Mycorrhizal Colonization in *Atriplex nummularia* Lind. Subjected to Desalinizador Reject

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ABSTRACT

This work has the objective of evaluating the mycorrhizal colonization of AMF *Claroideoglomus etunicatum* in *Atriplex nummularia* Lind. subjected to desalinator reject. The experiment was conducted in a greenhouse at the headquarters of Agronomic Institute of Pernambuco - IPA, Recife, Pernambuco, Brazil. The experimental design was randomized blocks with the treatments constituted in a factorial scheme of five levels of salinity in AC= 2.86 mS/cm; T1= 11.54 mS/cm; T2= 12.04 mS/cm; T3= 13.13 mS/cm and T4= 14.16 mS/cm, associated with the presence and absence of fungus, presence and absence of nutrient solution, and autoclaved and non-autoclaved soil. 8.0g of Hoagland & Arnon complete nutrient solution was added every fortnight. After five months, the roots of the treatments were collected and the root colonization was evaluated. It was found that in all treatments the association between *Claroiodeoglomus etunicatum* and *Atriplex nummularia* was beneficial. The correlation was positive for the treatment T4 (Reject + 14gNaCl) + AMF. Thus, it was observed that salinity had no negative effect on the association as well as on the growth of the vegetable.

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Keywords: Mutualism, plant growth, halophyte plants, AMF, saline reject.

1. INTRODUCTION

Salinity is an abiotic stress limiting plant growth and productivity worldwide, which can induce physiological changes and compromise plant growth and development [1].

Amidst this, some species of the *Chenopodiaceae* family are very tolerant to high salinity and many of them are of great economic and environmental importance (reference). *Atriplex nummularia* is one of the species tolerant to salinity, which has been used as a model plant in biosaline conditions due to its halophytic nature and also to extract salts from the soil and accumulate in the aerial part [2, 3].

Mycorrhiza is a mutualist association between certain soil fungi and plant roots. Its classification is divided into three groups according to its morphological and anatomical aspect of root colonization: ectomycorrhiza, endomycorrhiza and ectoendomycorrhiza. AMF are endomycorrhizal due to penetration of the internal mycelium into the intercellular and intercellular root cortex (reference). One of the main characteristics of this association is to increase the surface area of the root and to allow greater capacity of absorption of water and nutrients of the soil, providing a higher rate of growth and survival [4, 5, 6].

Therefore, the objective of this work is to analyze the mycorrhizal colonization of *Claroideoglomus etunicatum* in *Atriplex nummularia* Lind. irrigation with the desalinator reject.

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2. MATERIALS AND METHODS

The experiment was conducted in the period from November / 2018 to March / 2019 in a greenhouse at the headquarters of Agronomic Institute of Pernambuco - IPA, Recife, Pernambuco, Brazil.

The soil was obtained from the Experimental Station of the IPA of São Bento do Una, air dried, dewormed, homogenized and sieved in 2 mm mesh. Part of the soil was autoclaved at 120 °C for one hour. Then the sterile and the natural soils were transferred to the 80 polyethylene vessels.

In the Laboratory of Soil Fertility of the IPA a chemical analysis was carried out, determining P = 350 mg/dm³, pH (H_2O) = 7.8, Ca = 16 cmolc/dm³, Mg = 3.9 cmolc/dm³, Na = 3 cmolc/dm³, K = 0.7 cmolc/dm³ and Al = 0 cmolc/dm³. The physical characteristics of the soil were Dap = 1.29 g/cm³, Dr = 2.62 g/cm³, Coarse sand = 7%, Sand = 21%, Silte = 56%, Flocculation = 100%, Clay = 19%, Texture = Franco-silty, Residual humidity = 1.7%.

The reject for irrigation was obtained from the desalinator located in the municipality of Riacho das Almas, Pernambuco, Brazil. The physicochemical analysis was performed at the IPA Plant, Ration and Water Analysis Laboratory - LAPRA with the following characteristics: electrical conductivity = 11.541 µS/cm at 25 ºC, Ca^{+2} = 403 mg/L, Mg^{+2} = 393.09 mg/L, Na^{+} = 200 mg/L and K^{+} = 40 mg/L, RAS = 23.67, pH = 7.9, classification for irrigation = C4S4 (very high salinity water and high sodium concentration).

The seedlings of *Atriplex* were obtained with 120 days of age, multiplied by the cutting technique, and then planted in commercial substratum. After 30 days, the best seedlings were chosen to be transplanted into the vessels.

The AMF used was purchased from the AMF Inoculum Bank of the Laboratory of Soil Microbiology of IPA. During the transplantation into the vessels, the soil was inoculated - inoculum containing 50 g (specify (mycorrhizal roots with C. *etunicatum* or soil containing C. etunicatum, or spores of C. etunicatum) of *Claroideoglomus etunicatum*.

In all treatments, 8mL of ??? [7] was applied fortnightly. In addition, the temperature and humidity of the greenhouse were monitored daily.

The experimental design was randomized blocks consisting of a factorial scheme with 5 levels - AC: water – EC = 2.86 mS/cm; T1: reject – EC = 11.54 mS/cm; T2: reject plus 7g NaCl – EC = 12.4 mS/cm; T3: reject plus 14g NaCl – EC = 13.13 and T4: reject plus 21g NaCl – EC = 14.16 mS/cm, associated with two levels of the fungus (presence and absence), two levels of the nutrient solution (presence and absence) and two levels of the soil (autoclaved and non-autoclaved). A 5x2x2x2 factorial is then used, with two replicates, totaling 80 experimental units.

After five months of experiment, height was measured. Then the roots were collected, washed and sent to the Laboratory of Soil Microbiology - IPA, to perform the root coloration.

Root staining was performed using the methodology described by Phillips and Hayman [8], where five grams of secondary roots were removed from the plants, washed and placed in 10% KOH solution and heated in a 90 °C water bath, for ten minutes. The roots were then washed in running water to remove excess KOH, placed in 10% H2O2 solution for two minutes, washed in running water and placed in 1% HCl solution for five minutes. The HCl was then discarded and 0.05% trypan blue solution was added, heated at 90 °C for ten minutes, excess dye removed and the roots were placed in lactoglycerol.

The evaluation of the mycorrhizal colonization was through the technique of Giovanetti, and Mosse [9], by observing the fungal structures (~~hyphas~~hyphae, arbuscules, vesicles and ~~glomerosporos~~spores) in the interior of the roots, in the cortex region. Root segments of approximately one cm of stained sample were randomly selected and assembled in parallel in slices, in groups of 10 (10 slides with 10 root segments each).

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One hundred root segments were sufficient for evaluation by the method chosen. The root segments were fixed with lactoglycerol, covering the entire surface of the slides, which were then covered with cover slip, without forming air bubbles.

The method consists in evaluating the presence or absence of colonization in each segment and the result expressed in percentage of colonized roots.

For statistical analysis, the means between the treatments were compared through the analysis of variance Anova and the Tukey test at 5% probability. In addition, Pearson's (r) simple correlation analysis was performed between plant height and percentage of mycorrhizal colonization. These analyzes were performed using the software Statisctica version 10.

3. RESULTS AND DISCUSSION

During the conduction of the experiment, the average temperature and atmospheric humidity of the greenhouse was 32°C and 61%, respectively.

All treatments presented a significant difference between means at the significance level of $p < 0.001$. The ~~difference between the means of the treatments for the mycorrhizal colonization of Atriplex roots and for the height are~~difference between the means of the treatments for the mycorrhizal colonization of *Atriplex* roots and for the height is presented in Table 1.

The best result obtained for the colonization of *Claroideoglomus etunicatum* in the *Atriplex* was the treatment T3 (reject plus 14g NaCl - EC = 13.13 mS / cm) in the presence of AMF in autoclaved soil (9.6%).

The root colonization observed was of the *Paris* type, characterized by the intracellular growth of ~~hyphas~~hyphae, in a linear and longitudinal way along the cortical space. The fungal structures found in all treatments are shown in Figure 1.

Table 1. Percentage of mycorrhizal colonization and height of *Atriplex nummularia* Lind. cultivated under different conditions

Treatments	Colonization (%)	Height (cm)
AC= Water + AMF	8.7 ± 0,3 abcd	72 ± 5,0 bcd
AC= Water + AMF + SHA	8.2 ± 0,2 cd	80 ± 1,0 acdb
T1= Reject + AMF	8.4 ± 0,2 bcd	106 ± 22 ab
T1= Reject + AMF + SHA	9.0 ± 0,1 abcd	73 ± 3,0 bcd
T2= Reject + 7g NaCl + AMF	7.3 ± 0,4 f	88 ± 2,0 abc

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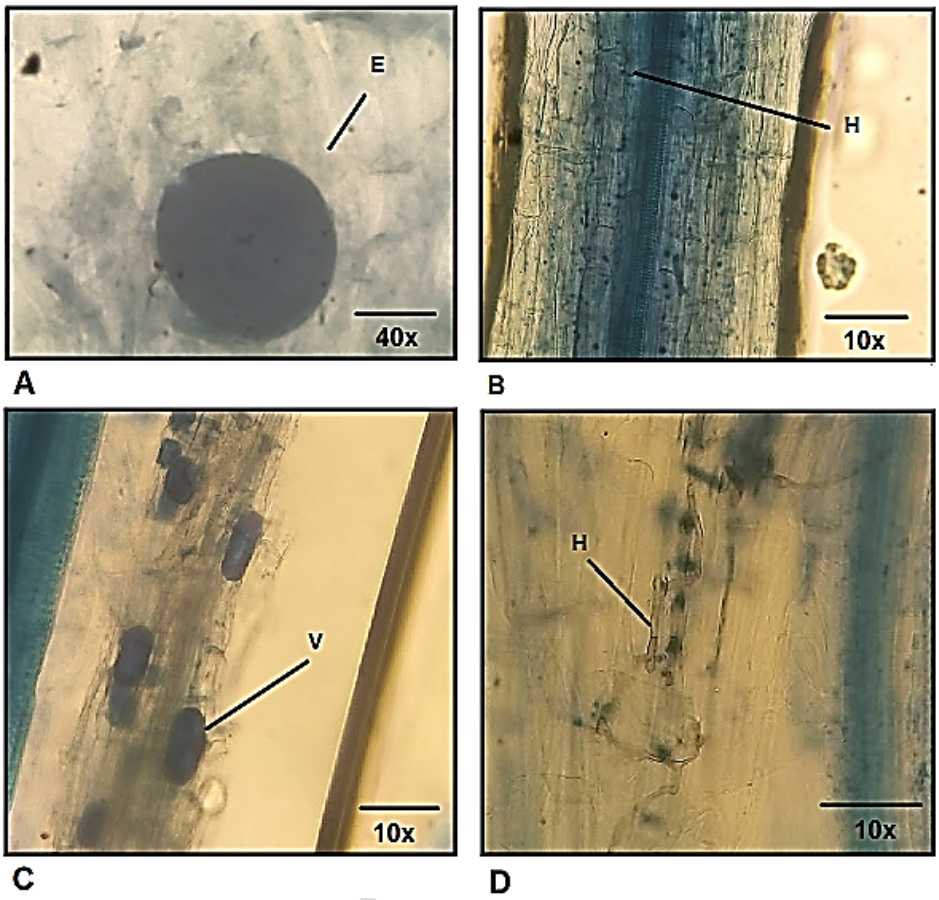
T2= Reject + 7g NaCl + AMF + SHA	8.1 ± 0,1 de	73 ± 5,0 bcd
T3= Reject + 14g NaCl + AMF	9.4 ± 0,1 ab	87 ± 1,0 abc
T3= Reject + 14g NaCl + AMF + SHA	9.3 ± 0,1 abc	70 ± 6,0 cd
T4= Reject + 21g NaCl + AMF	9.3 ± 0,2 abc	83 ± 2,0 abcd
T4= Reject + 21g NaCl + AMF + SHA	9.5 ± 0,1 ab	110 ± 2,0 a
* AC= Water + AMF	8.2 ± 0,1 de	102 ± 1,0 ab
* AC= Water + AMF + SHA	9.4 ± 0,1 abc	92 ± 1,0 abc
*T1= Reject + AMF	8.5 ± 0,1 abcd	70 ± 4,0 bcd
*T1= Reject + AMF + SHA	9.1 ± 0,2 abcd	82 ± 1,0 abcd
*T2= Reject + 7g NaCl + AMF	9.1 ± 0,2 abc	75 ± 3,3 abcd
*T2= Reject + 7g NaCl + AMF + SHA	8.8 ± 0,2 abcd	91 ± 3,0 abc
*T3= Reject + 14g NaCl + AMF	9.6 ± 0,2 a	87 ± 3,0 abc
*T3= Reject + 14g NaCl + AMF + SHA	8.1 ± 0,4 de	54 ± 6,0 d
*T4= Reject + 21g NaCl + AMF	9.3 ± 0,2 abc	72 ± 6,0 bcd
*T4= Reject + 21g NaCl + AMF + SHA	8.1 ± 0,2 de	75 ± 1,0 abcd

AMF = Arbuscular mycorrhizal fungus (Claroideoglomus etunicatum); SHA = Solution of Hoagland and Arnon (1950).
Different letters indicate a significant difference at the 5% probability level.
Values represent mean ± standard error.
* Autoclaved soil.

Furthermore, arbuscules were not observed; however, there were several septate hyphas in non-autoclaved soil of T3 and T4 treatments (reject plus 14g NaCl – EC = 14.16 mS/cm), which is not characterized by fungal ~~hyphas~~ hyphae (14 g NaCl - EC = 14.16 mS / cm).

It was observed by[10] that the percentage of mycorrhizal colonization in *Atriplex cordobensis* inoculated with *Funneliformis geosporum* in saline soils was 90%. In the work of [11], the colonization of *A. ~~numulária~~ numularia* roots by *G. intraradices* was well developed (77%), and only ~~hyphas~~ hyphae and internal vesicles were observed, the arbuscules were not found.

Figure 1. Structures of the *Claroideoglomus etunicatum* observed in the roots of *Atriplex nummularia* in non-autoclaved soil



(A) spore, (B) intracellular ~~hyphas~~hyphae, (C) vesicles, (D) ~~hyphas~~ hyphae septated from another fungus.

In the treatment with autoclaved and non-autoclaved soil, it was observed that, in the presence of AMF and in the combination AMF x SHA (Nutrition Solution of Hoagland & Arnon), mycorrhizal colonization increases according to the electrical conductivity, except for the AMF x SHA treatment in autoclaved soil. Thus, in accordance with Table 1, in the presence of the fungus, in autoclaved soil the following the following order is observed: AC (8.2%)>T1(8.5%)>T2(9.1%)>T3(9.3%), except for T4 (8.1%) and, in non-autoclaved soil: AC>(8.7)>T1(8.4)>T3(9.4) and T4(9.3), except for T2 (7.3%).

In the combination, for the non-autoclaved soil: AC (8.2%)>T1 (8.8%)>T2 (28.1%)>T3 (9.3%)>T4 (9.5%). And, finally, the autoclaved soil: AC (9.4%), T1 (9.1%), T2 (9.0%), T3 (8.1%) and T4 (8.1%).

In the work of Costa [12] the colonization with *Glomus etunicatum* (*Claroideoglomus etunicatum*), in sterile soil, was low, not exceeding 7% and in the non-disinfested soil reached 55% of colonization. These values were not observed in this study, because the percentage of colonization was higher in the treatments in non-autoclaved soil.

Hajiboland *et al.* [13], observed that the percentage of colonization in *Aeluropus littoralis* with *Claroidoglomus etunicatum* in the treatments with NaCl addition was reduced, showing saline irrigation water in three treatments: the control and addition of 50mM and 200 mM of NaCl, which resulted in colonization percentage of 33%, 16% and 10% respectively.

According to Taniguchi *et al.* [14], for the species ~~*Tamarox*~~ *Tamarix ramosissima*, tree species highly resistant to salt and drought, the colonization of AMF increased with EC of soil at low and medium salinity levels (0.4 to 4.3 dS m^{-1}) but decreased at high salinity levels (> 7 dS m^{-1}).

Soil pH ranged from 7.6 to 7.9. In relation to the pH presented by the autoclaved and non-autoclaved soil, [15] state that the pH near neutrality favors the *Glomus* species.

The correlation between height and colonization of Atriplex was significant at $p < 0.05$ level for the treatments with ~~*Claroideoglomus*~~ *C. etunicatum*. In non-autoclaved soil, with T2 + Fungus (R = -0.91) and T4 + Fungus (R = 0.90). For the autoclaved soil the correlation was as follows: in the treatment AC + Fungus (R = 1,00) and in T1 + Fungus (R = -0.97).

Asghari *et al.* [16] observed that *A. nummularia* at low salinity level 2.2 $dS.m^{-1}$ had a colonization percentage of 2%; even though, despite the low colonization, the inoculation promoted the growth of the plants and affected the nutrient absorption positively.

4. CONCLUSION

In view of the results obtained it is concluded that: the mycorrhizal association was beneficial for all treatments; the best treatment involving the percentage of mycorrhizal colonization consisted of T3 (reject plus 14g NaCl - EC = 13.13 mS / cm) in the presence of AMF ~~*Claroideoglomus*~~ *C. etunicatum* in autoclaved soil; for the height of the ~~*Atriplex*~~ *A. nummularia*, it was observed that the treatment T2 (reject plus 7g NaCl - EC = 12.4 mS / cm), in the presence of fungus, in the non-autoclaved soil, presented better result; when positively correlated, the height with the percentage of colonization,

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the most significant treatments were T4 (reject plus 21g NaCl - EC = 14.16 mS / cm) in the presence of ~~*Claroideoglomus*~~ *C. etunicatum* in non-autoclaved soil and AC (water - EC = 2.86 mS / cm) in the presence of fungus in autoclaved soil; in non-autoclaved soil the presence of microorganisms may have promoted a combination of beneficial associations that favored the growth of Atriplex; in autoclaved soil, there is a possibility of having suffered with the abiotic stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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