1. Original research papers

Influence of Arbuscular Mycorrhizal Fungus in the Development of Salicornia in Saline Water

ABSTRACT

The objective of this work was to evaluate the effect of the inoculation of the arbuscular mycorrhizal fungus (AMF) Claroideoglomus etunicatum on the development of Salicornia ramosissima subjected to five treatments: water, desalinator reject, reject plus one dose (7g) of NaCl, reject plus two doses of NaCl and reject plus three doses of NaCl. The experiment was conducted under greenhouse conditions at the Agronomic Institute of Pernambuco - IPA, in a completely randomized design, with autoclaved and non-autoclaved soil, with three replications. A dose corresponding to 1.0 mL/ kg of Hoagland & Arnon complete nutrient solution soil was added to each well and watered daily with the corresponding treatments. In the conduction of the experiment, polyethylene vessels with 8 kg of saline soil were used. The results showed that the reject with the intermediate doses (Reject + 1NaCl and Reject + 2NaCl) was more significant for the growth (17.16 cm and 17.37 cm respectively) of Salicornia ramosissima.

Keywords: Salicornia, inoculation, reuse, salinity.

1. INTRODUCTION

In Brazil, the scarcity of water is quite visible, especially in the semi-arid region of the Northeast, which corresponds to 58% of the territory. Water used in irrigation in this region has a high salt content, both in surface and underground waters, in small and medium dams (surface) and wells (groundwater). In addition, the availability of water for human consumption and for agricultural practice has been gradually reduced in both

quality and quantity, thus necessitating the use of alternative water of inferior quality to meet the demand of agricultural irrigation in these regions [1].

To minimize this problem, reverse osmosis water treatment plants were installed in several rural communities in the Northeast in order to obtain drinking water for families through the desalination of brackish water from wells. However, in the desalination process it generates, in addition to drinking water, a highly saline and high pollutant reject [2].

Desalination reject can pose a serious environmental threat due to its salinity; therefore, systems were developed aiming at the use of evaporation tanks for the creation of fish, particularly of the genus Tilapia, along with the irrigation of salt tolerant forage plants. However, this technique can transform an environmental problem (the discarding of desalination reject) into a water source for new economic activities [3].

According to [4], as an example of halophyte, there is Salicornia which was introduced in the European market as a culture that develops in the presence of high salt concentration with shoots without leaves. It resembles green asparagus, is in high demand in the gourmet market, not only for its salty taste, but also for its nutritional value in terms of minerals, antioxidants and vitamins. Even so, little information is available on cultivation conditions.

The studies of [5] confirmed the beneficial effect of arbuscular mycorrhizal fungi on the most varied plant species and conditions, stimulating plant growth as a consequence of the effect on their nutrition.

In order to contribute to minimizing the negative effects of the inappropriate use of the desalinator reject, an experiment was proposed to analyze the effect of the association of arbuscular mycorrhizal fungi on the development of Salicornia ramosissima submitted to different levels of salinity.

2. MATERIALS AND METHODS

The experiment was conducted from November 2017 to March 2018, in a greenhouse, at the Agronomic Institute of Pernambuco - IPA, located at Avenue General San Martin, 1371 - Bongi, Recife, Pernambuco, Brazil.

The experimental design was completely randomized, in a 2 x 2 x 2 x 5 factorial arrangement, corresponding to autoclaved and non-autoclaved soil, inoculated and non inoculated plants, presence and absence of nutrient solution of [6] (fortnightly) and 5 levels of irrigation treatment: water (absolute control), desalinator waste (control) and three salinity combinations, whose concentrations were obtained from addition of sodium chloride (NaCl) to the reject, calculated according to Richards (1954), namely: 7g – EC=12.612 $\mu S/cm^2$; 14g = EC=13.744 $\mu S/cm^2$ and 21g = EC=14. 746 $\mu S/cm^2$, with three replicates.

The soil used in the experiment was collected at the IPA Experimental Station, in the city of São Bento do Una, Pernambuco, which had the following characteristics: sandy texture, soil density – 1.34 g/cm^3, pH – 7.60, P - 209 mg/dm^3 and Ca, Mg, Na and K – 33.60, 6.00, 12.00 and 0.70 $cmolc/dm^3$ respectively. The average temperature inside the greenhouse during the experiment was 34ºC. According to the classification of Köeppen, the region presents an As' (Tropical Wet) climate. The mean relative air humidity inside the greenhouse was 56.6%.

The reject used in the experiment was collected in a desalinator located in the city of Riacho das Almas, Pernambuco, Brazil, Electric Conductivity - uS/cm^2 at 25ºC = 10905.00, pH = 7.0 and Ca, Mg, Na and K = 139.83, 304.94, 2760.00 and 22.00 $cmolc/dm^3$ respectively.

In the experiment, 5 cm small cutted pieces of Salicornia ramosissima were planted with commercial substrate in germination trays with 128 cells, for a period of 30 days. After 30 days of rooting, the plants were transferred to the polyethylene pots with 8 kg of air-dried soil, dewormed, homogenized and sieved in 2 mm mesh, where 50% of the soil was sterilized by autoclaving.

The inoculation with Arbuscular Mycorrhizal Fungus (AMF) was performed when the rooted plants were transported to the vessels, adding 50g of inoculum of Claroideoglomus etunicatum from the AMF Inoculum Bank of the Laboratory of Soil Microbiology (IPA).

After four months, the roots were collected, separating them at the height of the plant colon and washed with deionized water, determining the weight of the root fresh matter (RFM).

The root staining for observing the colonization was carried out using the methodology described by [7] modified where five grams of secondary roots were removed from the plants, washed, and placed in a solution of KOH at 10% and heated in a water bath at 90°C for 10 minutes. The roots were then washed in running water to remove the excess of KOH, placed in an H_2O_2 solution at 10% for 2 minutes, washed in running water again and placed in an HCl solution at 1% for 5 minutes. The HCl was discarded and a trypan blue solution at 0.05% was added, heated at 90°C for 10 minutes, excess dye was removed and the roots were placed in lactoglycerol.

The evaluation of the mycorrhizal colonization was done through the technique of [8], by observing the fungal structures (hyphae, arbuscule, vesicles and glomerospores) inside the roots, in the cortex region. Root segments of approximately 1cm of the stained sample were randomly selected and assembled in parallel slides, in groups of 10 (10 slides with 10 root segments each). One hundred segments of roots were sufficient for evaluation. The root segments were fixed with lactoglycerol, covering the entire surface of the slide, which were then covered with coverslip, without forming air bubbles.

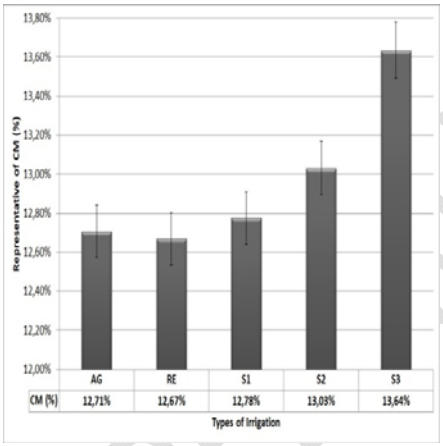
The method consisted in evaluating the presence or absence of colonization in each segment and the result expressed in percentage of colonized roots.

The data were submitted to individual and joint statistical analysis, pertinent to the studied variables. The variance was tested by Analysis of Variance (Anova) using the statistical software Minitab.

3. RESULTS AND DISCUSSION

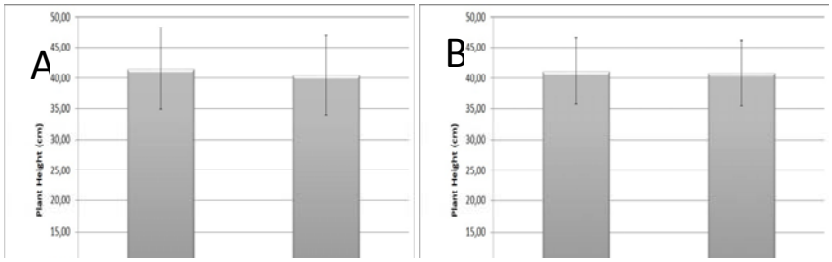
The roots of Salicornia ramosissima inoculated with Claroideoglomus etunicatum showed characteristic structures of AMF at all levels of salinity, including water irrigation (absolute control), respectively 39%, 37%, 43%, 37% and 44%. Therefore, for the increase or decrease of mycorrhizal colonization (MC), these levels did not present significant statistical difference. However, the reject + 3NaCl (highest dose) showed the best mycorrhizal colonization - 44% (Fig. 1). [9] studying sapiens' seedlings with different levels of salinities, noticed that mycorrhizal root colonization was reduced. [10] working with melon also noticed the reduction of mycorrhizal colonization in the presence of salinity.

Fig. 1: Effect of saline irrigation (AG - water); RE (Reject); S1 (Reject + 1 NaCl); S2 (Reject + 2 NaCl) and S3 (Reject + 3NaCl) on the mycorrhizal colonization (MC) of Salicornia ramosissima



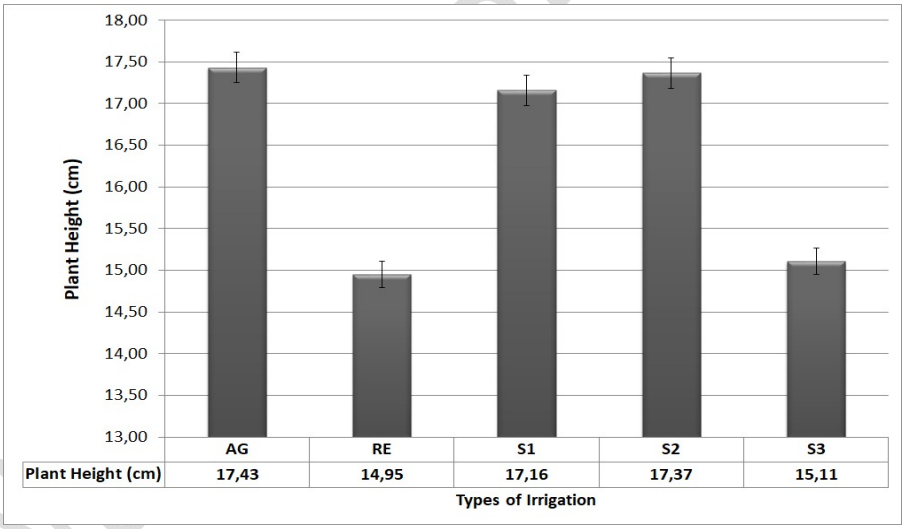
It can also be verified that in relation to *Salicornia* height there was no significant difference, both for the presence and absence of Claroideoglomus etunicatum and for the presence and absence of nutrient solution (Fig. 2A and 2B). [11] in an experiment under greenhouse conditions showed that the increase in soil salinity due to irrigation with saline water causes a reduction in plant height. [12] showed that using arugula with saline solution there was no difference in arugula growth as a function of the salinity of the nutrient solution.

Fig. 2: Relationship between the presence and absence of Claroideoglomus etunicatum (A) and Nutritive Solution (B) for the growth of Salicornia ramosissima



The increased salinity of irrigation water inhibited the growth of Salicornia (Fig. 3). A similar result was found by [13] in the growth of the sesame plant. Reduction of salinity growth has been attributed to osmotic stress caused by the reduction of external water potential and the ionic effect caused by the accumulation of ions in plant tissues [14].

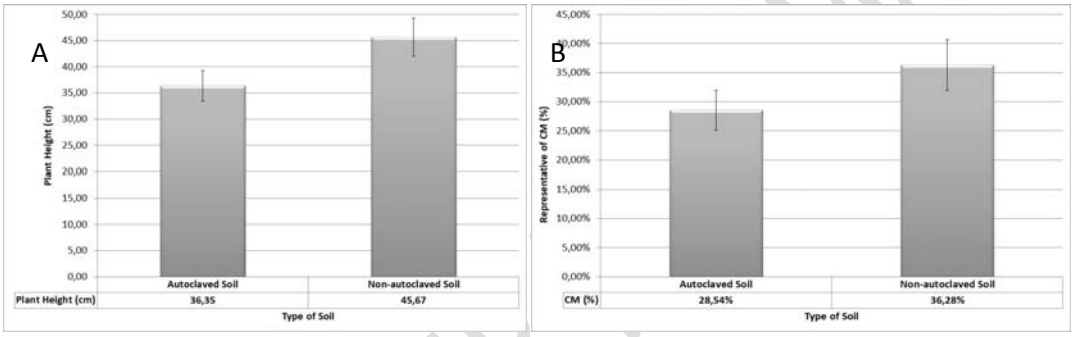
Fig. 3: Height of Salicornia ramosissima irrigated with AG (water), RE (Reject), S1 (Reject + 1NaCl), S2 (Reject + 2 NaCl) and S3 (Reject + 3



As shown in figures 4A and 4B, non-autoclaved soil is ideal for *Salicornia* growth compared to autoclaved soil and also for mycorrhizal colonization - MC. [15] evaluated the effects of the inoculation of native arbuscular mycorrhizal fungi (AMF) formed by the species Glomus etunicatum, G. glomerulatum, Scutellospora sp. and Acaulospora foveata, from the first community, and G. etunicatum, Entrophospora sp. and Scutellospora sp., from the second community, with autoclaved soil, on the growth and accumulation of nutrients in seedlings of the precocious dwarf cashew CCP 76. An

advantageous response was observed in the development of cashew tree seedlings at four months of sowing.

Fig. 4: Salicornia ramosissima height (A) and Mycorrhizal colonization (B) in the autoclaved and non-autoclaved soil



4. CONCLUSION

Based on these results it is concluded that high salinity (reject + 21g NaCl, EC = 14. 746 µS/ cm^2) reduces the growth of *Salicornia ramosissima* and the intensification of saline stress conditions (reject + 21g NaCl, EC = 14. 746 µS/ cm^2) increases mycorrhizal colonization (44%). The growth of Salicornia ramosissima (30.45 cm) is favored in the non-autoclaved soil. Mycorrhizal colonization showed results that are more significant in non-autoclaved soil. The addition of Hoagland & Arnon nutrient solution is not significant for Mycorrhizal colonization nor for the growth of Salicornia ramosissima.

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