Running title: Response of callistemon to salinity

Long-term effect of salinity on plant quality, water relations, photosynthetic parameters and ion distribution in Callistemon citrinus

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Abstract

The effect of saline stress on physiological and morphological parameters in *Callistemon citrinus* plants was studied to evaluate their adaptability to irrigation with saline water. *C. citrinus* plants, grown under greenhouse conditions, were subjected to two irrigation treatments lasting 56 weeks: control (C, 0.8 dS m$^{-1}$) and saline (S, 4dS m$^{-1}$). The use of saline water in *C. citrinus* plants decreased aerial growth, increased the root/shoot ratio and improved the root system (increased root diameter and root density), but flowering and leaf colour were not affected. Salinity caused a decrease in stomatal conductance and evapotranspiration, which may prevent toxic levels being reached in the shoot. Net photosynthesis was reduced in plants subjected to salinity, although this response was evident much later than the decrease in stomatal conductance. Stem water potential was a good indicator of salt stress in *C. citrinus*. The relative salt tolerance of Callistemon was related to the storage of higher levels of Na$^+$ and Cl$^-$ in the roots compared with the leaves, especially in the case of Na$^+$, which could have helped to maintain the quality of plants. The results show that saline water (around 4 dS m$^{-1}$) could be used for growing *Callistemon citrinus* plant commercially. However, the cumulative effect of irrigating with saline water for 11 months was a decrease in photosynthesis and intrinsic water use efficiency, meaning that the interaction of the salinity level and the time of exposure to the salt stress should be considered an important aspect in this species.

1. Introduction

As the competition for high quality water increases, the use of saline waters has become an option for irrigating salt tolerant ornamentals (Cassaniti *et al.* 2009). However, despite the importance of ornamental shrubs in Mediterranean areas, the salt tolerance of such species has received little attention (Bañón *et al.* 2005; Valdez-Aguilar *et al.* 2011). Given that NaCl is the most soluble and widespread salt, it is not
surprising that all plants have evolved mechanisms to regulate its accumulation and to preferentially select other nutrients commonly present in low concentrations (Munns 2002).

Callistemon genus is characterized by its good tolerance of environmental stresses, such as drought and salinity (Lippi et al. 2003; Vernieri et al. 2006), which explains its wide use in Mediterranean regions. Mugnai et al. (2009) tested the influence of several abiotic stresses commonly present in the Mediterranean environment (drought, salinity and negative physical soil properties) on C. citrinus, finding that it appeared to be particularly tolerant towards both water stress and root restriction conditions (reduced pot volume), but less resistant to salt stress, at least using irrigation water with 23 dS m$^{-1}$ (200 mM NaCl). It is well known that plant responses to salt depend on the time of exposure and the severity of the salt treatment (EC of the saline water used). Both factors must be considered when saline water is used as irrigation water, as the interaction of both parameters will determine the physiological and molecular changes that take place. Since the growing season also seems to affect the response of shrubs to salt (Valdez-Aguilar et al. 2011), the present research was carried out during the entire growing season (whole year) using a salt level (4 dS m$^{-1}$) similar to the levels of the irrigation water commonly applied in the Mediterranean horticultural sector (nurseries, growers, gardeners) (Pedrero et al. 2010).

This contribution is complementary to a previous publication on the effects of deficit irrigation on growth, dry matter accumulation, water relations and photosynthetic parameters in C. citrinus in greenhouse conditions (Álvarez & Sánchez-Blanco 2013). Early responses to water and salt stress are very similar, as both stresses reduce the ability to take up water, but different environmental stresses induce different responses, especially when applied on a long-term basis. Previous research results indicated that drought tolerant native plants are not necessarily salt tolerant and that salt tolerant plants are not necessarily drought tolerant (Kefu et al. 2003; Álvarez et al. 2012). The specific mechanisms involved in salt tolerance are of two main types: those minimizing the entry of salt into the plant, and those minimizing the concentration of salt in the cytoplasm. While halophytes have both types of mechanism (salt exclusion and salt compartmentalization in vacuoles), allowing them to grow for long periods of time, most glycophytes have a poor ability to exclude salt, leading to toxic levels in the transpiring leaves (Munns 2002), although these effects take time to develop.

The primary aim of our investigation was to quantify the changes in the growth rate, root morphology, ion uptake and water relations of C. citrinus under long term irrigation with saline water and throw light on the mechanisms the plants perform to confront salinity.
2. Materials and methods

2.1. Plant material and experimental conditions

Rooted cuttings of 2 year-old *Callistemon citrinus* (Curtis) Skeels, cv ‘Firebrand’ (Crimson Bottlebrush) grown in 14x12 cm pots by a specialized nursery were transplanted into 5 L plastic pots (20x16 cm) filled with an 8:7:1 (v/v/v) mixture of coconut fibre : black + sphagnum peat: perlite, amended with 2 g L\(^{-1}\) of Osmocote Plus (14:13:13 N,P,K plus microelements). Plants were placed inside a plastic greenhouse equipped with a cooling system, located at Santomera, Murcia, Spain (38°06′31.2″N; 1°02′13.7″W, 110 m altitude). The micro-climatic conditions, registered with a Hoboware Lite Data Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA) were 14.8 °C (mean minimum), 25.6 °C (mean maximum), and 19.3 °C (average) temperature; and 1.61 Kpa (mean maximum) and 0.80 Kpa (average) vapour pressure deficit. Additional information about evolution of the daily mean values of air temperature and vapour pressure deficit recorded inside de greenhouse during the experimental period is detailed elsewhere (Álvarez & Sánchez-Blanco, 2013).

All of the plants were watered daily for three weeks to field capacity prior to starting the treatments.

2.2. Treatments and experimental design

*C. citrinus* plants were grown in nursery conditions and subjected to two irrigation treatments (25 plants per treatment) using a computer-controlled drip irrigation system from March 2009 to April 2010. The irrigation treatments consisted of a control (C), where the electrical conductivity of the water was 0.8 dS m\(^{-1}\) and a saline treatment (S) using tap water with salt added until to reach 44 mM NaCl (4 dS m\(^{-1}\) S). All the plants were irrigated daily at 100% water holding capacity, and the irrigation amount was equal in both treatments as determined by noting when the leaching fraction for the controls reached 15-20% (v/v) of the applied water. One drip nozzle, delivering 2 L h\(^{-1}\) per pot, was connected to two spaghetti tubes (one on each side of every pot) and the duration of each irrigation episode was used to vary the amount of water applied, which depended on the season and on climatic conditions. The volume of water varied between 200 and 500 ml per pot and irrigation episode.
Significance between control and saline treatment was determined according to the two-sided Student’s t-test for unpaired samples, using Statgraphics Plus for Windows 5.1 software. Ratio and percentage data were subjected to an arcsine square-root transformation before statistical analysis to ensure homogeneity of variance. Significant differences between parts of the plants were analyzed by one-way ANOVA and means were separated with Duncan’s Multiple Range Test (P ≤ 0.05).

2.3. Growth and plant water measurements

At the beginning and at the end of the experimental period ten plants per treatment were harvested and separated into leaves, stems and roots. These were then oven-dried at 80 °C until they reached a constant weight to measure the respective dry weights (DW). Stem diameter (mm), and leaf area (cm²), using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK), were determined in the same plants.

Root system was cleaned by low pressure water applied through a flat nozzle. The cleaned root systems were then placed in a metacrylate tray coupled to a double scanner connected to a computer with a root system analyser (Winrhizo LA 1600 Regent Inc., USA). The root systems were oven-dried at 80°C to measure their DW immediately after the root length and root volume measurements. Roots were classified into three diameter classes: fine (<0.5 mm), medium (0.5–2.0 mm) and coarse (>2 mm). Root density was determined by dividing the dry weight by root volume.

At the beginning and at the end of the saline period, these ten plants per treatment after being separated into leaves, stems and roots, washed with distilled water and dried at 80 °C, were stored at room temperature for inorganic solute analyses. The concentration of Cl⁻ was analysed by a chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained when mixing 100 mg of dry vegetable powder with 40 ml of water before shaking for 30 min and filtering. The concentrations of Na⁺ were determined in a digestion extract with HNO₃:HClO₄ (2:1, v/v) by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL Thermo, England). The absorption rate of Na⁺ and Cl⁻ ions by the root system (J) was calculated in ten plants per treatment, using the formula described by Pitman (1975):

\[ P = \frac{(M_2 - M_1)}{(WR \times t)} \]
where $M_1$ and $M_2$ correspond to a concentration in mmol of Na\(^+\) or Cl\(^-\) in the total plant at the beginning and at the end of saline period, respectively, $t$ corresponds to time in days and $WR$ is the logarithmic mean root biomass, calculated as 
\[
WR = \frac{WR_2 - WR_1}{Ln\left(\frac{WR_2}{WR_1}\right)}
\]
with $WR_1$ and $WR_2$ are the root DW at the beginning and at the end of saline period respectively.

Throughout the experiment, plant height and number of inflorescences per plant were measured in 25 plants per treatment every 1-2 weeks. The relative growth rate was calculated as the rate of increase of height per unit of initial plant height. Leaf colour was measured at the end of the experimental period with a Minolta CR-10 colorimeter, which provided the colour coordinates lightness ($L^*$), chroma ($C^*$) and hue angle ($h^\circ$) (McGuire 1992), using three mature leaves for each plant and ten plants per treatment.

Moreover, one pot of each treatment was placed on a balance with a MITRA programmer that recorded the weight every half an hour, thus giving the evapotranspiration (ET) throughout the experimental period. Daily ET was measured using the difference in weights (weight after irrigation and weight before irrigating again).

During the experiment, leaf water potential ($\Psi_l$) and stem water potential ($\Psi_s$) were measured in eight plants per treatment in mature leaves at midday. $\Psi_l$ was estimated according to the method described by Scholander et al. (1965), using a pressure chamber (Soil Moisture Equipment Co, Santa Barbara, CA, USA), for which leaves were placed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s\(^{-1}\) (Turner 1988). $\Psi_s$ was measured in non-transpiring leaves that had been bagged with both a plastic sheet and aluminium foil for at least 1 h before measurement in order to prevent leaf transpiration; in this way, leaf water potential equalled stem water potential (Begg & Turner 1970). Leaf osmotic potential at full turgor ($\Psi_{100s}$) was measured in five plants per treatment, using excised leaves with their petioles placed in distilled water overnight to reach full saturation. Leaves from the $\Psi_{100s}$ measurements were then frozen in liquid nitrogen (-196 °C) and stored at -30 °C. After thawing, the osmotic potential was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al. (1991). Leaf stomatal conductance ($g_s$) and the net photosynthetic rate ($P_n$) were determined at midday in eight plants per treatment using a gas exchange system (LI-6400, LI-COR Inc., Lincoln, NE, USA). The $P_n/g_s$ ratio was used as an estimation of the intrinsic water use efficiency.
3. Results

3.1. Plant quality

*C. citrinus* plants submitted to the saline treatment showed a reduction in the leaf dry weight (DW, 24%) and leaf area (12%) at the end of the experimental period, while there were no significant decreases in stem diameter or stem and total dry weight compared with control plants. Higher root to shoot ratio was found in the saline treatment (Table 1).

Throughout the experiment, plant height was similar in the control and saline treatment (Fig. 1A). As regards the relative growth rate (RGR) as a function of plant height, three growth periods were evident during the growing season (May, August and March) in all plants, although the stressed plants only showed a lower RGR than the control 44 weeks after the beginning of treatments, in the third growth period (Fig. 1B).

Saline irrigation decreased total root length, a reduction observed in all root sizes (Table 2). Salinity increased the percentage of roots with a diameter higher than 0.5 mm and decreased those with a diameter lower than 0.5 mm, compared with the control. Salinity lowered root volume, although root DW was not modified, with the result that root density increased in these plants.

The number of inflorescences per plant was similar in the control and saline treatment during most of the experiment and only at the end of the experiment did the plants irrigated with saline water show a higher number of inflorescences per plant than the control plants (Fig. 2). The colorimetric values measured suggest that this salt level did not affect leaf colour (Table 1).

3.2. Plant water relations and osmotic adjustment

Daily evapotranspiration varied during the experiment according to environmental conditions (Fig. 3). Daily ET in both treatments reached its maximum value during summer and the minimum in winter, coinciding with maximum and minimum climatic requirements (Data shown in Álvarez & Sánchez-Blanco, 2013). No pronounced differences in water consumption were observed during most of the experiment between the control and saline treatments plants. Salinity only affected the ET of callistemon
plants at the end of the experiment, when smaller values of ET were observed in saline treated plants compared with the control.

The seasonal values of the leaf water potential ($\Psi_l$) showed greater variability than stem water potential ($\Psi_s$) (Fig. 4A). Leaf water potential at midday was closely related with environmental factors and showed maximum values in December and minimum values in August (In control plants, mean $\Psi_l$ value was -0.44 Mpa in December and -2.03 MPa in August while in saline plants, the corresponding values were -0.84 and -2.19 MPa respectively).

Leaf and stem water potential values were in general higher in the control than in the salt treatment (Fig. 4A), although differences in $\Psi_l$ between treatments were noted from the beginning of the experiment (June, 8 weeks after the beginning of the saline irrigation onwards), while differences in $\Psi_s$ were not observed until 19 weeks after the beginning of the treatments, September.

Differences in $\Psi_{100s}$ between the saline and the control treatments plants were found during the experimental period, pointing to a slight degree osmotic adjustment in this treatment of around 0.3 MPa (Fig. 4B).

3.3. Stomatal conductance and photosynthetic parameters

The values of the stomatal conductance and photosynthesis net rate during the period can be seen in Fig. 5. The seasonal pattern of $g_s$ consisted of a minor summer depression in both treatments, particularly in the salt treatment, and a more pronounced winter depression regardless of the EC of irrigation water applied (Fig. 5A). Such a reduction was also observed in $P_n$ in winter when both treatments had lower values than during the rest of the experiment, although no summer reduction was observed in $P_n$ (Fig. 5B). The smallest values of $P_n$ and $g_s$ were found in winter, confirming that plants are most sensitive to gas exchange parameters in this period.

Irrigating plants with water of 4 dS m$^{-1}$ EC produced a reduction in stomatal conductance with respect to the control, a reduction that was more marked in summer and, especially, towards the end of the experiment (Fig. 5A). Such reductions with respect to the control plants were not observed in the photosynthesis levels in saline treatments until 47 weeks after beginning of the saline irrigation (Fig. 5B).
In general, saline treatment plants showed higher $P_n/g_s$ ratios (intrinsic water use efficiency) than control plants throughout the experimental period, except at the end of the experiment when these differences between treatments disappeared (Fig. 5C).

3.4. Mineral distribution throughout the plant

The $\text{Na}^+$ and $\text{Cl}^-$ concentrations measured in the leaves, the stems and roots at the end of the experimental period are presented in Table 3. The concentrations of both $\text{Na}^+$ and $\text{Cl}^-$ increased with salinity in all parts of the plants, except the stem, where only $\text{Na}$ was seen to accumulate. In both treatments, the rate of $\text{Cl}^-$ absorption by root was higher than the corresponding rate of $\text{Na}^+$ absorption (Table 4). More $\text{Cl}^-$ was accumulated than $\text{Na}^+$ in all parts of the plants, especially the leaves. In control plants, mean $\text{Na}^+/\text{Cl}^-$ ratios were 0.09 for leaves, 0.18 for stem and 0.51 for roots while in saline plants, the corresponding ratios were 0.13, 0.34 and 0.54 respectively. As regards their distribution, $\text{Na}^+$ and $\text{Cl}^-$ concentrations were higher in roots than in stem and leaves in both treatments.

The tendency of the species to accumulate $\text{Na}^+$ and $\text{Cl}^-$ preferentially in a given part of the plant (the roots or the leaves) was investigated by calculating the slope of the linear regression between the increasing $\text{Na}^+$ and $\text{Cl}^-$ concentration in the irrigation water and their relative concentration in the plant tissues (Table 5). The accumulation of $\text{Na}$ in the stem and especially in root system showed a higher slope compared with the leaves. For $\text{Cl}^-$ accumulation higher slopes were found for leaves and root system, compared to stem.

4. Discussion

Reductions in plant growth and dry matter accumulation due to salinity have been described in several ornamental species (Bañón et al. 2005; Álvarez et al. 2012; Cassaniti et al. 2012); although the exact reaction to salt stress varies widely among species. For example, for the same level of salinity (44 mM $\text{NaCl}$), Bougainvillea glabra and Eugenia myrtiflora did not reduce plant growth, while Cotoneaster lacteus showed a reduction of more than 50% (Cassinati et al. 2009).

In our experiment, saline water irrigation had not effect on plant height and only affected the leaf DW and leaf area. Although salinity inhibited the relative growth rate as a function of plant height (RGR) in
*C. citrinus*, such a reduction was only evident a long time after the beginning of the treatments (10 months), confirming that the duration of the salt stress is also an important factor. Salts take time to accumulate inside plants before the concentrations reach toxic levels and affect plant function (Munns & Tester 2008). Ionic stress affect growth much later than osmotic stress, when salt reaches toxic concentrations in the old leaves. Plant growth was also reduced in *C. citrinus* submitted to deficit irrigation in otherwise similar experimental conditions (Álvarez & Sánchez-Blanco 2013), but the reduction was earlier than in saline conditions.

In contrast, the root system of *C. citrinus* plants was less affected by salt than by water stress, (Álvarez & Sánchez-Blanco 2013). The root to shoot ratio increased as a result of irrigating with saline water because the reductions in shoot growth were not matched by an equivalent loss of root development. This response was also observed in *Callistemon laevis* (Álvarez *et al.* 2009) and in *Callistemon citrinus* (Álvarez & Sánchez-Blanco 2013) plants exposed to water stress. In saline conditions shoot growth is more sensitive than root growth, a response that frequently occurs in drying soil (Franco *et al.* 2011) and which is related with greater water use efficiency by the plant. Under salinity, this trait may therefore present the advantage of limiting the capacity of the plant to accumulate toxic ions in the aerial part (Munns & Tester 2008). Gómez-Bellot *et al.* (2013) also reported increases in the root to shoot ratio in *Evonimous* plants irrigated with a NaCl solution with the same EC (4 dS m⁻¹).

*C. citrinus* plants grown in saline conditions showed an altered root system morphology, decreasing root length in all root sizes and increasing the percentage of thick roots respect to the thin ones compared with the control. These responses were observed in *Evonimous* plants irrigated with NaCl solution and reclaimed water, when total root length was decreased, especially in thin (Ø≤0.5 mm) and medium thickness (0.5<Ø≤2.0 mm) roots (Gómez-Bellot *et al.* 2013). Croser *et al.* (2001) and Franco *et al.* (2011) also observed an increase in root diameter (hypertrophy) in response to salinity. The same behaviour was also found by Álvarez *et al.* (2011) and Álvarez & Sánchez-Blanco (2013) in *C. citrinus* under deficit irrigation, which means that the effect of water stress on the *C. citrinus* root system morphology was very similar to that of irrigation with saline water.

The reduced root volume induced by salt stress in our experiment may be regarded, as we have already said, as a favourable trait, limiting the capacity of the plants to accumulate toxic ions in the shoot (Munns 2002; Alarcón *et al.* 2006). The greater root density observed in these plants suggests greater robustness and, presumably, a higher accumulation of reserves (Cameron *et al.* 2006; Franco *et al.* 2006;...
Álvarez et al. 2011), which would improve plant resistance to saline situations and speed up the establishment of ornamental plants in gardening and landscaping (Franco et al. 2006; 2011).

In general, an increase in external NaCl concentrations induces an increase of Na\(^+\) and Cl\(^-\) in leaves, stem and roots compared with control plants of different ornamental species (Navarro et al. 2007; Álvarez et al. 2012). However, whether there is a greater Na\(^+\) and/or Cl\(^-\) concentration in roots or in leaves, or similar values in both organs, depends on the species in question (Cassaniti et al. 2009). In our study, C. citrinus showed a higher concentration of these ions in the root system than in the leaves, which has also been observed in Cestrum fasciculatum and Escallonia rubra, both salt tolerant species (Cassaniti et al. 2009).

The retention of either Na\(^+\) and/or Cl\(^-\) in roots or leaves has been proposed as a trait related to salt tolerance in plants (Boursier & Läuchli 1990; Pérez-Alfocea et al. 2000). In a saline environment, controlling the salt concentration of the aerial parts of plants, restricting entry through the roots and limiting transport to the shoots (retaining these ions in the root and lower stem) is an important mechanism that allows plants to survive and grow in the face of salinity (Colmer et al. 2005; Murillo-Amador et al. 2006). Salt tolerance in C. citrinus has been associated with the root storage of Na\(^+\) and Cl\(^-\), especially in the case of the Na\(^+\) ions. In our experiment, Na\(^+\) was withheld so effectively in the woody roots and stems that little reached the leaves. Thus Cl\(^-\), which continues to pass to the lamina, becomes the most significant toxic component of the saline solution, as other authors have verified (Munns and Tester, 2008). The translocated Cl\(^-\) in leaves (most of the Na\(^+\) being retained in the roots) probably contributed to what little decrease in growth was observed in C. citrinus plant. Indeed, chloride has been described to be more toxic than Na\(^+\) when it accumulates in excess in leaves (Fornes et al. 2007). The ability of some species to differentiate between Na\(^+\) and Cl\(^-\) retention and transport is a topic as yet poorly understood in woody species (Munns & Tester 2008, Cassinati et al. 2009).

In ornamental shrubs, a decrease in the growth rate alone is not enough to characterize their salt tolerance since other important traits, such as the number of flowers and leaf colour, contribute to their ornamental value (Francois 1982; Fornes et al. 2007; Cassaniti et al. 2012). The reduction in leaf growth was not accompanied by colour modifications or flowering reductions and so salinity did not reduce the quality of callistemon as an ornamental plant. Plant subjected to saline stress may reduce flowering intensity, bring forward, or delay flowering and shorten the same (Fornes et al. 2007; Álvarez et al. 2012). In our experiment the increased number of inflorescences in plants subjected to saline stress
compared to control plants at the end of the experiment may have been due to early flowering rather than a higher intensity of flowering. According to Munns (2008), even moderate salinity stress, affects reproductive development, such as early flowering or a reduced number of florets. Similar responses have been cited by Zapryanova & Atanassova (2009), who observed that plants treated with NaCl have earlier and shorter blooming period than non-treated plants. Katerji et al. (2001) indicated that sensitivity to salinity was maximum during flowering, particularly during bud formation. Hence, the absence of reduced flowering in *C. citrinus* could be indicative of their relative tolerance to saline stress.

In our experiment, active periods of growth and inflorescence formation clearly affected evapotranspiration, as pointed out by Álvarez et al. (2011) and Álvarez & Sánchez-Blanco (2013) in previous studies in callistemon. Also, reductions in water consumption under saline conditions have been reported in some ornamental plants (Munns 2002; Navarro et al. 2007). In *C. citrinus* plants, ET was only inhibited at the end of the experiment, which may help to prevent toxic levels being reached in the shoot. ET reductions have been attributed to lower stomatal conductance in the short term and to the reduction in leaf area in the long term as salt injury becomes evident in the old leaves (Azza Maher et al. 2007; Ali et al. 2012). In *C. citrinus*, ET did not decrease until the effects of both factors became evident.

The changes in water flow could also explain the decreases in leaf water potential. In our conditions, water consumption (ET/d) was highest at the end of August, leading to lower leaf water potential values at midday, and the lowest values in December, when $\Psi_l$ values were the highest. Plants under saline irrigation exhibited slight dehydration throughout the experiment as indicated by the lower leaf and stem water potential. This would be due to a less available substrate water content and difficulty in taking up water from the substrate. An increase in the resistance to water flow from soil to plant in salt conditions has been observed in many species (Navarro et al. 2007; Álvarez et al. 2012).

According to Álvarez & Sánchez-Blanco (2013) stem water potential measured at midday is a good indicator of the water stress resulting from deficit irrigation in *C. citrinus* plants due to the small variability observed between bagged leaves. The same behaviour was observed with saline stress, as $\Psi_s$, identified differences between treatments earlier than $\Psi_l$, which did so only when the salt stress became more severe.

In addition, this salinity level pointed to a limited osmotic adjustment. This behaviour and the values of osmotic adjustment observed are within those reported for other studies on Mediterranean ornamental plants submitted to saline stress (Navarro et al. 2007). *C. citrinus* may behave as a typical Cl- includer,
compartmentalizing Cl within the leaf vacuoles, where it may be used as osmoticum to lower the osmotic potential necessary for the maintenance of leaf turgor (Koyro 2006).

As far as plant gas exchange is concerned, stomatal conductance was mostly limited by low winter temperatures in both treatments, which agrees with the results of Álvarez & Sánchez-Blanco (2013) who reported that stomata of callistemon are very sensitive to winter climatic conditions. In our study, salinity caused a decrease in stomatal conductance from the beginning of the experiment, especially in summer. Koyro (2006) suggested that this behaviour is an adaptative mechanism to cope with salt, especially during high transpiration periods. Decreases in gs due to salinity have been found in Viburnum (Bañón et al. 2012) and Evonymus (Gómez-Bellot et al. 2013). Stomatal responses to salinity are induced by the osmotic effect of the salt outside the roots and are probably regulated by root signals, as occurs in drying soils (Davies et al. 2005).

As indicated in the results, no pronounced differences in photosynthesis were observed during most the experiment between the control and saline treatment, despite the lower gs values observed in summer in saline-stressed plants. Eleven months after the beginning of saline treatment, Pn was seen to be negatively affected in plants subjected to salinity, although this response was much later than the decrease in gs values. The results of this study are consistent with the finding of Munns & Tester (2008), which suggests that rates of photosynthesis per unit leaf area in salt-treated plants are often unchanged, even though stomatal conductance is reduced (James et al. 2002). The long-term reduced net CO2 assimilation rates accompanying salinity have been attributed to stomatal closure, a decline in photosynthetic pigments and concurrent non-stomatal factors (i.e., reduced protein concentration) (Mugnai et al. 2009; Álvarez et al. 2012). A decrease in Pn due to salinity stress has also been reported in many other plant species, such as Viburnum tinus, a salt-sensitive species (Bañón et al. 2012) or in A. bettizickiana, a salt-tolerant ornamental plant (Ali et al. 2012). Differences in stomatal conductance between treatments do not seem to be followed by similar changes in photosynthetic rates. In this sense, C. citrinus plants submitted to a salt treatment are able to increase their intrinsic water use efficiency (Pn/gs) during the greatest part of the season, i.e. plants maintain similar photosynthesis rates despite reduced stomatal opening compared with the control. However, after a long period under salt conditions (47 weeks), Pn was proportionally more reduced than gs (decreased Pn/gs). A reduction in stomatal conductance was one of the causes of photosynthesis decline, although photoinhibition or increases in mesophyll resistance may have played a
role later when stress was more severe or prolonged (Flexas et al. 2004), which could delay plant recovery at the onset of the autumn or even cause permanent damage.

In conclusion, our results indicate that the use of saline water in C. citrinus plants slightly decreased aerial growth, increased the root/shoot ratio and improved the root system, but flowering and leaf colour were not affected and there were no toxicity symptoms. The salinity tolerance of callistemon was related to the limited Na$^+$ uptake from the substrate and to the higher ion concentration in the roots compared with leaves. These factors seem to contribute to the high salinity tolerance shown by C. citrinus, since a high photosynthetic rate would allow plants to maintain a high growth rate (hence, diluting Na$^+$ and Cl$^-$ in the leaves) as well as good osmotic adjustment through the synthesis and accumulation of compatible solutes. The fact that overall plant quality is maintained means that the use of saline water (around 4 dS m$^{-1}$) is feasible for growing this ornamental plant commercially, a consideration that is particularly relevant in arid and saline areas. However, for long periods, the cumulative effect of irrigating with saline water decreased $P_n$ and $P_n/g_s$, which could delay their recovery and even cause permanent damage, meaning that the coordination of the level of salinity and the time of exposure to the salt stress must be considered when using saline irrigation water.

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References


Table 1
Growth and leaf colour parameters at the end of the experiment in *C. citrinus* plants subjected to control and saline treatment. Values are the mean of ten plants.

P: probability level
ns: not significant

* $P \leq 0.05$.

Table 2
Root morphology at the end of the experiment in *C. citrinus* plants subjected to control and saline treatment. Values are the mean of ten plants.

P: probability level

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Table 3
Na$^+$ and Cl$^-$ concentrations at the end of the experiment in *C. citrinus* plants subjected to control and saline treatment. Values are the mean of ten plants.

Means within a row without a common lower case letter are significantly different by Student’s *t* test. Means within a column and ion without a common capital letter are significantly different by Duncan 0.05 test.

P: probability level

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Table 4
The absorption rate of Na$^+$ and Cl$^-$ ions by the root system (J) in *C. citrinus* plants subjected to control and saline treatment. Values are the mean of ten plants.

P: probability level
Table 5

Slopes of the linear regression between the Na+ and Cl- concentration in the irrigation water and their relative amounts in the plant tissues.

Means within a row without a common letter are significantly different by Duncan 0.05 test.

P: probability level

**P < 0.01 and ***P < 0.001.
**Figure legends**

**Fig. 1.** Height (A) and relative growth rate (B) of *C. citrinus* plants subjected to control and saline treatments. Values are means of 25 plants per treatment and the vertical bars indicate standard errors. Asterisks indicate significant differences between treatments according to Student's *t*-test (*P* ≤ 0.05).

**Fig. 2.** Number of inflorescences of *C. citrinus* plants subjected to control and saline treatments. Values are means of 25 plants per treatment and the vertical bars indicate standard errors. Asterisks indicate significant differences between treatments according to Student's *t*-test (*P* ≤ 0.05).

**Fig. 3.** Daily evapotranspiration of *C. citrinus* plants subjected to control and saline treatments.

**Fig. 4.** Leaf water potential and stem water potential (*Ψ*₁ and *Ψ*ₛ; A), and leaf osmotic potential at full turgor (*Ψ*₁₀₀s; B) in *C. citrinus* plants subjected to control and saline treatments. Values are means of eight plants (water potential) or five plants (osmotic potential) and the vertical bars indicate standard errors. For each studied day, * indicates significant differences between treatments for *Ψ*ₛ or *Ψ*₁₀₀s and + indicates significant difference between treatments for *Ψ*₁ according to Student's *t*-test (*P* ≤ 0.05).

**Fig. 5.** Stomatal conductance (gₛ; A), net photosynthetic rate (Pₙ; B) and intrinsic water use efficiency (Pₚ/gₛ; c) of *C. citrinus* plants subjected to control and saline treatments. Values are means of eight plants per treatment and the vertical bars indicate standard errors. Asterisks indicate significant differences between treatments according to Student's *t*-test (*P* ≤ 0.05).
Figures

Fig. 1

![Graph A: Plant height (cm)](image1)

![Graph B: Relative growth rate (cm cm^{-1} d^{-1})](image2)
Fig. 2

![Graph showing the number of inflorescences per plant over the months. The graph compares two conditions, labeled C and S, with data points for each month from April to May.]
Fig. 3

![Graph showing monthly evapotranspiration (ET) in ml d⁻¹ m⁻². The x-axis represents months from May to May, and the y-axis represents ET values from 0 to 700 ml d⁻¹ m⁻². There are two groups labeled C and S, indicated by filled and empty circles, respectively.](image-url)
Fig. 4

(A) Graph showing the water potential ($\Psi$) for different treatments. The graph includes lines and markers for different conditions.

(B) Graph showing the water potential ($\Psi_{\text{free}}$) with markers for different conditions over the months from April to May.
Table 1
Growth and leaf colour parameters at the end of the experiment in *C. citrinus* plants subjected to control and saline treatment. Values are the mean of ten plants.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem diameter (mm)</td>
<td>2.26 ± 0.12</td>
<td>2.04 ± 0.11</td>
</tr>
<tr>
<td>Leaf DW (g plant⁻¹)</td>
<td>31.77 ± 2.39</td>
<td>24.17 ± 1.39</td>
</tr>
<tr>
<td>Stem DW (g plant⁻¹)</td>
<td>45.70 ± 2.90</td>
<td>45.51 ± 2.48</td>
</tr>
<tr>
<td>Root DW (g plant⁻¹)</td>
<td>69.54 ± 9.30</td>
<td>59.55 ± 1.55</td>
</tr>
<tr>
<td>Total DW (g plant⁻¹)</td>
<td>158.96 ± 20.90</td>
<td>133.60 ± 6.19</td>
</tr>
<tr>
<td>Root to shoot ratio</td>
<td>1.98 ± 0.09</td>
<td>2.90 ± 0.23</td>
</tr>
<tr>
<td>Total leaf area (cm²)</td>
<td>1260 ± 88.52</td>
<td>1116 ± 136</td>
</tr>
<tr>
<td>Lightness (L*)</td>
<td>41.5 ± 2.9</td>
<td>41.1 ± 3.2</td>
</tr>
<tr>
<td>Chroma (C*)</td>
<td>17.9 ± 2.8</td>
<td>19.4 ± 3.5</td>
</tr>
<tr>
<td>angle hue (hº)</td>
<td>122.5 ± 6.5</td>
<td>124.6 ± 5.2</td>
</tr>
</tbody>
</table>

P: probability level
ns: not significant
* P ≤ 0.05.
Table 2
Root morphology at the end of the experiment in *C. citrinus* plants subjected to control and saline treatment. Values are the mean of ten plants.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>S</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Total root length (cm)</td>
<td>28332 ± 1453</td>
<td>10880 ± 1161</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>L ø 0-0.5 mm (cm)</td>
<td>15862 ± 594</td>
<td>4492 ± 289</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>L ø 0.5-2 mm (cm)</td>
<td>10015 ± 578</td>
<td>4946 ± 580</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>L ø +2 mm (cm)</td>
<td>2455 ± 292</td>
<td>1443 ± 294</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>L ø 0-0.5 mm (%)</td>
<td>56.07 ± 0.46</td>
<td>41.65 ± 0.95</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>L ø 0.5-2 mm (%)</td>
<td>35.32 ± 0.20</td>
<td>45.35 ± 0.27</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>L ø +2 mm (%)</td>
<td>8.61 ± 0.32</td>
<td>12.99 ± 0.69</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Root volume (cm³)</td>
<td>198.0 ± 18.9</td>
<td>140.7 ± 6.1</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Root density (g cm⁻³)</td>
<td>0.35 ± 0.02</td>
<td>0.42 ± 0.01</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

P: probability level
*P <0.05,**P <0.01 and ***P <0.001.
Table 3
Na⁺ and Cl⁻ concentrations at the end of the experiment in C. citrinus plants subjected to control and saline treatment. Values are the mean of ten plants.

<table>
<thead>
<tr>
<th>(mmol Kg⁻¹ DW)</th>
<th>Treatments</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Na⁺</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>30.8 ± 2.4 aA</td>
<td>75.1 ± 16.2 bA ***</td>
</tr>
<tr>
<td>Stem</td>
<td>70.0 ± 3.5 aA</td>
<td>164.9 ± 14.9 bA ***</td>
</tr>
<tr>
<td>Root</td>
<td>266 ± 35 aB</td>
<td>490 ± 46 bB **</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>336 ± 18 aA</td>
<td>544 ± 78 bA *</td>
</tr>
<tr>
<td>Stem</td>
<td>439 ± 49 aB</td>
<td>520 ± 66 aA ns</td>
</tr>
<tr>
<td>Root</td>
<td>557 ± 33 aC</td>
<td>839 ± 55 bB ***</td>
</tr>
</tbody>
</table>

Means within a row without a common lower case letter are significantly different by Student’s test. Means within a column and ion without a common capital letter are significantly different by Duncan 0.05 test.
P: probability level
★P <0.05, ★★P <0.01 and ★★★P <0.001.

Table 4
The absorption rate of Na⁺ and Cl⁻ ions by the root system (J) in C. citrinus plants subjected to control and saline treatment. Values are the mean of ten plants.

<table>
<thead>
<tr>
<th>J (mmol mg MS⁻¹ d⁻¹)</th>
<th>Treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Na⁺</td>
<td>3.799 ± 0.207</td>
<td>6.668 ± 0.378 ***</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>10.729 ± 0.711</td>
<td>15.039 ± 0.367 ***</td>
</tr>
</tbody>
</table>

P: probability level
★★★ P ≤ 0.001.

Table 5
Slopes of the linear regression between the Na⁺ and Cl⁻ concentration in the irrigation water and their relative amounts in the plant tissues.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Part of the plant</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Stem</td>
</tr>
<tr>
<td>Na⁺</td>
<td>1.304 ± 0.413 a</td>
<td>2.793 ± 0.345 b</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>6.076 ± 1.835 b</td>
<td>2.488 ± 0.676 a</td>
</tr>
</tbody>
</table>

Means within a row without a common letter are significantly different by Duncan 0.05 test.
P: probability level
★★ P <0.01 and ★★★ P <0.001.