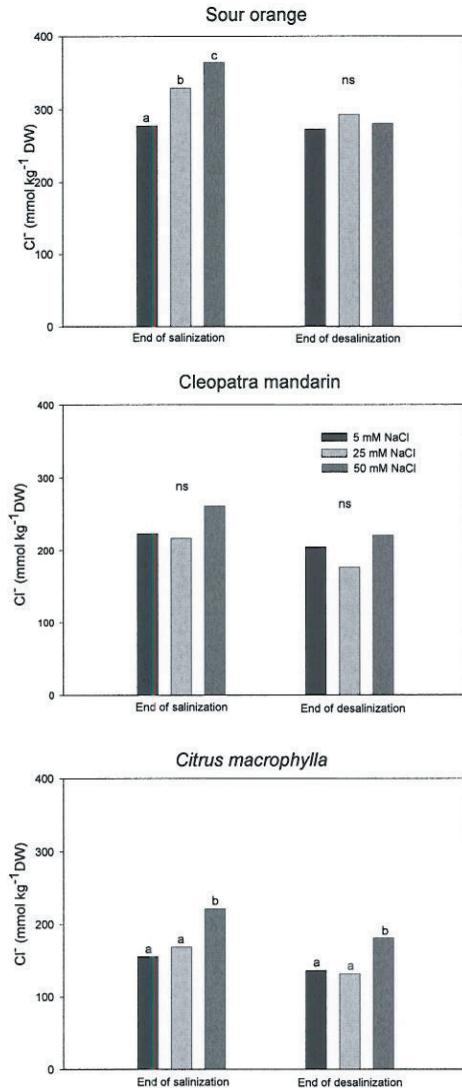
**Fig. 1.** Influence of salinity treatment on the evolution in leaf  $\text{Cl}^-$  concentration in sour orange, Cleopatra mandarin and *Citrus macrophylla* rootstocks, during the salinization and desalinization periods. At each date, values with the same letter are not significantly different at the 0.05 level according to an LSD test. Means without letters are not significantly different.

whereas those in the 50 mM NaCl treatment required about three months to return to such values. After three months of desalinization, all rootstocks pre-irrigated with 25 mM NaCl reached control Cl<sup>-</sup> concentrations in the leaves whereas only Cleopatra mandarin pre-irrigated with 50 mM NaCl reached control Cl<sup>-</sup> values.

Chloride can be accumulated in the root system as well as the leaves (2, 5, 28). Chloride uptake by roots and its concentration in the xylem is similar in susceptible and tolerant plants, but the size of the root system can significantly influence the total amount of Cl<sup>-</sup> accumulated in the plant (20). In each of the rootstocks, roots exhibited little capacity to inhibit the passage of Cl<sup>-</sup> to the shoots since they did not accumulate large concentrations of this ion (Fig. 2). At the end of the salinization period, Cl<sup>-</sup> concentrations in roots of sour orange were increased by 19% with 25 mM NaCl relative to the control and with sour orange and *C. macrophylla* by 30 and 42%, respectively, with 50 mM NaCl. In contrast, these increases were 230 and 200%, respectively, in the leaves of these two rootstocks (Fig. 1). Whereas roots of Cleopatra mandarin did not accumulate Cl<sup>-</sup> after three months of irrigation with either 25 or 50 mM NaCl (Fig. 2), the foliar Cl<sup>-</sup> concentrations increased by 156% relative to the control with 50 mM NaCl (Fig. 1). At the end of the salinization period, concentrations of Cl<sup>-</sup> in roots were similar to those in the leaves in both Cleopatra mandarin and sour orange plants irrigated with 50 mM NaCl. However, foliar Cl<sup>-</sup> concentrations in *C. macrophylla* were approximately twice those found in roots (Figs. 1 and 2). At the end of the desalinization period, Cl<sup>-</sup> concentrations in roots in sour orange returned to control levels, whereas the roots of *C. macrophylla* previously treated with 50 mM NaCl did not reach control Cl<sup>-</sup> values within this time.

During the salinization period, foliar Na<sup>+</sup> concentrations in the three rootstocks gradually increased under the 50 mM NaCl treatment and differences were significantly higher than those in the control plants. With the 25

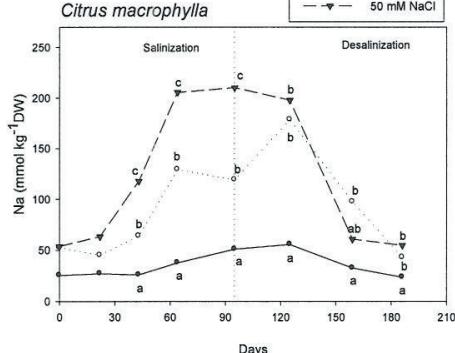
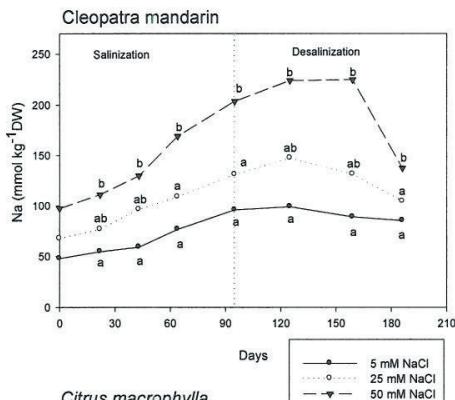
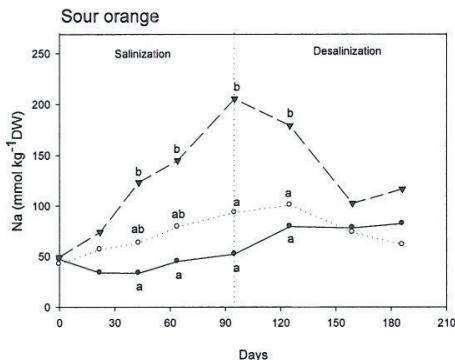
mM NaCl treatment, differences were only significant for *C. macrophylla* (Fig. 3). At the end of the salinization period, plants of sour orange and *C. macrophylla* irrigated with 50



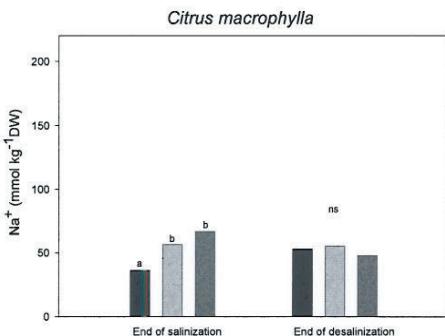
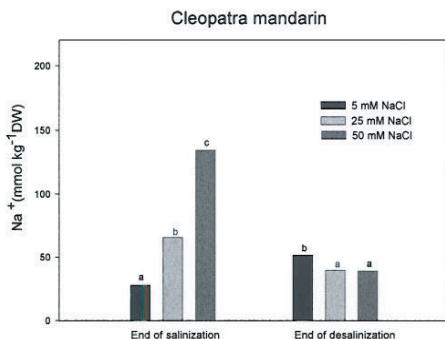
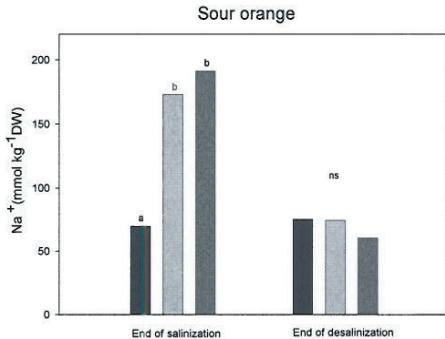
**Fig. 2.** Influence of the salinity treatment on the root concentration of Cl<sup>-</sup> in sour orange, Cleopatra mandarin and *Citrus macrophylla* rootstocks at the end of the salinization and desalinization periods. At each date, values with the same letter are not significantly different at the 0.05 level according to an LSD test. ns: not significant.

mM NaCl accumulated twice the Cl<sup>-</sup> concentration than Na<sup>+</sup> concentration (Figs. 1 and 3). In contrast, Cleopatra mandarin rootstock had

similar foliar concentrations of Cl<sup>-</sup> and Na<sup>+</sup>, indicating that it was a better excluder of Cl<sup>-</sup> ions than the other two rootstocks but that it was not good at excluding Na<sup>+</sup> ions (14, 26,



**Fig. 3.** Influence of the salinity treatment on the evolution of leaf Na<sup>+</sup> concentration in sour orange, Cleopatra mandarin and *Citrus macrophylla* rootstocks, during the salinization and desalinization periods. At each date, values with the same letter are not significantly different at the 0.05 level according to an LSD test. Means without letters are not significantly different.



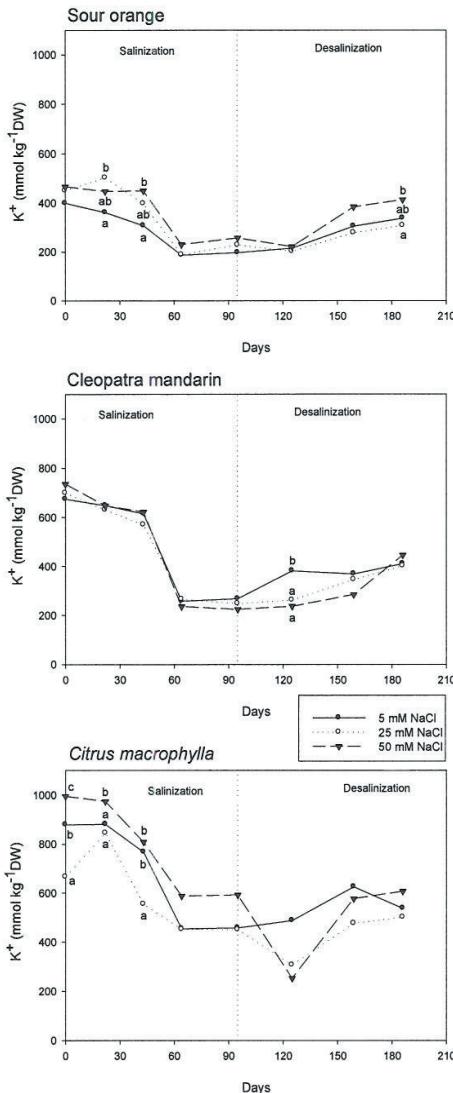
**Fig. 4.** Influence of the salinity treatment on the root concentration of Na<sup>+</sup> in sour orange, Cleopatra mandarin and *Citrus macrophylla* rootstocks at the end of the salinization and desalinization periods. At each date, values with the same letter are not significantly different at the 0.05 level according to an LSD test. ns: not significant.

31). Other studies have shown *C. macrophylla* to be good at excluding  $\text{Na}^+$  ions (10) and have shown sour orange to be capable of accumulating both  $\text{Cl}^-$  and  $\text{Na}^+$  (10, 29). During the desalination period, leaves of sour orange trees pre-irrigated with 50 mM NaCl quickly recovered to control  $\text{Na}^+$  concentrations whereas leaves of Cleopatra mandarin and *C. macrophylla* had not reached such values after three months (Fig. 3).

Roots of sour orange and *C. macrophylla* irrigated with 50 mM NaCl accumulated 174% and 83% more  $\text{Na}^+$ , respectively, than control roots at the end of the salinization period (Fig. 4). In contrast, leaves accumulated 292% and 303% more  $\text{Na}^+$ , respectively, for the same treatments (Fig. 3). Roots of Cleopatra mandarin in the 50 mM NaCl treatment accumulated 383% more  $\text{Na}^+$  relative to the control, whereas the leaves accumulated only 112% more. These results indicate that roots act as an important organ for chloride accumulation in Cleopatra mandarin, preventing this ion from reaching toxic levels in photosynthetic tissues.

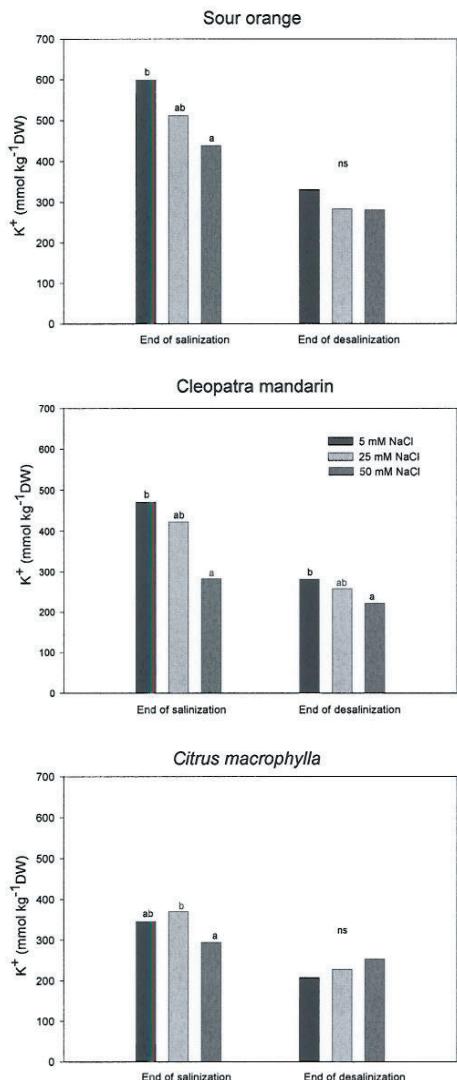
Under saline conditions, roots of each of the three rootstocks had a greater capacity to accumulate  $\text{Na}^+$  than to accumulate  $\text{Cl}^-$  (Figs. 2 and 4). The roots of sour orange had significantly higher  $\text{Na}^+$  concentrations than those of Cleopatra mandarin or *C. macrophylla*, showing that sour orange is good at excluding  $\text{Na}^+$  ions, as observed previously in plants with or without a scion (2, 11). In sour orange and *C. macrophylla*, there was a linear increase for both foliar  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations with time, but this increase may not have occurred in the roots, as a previous study has shown that a plateau was reached after a few days of saline exposure (8). Chloride and  $\text{Na}^+$  uptake were previously shown in sour orange and *C. macrophylla* to be driven by passive forces during the few days of saline treatment, after which the uptake and transport rates were greatly reduced for both ions (12).

Foliar  $\text{K}^+$  concentrations substantially decreased during the salinization period in all three rootstocks (Fig. 5). Unlike some plants which use  $\text{K}^+$  to regulate ion imbalance under



**Fig. 5.** Influence of the salinity treatment on the evolution of leaf  $\text{K}^+$  concentration in sour orange, Cleopatra mandarin and *Citrus macrophylla* rootstocks, during the salinization and desalination periods. At each date, values with the same letter are not significantly different at the 0.05 level according to an LSD test. Means without letters are not significantly different.

saline conditions (13), in our study plants did not apparently use it for osmotic adjustment since no differences were found between treat-



**Fig. 6.** Influence of the salinity treatment on the root concentration of  $K^+$  in sour orange, Cleopatra mandarin and *Citrus macrophylla* rootstocks at the end of the salinization and desalinization periods. At each date, values with the same letter are not significantly different at the 0.05 level according to an LSD test. ns: not significant.

ments at the end of the salinization period and concentrations were practically unchanged during the desalinization phase. The roots of sour orange and Cleopatra mandarin irrigated

during three months with 50 mM NaCl had 27% and 40%, respectively lower  $K^+$  concentrations than those in control roots. At the end of the desalinization period, sour orange and *Citrus macrophylla* roots had similar  $K^+$  concentrations in all treatments whereas Cleopatra mandarin roots pre-irrigated with 50 mM NaCl still had a lower concentration than that in the control roots.

The response of the saline treatments on plant weight depended on the rootstock and the salinity level (Table 1). Cleopatra mandarin did not modify leaf, stem and root dry weights by irrigation with either 25 or 50 mM NaCl. The weights of all tissues in sour orange significantly decreased after three months of irrigation with 25 or 50 mM NaCl whereas only the 50 mM NaCl treatment significantly influenced the dry weights of *C. macrophylla*. The higher salt tolerance observed in Cleopatra mandarin at both moderate and high salinity levels could be related to the lower leaf concentrations of  $Na^+$  and  $Cl^-$  compared with the other rootstocks. This salinity tolerance was not only related to  $Cl^-$  and  $Na^+$  uptake and transport, but also to plant vigor. Since a higher plant vigor has been shown to increase salinity damage (21), the slower growth of the Cleopatra mandarin trees in this and other studies (30) and the photosynthetic, anatomical and physiological properties of Cleopatra (20, 22) could be associated with its higher salinity tolerance.

Some authors have suggested that salt-tolerance in citrus depends on a number of inter-related mechanisms, including better compartmentation of  $Na^+$  and  $Cl^-$  in leaves, greater maintenance of root hydraulic conductivity and nutrient uptake, and better maintenance of root anatomy (12).

In conclusion, rootstocks differences were evident during the desalinization period. Cleopatra mandarin was the fastest rootstock and *C. macrophylla* the slowest to recover their foliar  $Cl^-$  concentrations during the desalinization period after being irrigated with moderate salinity. Three months were sufficient for full recovery of foliar  $Cl^-$  con-

**Table 1.** Influence of the salinity treatment on the dry weight of leaves, stems and roots (g) of sour orange, Cleopatra mandarin and *C. macrophylla* rootstocks, at the end of the salinization period.

Rootstock	NaCl (mM)	Leaf	Stem	Root
Sour orange	5	18.4 e	12.4 c	9.1 d
	25	10.4 d	7.4 b	7.1 c
	50	9.4 d	6.6 ab	5.1 b
Cleopatra mandarin	5	4.5 ab	6.3 ab	3.0 a
	25	4.2 a	5.3 a	3.0 a
	50	3.5 a	5.4 a	3.1 a
<i>Citrus macrophylla</i>	5	6.8 c	15.4 d	8.4 cd
	25	6.3 c	13.8 cd	7.2 c
	50	5.7 b	11.1 c	5.7 b

Mean separation within columns by LSD test ( $P \leq 0.05$ ). Values with the same letter are not significantly different.

centrations in Cleopatra mandarin when high salinity had previously been applied, whereas *C. macrophylla* and sour orange recovered more slowly. After three months of desalinization, Cleopatra mandarin and *C. macrophylla* plants did not recover to control values for  $\text{Na}^+$  concentration, whereas in sour orange recovery was rapid. It has been widely suggested that salinity damage is mainly associated with foliar  $\text{Cl}^-$  accumulation. According to our results, Cleopatra mandarin is the rootstock that recovered foliar  $\text{Cl}^-$  concentrations most rapidly, indicating that it should be considered as a good option for rootstock selection in environments where intermittent salinization/desalinization periods occur.

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## Effects of temperature and solar radiation on sap flow in dwarfing apple rootstocks

To elucidate the influence of temperature on water flow from soil to stem in a dwarfing rootstock, sap flow was measured under different temperature conditions in 1-year-old trees of apple rootstocks of different vigour. Sap flow was largely determined by solar radiation, and increased linearly as solar radiation increased when the temperatures above and below ground were held constant. The adjusted means of sap flow in the trees tested were not significantly influenced by the total leaf area, but were significantly influenced by the root mass. The adjusted means of sap flow at 30°C were two- to three-times greater than those at 20°C in all rootstocks tested, except one super-dwarfing rootstock, during the period of shoot extension. As the degree of rootstock vigour increased, the adjusted means of sap flow increased. The difference in sap flow between an invigorating rootstock and a dwarfing one increased under high temperature conditions. Because the degree of dwarfing was clearly expressed as the difference in sap flow under high temperature conditions, measurements of sap flow, as conducted in this study, will provide a useful tool for studying the mechanism of dwarfing. Abstract from Iwanami et al., 2011. *Journal of Horticultural Science and Biotechnology* 86 (3): 241-244.