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ABSTRACT

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

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

1. Introduction

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

It is well known that salinity provokes osmotic and ionic stresses in plants (Safdar et al., 2019). To cope with salt stress, halophytic plants develop mechanisms to reduce the toxicity of Na in the cytoplasm through restricting Na uptake, increasing the efflux of Na, and compartmentalizing Na in the vacuole (Yang and Guo, 2018). Plants also develop mechanisms to increase K in the cytoplasm to maintain a suitable K/Na and Ca/Na ratio and to prevent cellular damages and nutrient deficiency (Munns and Tester, 2008). The K ion is the major osmotically solute in the guard cells of open stomata and controls the influx of CO_2 and the efflux of water vapor and O_2, which can directly influence both photosynthetic and transpiration rates. The Ca ion acts as a second messenger in salt stress signaling (Munns and Tester, 2008).

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

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

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

2. Materials and methods

2.1. Plant material and germination

Mature fruits of *C. uvifera* were collected from Las Coloradas beach (provenance LC) (19º55'31,9" N, 77º41'14,1" W) and Punta Tomate beach (provenance PT) (21º.16'16.6'' N, 76º31'18.5'' W) in Cuba. The fruits were washed with tap water to eliminate surrounding pulp. The seeds were air dried at room temperature for 72 hours on cardboard trays. To break dormancy, the seeds were scarified in 95% sulfuric acid (H_2SO_4) for two hours and every 20 minutes the container was shaken to achieve the uniform action of the acid on the surface of the seeds (Bandou et al., 2006). Then, they were rinsed with abundant distilled water, kept in water for 24 hours and transferred to the sterile substrate incubating them for 5 to 8 days at room temperature before transplanting. The substrate is a sandy soil collected along the Yao River (20º15'32.3" N, 76º45'19.3" W) located in the town known as La China in Cuba. The sandy soil was sterilized at 121 °C and 1.2 kg/cm^2 pressure in an autoclave. The nutrient contents of the heat-sterilized sandy soil were as follows (ppm): 65 K, 25.4 Na, 80.19 Ca, 29.57 Mg, 3.1 Olsen-P, pH (H_2O) 7.5 and electrical conductivity (EC) 0.84 dS m^{-1}. The sandy soil was analyzed in the soil laboratory of the Ministry of Agriculture of Granma province (Cuba).

2.2. Fungal material, inoculation and experimental design

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

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

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

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

2.3. Photosynthesis and gas exchange parameters

The photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs) and sub-stomatal CO2 (Ci) were non-destructively measured using a portable open flow gas exchange system (ADC BioScientific. LCpro-SD, United Kingdom) from 9:00 to 11:00 a.m. **(civil time).** The fourth fully expanded leaf of each plant (ten plants per treatment, n = 10) was used for the measurements. Photosynthesis and gas exchange parameters were measured at a temperature of 35.5 ºC, a relative humidity of 79%, a CO_2 concentration of 414.54 ppm and an active photosynthetic irradiation of 1,950 µmol m^{-2} s^{-1}.

2.4. Chlorophyll fluorescence and content

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

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

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

2.5. Water status and plant biomass

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

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

RWC = [(FM - DM)/(TM - DM)] x100.

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

RMD = [Biomass of ECM plants - Biomass of non-ECM plants] ×100/Biomass of ECM plants.

2.6. Ectomycorrhizal colonization

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

2.7. Concentrations of Na, K and Ca

The roots, stems and leaves (ten plants per treatment, n = 10) were dried at 80 ºC until constant weight. They were crushed in a blade mill to particles smaller than 0.5 mm and 0.2 g were digested in a mixture of $HClO_4/HNO_3$ (v/v 1:5) diluted with 100 mL of distilled water. To determine the concentration of Na, K and Ca, each sample was analyzed in an atomic absorption spectrophotometer (PinAAcle. 900T. USA) in the Department of Plant Sciences, Faculty of Agriculture, University of Rostock (Germany).

2.8. Statistical analysis

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

3. Results

3.1. Root colonization and plant growth

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

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

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

3.2. Photosynthesis and gas exchange

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

3.3. Chlorophyll fluorescence and content

The three factors (provenance, inoculation and salinity) and their interactions had significant effects ($P \leq 0.05$) depending on parameters studied (Table 3). There was no significant effect of the provenance on these parameters except for SPAD values **($P<0.001$). However, the two factors (inoculation and salinity) and their interactions were significant for all parameters (Table 3).** In the absence of salt, basal fluorescence (Fo) values were significantly lower for ECM than non-ECM plants, whereas values of maximun fluorescence (Fm) were higher for ECM than non-ECM plants (Table 3). A Similar trend occurred for Fo and Fm values in ECM and non-ECM plants in the presence of salt (Table 3). In the absence of salt, Fv/Fm, PI_{ABS} and SPAD values were higher for ECM than non-ECM plants (Table 3). Increased salinity reduced the Fv/Fm and PI_{ABS} in non-ECM plants more than those in ECM plants. However, in ECM plants, increased salinity did not cause a significant decrease in Fv/Fm and PI_{ABS} parameters. Values of Fv/Fm and PI_{ABS} were higher in ECM than in non-ECM plants under saline conditions.

3.4. Plant water status

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

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

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

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

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

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

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

3.6. Relations between datasets

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

4. Discussion

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

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

The parameters A, gs, E and Ci were significant positive correlated with the improved total biomass in the leaves, stems and roots of ECM plants exposed to saline conditions. Indeed, the presence of ECM fungus, seagrape provenances had higher stomatal conductance, transpiration and photosynthetic rates, and sub-stomatal CO_2 concentration in the mesophyll than those of non-ECM plants under salt stress. This result suggests that ECM fungus can elevate the photosynthetic ability of seagrape seedlings thanks to an improving of the stomatal conductance and gas exchange capacity under salt stress. Our study is in consistence with work of Shi-Chu et al. (2019) which showed that increasing salt concentration led to a significant decrease of A *via* a decrease in the gs, which was less important in AM than non-AM alfalfa. The decrease of gs due to the stomatal closure is often related to the water status of plants (Mohamed et al., 2020). Under water deficit conditions due to salt stress, the plants reduce transpiration rate more in non-ECM than ECM plants by closing stomata to reduce water loss. Furthermore, in this study, a higher gs in the ECM *vs* non-ECM plants under salt stress was positively correlated to the increased of CO_2 diffusion through the stomata and water absorption. As a result, the photosynthesis and water status may be better in ECM plants. Measurements of RWC, Ψwf and Ψwx are considered informative on water status and transpiration rate in plants under salt stress (Larcher, 1995; Chen et al., 2017). It is also well known that the accumulation of salts in the root zone causes an increase in the osmotic potential and, consequently,–the water potential decreases in the rhizosphere (Augé et al., 2008). Here, despite their higher evaporative leaf surface, the ECM plants had higher RWC, Ψwf and Ψwx than non-ECM plants regardless of salinity and seagrape provenances,

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

Our study also indicated that the response of ECM plants to the ratio of Fv/Fm, PI_{ABS} and chlorophyll content was greater than those in non-ECM plants, suggesting that the ECM symbiosis could enhance the chlorophyll concentration and fluorescence of seagrape leaves, which is consistent with the results of other studies (Sheng et al., 2018). Indeed, plants trap photons in chlorophylls of PSII from sunlight and use them to split H_2O into O_2, H^+ and e- during light reaction phase of photosynthesis. Moreover, salt stress decreases the concentration of chlorophyll and inhibits the electron transport chain to produce ATP and NaDPH (Shi-Chu et al., 2019). Similarly, to our study, previous works showed that the AM fungus-plant symbiosis with a greater chlorophyll than that in non-AM, presented also higher rates of photosynthesis under salt stress (Elhindi et al., 2017).

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

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

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

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

Provenance of *C. uvifera*	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

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

Table 2. Effect of provenance, inoculation and salinity on stomatal conductance (gs), photosynthetic (A) and transpiration (E) rates and sub-stomatal CO_2 (Ci) in leaves of *C. uvifera* seedlings.

Provenance of *C. uvifera*	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

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

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 (PIabs) and chlorophyll quantification (SPAD) in leaves of *C. uvifera* seedlings.

Provenance of *C. uvifera*	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
		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
	Mycorrhizal	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

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

Table 4. Effect of provenance, inoculation and salinity on relative water content (RWC), foliar (Ψwf) and the xylem (Ψwx) water potential in leaves of *C. uvifera* seedlings.

Provenance of *C. uvifera*	Inoculation treatment	Salinity (dS m^{-1})	RWC (%)	Ψwf (MPa)	Ψwx (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

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

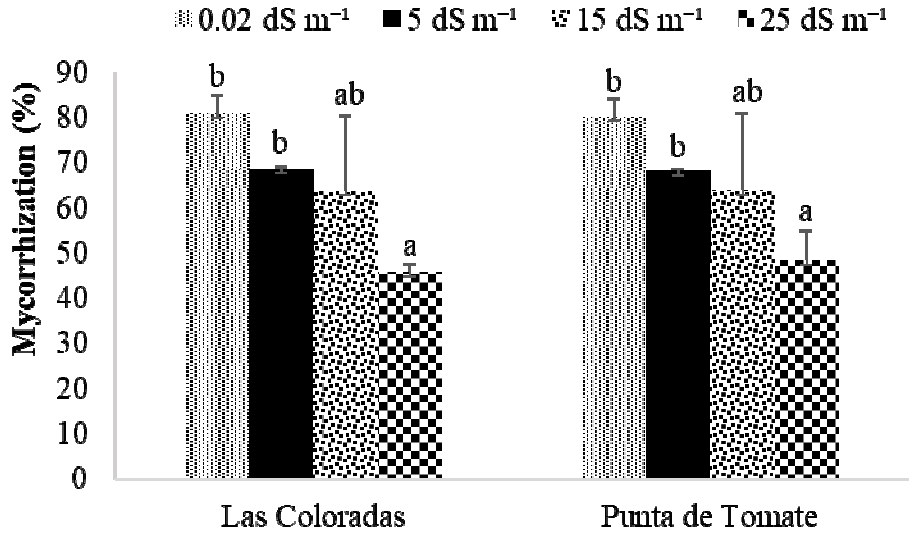

Figure 1. Effect of ectomycorrhizal inoculation and salinity on the mycorrhization of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$. Vertical bars indicate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

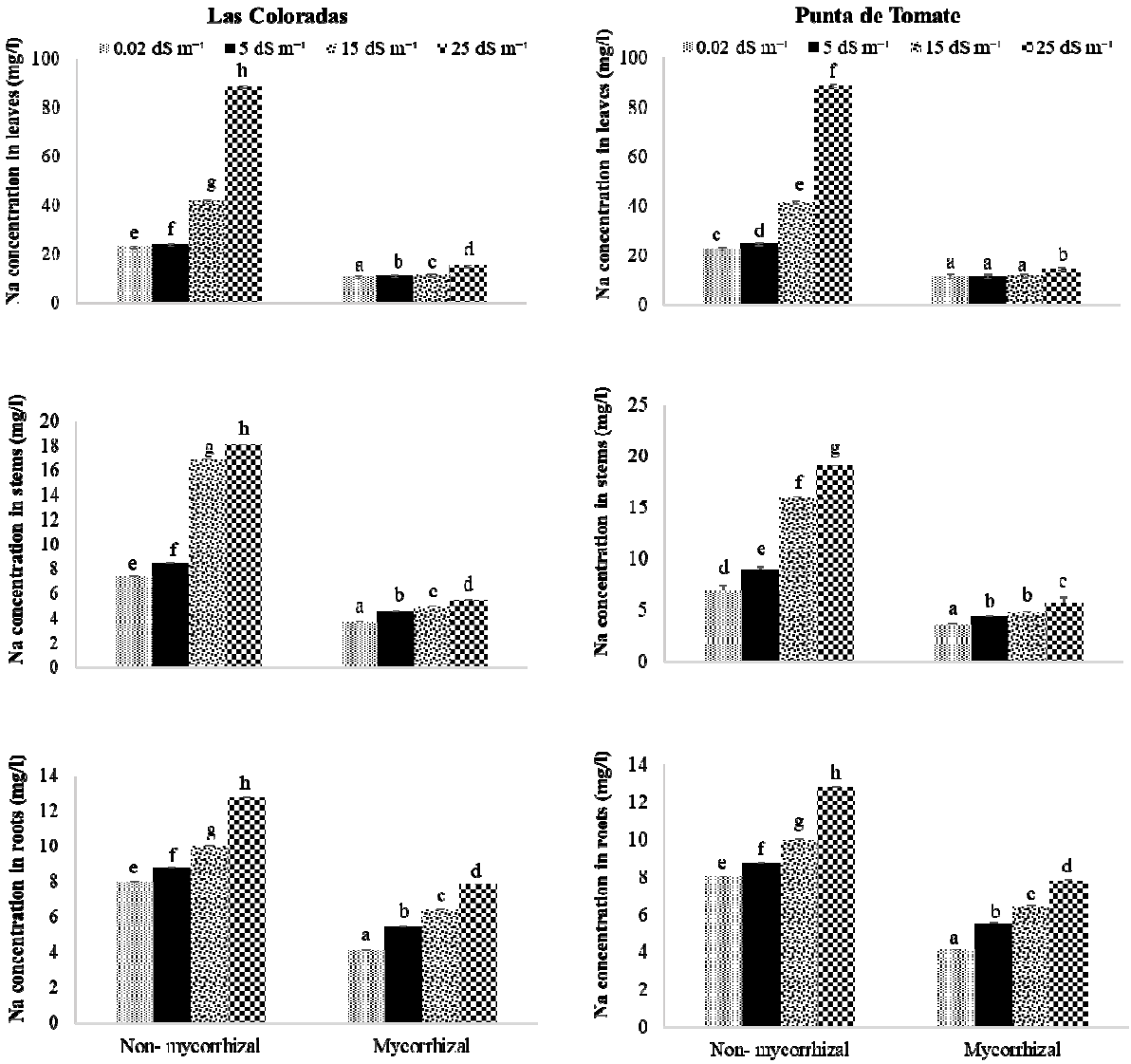

Figure 2. Effect of ectomycorrhizal inoculation and salinity on Na concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$.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

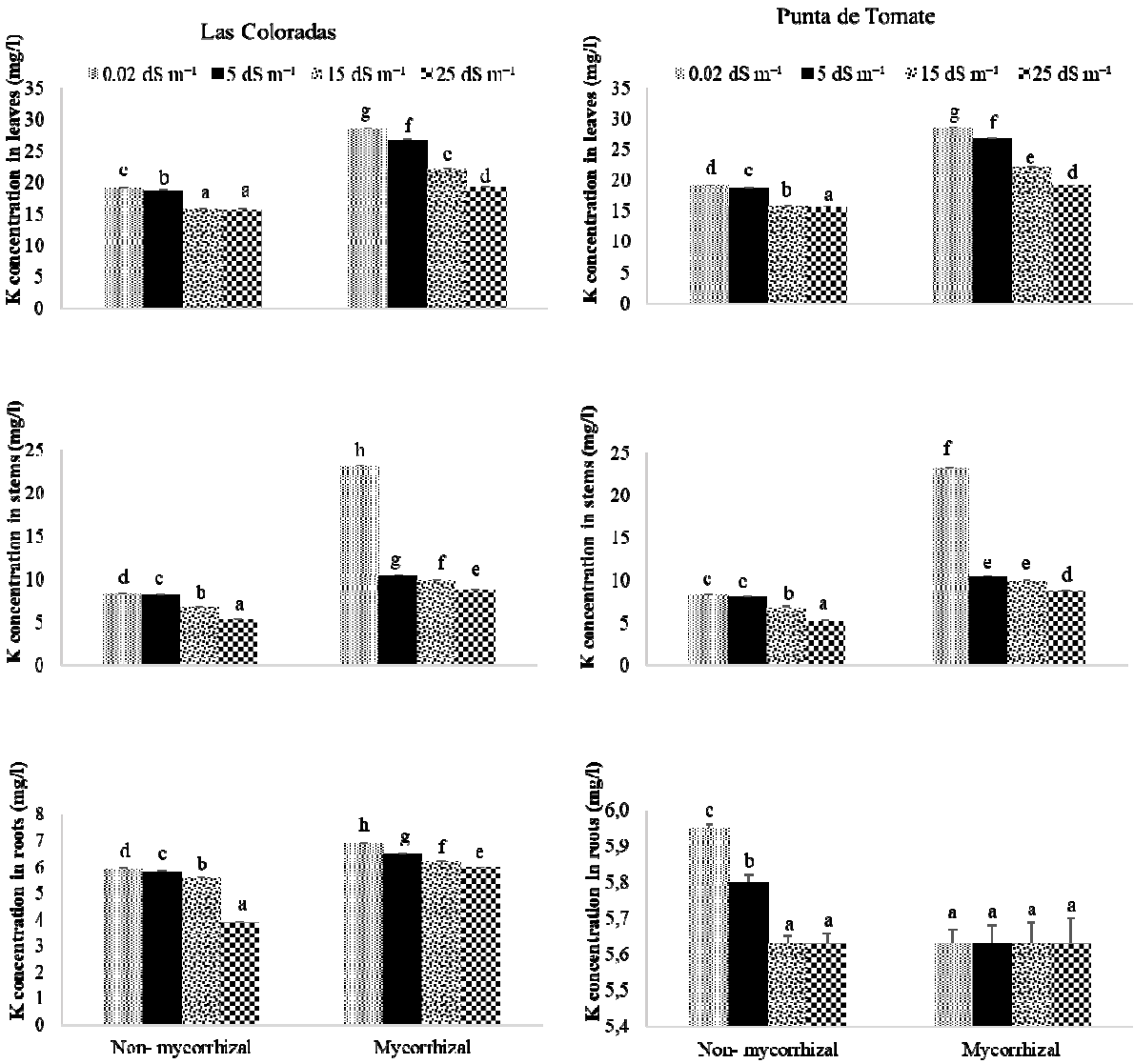

Figure 3. Effect of ectomycorrhizal inoculation and salinity on K concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$.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

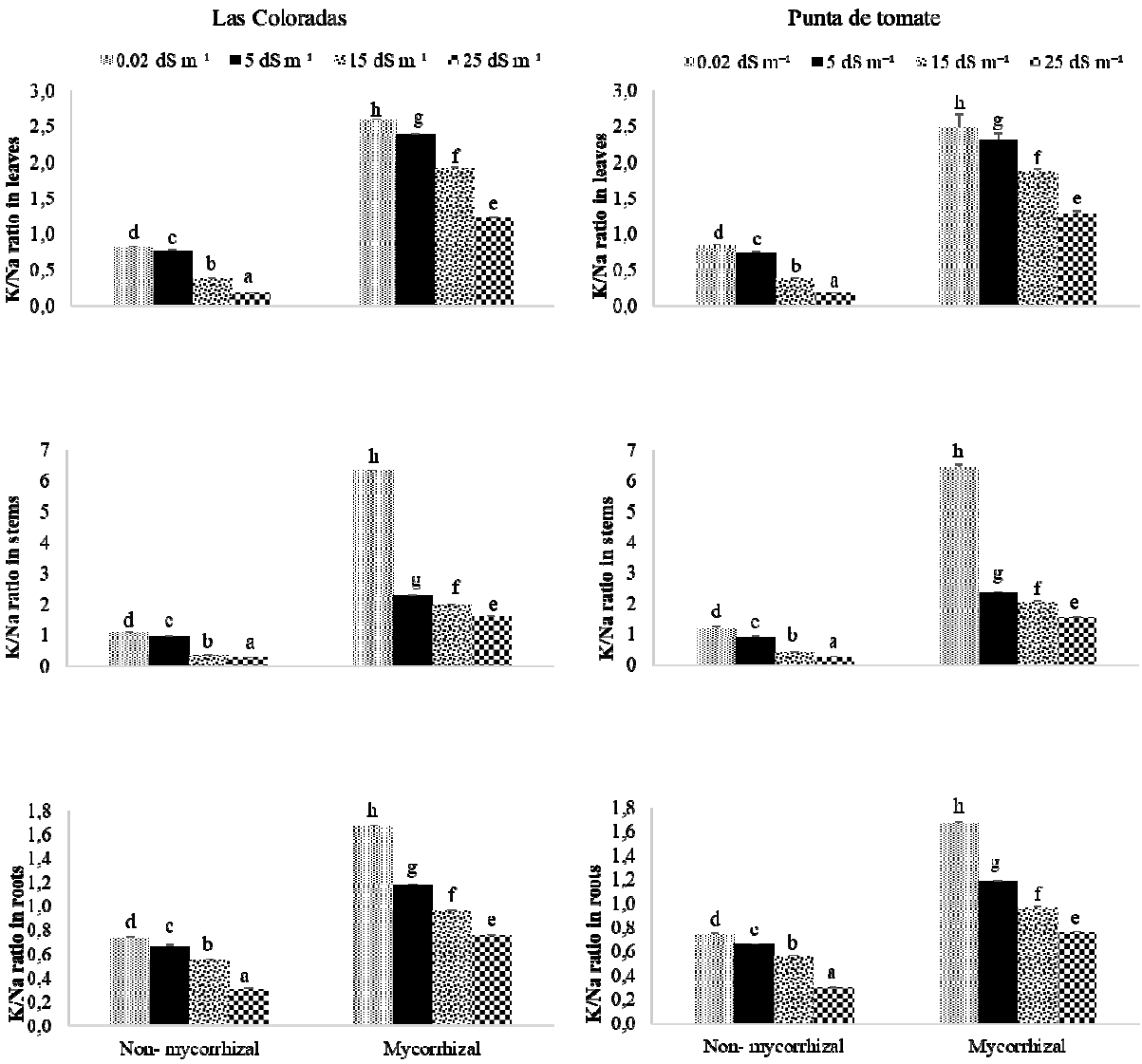

Figure 4. Effect of ectomycorrhizal inoculation and salinity on K/Na concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$.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

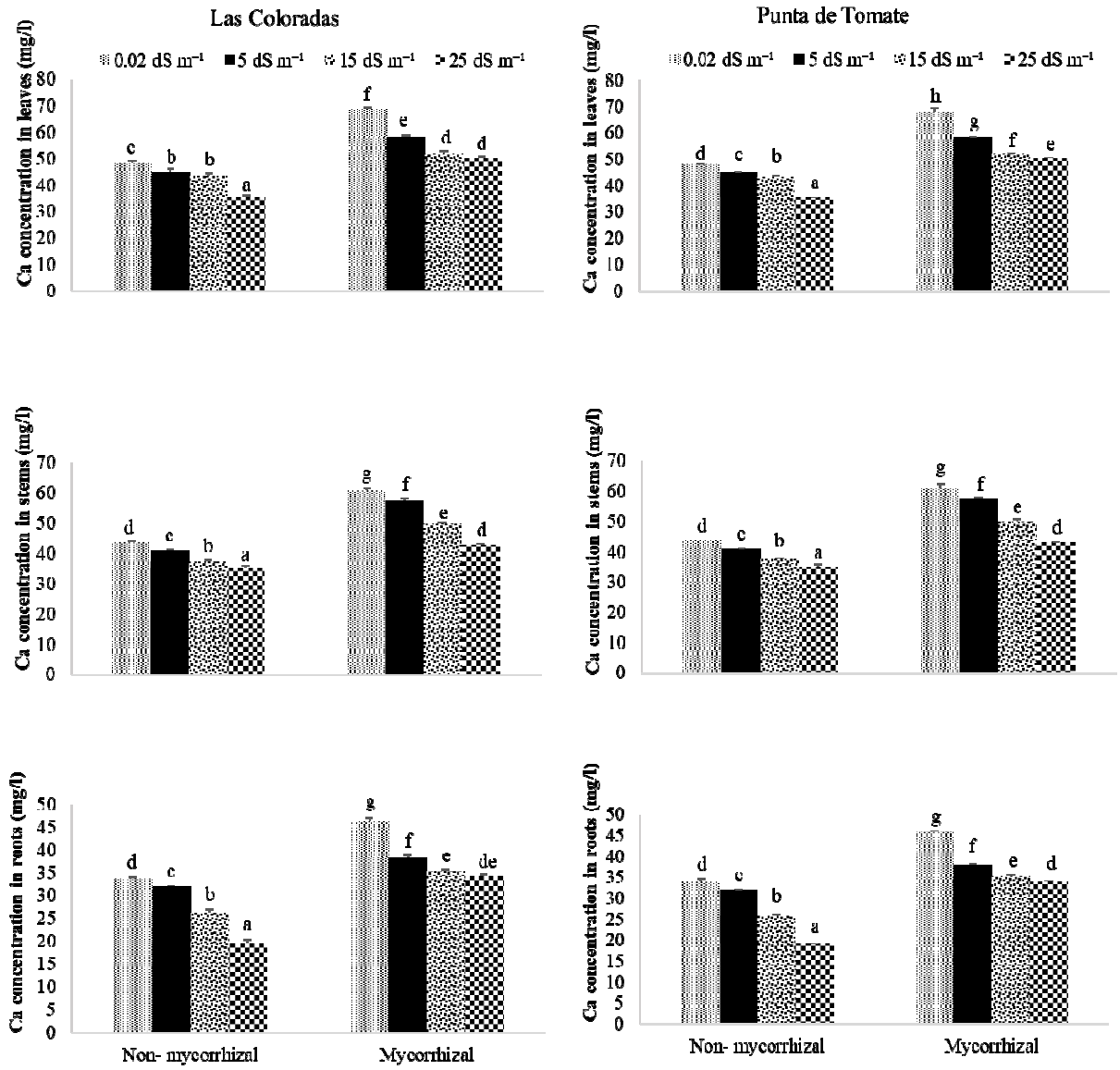

Figure 5. Effect of ectomycorrhizal inoculation and salinity on Ca concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$.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

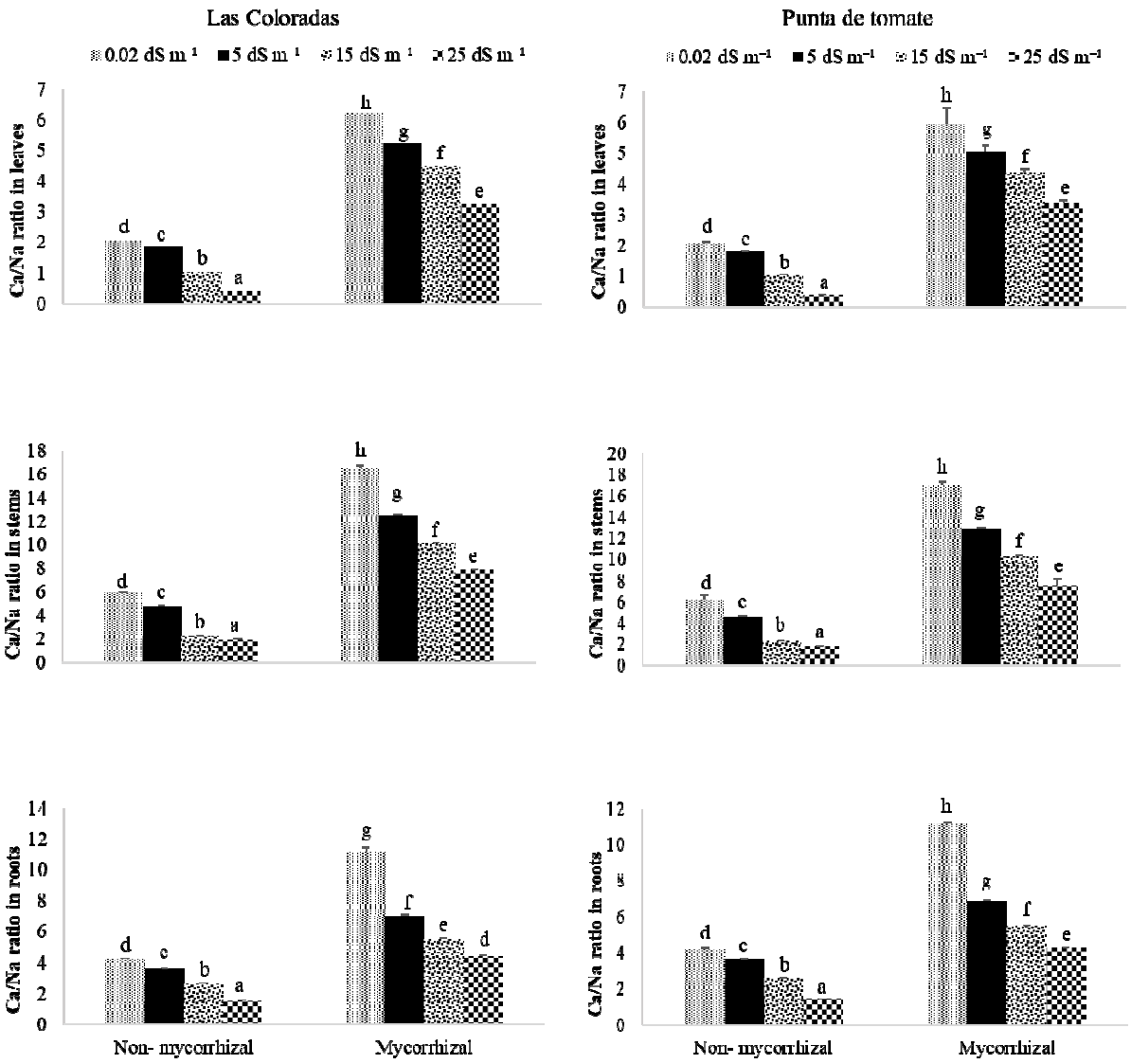

Figure 6. Effect of ectomycorrhizal inoculation and salinity on Ca/Na concentration in leaves, stems and roots of seedlings from each provenance (Las Coloradas and Punta Tomate) of *C. uvifera*. Bars topped with different letters are significant different according to the Tukey HSD test at $P \leq 0.05$.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

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