

1 **Running title:** Response of callistemon to salinity

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

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6 Sara Álvarez & M^a Jesús Sánchez-Blanco*

7 Departamento de Riego. Centro de Edafología y Biología Aplicada del Segura (CSIC). P.O. Box 164, E-

8 30100 Murcia, Spain

9

10 *Corresponding author. Tel.: +34 968396318

11 E-mail address: quechu@cebas.csic.es (MJ Sánchez-Blanco).

12

13 *Correspondence to:*

14 Dr. M^a Jesús Sánchez-Blanco

15 Centro de Edafología y Biología Aplicada del Segura (CSIC)

16 Departamento de Riego P.O. Box 164

17 E-30100 Espinardo (Murcia)

18 SPAIN

19

20 Phone: +34968396318

21 FAX: +34968396211

22 E-mail: quechu@cebas.csic.es

23

24 **Key words:** Biomass; evapotranspiration; gas exchange; ion uptake; ornamental potted plant; salinity.

25

26 **Abbreviations:** C, control; C*, chroma; DW, dry weight; EC, electrical conductivity; ET,
27 evapotranspiration; g_s , stomatal conductance; h° , hue angle; L, root length; L*, lightness; P, significance;
28 P_n , net photosynthesis; RGR, relative growth rate; S; saline treatment; Ψ_l , leaf water potential; Ψ_s , stem
29 water potential; Ψ_{100s} , leaf osmotic potential at full turgor.

30

31 **Abstract**

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

48

49 **1. Introduction**

50

51 As the competition for high quality water increases, the use of saline waters has become an option for
52 irrigating salt tolerant ornamentals (Cassaniti *et al.* 2009). However, despite the importance of ornamental
53 shrubs in Mediterranean areas, the salt tolerance of such species has received little attention (Bañón *et al.*
54 2005; Valdez-Aguilar *et al.* 2011). Given that NaCl is the most soluble and widespread salt, it is not

55 surprising that all plants have evolved mechanisms to regulate its accumulation and to preferentially
56 select other nutrients commonly present in low concentrations (Munns 2002).

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

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

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

85

86 **2. Materials and methods**

87

88 *2.1. Plant material and experimental conditions*

89

90 Rooted cuttings of 2 year-old *Callistemon citrinus* (Curtis) Skeels, cv 'Firebrand' (Crimson
91 Bottlebrush) grown in 14x12 cm pots by a specialized nursery were transplanted into 5 L plastic pots
92 (20x16 cm) filled with an 8:7:1 (v/v/v) mixture of coconut fibre : black + sphagnum peat: perlite,
93 amended with 2 g L⁻¹ of Osmocote Plus (14:13:13 N,P,K plus microelements). Plants were placed inside a
94 plastic greenhouse equipped with a cooling system, located at Santomera, Murcia, Spain (38°06'31.2"N;
95 1°02'13.7"W, 110 m altitude). The micro-climatic conditions, registered with a Hoboware Lite Data
96 Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA) were 14.8 °C (mean minimum), 25.6 °C
97 (mean maximum), and 19.3 °C (average) temperature; and 1.61 Kpa (mean maximum) and 0.80 Kpa
98 (average) vapour pressure deficit. Additional information about evolution of the daily mean values of air
99 temperature and vapour pressure deficit recorded inside de greenhouse during the experimental period is
100 detailed elsewhere (Álvarez & Sánchez-Blanco, 2013).

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

102

103 *2.2. Treatments and experimental design*

104

105 *C. citrinus* plants were grown in nursery conditions and subjected to two irrigation treatments (25
106 plants per treatment) using a computer-controlled drip irrigation system from March 2009 to April 2010.
107 The irrigation treatments consisted of a control (C), where the electrical conductivity of the water was 0.8
108 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).
109 All the plants were irrigated daily at 100% water holding capacity, and the irrigation amount was equal in
110 both treatments as determined by noting when the leaching fraction for the controls reached 15-20% (v/v)
111 of the applied water. One drip nozzle, delivering 2 L h⁻¹ per pot, was connected to two spaghetti tubes
112 (one on each side of every pot) and the duration of each irrigation episode was used to vary the amount of
113 water applied, which depended on the season and on climatic conditions. The volume of water varied
114 between 200 and 500 ml per pot and irrigation episode.

115

116 Significance between control and saline treatment was determined according to the two-sided Student' s t-
117 test for unpaired samples, using Statgraphics Plus for Windows 5.1 software. Ratio and percentage data
118 were subjected to an arcsine square-root transformation before statistical analysis to ensure homogeneity
119 of variance. Significant differences between parts of the plants were analyzed by one-way ANOVA and
120 means were separated with Duncan's Multiple Range Test ($P \leq 0.05$).

121

122 *2.3. Growth and plant water measurements*

123

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

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

134 At the beginning and at the end of the saline period, these ten plants per treatment after being
135 separated into leaves, stems and roots, washed with distilled water and dried at 80 °C, were stored at room
136 temperature for inorganic solute analyses. The concentration of Cl⁻ was analysed by a chloride analyzer
137 (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained when mixing
138 100 mg of dry vegetable powder with 40 ml of water before shaking for 30 min and filtering. The
139 concentrations of Na⁺ were determined in a digestion extract with HNO₃:HClO₄ (2:1, v/v) by Inductively
140 Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL Thermo, England).
141 The absorption rate of Na⁺ and Cl⁻ ions by the root system (J) was calculated in ten plants per treatment,
142 using the formula described by Pitman (1975):

143
$$P = \frac{(M_2 - M_1)}{(WR \times t)}$$

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

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

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

157 During the experiment, leaf water potential (Ψ_l) and stem water potential (Ψ_s) were measured in eight
158 plants per treatment in mature leaves at midday. Ψ_l was estimated according to the method described by
159 Scholander *et al.* (1965), using a pressure chamber (Soil Moisture Equipment Co, Santa Barbara, CA,
160 USA), for which leaves were placed in the chamber within 20 s of collection and pressurised at a rate of
161 0.02 MPa s^{-1} (Turner 1988). Ψ_s was measured in non-transpiring leaves that had been bagged with both a
162 plastic sheet and aluminium foil for at least 1 h before measurement in order to prevent leaf transpiration;
163 in this way, leaf water potential equalled stem water potential (Begg & Turner 1970). Leaf osmotic
164 potential at full turgor (Ψ_{100s}) was measured in five plants per treatment, using excised leaves with their
165 petioles placed in distilled water overnight to reach full saturation. Leaves from the Ψ_{100s} measurements
166 were then frozen in liquid nitrogen (-196°C) and stored at -30°C . After thawing, the osmotic potential
167 was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc.,
168 Logan, UT, USA), according to Gucci *et al.* (1991). Leaf stomatal conductance (g_s) and the net
169 photosynthetic rate (P_n) were determined at midday in eight plants per treatment using a gas exchange
170 system (LI-6400, LI-COR Inc., Lincoln, NE, USA). The P_n/g_s ratio was used as an estimation of the
171 intrinsic water use efficiency.

172

173 3. Results

174

175 3.1. Plant quality

176

177 *C. citrinus* plants submitted to the saline treatment showed a reduction in the leaf dry weight (DW,
178 24%) and leaf area (12%) at the end of the experimental period, while there were no significant decreases
179 in stem diameter or stem and total dry weight compared with control plants. Higher root to shoot ratio
180 was found in the saline treatment (Table 1).

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

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

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

194

195 3.2. Plant water relations and osmotic adjustment

196

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

202 plants at the end of the experiment, when smaller values of ET were observed in saline treated plants
203 compared with the control.

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

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

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

216

217 *3.3. Stomatal conductance and photosynthetic parameters*

218

219 The values of the stomatal conductance and photosynthesis net rate during the period can be seen in
220 Fig. 5. The seasonal pattern of g_s consisted of a minor summer depression in both treatments, particularly
221 in the salt treatment, and a more pronounced winter depression regardless of the EC of irrigation water
222 applied (Fig. 5A). Such a reduction was also observed in P_n in winter when both treatments had lower
223 values than during the rest of the experiment, although no summer reduction was observed in P_n (Fig.
224 5B). The smallest values of P_n and g_s were found in winter, confirming that plants are most sensitive to
225 gas exchange parameters in this period.

226 Irrigating plants with water of 4 dS m⁻¹ EC produced a reduction in stomatal conductance with respect
227 to the control, a reduction that was more marked in summer and, especially, towards the end of the
228 experiment (Fig. 5A). Such reductions with respect to the control plants were not observed in the
229 photosynthesis levels in saline treatments until 47 weeks after beginning of the saline irrigation (Fig. 5B).

230 In general, saline treatment plants showed higher P_n/g_s ratios (intrinsic water use efficiency) than
231 control plants throughout the experimental period, except at the end of the experiment when these
232 differences between treatments disappeared (Fig. 5C).

233

234 3.4. Mineral distribution throughout the plant

235

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

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

250

251 4. Discussion

252

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

258 In our experiment, saline water irrigation had not effect on plant height and only affected the leaf DW
259 and leaf area. Although salinity inhibited the relative growth rate as a function of plant height (RGR) in

260 *C. citrinus*, such a reduction was only evident a long time after the beginning of the treatments (10
261 months), confirming that the duration of the salt stress is also an important factor. Salts take time to
262 accumulate inside plants before the concentrations reach toxic levels and affect plant function (Munns &
263 Tester 2008). Ionic stress affect growth much later than osmotic stress, when salt reaches toxic
264 concentrations in the old leaves. Plant growth was also reduced in *C. citrinus* submitted to deficit
265 irrigation in otherwise similar experimental conditions (Álvarez & Sánchez-Blanco 2013), but the
266 reduction was earlier than in saline conditions.

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

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

286 The reduced root volume induced by salt stress in our experiment may be regarded, as we have
287 already said, as a favourable trait, limiting the capacity of the plants to accumulate toxic ions in the shoot
288 (Munns 2002; Alarcón *et al.* 2006). The greater root density observed in these plants suggests greater
289 robustness and, presumably, a higher accumulation of reserves (Cameron *et al.* 2006; Franco *et al.* 2006;

290 Álvarez *et al.* 2011), which would improve plant resistance to saline situations and speed up the
291 establishment of ornamental plants in gardening and landscaping (Franco *et al.* 2006; 2011).

292 In general, an increase in external NaCl concentrations induces an increase of Na⁺ and Cl⁻ in leaves,
293 stem and roots compared with control plants of different ornamental species (Navarro *et al.* 2007; Álvarez
294 *et al.* 2012). However, whether there is a greater Na⁺ and/or Cl⁻ concentration in roots or in leaves, or
295 similar values in both organs, depends on the species in question (Cassaniti *et al.* 2009). In our study, *C.*
296 *citrinus* showed a higher concentration of these ions in the root system than in the leaves, which has also
297 been observed in *Cestrum fasciculatum* and *Escallonia rubra*, both salt tolerant species (Cassaniti *et al.*
298 2009).

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

313 In ornamental shrubs, a decrease in the growth rate alone is not enough to characterize their salt
314 tolerance since other important traits, such as the number of flowers and leaf colour, contribute to their
315 ornamental value (Francois 1982; Fornes *et al.* 2007; Cassaniti *et al.* 2012). The reduction in leaf growth
316 was not accompanied by colour modifications or flowering reductions and so salinity did not reduce the
317 quality of callistemon as an ornamental plant. Plant subjected to saline stress may reduce flowering
318 intensity, bring forward, or delay flowering and shorten the same (Fornes *et al.* 2007; Álvarez *et al.*
319 2012). In our experiment the increased number of inflorescences in plants subjected to saline stress

320 compared to control plants at the end of the experiment may have been due to early flowering rather than
321 a higher intensity of flowering. According to Munns (2008), even moderate salinity stress, affects
322 reproductive development, such as early flowering or a reduced number of florets. Similar responses have
323 been cited by Zapryanova & Atanassova (2009), who observed that plants treated with NaCl have earlier
324 and shorter blooming period than non-treated plants. Katerji *et al.* (2001) indicated that sensitivity to
325 salinity was maximum during flowering, particularly during bud formation. Hence, the absence of
326 reduced flowering in *C. citrinus* could be indicative of their relative tolerance to saline stress.

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

335 The changes in water flow could also explain the decreases in leaf water potential. In our conditions,
336 water consumption (ET/d) was highest at the end of August, leading to lower leaf water potential values
337 at midday, and the lowest values in December, when Ψ_1 values were the highest. Plants under saline
338 irrigation exhibited slight dehydration throughout the experiment as indicated by the lower leaf and
339 stem water potential. This would be due to a less available substrate water content and difficulty in taking
340 up water from the substrate. An increase in the resistance to water flow from soil to plant in salt
341 conditions has been observed in many species (Navarro *et al.* 2007; Álvarez *et al.* 2012).

342 According to Álvarez & Sánchez-Blanco (2013) stem water potential measured at midday is a good
343 indicator of the water stress resulting from deficit irrigation in *C. citrinus* plants due to the small
344 variability observed between bagged leaves. The same behaviour was observed with saline stress, as Ψ_s
345 identified differences between treatments earlier than Ψ_1 , which did so only when the salt stress became
346 more severe.

347 In addition, this salinity level pointed to a limited osmotic adjustment. This behaviour and the values
348 of osmotic adjustment observed are within those reported for other studies on Mediterranean ornamental
349 plants submitted to saline stress (Navarro *et al.* 2007). *C. citrinus* may behave as a typical Cl⁻ includer,

350 compartmentalizing Cl within the leaf vacuoles, where it may be used as osmoticum to lower the osmotic
351 potential necessary for the maintenance of leaf turgor (Koyro 2006).

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

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

379 role later when stress was more severe or prolonged (Flexas *et al.* 2004), which could delay plant
380 recovery at the onset of the autumn or even cause permanent damage.

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

394

395 **Acknowledgements**

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399 **References**

- 400 Alarcón J.J., Morales M.A., Ferrández T., Sánchez-Blanco M.J. (2006) Effects of water and salt stresses
401 on growth, water relations and gas exchange in *Rosmarinus officinalis*. *Journal of Horticultural*
402 *Science and Biotechnology*, **81**, 845-853.
- 403 Ali A., Iqbal, N., Ali F., Afzal B. (2012) *Alternanthera bettzickiana* (Regel) G. Nicholson, a potential
404 halophytic ornamental plant: Growth and physiological adaptations. *Flora*, **207**, 318-321.
- 405 Álvarez S., Navarro A., Bañón S., Sánchez-Blanco M.J. (2009) Regulated deficit irrigation in potted
406 *dianthus* plants: effects of severe and moderate water stress on growth and physiological responses.
407 *Scientia Horticulturae*, **122**, 579-585.

408 Álvarez S., Navarro A., Nicolás E., Sánchez-Blanco M.J. (2011) Transpiration, photosynthetic responses,
409 tissue water relations and dry mass partitioning in *Callistemon* plants during drought conditions.
410 *Scientia Horticulturae*, **129**, 306-312.

411 Álvarez S., Gómez-Bellot M.J., Castillo M., Bañón S., Sánchez-Blanco M.J. (2012) Osmotic and saline
412 effect on growth, water relations, and ion uptake and translocation in *Phlomis purpurea* plants.
413 *Environmental and Experimental Botany*, **78**, 138-145.

414 Álvarez S., Sánchez-Blanco M.J. (2013) Changes in growth rate, root morphology and water use
415 efficiency of potted *Callistemon citrinus* plants in response to different levels of water deficit. *Scientia*
416 *Horticulturae*, **156**, 54-62. 10.1016/j.scienta.2013.03.024

417 Azza Mazher A.M., Fatma El-Quesni E.M., Farahat M.M. (2007) Responses of ornamental plants and
418 woody trees to salinity. *World Journal of Agricultural Sciences*, **3**, 386-395.

419 Begg J.E., Turner N.C. (1970) Water potential gradients in field tobacco. *Plant Physiol.* 46, 343-346.

420 Bañón S., Fernández J.A., Franco J.A., Torrecillas A., Alarcón J.J., Sánchez-Blanco M.J. (2004) Effects
421 of water stress and night temperature pre-conditioning on water relations and morphological and
422 anatomical changes of *Lotus creticus* plants. *Scientia Horticulturae*, **101**, 333-342.

423 Bañón S., Ochoa J., Fernández J.A., Sánchez-Blanco M.J., 2005. Paclobutrazol as an aid to reducing
424 some effects of salt stress oleander seedlings. *European Journal of Horticultural Science*, **70**, 43-49.

425 Bañón S., Ochoa J., Franco J.A., Alarcón J.J., Sánchez-Blanco M.J. (2006) Hardening of oleander
426 seedling by deficit irrigation and low air humidity. *Environmental and Experimental Botany*, **56**, 36-
427 43.

428 Bañón S., Miralles J., Ochoa J., Sánchez-Blanco M.J. (2012) The effect of salinity and high boron on
429 growth, photosynthetic activity and mineral contents of two ornamental shrubs. *Horticultural Science*
430 *(Prague)*, **39**, 188-194.

431 Boursier P., Läuchli A. (1990) Growth responses and mineral nutrient relations of salt stressed sorghum.
432 *Crop Science*, **30**, 1226-1233.

433 Cameron R.W.F., Harrison-Murray R.S., Atkinson C.J., Judd H.L. (2006) Regulated deficit irrigation: a
434 means to control growth in woody ornamentals. *Journal of Horticultural Science and Biotechnology*,
435 **81**, 435-443.

436 Cassaniti C, Leonardi C, Flowers T. (2009) The effects of sodium chloride on ornamental shrubs. *Scientia*
437 *Horticulturae*, **122**, 586-593.

438 Cassaniti C., Romano D., Flowers T.J. (2012) The Response of Ornamental Plants to Saline Irrigation
439 Water, Irrigation - Water Management, Pollution and Alternative Strategies, Dr Iker Garcia- Garizabal
440 (Eds), ISBN: 978-953-51-0421-6, InTech, Available from:
441 <http://www.intechopen.com/books/irrigation-water-management-pollution-and-alternative->
442 [strategies/theresponse- of-ornamental-plants-to-saline-irrigation-water](http://www.intechopen.com/books/irrigation-water-management-pollution-and-alternative-strategies/theresponse-of-ornamental-plants-to-saline-irrigation-water).

443 Colmer T.D., Muñoz R., Flowers T.J. (2005) Improving salt tolerance of wheat and barley: future
444 prospects. *Australian Journal of Experimental Agriculture*, **45**, 1425-1443.

445 Croser C., Renault S., Franklin J., Zwiazek J. (2001) The effect of salinity on the emergence and seedling
446 growth of *Picea mariana*, *Picea glauca*, and *Pinus banksiana*. *Environmental Pollution*, **115**, 9-16.

447 Davies W.J., Kudoyarova G., Hartung W. (2005) Long-distance ABA signaling and its relation to other
448 signaling pathways in the detection of soil drying and the mediation of the plant's response to drought.
449 *Journal of Plant Growth Regulation*, **24**, 285–95.

450 Flexas J., Bota J., Loreto F., Cornic G., Sharkey, T.D. (2004) Diffusive and metabolic limitations to
451 photosynthesis under drought and salinity in C₃ plants. *Plant Biology*, **6**, 269-279.

452 Fornes F., Belda R.M., Carrión C., Noguera V., García-Agustín P., Abad M. (2007) Pre-conditioning
453 ornamental plants to drought by means of saline water irrigation as related to salinity tolerance.
454 *Scientia Horticulturae*, **113**, 52-59

455 Franco J.A., Martínez-Sánchez J.J., Fernández J.A., Bañón S. (2006) Selection and nursery production of
456 ornamental plants for landscaping and xerogardening in semi-arid environments. *Journal of*
457 *Horticultural Science and Biotechnology*, **81**, 3-17.

458 Franco J.A., Bañón S., Vicente M.J., Miralles J., Martínez-Sánchez J.J. (2011) Root development in
459 horticultural plants grown under abiotic stress conditions – a review. *Journal of Horticultural Science*
460 *and Biotechnology*, **86**, 543–556.

461 Francois L.E. (1982) Salt tolerance of eight ornamental tree species. *Journal of the American Society of*
462 *Horticultural Science*, **107**, 66–68.

463 García-Legaz M.F., López-Gómez E., Mataix Beneyto J., Torrecillas A., Sánchez-Blanco M.J. (2005)
464 Effects of salinity and rootstock on growth, water relations, nutrition and gas exchange of loquat.
465 *Journal of Horticultural Science and Biotechnology*, **80**, 199-203.

466 Gómez-Bellot M.J., Álvarez S., Castillo M., Bañón S., Ortuño M.F., Sánchez-Blanco M.J. (2013) Water
467 relations, nutrient content and developmental responses of *Euonymus* plants irrigated with water of
468 different degrees of salinity and quality. *Journal of Plant Research*, DOI 10.1007/s10265-012-0545-z

469 Ibrahim K.M., Collins J.C., Collin H.A. (1991) Effects of salinity on growth and ionic composition of
470 *Coleus blumei* and *Salvia splendens*. *Journal of Horticultural Science*, **66**, 215-222.

471 James R.A., Rivelli A.R., Munns R., von Caemmerer S. (2002) Factors affecting CO₂ assimilation, leaf
472 injury and growth in salt-stressed durum wheat. *Functional Plant Biology*, **29**, 1393-1403.

473 Katerji N., van Hoorn J.W., Hamdy A., Mastrotrilli M. (2001) Salt tolerance of crops according to three
474 classification methods and examination of some hypothesis about salt tolerance. *Agricultural Water*
475 *Management*, **47**, 1-8.

476 Kefu Z., Hai F., San Z., Jie S. (2003) Study on the salt and drought tolerance of *Suaeda salsa* and
477 *Kalanchoe claigremontiana* under isoosmotic salt and water stress. *Plant Science*, **165**, 837-844.

478 Koyro H.W. (2006) Effect of salinity of growth, photosynthesis, water relations and solute composition of
479 the potential cash crop halophyte *Plantago coronopus* (L.) *Environmental and Experimental Botany*,
480 **56**, 136-146.

481 Lafuente M.T., Belver A., Guye M.G., Saltveit Jr. M.E. (1991) Effect of the temperature conditioning on
482 chilling injury of cucumber cotyledons. *Plant Physiology*, **95**, 443-449.

483 Lippi G., Serra G., Vernieri P., Tognoni F. (2003) Response of potted *Callistemon* species to high
484 salinity. *Acta Horticulturae*, **609**, 247-250.

485 Martínez- Ferri E., Balaguer L., Valladares F., Chico J.M., Manrique E. (2000) Energy dissipation in
486 drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. *Tree*
487 *Physiology*, **20**, 131-138.

488 Maxwell K., Johnson G.N. (2000) Chlorophyll fluorescence: A practical guide. *Journal of Experimental*
489 *Botany*, **51**, 659-668.

490 McGuire R.G. (1992) Reporting of objective colour measurements. *HortScience*, **27**, 1254-1255.

491 Mugnai S., Ferrante A., Petrognani L., Serra G., Vernieri P. (2009) Stres-Induced Variation in Leaf Gas
492 Exchange and Chlorophyll a Fluorescence in *Callistemon* Plants. *Research Journal of Biological*
493 *Sciences*, **4**, 913-921.

494 Munns R. (2002) Comparative physiology of salt and water stress. *Plant, Cell and Environment*, **25**, 239-
495 250.

- 496 Munns R., Tester M. (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, **59**, 651-
497 681.
- 498 Murillo-Amador B., Troyo-Diéguez E., García-Hernández J.L., López-Aguilar R., Ávila-Serrano N.Y.,
499 Zamora-Salgado S., Rueda-Puente E.O., Kaya C. (2006) Effect of NaCl salinity in the genotypic
500 variation of cowpea (*Vigna unguiculata*) during early vegetative growth. *Scientia Horticulturae*, **108**,
501 423-441.
- 502 Navarro A., Bañón S., Olmos E., Sánchez-Blanco M.J. (2007) Effects of sodium chloride on water
503 potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo*
504 plants. *Plant Science*, **172**, 473-480.
- 505 Pedrero F., Kalavrouziotis I., Alarcón J.J., Koukoulakis P., Asano T. (2010) Use of treated municipal
506 wastewater in irrigated agriculture—Review of some practices in Spain and Greece. *Agricultural*
507 *Water Management*, **87**, 1233-1241.
- 508 Percival G.C., Kearby I.P., Al-Habsib S. (2006) An assessment of the drought tolerance of *Fraxinus*
509 genotypes for urban landscaping plantings. *Urban Forestry & Urban Greening*, **5**, 17-27.
- 510 Pérez-Alfocea F., Balibrea M.E., Alarcón J.J., Bolarín M.C. (2000) Composition of xylem and phloem
511 exudates in relation to the salt tolerance of domestic and wild tomato species. *Journal of Plant*
512 *Physiology*, **156**, 367–374.
- 513 Pitman M.G. (1975) Ion transport in whole plants. Ion transport in plant cells and tissues. In: Baker D.A.,
514 Hall J.L. (Eds), North-Holland Publishing Co. Amsterdam, pp. 267-308.
- 515 Sánchez-Blanco M.J., Bolarín M.C., Morales M.A., Alarcón J.J., Torrecillas A. (1991) Salinity effect on
516 water relations in *Lycopersicum esculentum* and its wild salt-tolerance relative species, *L. pennellii*.
517 *Physiologia Plantarum*, **83**, 269-274.
- 518 Scholander P.F., Hammel H.T., Bradstreet E.D., Hemingsen E.A. (1965) Sap pressure in vascular plants.
519 *Science*, **148**, 339-346.
- 520 Sidari M., Mallamaci C., Muscolo M. (2008) Drought, salinity and heat differently affect seed
521 germination of *Pinus pinea*. *Journal of Forest Research*, **13**, 326-330.
- 522 Steudle E., Peterson C.A. (1998) How does water get through roots? *J. Exp. Bot.* **49**, 775-788.
- 523 Turner N.C. (1988) Measurement of plant water status by the pressure chamber technique. *Irrigation*
524 *Science*, **9**, 289-308.

- 525 Valdez-Aguilar L.A., Grieve C.M., Razak-Mahar A., McGiffen M., Merhaut D.J. (2011) Growth and ion
526 distribution is affected by irrigation with saline water in selected landscape species grown in two
527 consecutive growing-seasons: spring-summer and fall-winter. *HortScience*, **46**, 632-642.
- 528 Vernieri P., Mugnai S., Borghesi E., Petrognani L. Serra G. (2006) Non-chemical growth control of
529 potted *Callistemon laevis*. *Agricoltura Mediterranea*, **160**, 85-90.
- 530 Zapryanova N., Atanassova B. (2009) Effects of salt stress on growth and flowering of ornamental annual
531 species. *Biotechnol. & Biotechnol. Eq. Special edition/On.line*.177-
532

533 **Table 1**

534 Growth and leaf colour parameters at the end of the experiment in *C. citrinus*

535 plants subjected to control and saline treatment. Values are the mean of ten

536 plants

537 P: probability level

538 ns: not significant

539 * $P \leq 0.05$.

540

541 **Table 2**

542 Root morphology at the end of the experiment in *C. citrinus* plants subjected to

543 control and saline treatment. Values are the mean of ten plants

544 P: probability level

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

546

547 **Table 3**

548 Na^+ and Cl^- concentrations at the end of the experiment in *C. citrinus* plants

549 subjected to control and saline treatment. Values are the mean of ten plants.

550 Means within a row without a common lower case letter are significantly

551 different by Student's t_{test} . Means within a column and ion without a common

552 capital letter are significantly different by Duncan $_{0.05}$ test.

553 P: probability level

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

555

556 **Table 4**

557 The absorption rate of Na^+ and Cl^- ions by the root system (J) in *C. citrinus*

558 plants subjected to control and saline treatment. Values are the mean of ten

559 plants.

560 P: probability level

561 *** $P \leq 0.001$.

562

563 **Table 5**

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

566 Means within a row without a common letter are significantly different by

567 Duncan 0.05 test.

568 P: probability level

569 ** $P < 0.01$ and *** $P < 0.001$.

570

571 **Figure legends**

572

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

577

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

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

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585 **Fig. 4.** Leaf water potential and stem water potential (Ψ_l and Ψ_s ; A), and leaf osmotic potential
586 at full turgor (Ψ_{100s} ; B) in *C. citrinus* plants subjected to control and saline treatments. Values
587 are means of eight plants (water potential) or five plants (osmotic potential) and the vertical
588 bars indicate standard errors. For each studied day, * indicates significant differences between
589 treatments for Ψ_s or Ψ_{100s} and + indicates significant difference between treatments for Ψ_s ,
590 according to Student's *t*-test ($P \leq 0.05$).

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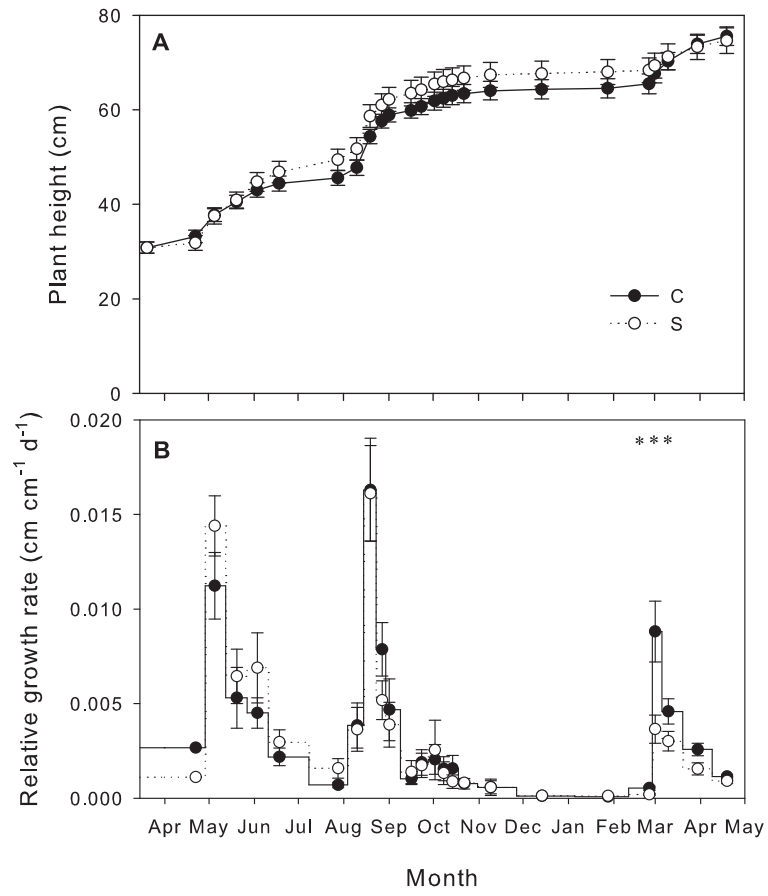
592 **Fig. 5.** Stomatal conductance (g_s ; A), net photosynthetic rate (P_n ; B) and intrinsic water use
593 efficiency (P_n/g_s ; c) of *C. citrinus* plants subjected to control and saline treatments. Values are
594 means of eight plants per treatment and the vertical bars indicate standard errors.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

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Figures

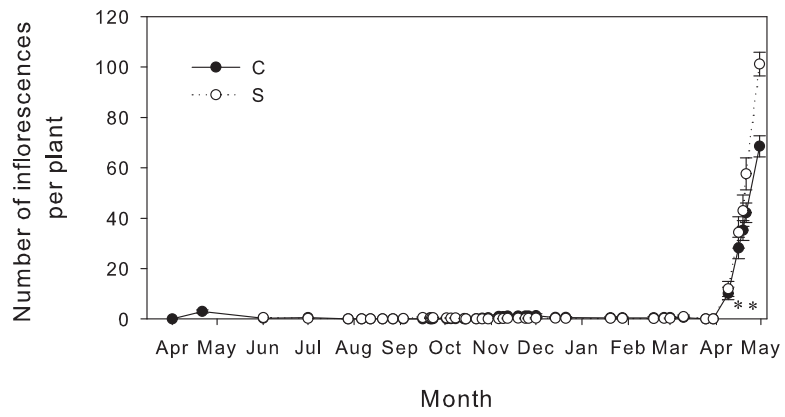
Fig. 1



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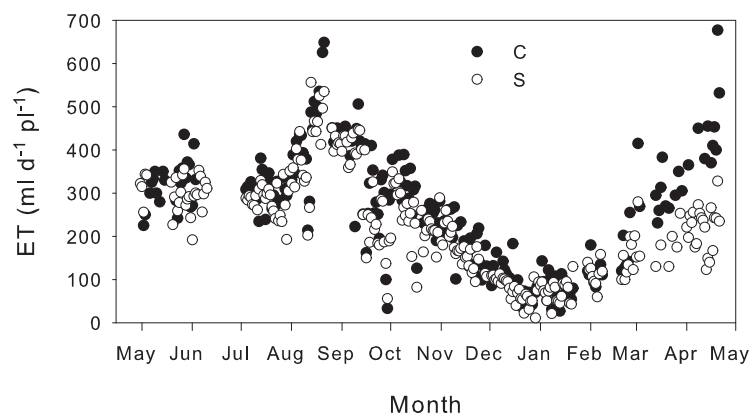
Fig. 2



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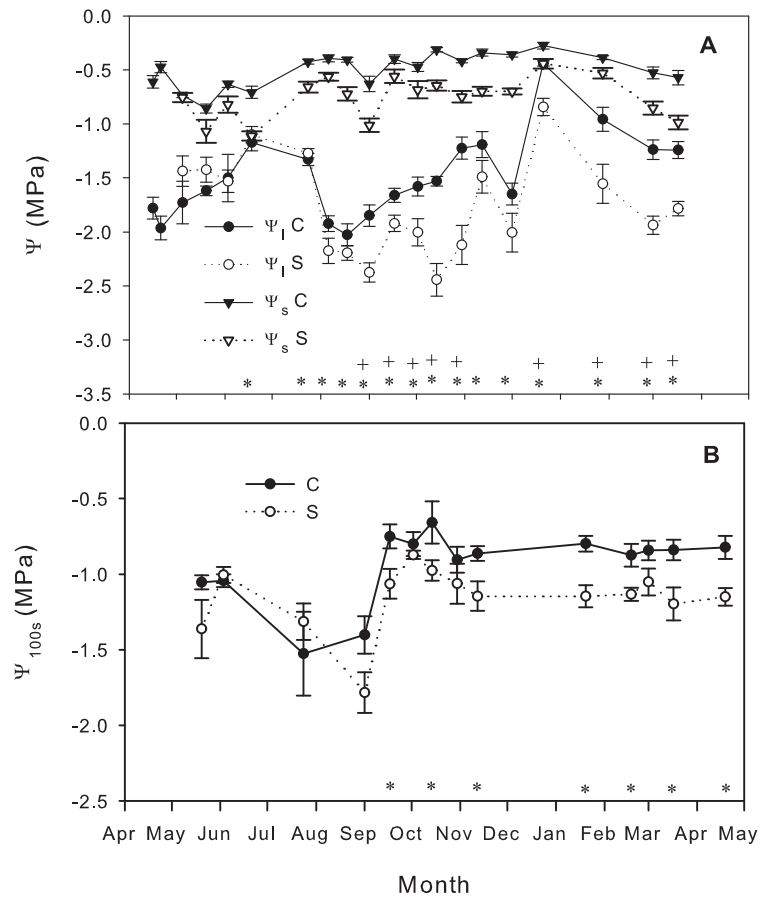
Fig. 3



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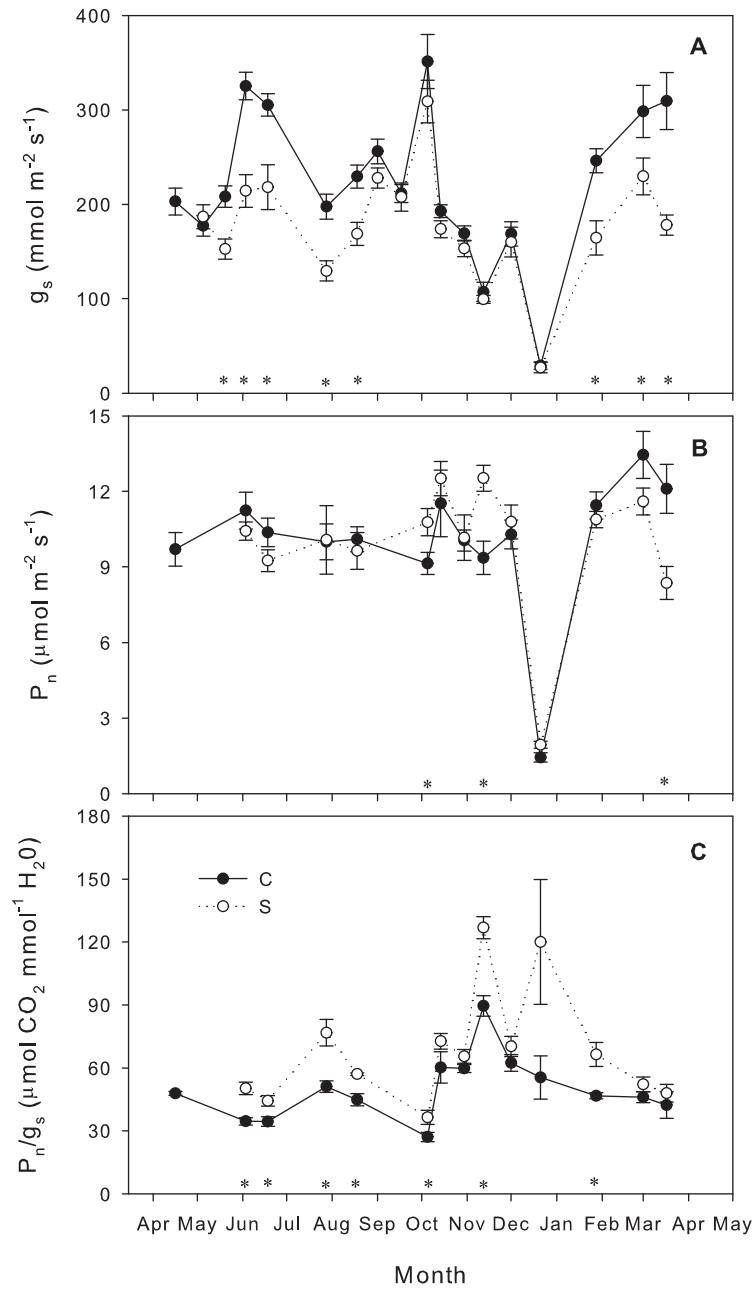
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Table 1
Growth and leaf colour parameters at the end of the
experiment in *C. citrinus* plants subjected to control and saline
treatment. Values are the mean of ten plants

Parameters	Treatments		P
	C	S	
Stem diameter (mm)	2.26 ± 0.12	2.04 ± 0.11	ns
Leaf DW (g plant ⁻¹)	31.77 ± 2.39	24.17 ± 1.39	*
Stem DW (g plant ⁻¹)	45.70 ± 2.90	45.51 ± 2.48	ns
Root DW (g plant ⁻¹)	69.54 ± 9.30	59.55 ± 1.55	ns
Total DW (g plant ⁻¹)	158.96 ± 20.90	133.60 ± 6.19	ns
Root to shoot ratio	1.98 ± 0.09	2.90 ± 0.23	*
Total leaf area (cm ²)	1260 ± 88,52	1116 ± 136	*
Lightness (L*)	41.5 ± 2.9	41.1 ± 3.2	ns
Chroma (C*)	17.9 ± 2.8	19.4 ± 3.5	ns
angle hue (h°)	122.5 ± 6.5	124.6 ± 5.2	ns

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P: probability level
ns: not significant
* P ≤ 0.05.

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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

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

P: probability level
 *P <0.05, **P <0.01 and ***P <0.001.

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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.

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

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.

J(mmol mg _{MS root} ⁻¹ d ⁻¹)	Treatment		P
	C	S	
Na ⁺	3.799 ± 0.207	6.668 ± 0.378	***
Cl ⁻	10.729 ± 0.711	15.039 ± 0.367	***

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.

Ion	Part of the plant			P
	Leaves	Stem	Root	
Na ⁺	1.304 ± 0.413 a	2.793 ± 0.345 b	6.289 ± 0.407 c	***
Cl ⁻	6.076 ± 1.835 b	2.488 ± 0.676 a	9.187 ± 0.610 b	**

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.