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1 **Growth and physiological responses of ectomycorrhizal *Coccoloba uvifera* (L.) L.**
2 **seedlings to salt stress**

3
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16 **ABSTRACT**

17 We studied the effect of the ectomycorrhizal (ECM) fungus *Scleroderma bermudense* on
18 growth, photosynthesis and transpiration rates, chlorophyll fluorescence and content, K/Na
19 and Ca/Na homeostasis, and water status of two provenances of *Coccoloba uvifera* L. (named
20 also seagrape) seedlings exposed to four levels of salinity (0.0, 54.7, 164.1 and 273.5 mM
21 NaCl equivalent to 0.02, 5, 15 and 25 dS m⁻¹, respectively). The results indicated that *S.*
22 *bermudense* improved the salt tolerance in seagrape seedlings. There were no differences in
23 terms of growth performance, nutritional and physiological functions between the two ECM
24 seagrape provenances in response to salt stress. The reduction of the Na concentration and the
25 increase of K and Ca favored a higher K/Na and Ca/Na ratio, respectively, in the tissues of
26 the ECM seedlings. Furthermore, the beneficial effects of ECM symbiosis on the
27 photosynthetic and transpiration rates, chlorophyll fluorescence and content, stomatal
28 conductance and water status resulted in the improved growth performance of the seagrape
29 provenances exposed to salt stress. From an ecological point of view, seagrape in symbiosis
30 with *S. bermudense* may be used in the development of ornamental plantings and coastal
31 windbreaks along beaches and roadsides in Cuba.

32 **Keywords:** Ectomycorrhiza, Seagrape, Photosynthesis, Chlorophyll, Water status

33 **1. Introduction**

34 Worldwide, 800 million hectares of soil are affected by salinity (Munns and Tester, 2008).
35 This represents more than 6% of the terrestrial globe and approximately 20% of the total
36 cultivated area. Land affected by salinity could increase to 50% of the total cultivated area by
37 2050 (Courel, 2019). Soil salinization is the excess accumulation of salts in the soil due to
38 natural and anthropogenic causes. This major constraint to cultivation predominates in arid
39 and semi-arid regions as well as in coastal regions, due to the inappropriate irrigation with a
40 low-quality groundwater, the recovery of salts by capillarity and the sea level rise related to
41 climate change (Gamalero et al., 2009; Gopalakrisnan and Kumar, 2021). High
42 concentrations of salts in soils cause an adverse impact on biodiversity, agricultural
43 production and sustainable development (Tester and Davenport, 2003).

44 It is well known that salinity provokes osmotic and ionic stresses in plants (Safdar et al.,
45 2019). To cope with salt stress, halophytic plants develop mechanisms to reduce the toxicity
46 of Na in the cytoplasm through restricting Na uptake, increasing the efflux of Na, and
47 compartmentalizing Na in the vacuole (Yang and Guo, 2018). Plants also develop
48 mechanisms to increase K in the cytoplasm to maintain a suitable K/Na and Ca/Na ratio and
49 to prevent cellular damages and nutrient deficiency (Munns and Tester, 2008). The K ion is
50 the major osmotically solute in the guard cells of open stomata and controls the influx of CO₂
51 and the efflux of water vapor and O₂, which can directly influence both photosynthetic and
52 transpiration rates. The Ca ion acts as a second messenger in salt stress signaling (Munns and
53 Tester, 2008).

54 Several strategies have been established to overcome salt-stress problems such as a selection
55 of salt-tolerant plants (Larcher, 1995), a desalination of soil by leaching excessive salts (Zeng
56 et al., 2014) and a mycorrhizal inoculation (Chen et al., 2017). There is considerable evidence
57 that arbuscular mycorrhizal (AM) fungi can improve plant growth and nutrition in soils
58 subject to a range of saline stress (Chen et al., 2017; Chandrasekaran et al., 2019; Shi-Chu et
59 al., 2019; Heidarianpour et al., 2020). The AM fungi can also enhance photosynthetic and
60 transpiration rates, chlorophyll fluorescence and content, stomatal conductance, antioxidant
61 metabolism, K/Na and Ca/Na ratio, and water status of the plants under salt stress (Lin et al.,
62 2017; Chandrasekaran et al., 2019; Li et al., 2019). In contrast, little is known about the role
63 of the ectomycorrhizal (ECM) symbiosis in enhancing salt tolerance of trees (Bandou et al.,
64 2006; Bois et al., 2006; Shi et al., 2017; Guerrero-Galán et al., 2019; Zwiazek et al., 2019).

65 ECM symbiosis develops a fungal mantle around root tip from which an extracellular hyphal
66 network facilitates the absorption of water and nutrients by the fungus. It develops also a
67 Hartig net between cortical cells where fungus and host plant interact: fungus provides water
68 and nutrients to the host plant, which, in return, provides carbohydrates to the fungus (Bâ et
69 al., 2012). It is also a key factor for improved tolerance of woody plants to salt stress through
70 the exclusion and compartmentation of Na in an extracellular hyphal network, improving K
71 for plant growth and the water status of plants through the activation of both fungal and plant
72 aquaporins. This leads to a higher K/Na ratio in ECM plants than in non-ECM plants under
73 salt stress (Bandou et al., 2006; Guerrero-Galán et al., 2019).

74 *Coccoloba uvifera* (L.) L. (Polygonaceae), also named seagrape, is a woody plant often
75 subject to high levels of salinity along the Atlantic, Caribbean and Pacific coasts of the
76 American tropics and subtropics (Parrota, 1994). It is an important ECM tree for edible fruits
77 and mushrooms, ornamental plantings and coastal windbreaks along Caribbean beaches and
78 roadsides (Bandou et al., 2006; Séné et al., 2015, 2018). In a previous study, Bandou et al.
79 (2006) showed that Na and Cl uptake together with a concomitant increase of P and K
80 absorption and a higher water status in ECM plants may be important salt-alleviating
81 mechanisms for one provenance of seagrape seedlings inoculated with *Scleroderma*
82 *bermudense* under salt stress. However, growth responses of tree seedlings to ECM
83 inoculation can vary from one provenance to another (Bâ et al., 1999), and some key
84 physiological mechanisms (i.e. stomatal conductance, transpiration and photosynthetic rates,
85 gas exchange, chlorophyll fluorescence and content, water status) by which *S. bermudense*
86 can improve the growth of seagrape seedlings under salt stress were not taken into account by
87 this study. Here, we analyzed some physiological parameters such as photosynthesis and
88 transpiration rates, chlorophyll fluorescence and content, K/Na and Ca/Na homeostasis, and
89 water status of the plants to assess the effect of the established *S. bermudense* on plant growth
90 of two provenances of seagrape under salt stress, in order to improve understanding of the
91 mechanisms regarding the alleviation of salt toxicity in ECM seagrape. This ECM fungus
92 was selected as inoculum because it dominated ECM fungal communities in the seagrape
93 coastal forests (Séné et al., 2015) and was the most tolerant ECM fungi to high NaCl
94 concentrations (Bâ et al., 2014).

95 **2. Materials and methods**

96 2.1. Plant material and germination

97 Mature fruits of *C. uvifera* were collected from Las Coloradas beach (provenance LC)
98 (19°55'31.9" N, 77°41'14.1" W) and Punta Tomate beach (provenance PT) (21°16'16.6" N,
99 76°31'18.5" W) in Cuba. The fruits were washed with tap water to eliminate surrounding
100 pulp. The seeds were air dried at room temperature for 72 hours on cardboard trays. To break
101 dormancy, the seeds were scarified in 95% sulfuric acid (H₂SO₄) for two hours and every 20
102 minutes the container was shaken to achieve the uniform action of the acid on the surface of
103 the seeds (Bandou et al., 2006). Then, they were rinsed with abundant distilled water, kept in
104 water for 24 hours and transferred to the sterile substrate incubating them for 5 to 8 days at
105 room temperature before transplanting. The substrate is a sandy soil collected along the Yao
106 River (20°15'32.3" N, 76°45'19.3" W) located in the town known as La China in Cuba. The
107 sandy soil was sterilized at 121 °C and 1.2 kg/cm² pressure in an autoclave. The nutrient
108 contents of the heat-sterilized sandy soil were as follows (ppm): 65 K, 25.4 Na, 80.19 Ca,
109 29.57 Mg, 3.1 Olsen-P, pH (H₂O) 7.5 and electrical conductivity (EC) 0.84 dS m⁻¹. The sandy
110 soil was analyzed in the soil laboratory of the Ministry of Agriculture of Granma province
111 (Cuba).

112 2.2. Fungal material, inoculation and experimental design

113 Mature sporocarps of *Scleroderma bermudense*, a gasteromycetous fungus, were collected **in**
114 sandy soil from under a stand of *C. uvifera* along the Las Coloradas beach. An herbarium
115 reference voucher UG-04 was given to the sporocarps. Spores were aseptically extracted
116 directly from the gleba using a spatula and kept in sealed boxes at 4°C until use. The spore
117 inoculum consisted of 0.12 g fresh weight of spores per plant.

118 Inoculation consisted of introducing 0.12 g of spores in a drain hole bored 2 cm above the
119 bottom of the surface of sterile sandy soil. Then, pre-germinated seeds with approximately 2
120 cm long tap roots were planted in the same hole, one per polyethylene bag (long 20 cm,
121 diameter 12 cm), with 1 kg of previously moistened and sterilized sandy soil.

122 The experiment was set up as completely 2x2x4 factorial design consisting of two
123 provenances (LC and PT) of *C. uvifera*, two ECM inoculation treatments (inoculated and
124 non-inoculated) and four salinity levels (0.0, 54.7, 164.1 and 273.5 mM NaCl equivalent to
125 0.02, 5, 15 and 25 dS m⁻¹, respectively) representative to the salt concentrations of sandy soils
126 along the beach. In all, 16 treatments were compared with ten replicates per treatment.
127 During the first two months, seedlings were well irrigated with tap water without NaCl to
128 achieve adequate mycorrhization. Then, over the course of 1 month, seedlings were subjected

129 to salinization at the four salt levels by adding NaCl until reaching the levels of
130 predetermined salinity for each treatment. For this purpose and to avoid osmotic shock, a
131 volume of 25 ml from 2 dS m⁻¹ of NaCl solution was gradually added to the soil every 3 days
132 to increase the initial EC from 0.02 (0 mM NaCl) to 5, 15 and 25 dS m⁻¹ during 4 weeks. The
133 sandy soil was leached with tap water every week to reduce salt accumulation. A volume of
134 25 ml of fresh salt solution was added immediately after each leaching to keep a constant
135 NaCl concentration in the soil. Salinity was controlled weekly by the method of estimating
136 salinity (Torres et al., 2001), using a portable conductivity meter (HANNA. HI 9033,
137 Rumania). Chandrasekaran et al. (2019) define soil salinity in three categories according to
138 USDA Natural Resources Conservation Service: low soil salinity has an EC_≤4 dS m⁻¹,
139 moderate soil salinity ranged from 4 to 8 dS m⁻¹, and higher than 8 dS m⁻¹ was high salinity.
140 The experiment was carried out over 6 months (from September 2018 to February 2019) in
141 nursery conditions at temperatures of 25°C-30°C with a period of exposure to sunlight of
142 approximately 12 hours in the open air (**civil time**). Seedlings were harvested after they had
143 been grown under salt stress conditions for 3 months.

144 2.3. Photosynthesis and gas exchange parameters

145 The photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs) and sub-
146 stomatal CO₂ (C_i) were non-destructively measured using a portable open flow gas exchange
147 system (ADC BioScientific. LCpro-SD, United Kingdom) from 9:00 to 11:00 a.m. (**civil**
148 **time**). The fourth fully expanded leaf of each plant (ten plants per treatment, n = 10) was
149 used for the measurements. Photosynthesis and gas exchange parameters were measured at a
150 temperature of 35.5 °C, a relative humidity of 79%, a CO₂ concentration of 414.54 ppm and
151 an active photosynthetic irradiation of 1,950 μmol m⁻² s⁻¹.

152 2.4. Chlorophyll fluorescence and content

153 The minimal fluorescence (F_o), maximum fluorescence (F_m) and variable fluorescence (F_v=
154 F_m-F_o) were measured with a portable chlorophyll fluorimeter (Hansatech Instruments.
155 RS232, United Kingdom) according to the manufacturer's instructions. For this, the fourth
156 fully expanded leaf of each plant (ten plants per treatment, n = 10) was used for the
157 measurements. Before measuring, the leaves were dark-adapted for 30 min using the clips
158 provided with the kit. After darkening the leaves, the F_o was recorded and a saturating pulse
159 of radiation (3500 μmol m⁻² s⁻¹ with the help of three light-emitting diodes of 650 nm) was

160 given for (from 10 μ s to 1 s) to determine Fm. The data obtained from Fo, Fm and Fv
161 permeated to determine two biophysical parameters that describe the photochemistry of PII:
162 the maximum quantum yield of photosystem II (Fv/Fm) and the performance index on
163 absorption basis (PIABS) (Table 5).

164

165 The chlorophyll content was determined on the fourth fully expanded leaf of each plant (ten
166 plants per treatment, n = 10) using SPAD-502 chlorophyll meter (Konica-Minolta, Japan)
167 from 9:00 to 11:00 a.m. (**civil time**) according to the manufacturer's instructions. The reading
168 is made in an arbitrary unit that is proportional to the leaf chlorophyll concentration (Jiang et
169 al., 2017).

170 2.5. Water status and plant biomass

171 The foliar (Ψ_{wf}) and the xylem (Ψ_{wx}) water potential were measured at midday (**civil time**)
172 with a Scholander pressure chamber (PMS. 615, USA) (Scholander et al., 1965) at the end of
173 the harvest. One and a half hours before measuring the xylem potential, the leaves were
174 covered with hermetic ziplock bags made of high-density polyethylene with a reflective
175 cover. The leaf area was determined with the area meter (ADC BioScientific. AM.350,
176 United Kingdom).

177 At the end of the harvest, the leaves, stems and roots of seedlings (ten seedlings per
178 treatment, n=10) were separated to measure the length of the stem, collar diameter and dry
179 weight (5 days at 80°C). The relative water content (RWC) was determined after measuring
180 the fresh mass (FM), dry mass (DM) and turgid mass (TM) in leaf discs (diameter 15 mm)
181 from the fourth fully expanded leaf of each plant (ten plants per treatment) was used for the
182 measurement. The turgid mass was the mass of the leaves after their saturation in water at 4
183 °C in the dark. The relative water content was calculated according to Morgan (1984) and
184 using the following formula:

$$185 \text{RWC} = [(FM - DM)/(TM - DM)] \times 100.$$

186 The relative mycorrhizal dependency (RMD) was calculated at a given NaCl level
187 (Plenchette et al., 1983) using the following formula:

$$188 \text{RMD} = [\text{Biomass of ECM plants} - \text{Biomass of non-ECM plants}] \times 100 / \text{Biomass of ECM}$$

189 plants.

190 2.6. Ectomycorrhizal colonization

191 To determine the percentage of mycorrhization, a sample of ten lateral roots (per seedling and
192 per treatment) was washed gently and dispersed in a petri dish containing water, and the
193 number of root tips of ECM roots and non-colonized roots was counted under a
194 stereomicroscope at $\times 100$ magnification. ECM colonization was evaluated [(number of ECM
195 roots/total number of roots) $\times 100$] and confirmed by microscope ($\times 400$) examination of root
196 tips to determine the presence of a fungal mantle and Hartig net, according to Bandou et al.
197 (2006).

198 2.7. Concentrations of Na, K and Ca

199 The roots, stems and leaves (ten plants per treatment, $n = 10$) were dried at $80\text{ }^{\circ}\text{C}$ until
200 constant weight. They were crushed in a blade mill to particles smaller than 0.5 mm and 0.2 g
201 were digested in a mixture of $\text{HClO}_4/\text{HNO}_3$ (v/v 1:5) diluted with 100 mL of distilled water.
202 To determine the concentration of Na, K and Ca, each sample was analyzed in an atomic
203 absorption spectrophotometer (PinAAcle. 900T. USA) in the Department of Plant Sciences,
204 Faculty of Agriculture, University of Rostock (Germany).

205 2.8. Statistical analysis

206 Prior to the ANOVA analysis, Kolmogorov-Smirnov and Shapiro Wilk's test, and Levene
207 and Bartlett's test were performed for normality and homogeneity of variances, respectively.
208 All data were subjected to two-way (inoculation and salinity) or three-way (provenance,
209 inoculation and salinity) analysis of variance, and mean values were compared using Tukey's
210 test with the InfoStat software version 2008 (Di Rienzo et al., 2008). The Pearson's
211 correlation coefficients between dependent variables were determined by the same software.

212 3. Results

213 3.1. Root colonization and plant growth

214 There was not a significant effect of provenances on ECM colonization (**$P = 0.886$**) (**S1 Table**).
215 Therefore, we analyzed the two factors (inoculation and salinity) and their interactions
216 (Figure 1). The two factors (inoculation and salinity) and their interactions had a significant
217 effect ($P \leq 0.05$) on mycorrhization (Figure 1). The ectomycorrhizas of the *S. bermudense*
218 were characterized by a white and smooth mantle and abundant mycelial strands. The ECM
219 colonization by the *S. bermudense* varied from 45.8% to 81.0% and from 48.4% to 80.2%

220 depending on the provenances of the seagrape. However, the extent of ECM colonization was
221 significantly affected by salt stress for each provenance, respectively (Figure 1).

222 The three factors (provenance, inoculation and salinity) and their interactions had significant
223 effects ($P \leq 0.05$) depending on studied **growth** parameters (Table 1). **There was no**
224 **significant effect of the provenance on these parameters. However, the two factors**
225 **(inoculation and salinity) and their interactions were significant for all parameters**
226 **(Table 1)**. The effects of salinity on seagrape seedlings growth after inoculation with *S.*
227 *bermudense* did not vary with the seagrape provenances (Table 1). The ECM colonization by
228 *S. bermudense* enhanced the seedlings growth regardless of the salt level. Stem and root
229 length, stem diameter, number of leaves and leaf area were much higher for ECM than for
230 non-ECM plants regardless of salt level (Table 1). Furthermore, the total biomass of ECM
231 plants increased by 68% compared with the non-inoculated plants whatever the provenance
232 of the seagrape and salt level. Although growth parameters declined in both ECM and non-
233 ECM plants as salinity increased, all of them remained superior to that of non-ECM plants at
234 each salt level. The ECM dependency on seagrape seedlings with *S. bermudense* showed an
235 increasing trend with increasing NaCl levels (Table 1). However, the ECM dependence did
236 not differ significantly among salt treatments. This result implies that although salt stress
237 affected ECM colonization, *S. bermudense* enhanced the salinity tolerance of seedlings
238 regardless the seagrape provenances.

239 3.2. Photosynthesis and gas exchange

240 The three factors (provenance, inoculation and salinity) and their interactions had significant
241 effects ($P \leq 0.05$) depending on parameters studied (Table 2). There was no significant effect
242 of the provenance on these parameters except for g_s ($P=0.001$). **However, the two factors**
243 **(inoculation and salinity) and their interactions were significant for all parameters**
244 **(Table 2)**. In the absence of salt, A, g_s , E and C_i parameters were higher for ECM than non-
245 ECM plants, suggesting an enhancing of photosynthesis and transpiration in seagrape
246 seedlings inoculated with *S. bermudense* regardless of the provenances (Table 2). In the
247 presence of salt, there was a significant decrease of A, g_s , E and C_i in the ECM and in the
248 non-ECM seagrape provenances. However, at each level of salinity, A, g_s , E and C_i
249 parameters were higher for ECM than non-ECM plants.

250 3.3. Chlorophyll fluorescence and content

251 The three factors (provenance, inoculation and salinity) and their interactions had significant
252 effects ($P \leq 0.05$) depending on parameters studied (Table 3). There was no significant effect
253 of the provenance on these parameters except for SPAD values ($P < 0.001$). **However, the two**
254 **factors (inoculation and salinity) and their interactions were significant for all**
255 **parameters (Table 3).** In the absence of salt, basal fluorescence (F_o) values were
256 significantly lower for ECM than non-ECM plants, whereas values of maximum fluorescence
257 (F_m) were higher for ECM than non-ECM plants (Table 3). A similar trend occurred for F_o
258 and F_m values in ECM and non-ECM plants in the presence of salt (Table 3). In the absence
259 of salt, F_v/F_m , PI_{ABS} and SPAD values were higher for ECM than non-ECM plants (Table 3).
260 Increased salinity reduced the F_v/F_m and PI_{ABS} in non-ECM plants more than those in ECM
261 plants. However, in ECM plants, increased salinity did not cause a significant decrease in
262 F_v/F_m and PI_{ABS} parameters. Values of F_v/F_m and PI_{ABS} were higher in ECM than in non-
263 ECM plants under saline conditions.

264 3.4. Plant water status

265 **The three factors (provenance, inoculation and salinity) and only the interactions of the**
266 **two factors (inoculation and salinity) had significant effects on all parameters (Table 4).**
267 The RWC values in the leaves were higher in ECM than non-ECM plants at all salinity levels
268 regardless of seagrass provenances (Table 4). The RWC declined with exposure to salinity
269 (Table 4). In non-ECM plants, the reduction in the RWC was 24% in provenance LC and
270 27% in provenance PT, whereas in ECM plants, the reduction was 20% in provenance LC
271 and 22% in provenance PT.

272 In the absence of salt, leaf and xylem water potentials were higher for ECM than non-ECM
273 plants regardless the provenances, suggesting an improvement of water status of seagrass
274 seedlings inoculated with *S. bermudense* (Table 4). Salt stress however, reduced leaf and
275 xylem water potentials in both ECM and non-ECM plants (Table 4).

276 3.5. Concentrations of Na, K, Ca, K/Na and Ca/Na ratio in leaves, stems and roots

277 There was not a significant effect of provenances on Na, K, Ca, K/Na and Ca/Na ratio in
278 leaves, stems and roots (S1 table). Therefore, we analyzed the two factors (inoculation and
279 salinity) and their interactions. They had a significant effect ($P \leq 0.05$) on Na in leaves, stems
280 and roots (Figure 2). In the absence of salt, there was less Na in leaves, stems and roots of
281 ECM than non-ECM plants (Figure 2). Under saline conditions, non-ECM plants

282 accumulated more Na than ECM plants at a given NaCl level regardless of seagrape
283 provenance. The seagrape provenances did not differ regarding Na content of ECM plants
284 (Figure 2). Na content was apparently higher in the leaves than in the stems and roots for all
285 salt treatments.

286 The two factors (inoculation and salinity) and their interactions had significant effects ($P \leq$
287 0.05) on K (Figures 3) and K/Na (Figure 4) in leaves, stems and roots. The K content in
288 leaves, stems and roots was higher in ECM than in non-ECM plants regardless of the salinity
289 level and the seagrape provenance (Figure 3). As a consequence of this, the K/Na ratio
290 increased in the leaves, stems and roots of ECM plants compared to non-ECM plants (Figure
291 4).

292 The two factors (inoculation and salinity) and their interactions had a significant effect ($P \leq$
293 0.05) on Ca (Figures 5) and Ca/Na (Figures 6) in leaves, stems and roots (Figures 5 and 6).
294 There was more Ca in the leaves, stems and roots in ECM than non-ECM plants regardless of
295 salinity (Figure 5). Therefore, the ratio Ca/Na ratio in leaves, stems and roots was higher in
296 ECM than non-ECM plants (Figure 6).

297 3.6. Relations between datasets

298 Most of the morphological and physiological variables were significant positive or negative
299 correlated between them (S2 table). For example, A was positively correlated to Fv/Fm ($r =$
300 0.89, $P \leq 0.01$), Fv/Fo ($r = 0.85$, $P \leq 0.01$), PI ($r = 0.83$, $P \leq 0.01$) and total biomass ($r = 0.35$, P
301 ≤ 0.05) (S2 table). The parameter E was also positively correlated to RWC ($r = 0.59$, $P \leq$
302 0.01), and negatively correlated to the foliar Ψ_{wf} ($r = -0.94$, $P \leq 0.01$) and Ψ_{wx} ($r = -0.94$, $P \leq$
303 0.01). The *C. uvifera* seedlings showed a negative correlation between Na leaves and K
304 contents in leaves ($r = -0.81$, $P \leq 0.01$), shoots ($r = -0.47$, $P \leq 0.01$) and roots ($r = -0.76$, $P \leq$
305 0.01). There was also a negative correlation between Na accumulation in leaves and K/Na
306 ratios in leaves ($r = -0.92$, $P \leq 0.01$), shoots ($r = -0.49$, $P \leq 0.01$) and roots ($r = -0.70$, $P \leq 0.01$).

307 4. Discussion

308 In the present study, salinity caused a reduction in ECM colonization, which is in line with a
309 previous study on seagrape (Bandou et al., 2006). The reduction of the ECM colonization by
310 the *S. bermudense* with increasing NaCl levels, tended to reduce total biomass of the ECM
311 seedlings compared with the non-ECM plants, regardless of seagrape provenances. As a

312 consequence of this, ECM dependency apparently increased with increasing NaCl levels.
313 This suggests that ECM plants mitigated salt stress in both seagrape provenances and
314 indicates a high symbiosis efficiency of *S. bermudense* once it was established. Indeed, it is
315 well known that the ECM symbiosis plays a major role in helping tree seedlings to survive
316 under salt conditions (Bandou et al., 2006; Bois et al., 2006; Zwiazek et al., 2019; Guerrero-
317 Galán et al., 2019; Thiem et al., 2020). A similar effectiveness of the ECM symbiosis was
318 also reported in other tree species, including *Pinus* spp. (Bandou et al., 2006; Bois et al.,
319 2006; Zwiazek et al., 2019; Guerrero-Galán et al., 2019). For example, Zwiazek et al. (2019)
320 found that *Pinus concorta* was dependent on ECM fungi that could be helpful in alleviating
321 effects of NaCl in urban soils. Bandou et al. (2006) had also shown an increase of ECM
322 dependency with an increasing salt level within in one seagrape provenance from
323 Guadeloupe.

324 The parameters A, gs, E and Ci were significant positive correlated with the improved total
325 biomass in the leaves, stems and roots of ECM plants exposed to saline conditions. Indeed,
326 the presence of ECM fungus, seagrape provenances had higher stomatal conductance,
327 transpiration and photosynthetic rates, and sub-stomatal CO₂ concentration in the mesophyll
328 than those of non-ECM plants under salt stress. This result suggests that ECM fungus can
329 elevate the photosynthetic ability of seagrape seedlings thanks to an improving of the
330 stomatal conductance and gas exchange capacity under salt stress. Our study is in consistence
331 with work of Shi-Chu et al. (2019) which showed that increasing salt concentration led to a
332 significant decrease of A *via* a decrease in the gs, which was less important in AM than non-
333 AM alfalfa. The decrease of gs due to the stomatal closure is often related to the water status
334 of plants (Mohamed et al., 2020). Under water deficit conditions due to salt stress, the plants
335 reduce transpiration rate more in non-ECM than ECM plants by closing stomata to reduce
336 water loss. Furthermore, in this study, a higher gs in the ECM *vs* non-ECM plants under salt
337 stress was positively correlated to the increased of CO₂ diffusion through the stomata and
338 water absorption. As a result, the photosynthesis and water status may be better in ECM
339 plants. Measurements of RWC, Ψ_{wf} and Ψ_{wx} are considered informative on water status and
340 transpiration rate in plants under salt stress (Larcher, 1995; Chen et al., 2017). It is also well
341 known that the accumulation of salts in the root zone causes an increase in the osmotic
342 potential and, consequently, the water potential decreases in the rhizosphere (Augé et al.,
343 2008). Here, despite their higher evaporative leaf surface, the ECM plants had higher RWC,
344 Ψ_{wf} and Ψ_{wx} than non-ECM plants regardless of salinity and seagrape provenances,

345 suggesting that the extensive hyphal extension developed by *S. bermudense* allowed higher
346 water absorption and hydraulic conductivity in roots of seagrape even when water potential is
347 low (Bandou et al., 2006; Augé et al., 2008; Lehto and Zwiazek, 2011).

348 Our study also indicated that the response of ECM plants to the ratio of Fv/Fm, PI_{ABS} and
349 chlorophyll content was greater than those in non-ECM plants, suggesting that the ECM
350 symbiosis could enhance the chlorophyll concentration and fluorescence of seagrape leaves,
351 which is consistent with the results of other studies (Sheng et al., 2018). Indeed, plants trap
352 photons in chlorophylls of PSII from sunlight and use them to split H₂O into O₂, H⁺ and e-
353 during light reaction phase of photosynthesis. Moreover, salt stress decreases the
354 concentration of chlorophyll and inhibits the electron transport chain to produce ATP and
355 NaDPH (Shi-Chu et al., 2019). Similarly, to our study, previous works showed that the AM
356 fungus-plant symbiosis with a greater chlorophyll than that in non-AM, presented also higher
357 rates of photosynthesis under salt stress (Elhindi et al., 2017).

358 Here, results show that ECM plants kept a higher K/Na ratio in leaves, shoots and roots and
359 confirmed that K competed for the absorption site of Na on the cell membrane (Garcia and
360 Zimmermann, 2014). Therefore, ECM symbiosis can facilitate K whereas preventing Na
361 absorption and translocation in shoot and leaves of seagrape seedlings to maintain a high
362 cytosolic K/Na ratio which is a key feature of plant salt tolerance. Our results were in
363 consistence with works showing that the ECM symbiosis enhances the growth of host plants
364 by promoting uptake water and nutrients by the host under salt stress (Bandou et al., 2006;
365 Bois et al., 2006; Guerrero-Galán et al., 2019). In this respect, concentrations of Na clearly
366 decreased and K content increased in ECM-plants compared to non-ECM plants,
367 concomitantly with an increasing of the ratio K/Na ratio to maintain higher cell turgor
368 (Bandou et al., 2006; Chen et al., 2017). Moreover, K ion is involved in regulating stomatal
369 opening and osmotic potential in the vacuoles. The results here showed that competition of K
370 due to colonization by *S. bermudense* may induce the decrease of Na, thus enhancing salt
371 tolerance of ECM plants. Alongside with Ca, K is also an essential nutrient for plant growth
372 (Evelin et al., 2019). Indeed, in the present study, the ECM plants had higher concentrations
373 of K and Ca than non-ECM plants particularly under salt stress conditions. The Ca ion can
374 also act as a second messenger in salt stress signaling (Munns and Tester, 2008; Evelin et al.,
375 2019). However, the absorption of Ca could be limited through a competition with an
376 elevated Na concentration in the rhizosphere under salt stress (Evelin et al., 2019). Less Ca
377 absorption and translocation than Na within the plant leads to a decrease in the Ca/Na ratio in

378 salt stressed plants. Evelin et al. (2012, 2019) suggested that the Ca/Na ratio could also be
379 increased by AM colonization and also suggested that Ca/Na ratio could be an indicator of
380 salt tolerance in plants. This statement is consistent with the present study, which showed a
381 higher Ca/Na ratio in ECM than non-ECM plants. However, the mechanism involved is not
382 well known and needs further works (Evelin et al., 2019).

383 In conclusion, the results indicate that the ECM fungus *S. bermudense* improved the salt
384 tolerance in seagrape seedlings. There was no difference in terms of growth performance or
385 nutritional and physiological functions between the two ECM seagrape provenances in
386 response to salt stress. The reduction of the Na concentration and the increase of K and Ca
387 favored a higher K/Na and Ca/Na ratio, respectively, in the tissues of the ECM seedlings.
388 Additionally, the beneficial effects of ECM symbiosis on the photosynthetic and transpiration
389 rates, chlorophyll fluorescence and content, stomatal conductance and water status resulted in
390 the improved growth performance of the seagrape provenances exposed to salt stress. From
391 an applied point of view, transplanting of the ECM seagrape to such degraded sites not only
392 may benefit the individual plant but, more importantly, may result in the development of
393 ornamental plantings and coastal windbreaks along beaches and roadsides in Cuba.

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521 **Table 1.** Effect of provenance, inoculation and salinity on morphological variables in *C. uvifera* seedlings.

Provenance of <i>C. uvifera</i>	Inoculation treatment	Salinity (dS m ⁻¹)	Stem length (cm)	Stem diameter (mm)	Number of leaves	Root length (cm)	Total biomass (g)	Leaf area (cm ²)	ECM dependency (%)		
Las Coloradas	Non-mycorrhizal	0.02	16.10±1.30fg	3.30±0.24cd	5.60±0.55efg	26.80±1.79ab	2.76±0.22cd	100.36±31.48bc	-		
		5	13.68±0.84cdef	3.00±0.56bc	4.20±0.45cd	27.90±2.75abc	2.41±0.45bc	72.38±21.45c	-		
		15	11.04±1.01abc	2.31±0.21a	3.40±0.55bc	30.40±2.79bcd	1.26±0.30a	41.77±6.05d	-		
		25	9.04±0.43a	2.17±0.23a	2.40±0.55ab	27.04±1.39ab	0.97±0.27a	41.97±8.09d	-		
	Mycorrhizal	0.02	19.42±2.10h	4.94±0.21g	7.40±0.55i	41.00±3.59e	4.04±0.60e	237.71±43.05a	30.62±8.95abc		
		5	18.20±2.02gh	4.11±0.14ef	6.80±0.45hi	40.00±2.45e	3.06±0.37d	142.75±22.95ab	19.29±21.89a		
		15	14.20±1.45def	3.64±0.22de	5.80±0.45fgh	37.20±4.48de	2.27±0.21b	97.21±3.24abc	43.86±14.84bc		
		25	12.58±1.11bcde	3.03±0.13c	5.20±0.45def	37.30±3.53de	1.94±0.37b	81.26±17.31bc	47.56±20.39bc		
		Punta de Tomate	Non-mycorrhizal	0.02	15.08±1.59ef	3.92±0.14cd	5.40±0.55ef	30.50±1.41bcd	2.78±0.16cd	99.92±32.03bc	-
				5	12.96±0.53bcde	3.33±0.18cd	5.00±0.00def	31.00±2.15bcd	2.41±0.31bc	71.71±21.36c	-
				15	10.92±0.04ab	2.43±0.19ab	3.20±0.45abc	28.10±3.65abc	1.25±0.22a	40.90±5.08d	-
				25	8.98±0.36a	1.92±0.19a	2.20±0.45a	20.82±3.19a	0.97±0.14a	42.03±7.93d	-
Mycorrhizal	0.02		18.92±1.43h	4.60±0.26fg	7.00±0.00i	35.60±4.52cde	3.98±0.32e	239.02±42.39a	29.88±7.17abc		
	5		17.92±1.40gh	4.03±0.50ef	6.60±0.55ghi	36.90±4.42de	3.01±0.28d	141.36±20.18ab	19.82±10.62ab		
	15		13.90±0.96def	2.98±0.14cd	5.60±0.55efg	34.50±6.43bcde	2.37±0.23bc	96.84±2.92abc	46.99±10.91bc		
	25		11.92±0.47bcd	2.93±0.18bc	4.60±0.55de	33.84±2.45bcde	1.93±0.26b	80.52±17.25bc	48.77±8.95c		
Provenance		P= 0.091	P= 0.059	P= 0.162	P= 0.013	P= 0.995	P= 0.914	P= 0.739			
Inoculation		P< 0.001	P< 0.001	P< 0.001	P< 0.001	P< 0.001	P< 0.001	P< 0.001			
Salinity		P< 0.001	P< 0.001	P< 0.001	P= 0.002	P< 0.001	P< 0.001	P< 0.001			
Provenance x Inoculation		P= 0.933	P= 0.009	P= 0.064	P= 0.048	P= 0.954	P= 0.980	P= 0.739			
Provenance x Salinity		P= 0.901	P= 0.995	P= 0.103	P= 0.164	P= 0.958	P= 0.999	P= 0.977			
Inoculation x Salinity		P= 0.123	P= 0.003	P= 0.023	P= 0.182	P< 0.001	P< 0.001	P< 0.001			
Provenance x Inoculation x Salinity		P= 0.852	P= 0.335	P= 0.263	P= 0.047	P= 0.635	P= 0.702	P= 0.572			

522 Different letters indicate significant differences for P ≤ 0.05. Values are the means±SD (n=10).

524 **Table 2.** Effect of provenance, inoculation and salinity on stomatal conductance (gs),
 525 photosynthetic (A) and transpiration (E) rates and sub-stomatal CO₂ (Ci) in leaves of *C.*
 526 *uvifera* seedlings.

Provenance of <i>C. uvifera</i>	Inoculation treatment	Salinity (dS m ⁻¹)	gs (mol m ⁻² s ⁻¹)	E (mol m ⁻² s ⁻¹)	A (μmol m ⁻² s ⁻¹)	Ci (vpm)
Las Coloradas	Non-mycorrhizal	0.02	0.07±0.02cd	0.96±0.22cd	1.26±0.09d	374.80±1.23e
		5	0.05±0.01bc	0.54±0.14ab	0.86±0.07c	345.80±1.03c
		15	0.04±0.01ab	0.49±0.02ab	0.50±0.03b	325.10±0.88b
		25	0.04±0.0048ab	0.43±0.04a	0.22±0.02a	274.50±1.08a
	Mycorrhizal	0.02	0.16±0.02f	2.61±0.25e	1.96±0.02e	432.10±1.85g
		5	0.13±0.0048ef	1.93±0.06de	1.78±0.13e	431.00±1.25g
		15	0.07±0.01cde	1.07±0.09cd	0.89±0.05c	394.80±1.23f
		25	0.06±0.0032c	0.71±0.05bc	0.57±0.01b	361.90±1.60d
Punta de Tomate	Non-mycorrhizal	0.02	0.06±0.02c	0.95±0.26cd	1.25±0.11d	376.10±1.20e
		5	0.04±0.01ab	0.52±0.16ab	0.83±0.08c	346.40±0.84c
		15	0.03±0.01a	0.47±0.07a	0.51±0.05b	323.80±0.79b
		25	0.03±0.01a	0.41±0.06a	0.19±0.05a	273.90±0.88a
	Mycorrhizal	0.02	0.15±0.02f	2.60±0.27e	1.91±0.02e	431.70±1.57g
		5	0.12±0.01def	1.91±0.08de	1.79±0.15e	430.80±1.03g
		15	0.06±0.01bc	1.05±0.09cd	0.87±0.09c	396.10±0.74f
		25	0.06±0.01cd	0.69±0.08bc	0.54±0.05b	363.10±0.88d
Provenance		P= 0.001	P= 0.476	P= 0.335	P= 0.231	
Inoculation		P < 0.001	P < 0.001	P < 0.001	P < 0.001	
Salinity		P < 0.001	P < 0.001	P < 0.001	P < 0.001	
Provenance x Inoculation		P= 0.076	P= 0.930	P= 0.777	P= 0.231	
Provenance x Salinity		P= 0.702	P= 0.998	P= 0.911	P= 0.876	
Inoculation x Salinity		P < 0.001	P < 0.001	P < 0.001	P < 0.001	
Provenance x Inoculation x Salinity		P= 0.257	P= 0.510	P= 0.462	P= 0.032	

527 Different letters indicate significant differences for P ≤ 0.05. Values are the means±SD
 528 (n=10).

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Table 3. Effect of provenance, inoculation and salinity on minimal fluorescence (Fo), maximum fluorescence (Fm), maximum quantum yield of photosystem II (Fv/Fm), performance index on absorption basis (PI_{abs}) and chlorophyll quantification (SPAD) in leaves of *C. uvifera* seedlings.

Provenance of <i>C. uvifera</i>	Inoculation treatment	Salinity (dS m ⁻¹)	Fo (bits)	Fm (bits)	Fv/Fm (bits)	Fv/Fo (bits)	PI _{abs}	Chlorophyll quantification (SPAD)	
Las Coloradas	Non-mycorrhizal	0.02	310.40±14.47c	1496.00±72.95c	0.79±0.02cde	3.84±0.43de	2.05±0.0034c	54.26±0.77e	
		5	374.20±32.94d	1442.80±10.69bc	0.74±0.03bc	2.88±0.35bc	1.29±0.11b	51.78±0.84d	
		15	378.60±34.22d	1341.60±38.00b	0.72±0.03b	2.57±0.41ab	1.00±0.08b	46.20±0.66b	
		25	383.20±25.06d	1018.40±64.90a	0.62±0.05a	1.68±0.34a	0.28±0.02a	40.02±0.29a	
	Mycorrhizal	0.02	270.20±7.85ab	1809.60±124.06e	0.85±0.01fg	5.71±0.67hi	5.12±0.80f	60.76±0.69g	
		5	284.80±19.61abc	1649.00±10.79d	0.83±0.01efg	4.81±0.44fgh	3.94±0.20e	56.86±0.66f	
		15	288.40±8.56abc	1509.20±7.50c	0.81±0.01ef	4.24±0.18efg	3.41±0.20e	54.00±0.50e	
		25	301.60±12.74bc	1412.40±46.96bc	0.79±0.02cde	3.69±0.34cde	2.81±0.15d	48.64±0.69c	
	Punta de Tomate	Non-mycorrhizal	0.02	306.20±15.02bc	1491.20±73.21c	0.80±0.02de	3.89±0.45ef	2.10±0.05c	53.80±0.77e
			5	370.80±31.52d	1438.60±11.37bc	0.74±0.02bcd	2.90±0.35bcd	1.30±0.10b	50.89±0.96d
			15	376.00±33.30d	1336.60±39.78b	0.72±0.03b	2.59±0.42ab	1.01±0.08b	45.40±1.01b
		Mycorrhizal	25	380.80±24.49d	1014.60±65.23a	0.62±0.05a	1.68±0.34a	0.25±0.02a	39.26±0.69a
0.02			262.00±7.38a	1806.80±123.13e	0.86±0.02g	5.91±0.68i	5.08±0.78f	59.83±0.95g	
5			281.80±18.43abc	1644.20±10.62d	0.83±0.01efg	4.85±0.42gh	3.90±0.23e	55.92±0.71f	
15			286.60±9.61abc	1501.80±7.56c	0.81±0.01ef	4.25±0.20efg	3.44±0.34e	53.31±0.51e	
25			298.80±12.74abc	1403.00±35.60bc	0.79±0.01cde	3.70±0.31cde	2.78±0.20d	47.78±0.54c	
Provenance		P= 0.451	P= 0.687	P= 0.781	P= 0.631	P= 0.925	P < 0.001		
Inoculation		P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001		
Salinity		P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001		
Provenance x Inoculation		P= 0.932	P= 0.950	P= 0.980	P= 0.817	P= 0.831	P= 0.576		
Provenance x Salinity		P= 0.990	P= 0.999	P= 0.998	P= 0.964	P= 0.996	P= 0.914		
Inoculation x Salinity		P= 0.001	P < 0.001	P < 0.001	P= 0.522	P= 0.017	P < 0.001		
Provenance x Inoculation x Salinity		P= 0.741	P= 0.407	P= 0.543	P= 0.867	P= 0.625	P= 0.358		

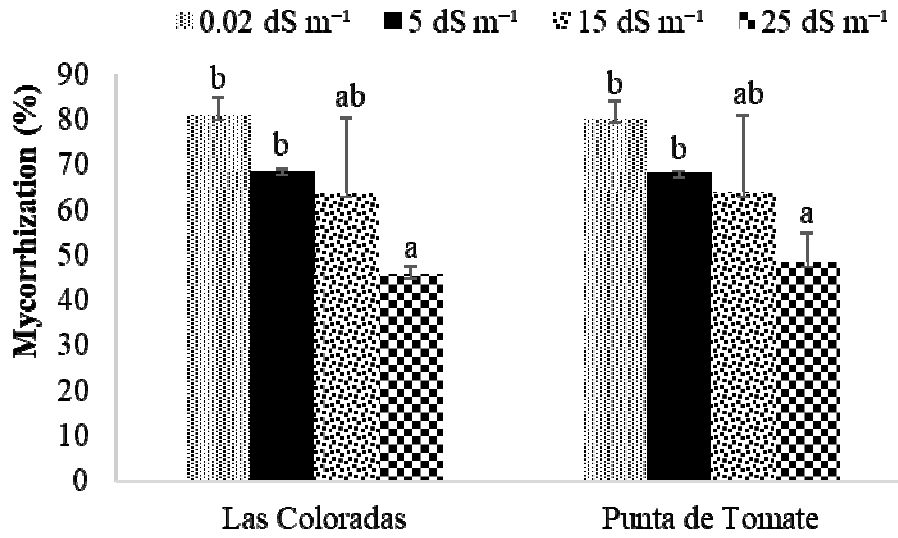
533 Different letters indicate significant differences for P ≤ 0.05. Values are the means±SD (n=10).

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535 **Table 4.** Effect of provenance, inoculation and salinity on relative water content (RWC),
 536 foliar (Ψ_{wf}) and the xylem (Ψ_{wx}) water potential in leaves of *C. uvifera* seedlings.

Provenance of <i>C. uvifera</i>	Inoculation treatment	Salinity (dS m ⁻¹)	RWC (%)	Ψ_{wf} (MPa)	Ψ_{wx} (MPa)
Las Coloradas	Non-mycorrhizal	0.02	77.09±1.37efgh	-0.70±0.00b	-0.50±0.00b
		5	88.60±4.04ij	-1.50±0.00d	-1.03±0.02d
		15	68.88±2.87cde	-2.50±0.00f	-1.94±0.06f
		25	58.24±7.17ab	-4.00±0.00h	-3.12±0.10h
	Mycorrhizal	0.02	93.74±3.70jk	-0.51±0.01a	-0.32±0.01a
		5	98.46±5.32k	-0.98±0.04c	-0.74±0.04c
		15	78.78±4.34fgh	-2.00±0.00e	-1.44±0.03e
		25	74.57±2.09defg	-3.30±0.00g	-2.40±0.07g
Punta de Tomate	Non-mycorrhizal	0.02	69.09±2.93cde	-0.72±0.04b	-0.55±0.04b
		5	80.60±2.54ghi	-1.53±0.05d	-1.06±0.03d
		15	60.88±4.38bc	-2.54±0.05f	-1.97±0.07f
		25	50.24±5.68a	-4.05±0.05h	-3.24±0.04h
	Mycorrhizal	0.02	85.74±5.15hij	-0.53±0.05a	-0.32±0.03a
		5	90.46±3.84jk	-1.01±0.06c	-0.73±0.08c
		15	70.78±5.90def	-2.04±0.05e	-1.48±0.04e
		25	66.77±0.72bcd	-3.35±0.05g	-2.48±0.05g
Provenance		P < 0.001	P < 0.001	P < 0.001	
Inoculation		P < 0.001	P < 0.001	P < 0.001	
Salinity		P < 0.001	P < 0.001	P < 0.001	
Provenance x Inoculation		P= 0.979	P= 0.999	P= 0.077	
Provenance x Salinity		P= 0.999	P= 0.303	P= 0.002	
Inoculation x Salinity		P= 0.008	P < 0.001	P < 0.001	
Provenance x Inoculation x Salinity		P= 0.477	P= 0.358	P= 0.292	

537 Different letters indicate significant differences for $P \leq 0.05$. Values are the means±SD
 538 (n=10).
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Figure 1. Effect of ectomycorrhizal inoculation and salinity on the mycorrhization of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviations of mean values ($n = 10$).

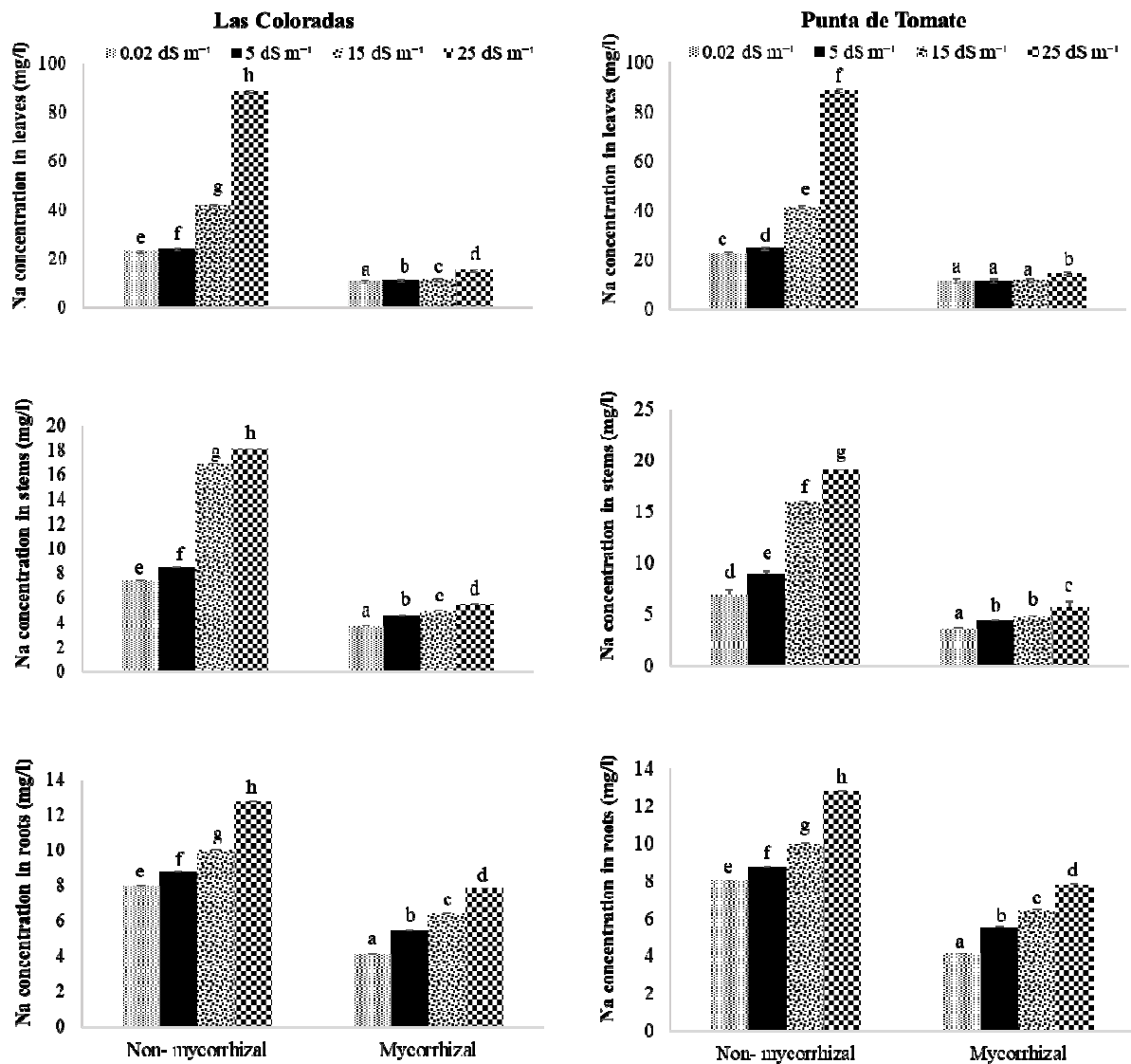


Figure 2. Effect of ectomycorrhizal inoculation and salinity on Na concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviations of mean values ($n = 10$).

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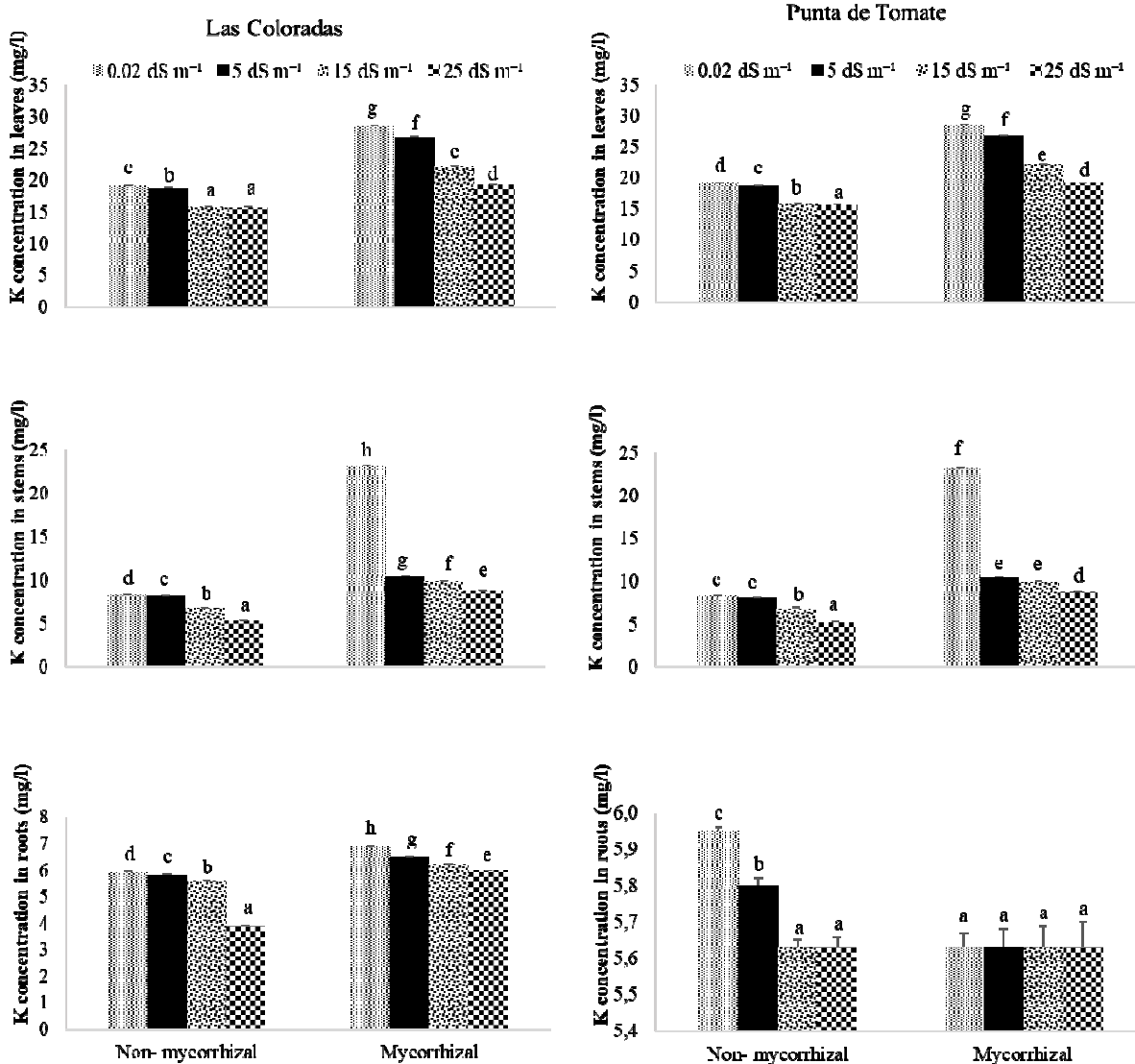


Figure 3. Effect of ectomycorrhizal inoculation and salinity on K concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviations of mean values ($n = 10$).

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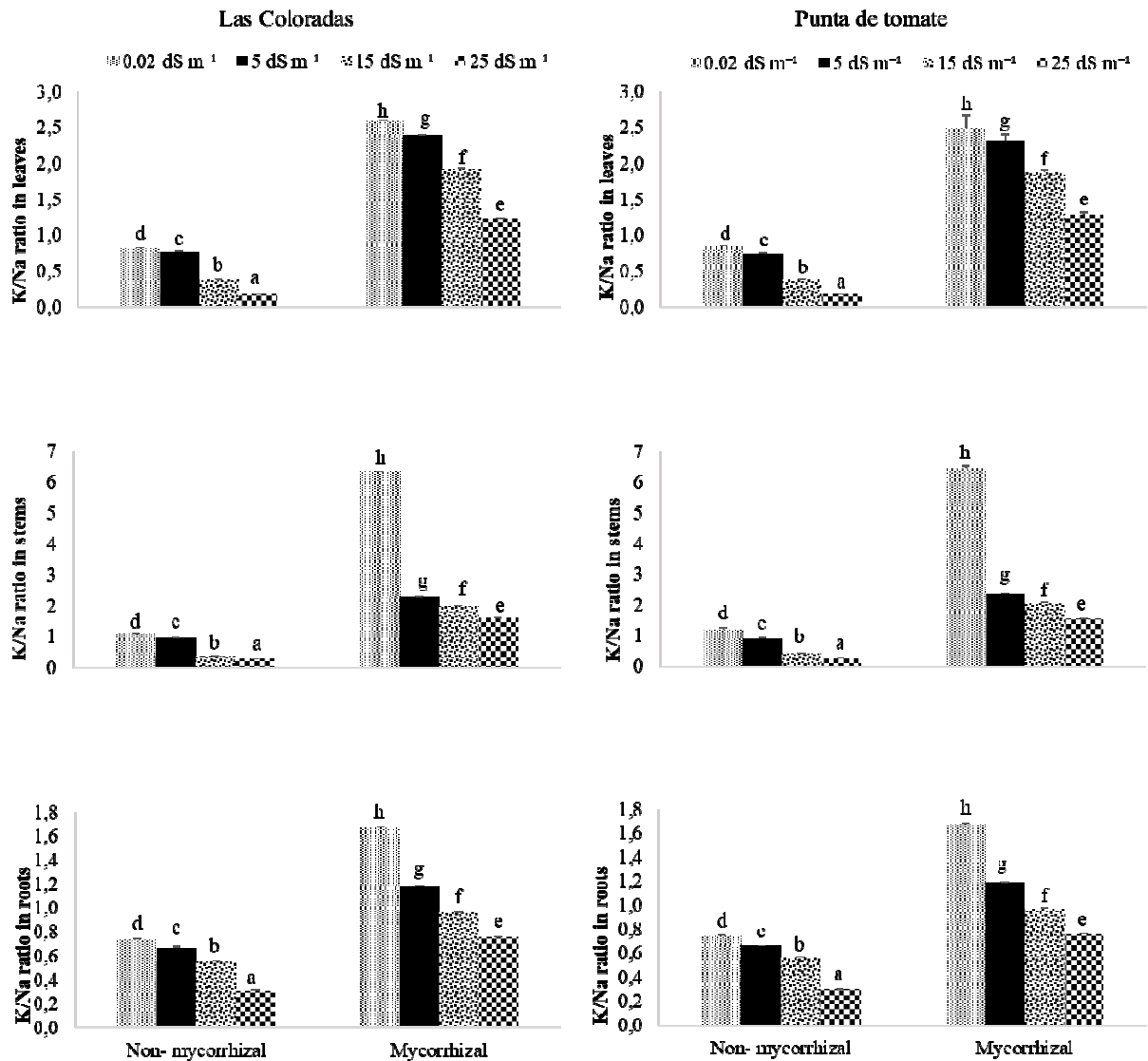


Figure 4. Effect of ectomycorrhizal inoculation and salinity on K/Na concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviations of mean values ($n = 10$).

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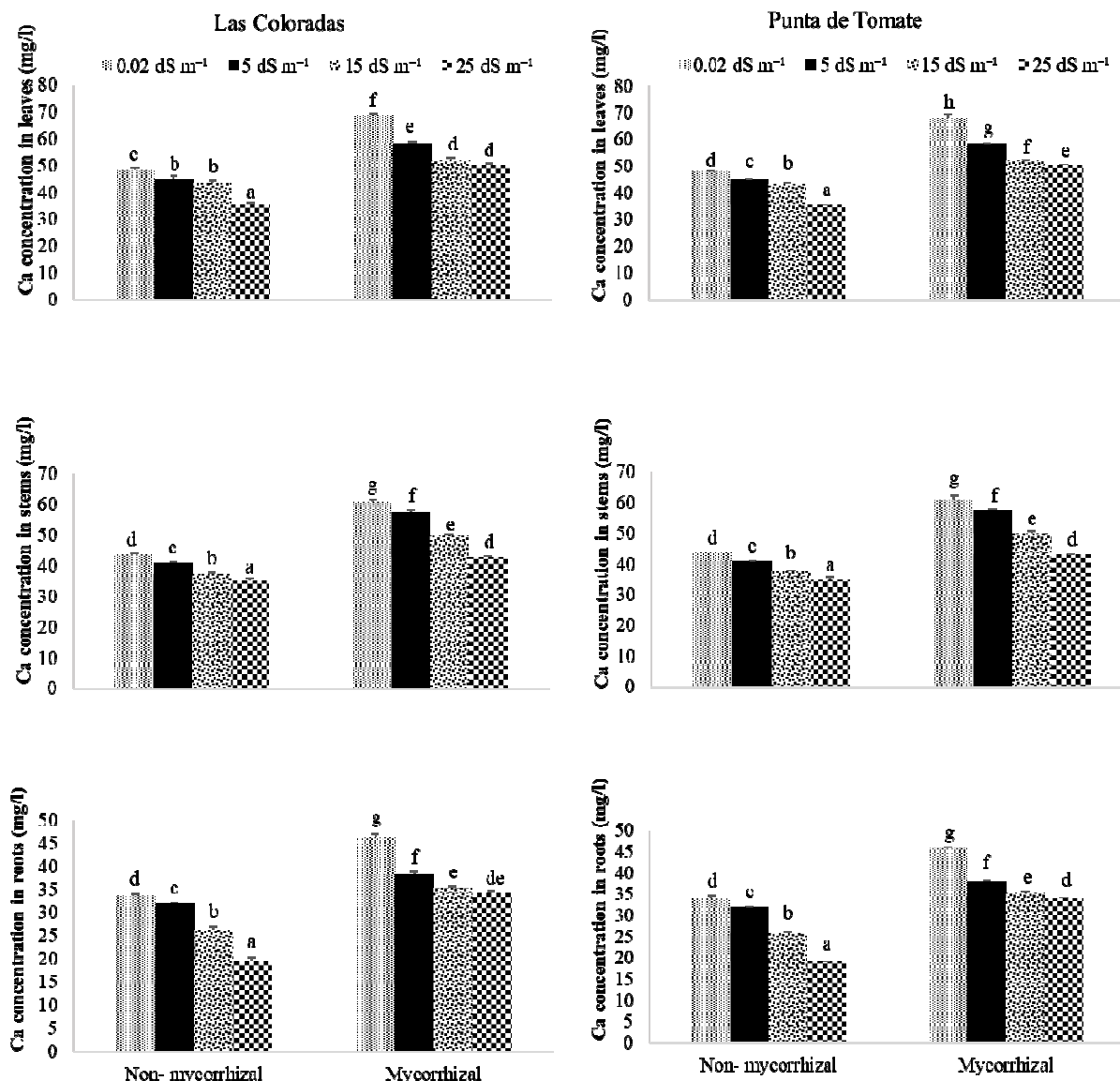


Figure 5. Effect of ectomycorrhizal inoculation and salinity on Ca concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviations of mean values ($n = 10$).

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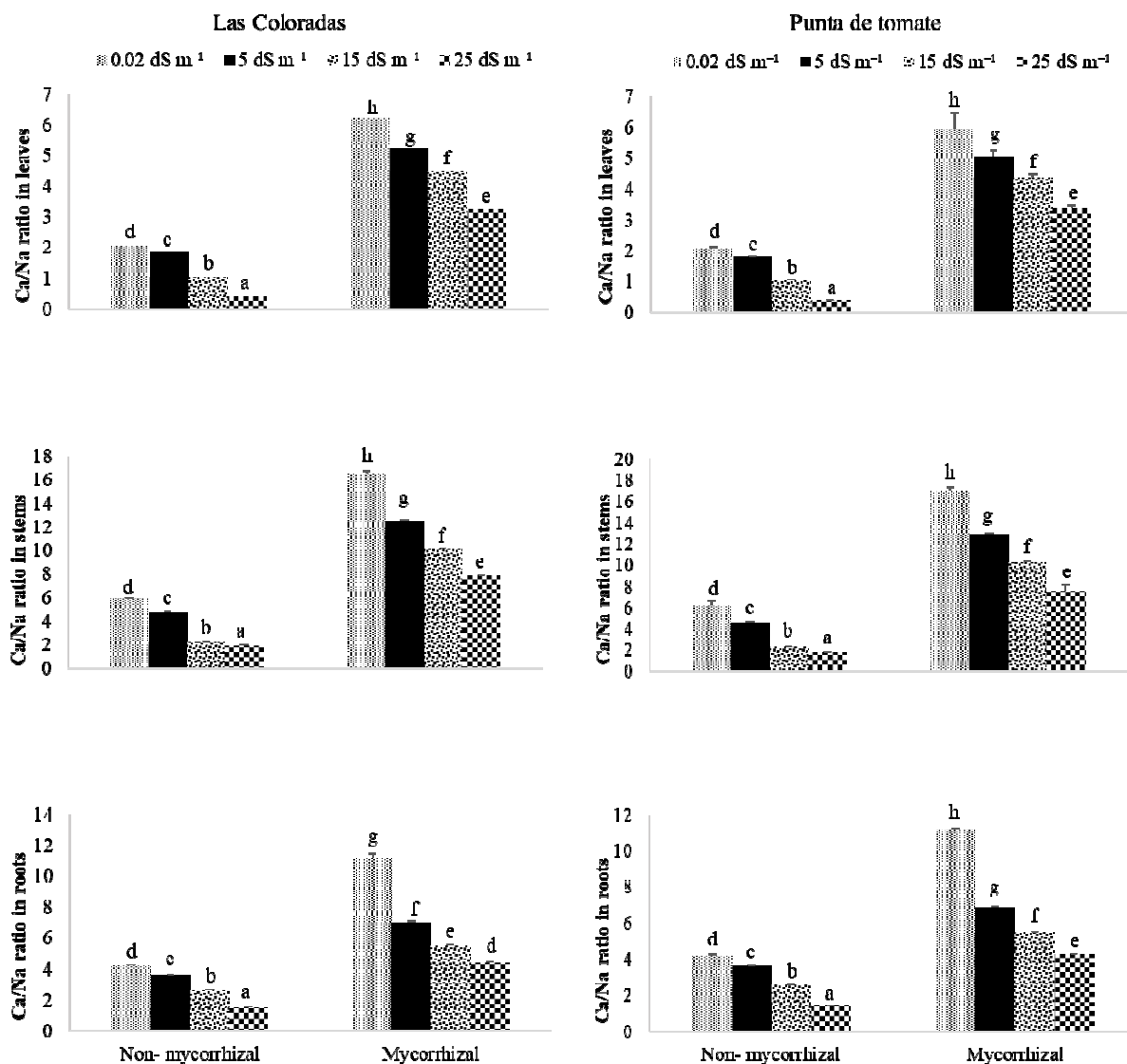


Figure 6. Effect of ectomycorrhizal inoculation and salinity on Ca/Na concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$. Vertical bars indicate standard deviations of mean values ($n = 10$).