Article

Photosynthetic rate and biomass production by inoculation of *Scleroderma* sp. with different concentrations of NaCl in pecan tree

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Abstract

The pecan tree [*Carya illinoinensis* (Wangenh) K. Koch] is one of the fruit trees established in northern Mexico, where there is a considerable area of soils with different degrees of salinity, to which the tree is sensitive. In seedlings inoculated with *Scleroderma* sp., the effect of NaCl was evaluated at concentrations of 0, 20, 25, 30 and 35 mM, including a control without inoculation and without NaCl. The experiment was carried out at the University Regional Unit of Arid Zones in Bermejillo, Durango, Mexico, under greenhouse conditions. The seedlings were watered twice a week, during 2018. The plants inoculated and under non-saline conditions, showed the highest photosynthesis and transpiration, followed by the control seedlings. The concentration of Na^+ in root, stem and leaf was lower in the control and in the inoculated seedlings and without NaCl. The concentration of K in inoculated seedlings and under saline and non-saline conditions was like the control. The k/Na ratio was higher in the control and in the inoculated seedlings and under non-saline conditions and decreased as NaCl concentration increased, particularly in the root. The control seedlings showed higher dry weight in root, stem and leaf than those inoculated with *Scleroderma* sp. and under salt stress, but it was lower than in those inoculated with the fungus and under non-saline conditions. In seedlings under saline stress of 20 and 35 mM, the decrease in dry weight was 8.5 and 47%, compared to the control. Walnut seedlings inoculated with *Scleroderma* sp. and under non-saline condition, they showed better physiological response and biomass accumulation and not under saline condition.

Keywords: mycorrhizae, seedling behavior, stressful medium.

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Introduction

The pecan tree is a species whose native ancestors developed spontaneously near rivers or streams where the soils are of alluvial origin, deep and fertile, with high moisture retention capacity and with good drainage (Campos *et al*., 2017). Currently, most of the commercial pecan tree orchards, established in Mexico are located in areas distant from those ecological niches, in which the physical and chemical conditions of the soils in which they grow are not entirely adequate. In northern Mexico, a considerable number of pecan tree orchards have been established in saline areas (Moreno *et al*., 2015).

This condition limits the productivity of this species, which is considered susceptible to salinity, Campos *et al*. (2017), since the tolerance threshold, determined in irrigated adult orchards, is 2 to 3 dS m^{-1}, when Na is the dominant cation (Miyamoto and Nesbitt, 2011). In pecan tree seedlings, photosynthesis, stomatal conductance and biomass decrease from 2 dS m^{-1} of NaCl in irrigation water (Campos *et al*., 2017). Ionic retention in root or stem is one of the mechanisms that some plants have to tolerate salinity (Ma *et al*., 2014). However, in this species, this characteristic has not been observed.

The genus *Carya*, as in most forest trees, has established a mutual relationship with native ectomycorrhizal fungi, necessary for its development and survival (Bonito *et al*., 2011). The fungus has the function of increasing its host's access to nutrients (Bonito *et al*., 2011), as well as increasing the plant's capacity to develop under water stress conditions (Syvertsen and Levy, 2005; Estrada *et al*., 2013; Yang *et al*., 2014; Babuin *et al*., 2016; Scagel *et al*., 2017; Behrozz *et al*., 2019).

Inoculation with ectomycorrhizal fungi is an important tool to improve the functioning and ensure the production of biomass in forest species, under saline conditions (Ma *et al*., 2014), as they decrease the entry of Na and Cl (Smith *et al*., 2010). Although in other species such as aspen and birch, this does not happen (Yi *et al*., 2008). In *Carya illinoinensis*, the information on the response of the plant inoculated with ectomycorrhizae under salinity conditions is limited.

The presence of Na in orchards planted with pecan tree, and the absence of salinity-tolerant rootstocks, for new plantations, leads to the search for options to counteract this problem, without negatively affecting the soil ecosystem. The objective of the present study was to evaluate the photosynthetic rate and biomass production, in response to inoculation with *Scleroderma* sp., with different concentrations of NaCl in pecan tree seedlings.

Materials and methods

Obtaining *Scleroderma* sp.

The orchard where the specimens of fruiting bodies of *Scleroderma* sp. were collected is 40 years old, and the trees are planted in a real frame at a distance of 12 m x 12 m. It is located in the municipality of Matamoros, Coahuila, whose geographical coordinates are 2.5° 25' north latitude, 108° 18' west longitude, with an elevation of 1 200 masl. The search and collection of the specimens was carried out from May to November 2018.

The tours were carried out in blocks of 10 000 m^2 in 5 sites of the orchard, whose area is 20 ha. For each collection, photographic records and observations such as size, color and shape were made. The colors of the fungi were recorded using color guide (Munsell, 1975). The specimens were placed on waxed paper and introduced and transported in portable cooler to the microbial ecology laboratory of the Faculty of Biological Sciences, of the Juárez University of the State of Durango, Mexico. To identify microscopic structures, temporary preparations with 5% KOH and Melzer reagent were analyzed in Velab VE-B3 and ZEISS Scope A1 microscopes. Specimens were identified according to the taxonomic keys (Guzmán, 2013).

Isolation of the strain of *Scleroderma* sp.

Fragments of the fungus were obtained from the central part of the gleba under aseptic conditions in a laminar flow hood, disinfected with 70% alcohol and 10% chlorine. The fragments were transferred to Petri dishes, with nutritive medium and incubated at a temperature of 25 °C, until the beginning of the growth of the mycelium.

Preparation of the fungal inoculum

The strains were reseeded in potato dextrose agar (PDA). Subsequently, the colonies were fragmented into small squares of 5 x 5 and transferred to 250 ml flasks, which contained 100 ml of biotin aneurin and folic acid (BAF) and 15 g of dextrose. The flasks were kept in mechanical stirring at 150 rpm and 25 °C for two weeks. The nutrient medium was removed using distilled water. Serial dilutions of the active mycelium were performed to obtain a concentration of 2.5×10^8 colony-forming units per milliliter, which was used in the inoculation of the seedlings.

Treatment design

The seeds of landrace pecan tree were collected in November 2018, in the orchard where the fungus was collected. They were then stratified in sawdust for a month at a temperature of 5 °C. Later they were immersed in water for 48 h as a pre-germinative treatment and sown in a wooden box for seedbed, under greenhouse conditions at 30 °C in the Regional University Unit of Arid Zones, Bermejillo, Durango, Mexico, whose coordinates are 25.8° latitude and 103.6° west longitude, with a height of 1 120 masl.

Sand was used as a substrate and irrigations with distilled water were applied every third day. Once the plants reached a size of approximately 15 cm, they were transferred to pots with a capacity of 9 kg that contained substrate composed of sand, peat moss and soil in a ratio of 40:20:40, which was sterilized with methyl bromide. Twenty-five homogeneous seedlings inoculated and without mycorrhizae received treatments with NaCl (0, 20, 25, 30 and 35 mM), at the time of transplantation into pots. Five non-inoculated seedlings corresponding to the control received irrigation without NaCl.

Inoculation was carried out at the base of the stem of the seedlings using a pipette of 10 ml. The treatments were applied considering a completely randomized design with five repetitions, a tree was considered as repetition. The MSD test (0.05) was used to compare means between treatments. The seedlings were irrigated every third day, using 300 ml of distilled or saline water.

Variables evaluated

The variables stomatal conductance, photosynthesis and transpiration were evaluated every week, starting one month after carrying out the treatments, using an infrared gas analyzer (LI-COR 6400®, Lincoln, NE, USA) once a week. At the end of the experiment and when the treated plants manifested considerable symptoms due to salt damage, which occurred three months after the treatments were established, they were collected and fractionated into root, stem and leaf.

The samples were dried in a drying oven (ThermoScientific. Model Precision, OV700F) at 65 °C. To constant weight and the dry weight of these tissues was determined. The content of Na and K was determined by digestion, carried out by wet route, with a nitric-perchloric mixture. Subsequently, the readings were made using an atomic absorption spectrophotometer (Analyst 200, Perkin Elmer®, USA).

Results and discussion

Photosynthesis

Control seedlings, not inoculated with *Scleroderma* sp. and developed in a non-saline medium showed the lowest photosynthetic rate (Table 1). In the pecan trees inoculated and under non-saline condition, this variable increased 10%, with respect to the control. However, concentrations of 20 and 25 mMol of $NaCl^+$ in the water reduced photosynthesis by 5 and 19% compared to the control. Similarly, the increase in saline concentrations to 30 and 35 mM significantly reduced the photosynthetic rate by 31% and 42% respectively.

Table 1. Photosynthetic rate, stomatal conductance and transpiration in pecan tree seedlings not inoculated and inoculated with *Scleroderma* sp. and irrigated with different concentrations of sodium chloride.

NaCl (mM)	Photosynthesis (μmol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})
Control without inoculum	6.3 ±0.6 a^z	0.08 ±0.008 a	1.88 ±0.25 a
0 with inoculum	7 ±0.57 a	0.08 ±0.007 a	2.18 ±0.24 a
20 with inoculum	6 ±0.18 a	0.08 ±0.009 a	1.68 ±0.23 a
25 with inoculum	5.1 ±0.08 ab	0.07 ±0.01 a	1.69 ±0.25 a
30 with inoculum	4.8 ±0.3 b	0.05 ±0.003 b	1.22 ±0.08 b
35 with inoculum	4 ±0.63 b	0.05 ±0.003 b	1.2 ±0.15 b

^{z}= means (n= 5, ± standard error) with different letters within each column are statistically different (MSD; $p \leq 0.05$).

The effect of saline stress on the decrease in the photosynthetic rate found here has also been observed in the species of pine *Picea mariana*, *Picea glauca* and *Pinus banksiana* inoculated with mycorrhizae of the genus *Hebeloma crustuliniforme* or *Laccaria bicolor* and developed under saline condition (Nguyen *et al*., 2006). This negative effect is since saline stress restricts the formation of chlorophyll (Moreno *et al*., 2015) and involves the destruction of photosystem II, disrupting electron transport in the photosynthetic apparatus of plants (Sheng *et al*, 2008). On the other hand, symbiosis with mycorrhizae has been found to increase photosynthesis since salt interferes less in the synthesis of chlorophyll than in non-inoculated plants (Smith *et al*., 2010).

Stomatal conductance

The stomatal conductance in the control seedlings, without inoculum and without $NaCl^+$ was similar to that observed in the seedlings inoculated and grown in non-saline medium, as well as in those treated with 20 mM of $NaCl^+$ (Table 1). This variable decreased 12.5% in pecan trees inoculated and irrigated with water containing 25 mM of NaCl. Treatments with 30 and 35 mM of NaCl showed a significant decrease of 37.5% compared to the control. The increase in Na and Cl has also been observed in pecan tree seedlings without inoculation and with NaCl stress in all salinity treatments (Campos *et al*., 2017).

In apple tree seedlings inoculated with *Glomus versiforme* and treated with 2, 4 and 6% of NaCl, the turgor of the leaf increased in relation to those not inoculated, however, it decreased as saline stress increased. The results found in this study are similar to those observed by Muhsin and Zwiazek (2002). They observed a decrease in stomatal conductance in fir seedlings without inoculation and inoculated with *Hebeloma crustuliniforme* and treated with 25 mM of NaCl. However (Birhane *et al*., 2012) observed different response in mycorrhized seedlings with the genus *Glomus* and in saline condition, in which greater stomatal conductance due to the greater availability of water was observed.

Pecan tree seedlings treated with Na_2SO_4 had a high concentration of proline in the leaf (Moreno *et al*., 2015). This response has been found in plants with mycorrhizae at the root, which must maintain the balance between the rhizosphere and the external environment, thus influencing stomatal conductance (Evelin *et al*., 2009; Lee *et al*., 2010),

Transpiration

The seedlings inoculated and treated with saline solution had transpiration values lower than the control, without inoculum and without $NaCl^+$ (Table 1). Under saline condition equivalent to 20 and 25 mM, transpiration decreased by approximately 10%, in relation to the control. However, when increasing the salinity gradient to 30 and 35 Mm, transpiration decreased significantly by 35 and 36%, respectively. The pecan trees inoculated and grown under non-saline condition showed the highest transpiration, equivalent to 14% with respect to the control. In this study the presence of *Scleroderma* sp. did not improve physiological drought in seedlings treated with NaCl.

This could be due to the fact that the NaCl in the medium exerted an osmotic effect, which decreased the availability of free water, increasing the water deficit of the leaf as mentioned by (Syvertsen and Levy, 2005). Similarly, in the seedlings of *Populus tremuloides* Michx., and *Betula papyrifera* Marsh inoculated by Yi *et al*. (2008) with *Hebeloma crustuliniforme* or *Laccaria bicolor* and treated with 25 mM NaCl for 6 weeks, transpiration, as in this study, was not affected under the same concentration of NaCl. However, in birch seedlings under the same saline condition and treated with the same mycorrhizae by Muhsin and Zwiazek (2002), transpiration decreased.

Concentration of Na in tissue

The lowest concentration of Na^+ in the tissues was observed in the control seedlings, without *Scleroderma* sp. and in the absence of NaCl, as well as in seedlings inoculated and treated with salt (Figure 1). This did not happen in seedlings inoculated and irrigated with saline solution, in which Na^+ increased significantly in the three types of tissue, being 74, 77 and 43% higher in root, stem and leaf for the saline condition of 35 mM. The root showed the least amount of Na and the leaf the most.

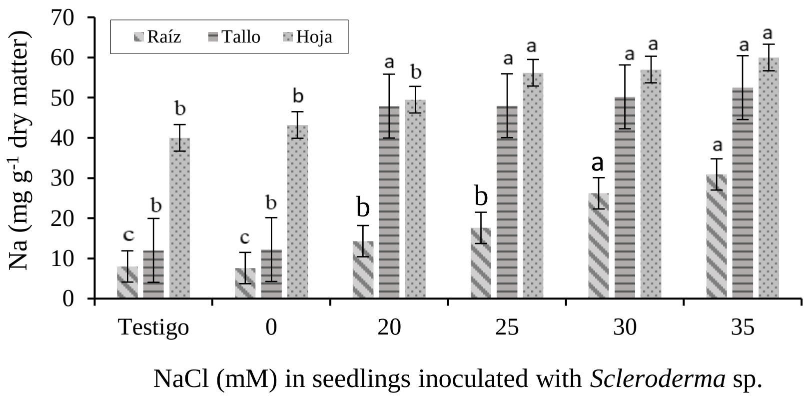

Figure 1. Sodium concentration in root, stem and leaf in seedlings of pecan tree without inoculation and inoculated with *Scleroderma* sp. and irrigated with different sodium chloride concentrations. Vertical bars (n= 5, ± standard error) within treatments with unequal letters are statistically different (MSD; $p \leq 0.05$).

Colonization of plants with mycorrhizae can increase the absorption of K under saline conditions, thus preventing the translocation of Na into the stem (Giri *et al*., 2007); Porcel *et al*., 2016). In this study, this did not happen, as the concentration of Na^+ in the seedling tissues increased as the saline condition increased. Although in fir seedlings treated with 25 mM of NaCl and inoculated with *Hebeloma crustuliniforme*, less Na was observed in root and stem than in untreated ones.

Considerable differences in sodium concentration between plants with and without mycorrhizae suggest that mechanisms to improve tolerance to saline stress in these inoculated plants may be related to Na exclusion (Feng *et al*., 2006). Therefore, the different responses of plants to saline stress will depend on plant-microorganism capacity to tolerate the inhibitory effect caused by salinity (Bandou, 2006).

Concentration of K in tissue

The concentration of K in the root and stem of seedlings with *Scleroderma* sp. under saline condition was similar to the control without inoculation and without NaCl (Figure 2). However, in the leaves, the treatments had a significant effect, since, in the seedlings treated with 30 and 35 mM of NaCl, they showed the highest concentration of K, which was 37 and 47% higher than in the control. When the concentration of Na in the medium increases, plants absorb more Na^+ than potassium (Ruiz *et al*., 2012; Bassil *et al*., 2012).

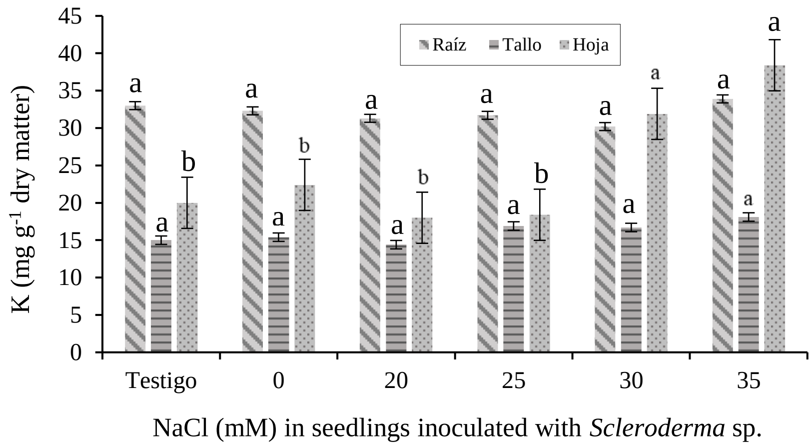

Figure 2. Concentration of potassium in root, stem and leaves of pecan tree seedlings without inoculation and inoculated with mycorrhiza *Scleroderma* sp. and irrigated with different concentrations of sodium chloride. Vertical bars (n=5, ± standard error) within treatments with unequal letters are statistically different (MSD; $p\leq 0.05$).

Although the concentration of Na was significant from 25 mM in the solution. Despite the above, the high concentration of K in the foliage, found in the present study, is similar to that observed by Bandou *et al*. (2006). In seedlings of sea grape *Coccoloba uvifera*, inoculated and under saline condition, although this species exhibited high levels of potassium in all tissues, in all levels of salinity studied. The authors also mention that *Scleroderma bermudense* seemed to protect seedlings from saline stress by increasing the K/Na ratio to maintain a high turgor.

K/Na ratio in tissue

The highest K^+/Na^+ ratio in root was observed in the control seedlings, as well as in those inoculated with *Scleroderma* sp. treated with non-saline solution (Figure 3). This ratio gradually decreased in those treated with NaCl as the concentration increased. The highest K/Na ratio was also observed in the stems of the control seedlings as well as in those inoculated but without NaCl, in which it significantly decreased in all treatments with NaCl. A similar situation was observed in the leaves, except in those of the plants treated with 30 and 35 mM of NaCl, which were significantly higher than those developed in the two immediate lower NaCl concentrations.

Na^+ competes with K^+ and when the concentration of Na^+ in the medium is high, plants tend to take more of this ion and less K^+ (Chen *et al*., 2007; Evelin, 2009). In this research, plants with mycorrhizae and under stress caused by the presence of NaCl showed a low K^+/Na^+ ratio in their tissues. These results are different from those found in *Acacia nilotica* plants with mycorrhizae, in which a high K absorption was observed, shown in the stem, compared to non-mycorrhized ones (Giri *et al*., 2007; Garcia *et al*., 2014). In another study with poplars inoculated with mycorrhizae and under saline condition, a greater absorption of K was observed, thus improving the ability to maintain a self-regulation of K^+/Na^+, crucial condition to tolerate salinity conditions, as mentioned by (Li *et al*., 2012).

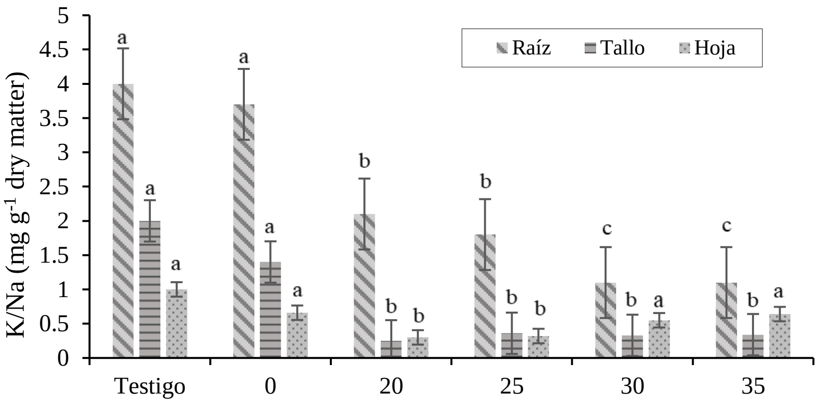

Figure 3. K/Na ratio in root, stem and leaf of pecan tree without inoculation and inoculated with mycorrhiza *Scleroderma* sp. and irrigated with different concentrations of salinity. Vertical bars (n=5, ± standard error) within treatments with unequal letters are statistically different (MSD; $p\leq 0.05$).

Dry root weight

Treatments with NaCl significantly affected the dry weight of the root (Figure 4). Seedlings inoculated and irrigated with salt-free water and the control showed the highest dry weight compared to seedlings inoculated with *Scleroderma* sp. and treated with NaCl. This variable decreased as the concentration of NaCl in the medium increased. The dry weight of the root in seedlings grown in the saline medium with 20 mM decreased by 39%, being of 43% for those grown with 25 and 30 mM. While those treated with 35 mM showed a decrease in dry weight of 55%.

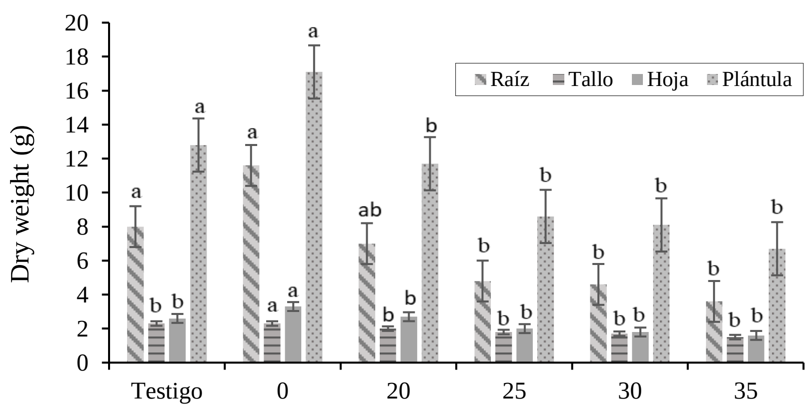

Figure 4. Dry weight of root, stem and leaves in seedlings of pecan tree without inoculation and inoculated with mycorrhiza *Scleroderma* sp. and irrigated with different concentrations of salinity. Vertical bars (n=5, ± standard error) within treatments with unequal letters are statistically different (MSD; $p\leq 0.05$).

Dry stem weight

The greatest dry weight of the stem was observed in the seedlings inoculated and without NaCl, which exceeded the control by 31% (Figure 4). On the other hand, the treatments with NaCl in concentrations of 20, 25, 30 and 35, induced a reduction of 13, 21, 26 and 34% compared to the control.

Dry weight of leaves

Seedlings inoculated and developed under saline condition showed a decrease in the dry weight of the leaf, compared to the control (Figure 4). This decrease was 30 and 38% in treatments with the highest concentration of NaCl. The highest dry weight was observed in the seedlings inoculated and developed under non-saline condition, which was 21% higher than the control.

Dry seedling weight

The control seedlings showed greater dry weight than those inoculated with *Scleroderma* sp. and under saline stress, but it was lower than in those inoculated with the fungus and under non-saline condition (Figure 4). Under saline stress of 20 and 35 mM, the decrease of this variable was 8.5 and 47% compared to the control, but it was 31 and 60% in relation to seedlings inoculated and without saline stress. The total dry weight showed a downward trend as the salinity level increased.

The results found here are similar to those observed by (Yi *et al*., 2008) in seedlings of *Populus tremuloides* Michx. and *Betula papyrifera* Marsh inoculated with *Hebeloma crustuliniform*e and *Laccaria bicolor* and stressed with similar concentrations of NaCl for six weeks. Similar results were found by Zwiazek *et al*. (2019) on the reduction in dry weight of root and stem, in seedlings of *Pinus contorta*, developed in soil with the presence of various genera of mycorrhizae and treated with 60 and 90 mM NaCl.

Similar observations have been made by Bandou *et al*. (2006) in sea grape seedlings, *Coccoloba uvifera* inoculated with *Scleroderma bermudense*, in which stress with NaCl decreased biomass at all salinity levels. Likewise, in basil inoculated with *Rhyzophagus irregularis*, concentrations of 91 and 181 mM of NaCl decreased dry weight in the order of 20 and 38%, This response may be related to the osmotic effect of salt, which limits the absorption of water and nutrients, or to the direct toxicity of the ion (Evelin *et al*., 2009).

On the other hand, the positive effect of mycorrhizae on growth and biomass in plants under conditions of saline stress has been observed. In seedlings of *Populus tomentosa*, inoculated with *Glomus constrictum* by Lu *et al*. (2014) and treated with 100 mmol L^{-1} of NaCl, the total dry weight was higher than the control, without inoculation and without saline stress. Similar results have been observed by Parvin *et al*. (2020) in rice inoculated with *Funnelliformis mosseae*, *Acaulospora laevis* and *Gigaspora margarita* and under saline stress, whose increase in dry weight of the stem and leaves occurred both in the individual and group presence.

At the same time, the seedlings of *Acacia auriculiformis* inoculated with mycorrhizae showed greater growth than those not inoculated, under conditions of saline stress (Giri *et al*., 2003). In this study, a higher dry weight was observed in seedlings mycorrhized and without NaCl, compared to the control, without inoculation and without saline stress. Similar results have been observed in other deciduous species (Ruiz *et al*., 2012; Estrada *et al*., 2013; Babuin *et al*., 2016).

The decrease of Na in the plant, while the absorption of nutrients increases, maintaining high rate of transpiration and hydraulic conductance of the root may be an important mechanism of resistance in mycorrhized plants growing in saline conditions (Muhsin and Zwiazek, 2002). Likewise, the response to salinity in plants mycorrhized and under saline stress varies depending on the relationship between the host and the plant species (Ma *et al*., 2014).

Conclusions

Under non-saline conditions, pecan tree seedlings not inoculated and inoculated with *Scleroderma* sp. showed the highest photosynthesis, stomatal conductance, transpiration, and biomass. However, the presence of NaCl in any concentration in the medium caused an adverse effect on the variables studied here. It is suggested in future studies to increase the concentration of the fungus and increase the number of applications, under controlled conditions.

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