Effect of the ectomycorrhizal fungus *Scleroderma bermudense* inoculation on growth and physiological response of *Coccoloba uvifera* (L.) L. seedlings under salt stress

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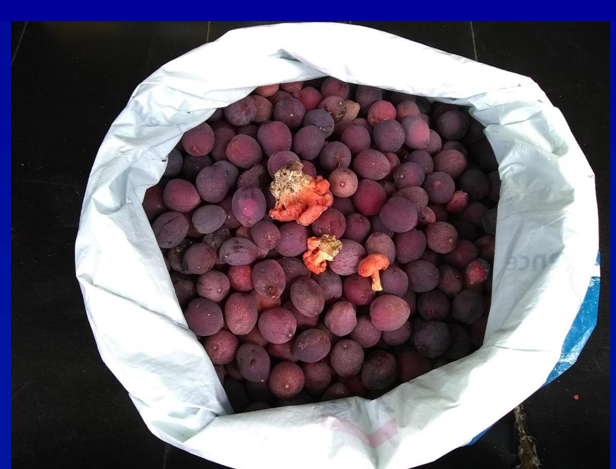
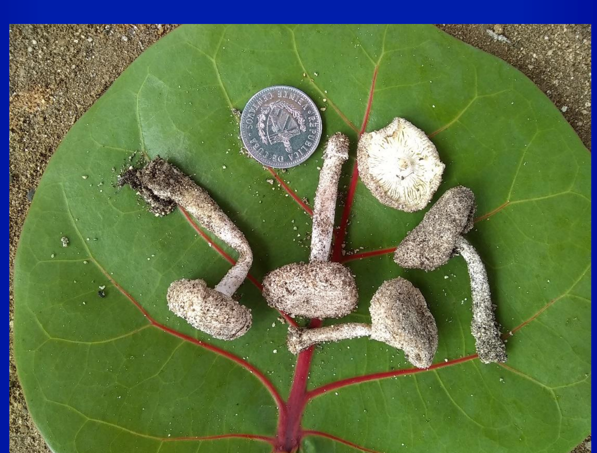
Introduction

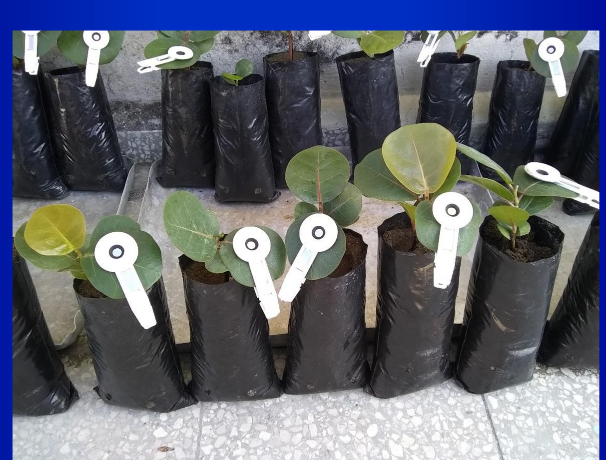
Coccoloba uvifera (L.) L. (Polygonaceaea), also named seagrape, is a woody plant often subject to high levels of salinity along the Atlantic, Caribbean and Pacific coasts of the American tropics and subtropics. It is an important ECM tree for edible fruits and mushrooms, ornamental plantings and coastal windbreaks along Caribbean beaches and roadsides

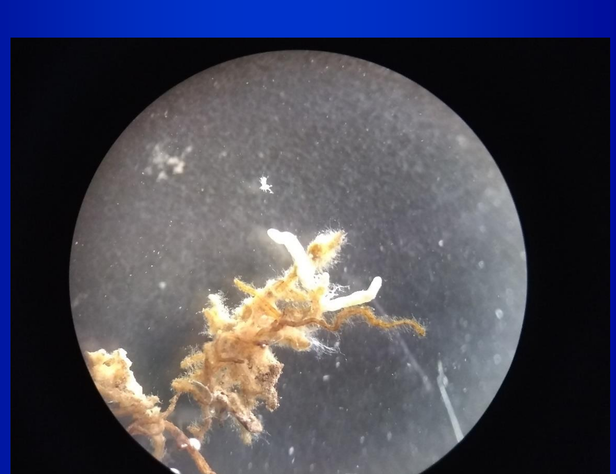
We analyzed growth and physiological parameters such as photosynthesis and transpiration rates, chlorophyll fluorescence and water status of the plant to assess the effect of the established S. bermudense on two provenances of seagrape seedlings in order to improve understanding of the mechanisms regarding the alleviation of salt toxicity in ECM seagrape.

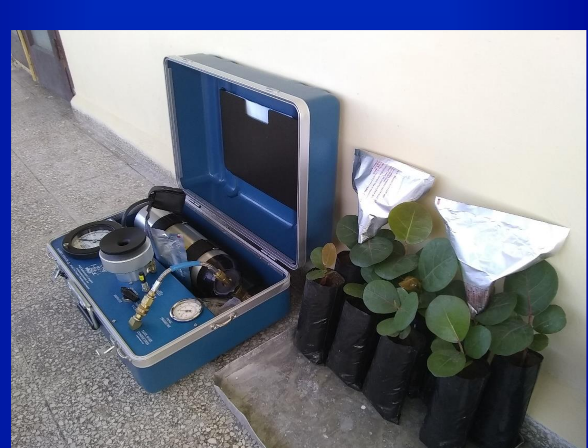
Materials and Methods











Fungal material, inoculation and experimental design

Mature sporocarps of *Scleroderma bermudense*, a gasteromycetous fungus, were collected in the Playa Las Coloradas' Beach. An herbarium reference voucher UG-04 is given to the sporocarps.

The experiment was set up as a completely 2x2x4 factorial design consisting of two provenances of *C. uvifera* (Las Coloradas and Punta de Tomate), two ectomycorrhizal inoculation treatments (Inoculated and Non- inoculated) and four salinity levels (0.02, 5, 15 and 25 dS m-1). In all, 16 treatments were compared with ten replicates per treatment.

Photosynthesis and gas exchange parameters

The photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs) and sub-stomatal CO2 (Ci) were non-destructively measured.

Chlorophyll fluorescence and content

The basal fluorescence (Fo), maximum fluorescence (Fm), maximum quantum efficiency of photosystem II (Fv/Fm), ratio variable fluorescence basal fluorescence (Fv/Fo), and performance index (PIabs) were measured.

Plant biomass and leaf water status

The foliar water potential (Ψwf) and the xylem water potential (Ψwx), the leaf area, length of stem, collar diameter and dry weight. The relative water content (RWC) the fresh mass (FM), dry mass (DM) and turgid mass (TM). The relative water content (RWC) were measured

Ectomycorrhizal colonization

The relative mycorrhizal dependency (RMD) and percentage of mycorrhization, and ECM colonizatio

Results

Effect of provenance, mycorrhization and salinity on stomatal conductance (gs), photosynthetic (A) and transpiration (E) rates and sub-stomatal CO2 (Ci) in leaves of C. uvifera seedlings.

Provenance of C. uvifera	Inoculation treatment	Salinity (dS/cm^{-1})	gs ($mol\ m^{-2}\ s^{-1}$)	E ($mol\ m^{-2}\ s^{-1}$)	A ($\mu mol\ m^{-2}\ s^{-1}$)	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

Effect of provenance, mycorrhization and salinity on morphological variables in C. uvifera seedlings.

Provenance of C. uvifera	Inoculation treatment	Salinity (dS/cm^{-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	0,00±0,00a
		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	0,00±0,00a
		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	0,00±0,00a
		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	0,00±0,00a
	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±8.95bcd
		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±21.89b
		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±14.84cd
		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±20.39cd
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	0,00±0,00a
		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	0,00±0,00a
		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	0,00±0,00a
		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	0,00±0,00a
	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±7.17bcd
		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±10.62bc
		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±10.91cd
		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±8.95d

Effect of provenance, mycorrhization and salinity on water status in leaves of C. uvifera seedlings.

Provenance of C. uvifera	Inoculation treatment	Salinity (dS/cm^{-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

Conclusions

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 and physiological functions between the both ECM seagrape provenances in response to salt stress. Additionally, the beneficial effects of ECM symbiosis on the photosynthetic and transpiration rates, chlorophyll fluorescens content, stomatal conductance and water status resulted in the improved growth performance of seagrape provenances exposed to salt stress. From an applied point of view, transplanting 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.