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ORIGINAL PAPER

The ectomycorrhizal fungus *Scleroderma bermudense* alleviates salt stress in seagrape (*Coccoloba uvifera L.*) seedlings

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Abstract The purpose of this study was to test the capacity of the ectomycorrhizal (ECM) fungus, *Scleroderma bermudense*, to alleviate saline stress in seagrape (*Coccoloba uvifera* L.) seedlings. Plants were grown over a range (0, 200, 350 and 500 mM) of NaCl levels for 12 weeks, after 4 weeks of non-saline pre-treatment under greenhouse conditions. Growth and mineral nutrition of the seagrape seedlings were stimulated by *S. bermudense* regardless of salt stress. Although ECM colonization was reduced with increasing NaCl levels, ECM dependency of seagrape seedlings increased. Tissues of ECM plants had significantly increased concentrations of P and K but lower Na and Cl concentrations than those of non-ECM plants. Higher K concentrations in the leaves of ECM plants suggested a higher osmoregulating capacity of these plants. Moreover, the water status of ECM plants was improved despite their higher evaporative leaf surface. The results suggest that the reduction in Na and Cl uptake together with a concomitant increase in P and K absorption and a higher water status in ECM plants may be important salt-alleviating mechanisms for seagrape seedlings growing in saline soils.

Keywords *Coccoloba uvifera* · Ectomycorrhizal dependency · Mineral uptake · Salt stress · Water status

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Introduction

Salinity affects more than 7% of the Earth's land area (Parida and Das 2005). Most of this salinity is natural but the extent of saline soils is increasing in a significant proportion of cultivated agriculture land because of land clearing or irrigation (Munns 2005). It is considered as one of the most significant environmental factors limiting plant growth and productivity (Tian et al. 2004). Salinity affects plant growth and survival of glycophytes (Munns 2005). To overcome salt-stress problems, it is

possible to select salt-tolerant plants, to use biological processes such as mycorrhizal interactions, or to desalinate soil by leaching excessive salts (Al-Karaki et al. 2001; Feng et al. 2002; Munns 2005; Parida and Das 2005). The desalination of soils is not economically viable for sustainable agriculture. Although the results are somewhat variable, salt-tolerant plants develop a plethora of strategies to cope with salt stress including selective accumulation or exclusion of ions, control of ion uptake by roots and transport into leaves, compartmentalization, and recirculation of ions at the cellular and whole-plant levels (Berthomieu et al. 2003; Parida and Das 2005). There is considerable evidence that arbuscular mycorrhizal (AM) fungi can improve plant growth and nutrition in soils subject to a range of saline stress (Juniper and Abbott 1993; Gupta and Krishnamurthy 1996; Al-Karaki 2000; Ruiz-Lozano and Azcon 2000; Al-Karaki et al. 2001; Yan-Melo et al. 2003; Giri and Mukerji 2004; Tian et al. 2004). Mycorrhiza can help moderately salt-tolerant plants to prevent Na and Cl translocation to shoot and leaf tissues (Giri and Mukerji 2004), and improved salt tolerance after mycorrhizal colonization may also result from more efficient P uptake by mycorrhizal plants in P-deficient soils, leading to increased growth and subsequent dilution of toxic ion effects (Juniper and Abbott 1993). In contrast, little is known about the role of the ectomycorrhizal (ECM) symbiosis in enhancing salt tolerance of trees (Dixon et al. 1993; Mushin and Zwiazek 2002), despite several in vitro studies showing that salt-tolerant ECM fungi may be interesting for their potential use under field conditions (Hutchison 1990; Chen et al. 2001; Kernaghan et al. 2002; Bois et al. 2006).

Coccoloba uvifera (L.) L. (Polygonaceae), also named seagrape, is a small tree widely distributed along the Atlantic, Caribbean and Pacific coasts of the American tropics and subtropics (Parrota 1994). It is an important tree for edible fruits, ornamental plantings and coastal windbreaks along Caribbean beaches and roadsides. Seagrape is considered as a drought-hardy and non-halophytic woody plant relatively tolerant to salt, growing often in pure stands within well-drained sandy soils that are slightly to moderately alkaline (Parrota 1994; Larcher 1995). This tree is naturally associated with ECM fungi belonging to the genera *Amanita, Russula, Lactarius, Xerocomus, Inocybe* and *Scleroderma* (Kreisel 1971; Pegler 1983; Miller et al. 2000; Guzman et al. 2004; Bandou 2005). Although the ECM status of seagrape was reported, the role of ECM fungi in seagrape growth and nutrition remains unknown. In the present study, we tested the capacity of an ECM fungus naturally associated with seagrape, *Scleroderma bermudense*, to alleviate saline stress and to stimulate growth and nutrition in seagrape seedlings under greenhouse conditions.

Materials and methods

The substrate used was a mixture of heat-sterilized pouzzolane (crushed volcanic rock with particle size average 2-mm) and vermiculite (4:1; v/v). The nutrient contents of the heat-sterilized crushed volcanic rock were the following (ppm): 4.28 K, 15.67 Na, 6.36 Ca, 4.99 Mg, 1.26 NH_4^+, 2.75 NO_3^- (H_2O-extractable), 0.12 Olsen-P. pH (H_2O) was 8.41, pH (KCl) 7.2, total salt 0.11 g/l and electrical conductivity 0.036 mS/cm.

The ECM fungus used, *S. bermudense* (UAG sp. 6), was isolated from one sporocarp collected from under a seagrape stand along Viard's Beach in Guadeloupe, Lesser Antilles. A herbarium reference voucher (BA 03.10.31.01) is given to the sporocarp. Cultures were maintained at 30°C on agar MMN medium (Marx 1969). Glass jars (1·l) were half-filled with a mixture of vermiculite and peat moss (4:1; v/v) and autoclaved (120°C, 20 min). The substrate was moistened with 300-ml liquid MMN medium and autoclaved again. Ten plugs (0.5 cm diameter) of *S. bermudense* were aseptically removed from the edge of 1 month-old cultures and inoculated per jar. The substrate was well-colonized by the ECM fungus after 1–2 months at 30°C. The resulting fungal inoculum was mixed with the heat-sterilized pouzzolane (1:10; v/v), and 500 g portions of the mixture were transferred into black plastic pots (11 cm deep, 9 cm diameter). Non-inoculated controls received a mixture of non-inoculated moistened vermiculite-peat moss with nutrient solution. Pots were filled to within 1 cm of the rim. Substrate leakage was prevented by placing a wad of polyester fiber at the bottom of each pot.

Mature fruits of seagrape were collected in June 2004 along Viard's Beach in Guadeloupe. Seeds from fruits were washed with tap water to eliminate surrounding pulp and then air-dried at room temperature 5 days before use. To break dormancy, seeds were scarified in 95% sulfuric acid for 3 h, rinsed with sterilized demineralized water, transferred aseptically to 8‰ water agar in Petri dishes, and incubated for 5–8 days at 32°C in the dark. Seeds with approximately 2-cm long tap roots were planted two per pot. After emergence, the seedlings were thinned to one per pot. During the first 4 weeks, plants were grown under well-watered conditions with tap water without NaCl to avoid salt effects on ECM establishment. Then, plants were subjected to four salt levels by adding NaCl (0, 10, 20 and 30‰ equivalent to 0, 200, 350 and 500 mM) to pots. The NaCl concentration range was based on field assessment of soil salinity in seagrape stands. To avoid osmotic shock, the soil was salinized step-wise by increasing the concentration per every 3 days during 4 weeks. The pots were leached with tap water every week to reduce salt accumulation. Fresh salt solution was added immediately after each leaching to keep a constant NaCl concentration in the soil.

Salt levels were controlled using a temperature-compensated hand refractometer (ATAGO ATC-S, Inc., Bellevue, WA, USA). The plants were supplied each month with a Long Ashton's nutrient solution (Hewitt 1966) without P, and were grown in a greenhouse receiving approximately 205 W m^{-2} between February and June 2005, at 25°C–35°C with a day-length of about 12 h. Plants were harvested after they had been grown under salt stress conditions for 12 weeks.

At the end of the experiment, minimal leaf water potentials were measured at midday using a Scholander pressure chamber (PMS Instrument Co., USA), and leaf areas were determined using a CI-203 area meter (CID, Inc., USA.). Shoots and roots were harvested separately. Height, collar diameter and dry weight (7 days at 80°C) of leaves, shoots and roots were evaluated. For each inoculated and non-inoculated treatment, a sample of ten lateral roots was washed gently, dispersed in water, and numbers of root tips of ECM roots and non-colonized roots were counted under a stereomicroscope at ×100 magnification for each lateral root to determine the percentage of ECM colonization (number of ECM roots/total number of roots×100). ECM colonization was evaluated according to the grid-line intersection method modified by Brundrett et al. (1996) and was confirmed by microscope (×400) examination of root tips to determine the presence of a fungal mantle and Hartig net. The relative ECM dependency was calculated (biomass of ECM plants−biomass of non-ECM plants×100/biomass of ECM plants) at a given NaCl level (Plenchette et al. 1983). After drying and grinding, leaf, shoot and root samples were heated at 500°C and digested in hydrochloric acid for determination of P, K and Na. P was determined by colorimetry (Technicon) according to Novozamsky et al. (1983). K and Na were determined using flame photometry (Ryan et al. 1996). Cl contents in leaf, shoot and root samples were analyzed in the aqueous extract by colorimetry (Technicon) according to Zall et al. (1956).

The experiment was set up as a completely randomized 2×4 factorial design consisting of two inoculum treatments, four salt levels and ten replicates per treatment. Three replicates per treatment were used for mineral analyses. All data were subjected to two-way analysis of variance, and mean values were compared using Newman–Keuls multiple range test (Gagnon et al. 1989). Percentages of ECM colonization were calculated from arc sin (square root) transformed data.

Results

None of the seagrape seedlings from non-inoculated treatments were colonized by *S. bermudense* (Table 1). No contamination by other ECM fungi was observed on the inoculated seedlings. Ectomycorrhizas of the *S. bermudense* were characterized by a white and smooth mantle, sclerotia, and abundant mycelial strands. Seagrape seedlings inoculated with *S. bermudense* had root length colonization of 89.4%, but the extent of ECM colonization was significantly affected by salt treatments.

ECM colonization by *S. bermudense* improved seedling growth regardless of the salt level (Table 1). Collar diameter did not differ significantly between inoculated and non-inoculated plants, nor did height between inoculated and non-inoculated plants at 500 mM NaCl. Leaf, shoot and root dry weights were higher in ECM than in non-ECM plants. The overall biomass of ECM plants increased by 120% relative to the controls, regardless of salt stress. Salinity reduced the biomass of both ECM and non-ECM plants at all salt levels compared to the controls. At the highest salt level (500 mM), the ECM effect on total plant biomass production was 215% compared to the control plants. The ECM dependency of seagrape seedlings on *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, the promoting effect of *S. bermudense* on enhancing salinity tolerance of seagrape seedlings was not depressed.

The number of leaves and leaf areas were much higher for ECM than for non-ECM plants regardless of salt level (Table 2). Although leaf area declined in both ECM and non-ECM plants as salinity increased, leaf area of ECM plants remained superior to that of non-ECM plants whatever the salt level. Water status of plants was quantified by measuring minimum leaf water potential. In the absence of salt, minimum leaf water potentials were higher for ECM than non-ECM plants, suggesting an improvement of water status of plants inoculated with *S. bermudense*. They were also affected by salt stress in both ECM and non-ECM plants as salinity increased (Table 3), but they declined for 1.5-fold for non-ECM and for 2-fold for ECM plants except at 500 mM NaCl.

Na and Cl concentrations in leaves, shoots and roots of both ECM and non-ECM plants did not increase significantly as salinity increased from 200 to 500 mM (Tables 3 and 4). However, Na and Cl contents were generally much higher for non-ECM than ECM plants at a given NaCl level. Na and Cl contents were apparently higher in the leaves than in the shoots and roots at all salt treatments. There were significant negative correlations between Na and Cl concentrations in the leaves, shoots and roots and total biomass of seagrape seedlings (Table 5).

Only P and K contents in leaves were higher in ECM than in non-ECM plants regardless of the salinity level

Table 1 Effects of inoculation with *Scleroderma bermudense* and NaCl levels on growth variables and ectomycorrhizal (ECM) colonization in seedlings of *Coccoloba uvifera* at 3 months

ECM status	NaCl levels (mM)	Height (cm)	Collar diameter (mm)	Leaf (g)	Shoot (g)	Root (g)	Total biomass (g)	ECM colonization (%)	ECM dependency (%)
Non-inoculated	0	6.16b	3.71f	0.49b	0.35b	0.52b	1.38b	0.00a	–
	200	4.97a	2.27b	0.29a	0.16a	0.29a	0.75a	0.00a	–
	350	5.47a	1.97a	0.26a	0.14a	0.19a	0.57a	0.00a	–
	500	4.99a	2.01a	0.24a	0.13a	0.16a	0.57a	0.00a	–
Inoculated	0	7.23d	3.88f	1.07d	0.48d	1.56e	3.12f	89.40d	55.78a
	200	6.76cd	3.21e	1.15d	0.43cd	1.06d	2.65e	66.60c	71.70c
	350	7.01d	2.96d	1.13d	0.41c	0.75c	2.30d	46.30b	75.22c
	500	6.25bc	2.65c	0.82c	0.31b	0.83c	1.87c	41.60b	69.52c
ECM status		S	S	S	S	S	S	S	S
NaCl levels		S	S	S	S	S	S	S	S
ECM status × NaCl levels		NS	S	S	S	S	S	S	S

S, significant at $P \leq 5\%$; NS, not significant
Those means in a column not sharing a similar letter differ significantlt at $P \leq 5\%$ by use of Newman–Keuls multiple range test

(Tables 6 and 7). Overall, P contents in the leaves, shoots and roots increased with increasing soil salinity in ECM and non-ECM plants (Table 6). There was more P in the leaves and shoots than in the roots of ECM plants. However, P increased much more in ECM than in non-ECM plants at a given salt stress level. Accumulation of K in the shoots and roots seemed to be similar in ECM and non-ECM plants whatever the salt level, while there was more K in the leaves of ECM plants (Table 7). There were significant positive correlations between K and P concentrations in leaves, shoots and roots and total biomass of seagrape seedlings (Table 5).

Discussion

The beneficial effects of *S. bermudense* on seagrape seedlings occurred not only during NaCl stress but also under non-stress conditions, suggesting that the ECM effect in improving plant growth was not a specific process induced by saline stress. These results recall observations on the beneficial effect of AM fungi on plant growth under salt stress conditions (Al-Karaki et al. 2001; Yan-Melo et al. 2003; Giri and Mukerji 2004; Tian et al. 2004). Although ECM colonization by *S. bermudense* was reduced with increasing NaCl levels, ECM dependency of seagrape seedlings was increased. This suggests that the activity of the ECM symbiosis between seagrape seedlings and *S. bermudense* was strengthened under saline stress conditions once the symbiosis was established. Similar effectiveness of the AM symbiosis was reported by Giri et al. (2003) where *Sesbania aegyptiaca* and *S. grandiflora* were dependent on *Glomus macrocarpum* not only for acclimation but also for continuous nutrient uptake during progressive growth stages under salt stress conditions. Tian et al. (2004) have also indicated that AM dependency of cotton plants increases with increasing NaCl levels.

Salinity reduces the ability of plants to take up water and causes the accumulation of Na and Cl that can reach toxic levels in leaves (Munns 2002). To tolerate osmotic and toxic effects associated with the increased ion concentrations, plants can regulate their ion content by salt exclusion,

Table 2 Effects of inoculation with *S. bermudense* and NaCl levels on the number of leaves, leaf area and minimal leaf water potential in seedlings of *C. uvifera* at 3 months

ECM status	NaCl levels (mM)	Number of leaves	Leaf area (cm^2)	Minimal leaf water potential (−bars)
Non-inoculated	0	3.60d	33.77b	9.20e
	200	2.90c	18.77a	13.40bc
	350	2.30b	15.62a	14.00b
	500	1.90a	15.77a	14.60ab
Inoculated	0	4.10e	80.96e	5.90f
	200	4.20e	76.44d	11.15d
	350	3.80de	68.92d	12.25cd
	500	3.60d	56.05c	15.50a
ECM status		S	S	NS
NaCl levels		S	S	S
ECM status×NaCl levels		S	S	S

S, significant at $P \leq 5\%$; NS, not significant
Those means in a column not sharing a similar letter differ significantly at $P \leq 5\%$ by use of the Newman–Keuls multiple range test

Table 3 Changes in sodium (Na) concentrations in the leaves, shoots and roots of *C. uvifera* seedlings in response to NaCl levels and inoculation with the ectomycorrhizal (ECM) fungus, *S. bermudense*, at 3 months

ECM status	NaCl levels (mM)	Leaf (%)	Shoot (%)	Root (%)
Non-inoculated	0	0.458a	0.081a	0.200a
	200	1.763c	0.497c	0.477c
	350	1.974c	0.438c	0.486c
	500	1.928c	0.492c	0.564d
Inoculated	0	0.261a	0.056a	0.218a
	200	1.306b	0.259b	0.234a
	350	1.295b	0.295b	0.229a
	500	1.128b	0.280b	0.322b
ECM status		S	S	S
NaCl levels		S	S	S
ECM status×NaCl levels		S	S	S

S, significant at $P \leq 5\%$
Those means in a column not sharing a similar letter differ significantly at $P \leq 5\%$ by use of the Newman–Keuls multiple range test

rejection, compartmentalization and/or recirculation (Larcher 1995; Berthomieu et al. 2003). In our study, ECM plants showed considerably reduced Na and Cl uptake into roots compared to non-inoculated plants. Moreover, ECM inoculation of plants lowered Na and Cl translocation to shoot and leaf tissues. The marked differences in Na and Cl concentrations between ECM and non-ECM plant tissues suggest that the mechanisms of enhanced tolerance to salt stress in ECM plants were related to the exclusion of Na and Cl by the ECM plants growing in the NaCl treatments. One of the main mechanism for enhanced salinity tolerance in ECM plant was also proposed to be the exclusion of Na and Cl (Giri and Mukerji 2004; Tian et al. 2004). However, some studies have a lack of change in Na content in AM plants, which may be explained by the dilution effects of plant growth enhancement caused by the symbiosis (Al-Karaki 2000; Al-Karaki et al. 2001; Feng et al. 2002). It is clear that different responses of plants to salt stress will depend on the salinity tolerance of both the host plant species and the associated mycorrhizal fungi.

In addition to the exclusion of Na and Cl, another possible mechanism whereby mycorrhiza protect host plants against salinity could be enhanced P uptake (Ruiz-Lozano and Azcon 2000; Giri and Mukerji 2004). Salt stress induces P deficiency by reducing P mobility and uptake (Munns 2002). It is well-known that mycorrhiza can be helpful for plant growth in saline soil by overcoming the

Table 5 Correlation coefficients (r) between nutrient concentrations (K, P, Na and Cl) and total biomass of inoculated and/or non-inoculated *C. uvifera* seedlings

	Inoculated[a]	Non-inoculated[a]	Inoculated plus non-inoculated[b]
K	0.65*	0.50	0.91*
P	−0.85*	0.25	0.55*
Na	−0.70*	−0.94*	−0.74*
Cl	−0.71*	−0.91*	−0.79*

*significant ($P \leq 5\%$)
[a] 10 *df*
[b] 22 *df*

Table 4 Changes in chloride (Cl) concentrations in leaves, shoots and roots of *C. uvifera* seedlings in response to NaCl levels and colonization by the ectomycorrhizal (ECM) fungus, *S. bermudense*, at 3 months

ECM status	NaCl levels (mM)	Leaf (%)	Shoot (%)	Root (%)
Non-inoculated	0	0.760a	0.447ab	0.393a
	200	3.510c	1.967c	1.148c
	350	3.920c	2.030c	1.200c
	500	3.567c	2.253c	1.417d
Inoculated	0	0.423a	0.330a	0.273a
	200	2.160b	0.920b	0.570b
	350	1.930b	0.853ab	0.650b
	500	1.850b	0.833ab	0.597b
ECM status		S	S	S
NaCl levels		S	S	S
ECM status×NaCl levels		S	S	S

S, significant at $P \leq 5\%$
Those means in a column not sharing a similar letter differ significantly at $P \leq 5\%$ by use of the Newman–Keuls multiple range test

Table 6 Changes in phosphorus (P) concentrations in the leaves, shoots and roots of *C. uvifera* seedlings in response to NaCl levels and colonization by the ectomycorrhizal (ECM) fungus, *S. bermudense*, at 3 months

ECM status	NaCl levels (mM)	Leaf (%)	Shoot (%)	Root (%)
Non-inoculated	0	0.037ab	0.028a	0.032b
	200	0.030a	0.027a	0.017a
	350	0.041abc	0.035ab	0.028ab
	500	0.061bc	0.042bc	0.038b
Inoculated	0	0.064c	0.052c	0.041b
	200	0.110d	0.108d	0.065c
	350	0.114d	0.126e	0.076cd
	500	0.128d	0.129e	0.078d
ECM status		S	S	S
NaCl levels		S	S	S
ECM status×NaCl levels		S	S	S

S, significant at $P \leq 5\%$
Those means in a column not sharing a similar letter differ significantly at $P \leq 5\%$ by use of the Newman–Keuls multiple range test

Table 7 Changes in potassium (K) concentrations in the leaves, shoots and roots of *C. uvifera* seedlings in response to NaCl levels and colonization by the ectomycorrhizal (ECM) fungus, *S. bermudense*, at 3 months

ECM status	NaCl levels (mM)	Leaf (%)	Shoot (%)	Root (%)
Non-inoculated	0	0.847a	0.780cd	0.687ab
	200	0.817a	0.613abc	0.447a
	350	0.830a	0.570a	0.640ab
	500	0.913a	0.587ab	0.610ab
Inoculated	0	2.013c	0.860d	0.790b
	200	1.800c	0.763bcd	0.830b
	350	1.973c	0.817d	0.743b
	500	1.473b	0.777b	0.830b
ECM status		S	S	S
NaCl levels		S	S	S
ECM status×NaCl levels		S	S	S

S, significant at $P \leq 5\%$

Those means in a column not sharing a similar letter differ significantly at $P \leq 5\%$ by use of the Newman–Keuls multiple range test

P binding capacity of the soil (Ruiz-Lozano et al. 1996; Al-Karaki et al. 2001; Feng et al. 2002). ECM plants had higher concentrations of P than non-inoculated plants particularly under salt stress conditions. This could be due to the extended network of hyphae beyond the depletion zone around roots that acquire P and therefore suppress the adverse effect of salinity stress. These results suggest that *S. bermudense* can alleviate the deleterious effects of NaCl stress on plants by mechanisms, which may be also related to the improvement of P nutrition. Nevertheless, some works showed that salt tolerance of AM plants appear to be independent to the acquisition of P in plants (Ruiz-Lozano et al. 1996; Feng et al. 2002; Tian et al. 2004). Ruiz-Lozano et al. (1996) have indicated that the mechanisms underlying AM plants growth improvement under saline soils are based on physiological processes rather than on nutrient uptake.

If Na and Cl are sequestered into plant vacuoles, K and organic solutes (e.g. glycine betaine, proline) should accumulate in the cytoplasm and organelles to balance the osmotic pressure of intra-vacuolar ions (Munns 2002). These compounds are in fact accumulated under salt stress and found at high concentrations in plants adapted to saline soils. It is noteworthy that K concentrations were higher in leaves of ECM seagrape seedlings than in the controls at all salinity levels. *S. bermudense* seemed to protect seedlings by increasing the K/Na ratio to maintain higher turgor under salt stress. It is well-known that high concentrations of K and low concentrations of Na can play a major role in salt stress tolerance by regulating stomatal opening and osmotic potential in the vacuoles (Zhu 2003; Munns 2005). Ojala et al. (1983) found AM formation by onion maintained a high K/Na in shoots and bulbs under salt stress, and mycorrhizal *Acacia auriculiformis* plants were also reported to accumulate more K than Na at different salinity levels (Giri et al. 2003). Nevertheless, the role of K in balancing intracellular ion compartmentalization is sometimes contradictory under saline stress conditions. Some studies indicate that plants subjected to increased salinity accumulated less K because Na uptake competes with the uptake of K, leading to decreased K levels (Larcher 1995; Parida and Das 2005).

In the case of AM symbiosis, the contribution of mycorrhizal fungi to plant growth is not limited to the improvement in mineral nutrition but also to the changes in the morphological characters of leaves and the water status of plants (Augé 2001). It is well-known that salinity provokes a reduction in the osmotic potential of the soil solution, reducing plant-available water (Munns 2002). Measurements of minimal leaf water potentials are considered informative on evapotranspiration (Larcher 1995). In general, the ECM plants had higher minimal leaf water potential so independent of salinity. However, *S. bermudense* improved the water status of seagrape seedlings despite their higher evaporative leaf surface. Nevertheless, in several reports where root systems are constrained to relatively small soil volumes, leaf water potential decreases more quickly in AM plants than in non-AM plants with exposure to drought because AM plants are larger and extract soil water more quickly (Augé 2001). Further studies should be done to elucidate the physiological mechanisms (stomatal conductance, water use efficiency, transpiration, photosynthetic rate, osmotic adjustment) by which *S. bermudense* improved the water status of seagrape seedlings.

In conclusion, the results of this study indicate that seagrape tolerance to salt stress is considerably enhanced by *S. bermudense*. Reductions in Na and Cl uptake and enhanced P nutrition may be significant in helping the ECM plants to grow under saline conditions. Furthermore, the higher accumulation of K in the leaves of ECM plants could render ECM plants more tolerant to osmotic stress induced by exposure to salt. The overall improved water status of seagrape seedlings related to ECM inoculation with *S. bermudense* may also be a contributing factor. Our results should be taken cautiously as the experiment was a greenhouse study with 3 month-old seedlings. From an ecological point of view, the ECM symbiosis may be an important factor for the establishment and survival of seagrape stands under saline stress along the Caribbean's Beach. Moreover, transplanting ECM-inoculated seagrape to such degraded sites not only benefits the individual plant but, more importantly, may result in the development of ornamental plantings and coastal windbreaks along Caribbean beaches and roadsides.

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