

*Full Length Research Paper*

# Arbuscular mycorrhizal fungi (AMF) promote the growth of the pioneer dune plant of coastal areas

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The establishment of economic enterprises and the demand for coastal areas for leisure purposes exert great pressure on the dunes, stripping them of vegetation and causing the aggravation of coastal erosion processes. The use of arbuscular mycorrhizal fungi (AMF) is a viable alternative to restore dunes, giving their ability to improve soil conditions and plant growth under unfavorable conditions. The aim of this study was to evaluate the effect of AMF on the growth of *Canavalia rosea* (Sw.) DC. For the experiment, two treatment groups were set up (16 pots with arbuscular mycorrhizal fungi inoculum and 16 pots without) over a period of 10 weeks. It was found that *C. rosea* (Sw.) DC. responded positively to the inoculation with the AMF and the percentages of colonization were 6.4 and 10.2% in the eighth and tenth week of plant growth, respectively. Also, the growth of *Canavalia rosea* (Sw.) DC. increased significantly with AMF throughout the experiment. This proved the efficacy of arbuscular mycorrhizal fungi as promoters of dune plants' growth, and as potential strategy for the rehabilitation of dunes in the coastal areas.

**Key words:** *Canavalia rosea*, inoculation, mycorrhizal colonization, dune restoration, re-plant.

## INTRODUCTION

The coastal dunes are very important dynamics ecosystems playing different ecological roles such natural barriers and protection of coastal areas from extreme sea activities, natural boundary of the shoreline movement and preservation of beaches (Henrico et al., 2020). There is a pioneer vegetation cover associated with coastal dunes assuming a primordial role in soil (Sigren et al., 2014), protecting it against wind (Dewhurst, 2002; Bar et al., 2016), allowing the protection and maintenance of the

coastline (Gomes-Neto et al., 2004) and reducing the occurrence of erosion (Dewhurst, 2002; Sigren et al., 2014; White et al., 2019).

The coastal dunes are affected by various natural and anthropogenic factors (White et al., 2019). The intentional or accidental introduction of alien species in dunes (Sun et al., 2017; Malavasi et al., 2018; Marzialetti et al., 2019) increase in the establishment of economic enterprises, inadequate land parceling (Maueua et al., 2007) and the

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demand for coastal areas for leisure purposes (Langa, 2007; Teixeira et al., 2016) exert great pressure on the dunes, stripping them of vegetation (Langa, 2007; Sperandii et al., 2019) and causing the aggravation of coastal erosion processes (Maueua et al., 2007; Bar et al., 2016).

The escalation of coastal erosion processes affected at least 55% of coastal dune around the world (Bar et al., 2016) will lead to the destruction and loss of habitats, soil erosion and depletion, water pollution, alteration of the coastline configuration, destruction of infrastructure and loss of investment (Hoguan, 2007; Gracia et al., 2018). Can also cause the extinction of plant and animal species occurring in coastal dunes (Prisco et al., 2013), and it is estimated that 85% of the existing coastal dunes are under threat (Henrico et al., 2020).

Around the world diverse techniques have been applied to restore coastal dunes, but the majority are expensive to implement (Teixeira et al., 2016). One of the interventions used to reduce the observed erosion process was the re-colonization of the dunes (Langa, 2007; Assis et al., 2016). Studies showed that using plants in coastal dune restoration has an enormous potential to reduce erosion under wind and wave action (Sigren et al., 2014). However, this was difficult and slow (Bécard et al., 2004; Teixeira et al., 2016), because of the high mortality rates of seedlings as a consequence of the stress to which they were subjected on the dunes (Gomes-Neto et al., 2004; Teixeira et al., 2016).

The use of mycorrhizae is a viable alternative to restore dunes (Bécard et al., 2004; Asmelash et al., 2016; Assis et al., 2016), giving their ability to improve soil conditions and plant growth under unfavorable conditions (Bever, 2003; Al-Karaki, 2013; Amir et al., 2013; Winagraski et al., 2019), and it is known that AMF are highly prevalent in coastal dune plants (Sigren et al., 2014). Thus, since AMF offered a possible solution to the identified problem, it was relevant to study their possible effects on the growth of *Canavalia rosea* (Sw.) DC., a typical dune pioneer, which was of extreme importance in the stabilization process of dunes (Kitajima et al., 2008; Mendoza-González et al., 2014) and widely used to control soil erosion in several countries due to dense cover, root binding the substrate and the quick growth (Mendoza-González et al., 2014). Therefore, the present study aimed to provide information about the mycorrhizal association with *C. rosea*, as no study has been published to date on mycorrhizal association with this plant. Also, to evaluate the effect of the use of AMF inoculum on *C. rosea* growth and to determine the percentage of colonization in its roots.

## MATERIALS AND METHODS

### Study area

The experiment was conducted in the greenhouse and in the Plant Physiology Laboratory of the Department of Biological Sciences of

the Eduardo Mondlane University, Maputo, Mozambique, over a period of three months. The experimental design was completely randomized, with two treatments (with inoculum-propagules of *Glomus intraradices* N.C. Schenck and G.S. Sm. and control) with eight replicates for each treatment.

### Sampling and laboratory procedure

The seeds were collected from dry pods of *C. rosea* (Sw.) DC on the dunes of Muntanhana beach in Maputo. The soil was collected to a depth of about 20 cm and then autoclaved at 120°C for 90 min to eliminate microorganisms in the soil that could influence the results of the experiment (Miyasaka et al., 2003). The samples were immersed in 40% sodium hypochlorite for 15 min, to disinfect them according to the protocol described by Zorato et al. (2001). Subsequently, the samples were immersed in 100% sulfuric acid for 110 min to break dormancy (Hartmann et al., 2001). The seeds were pre-germinated in Petri dishes lined with filter paper moistened with distilled water, and those with a radicle equal to or greater than 2 mm in length were used for sowing.

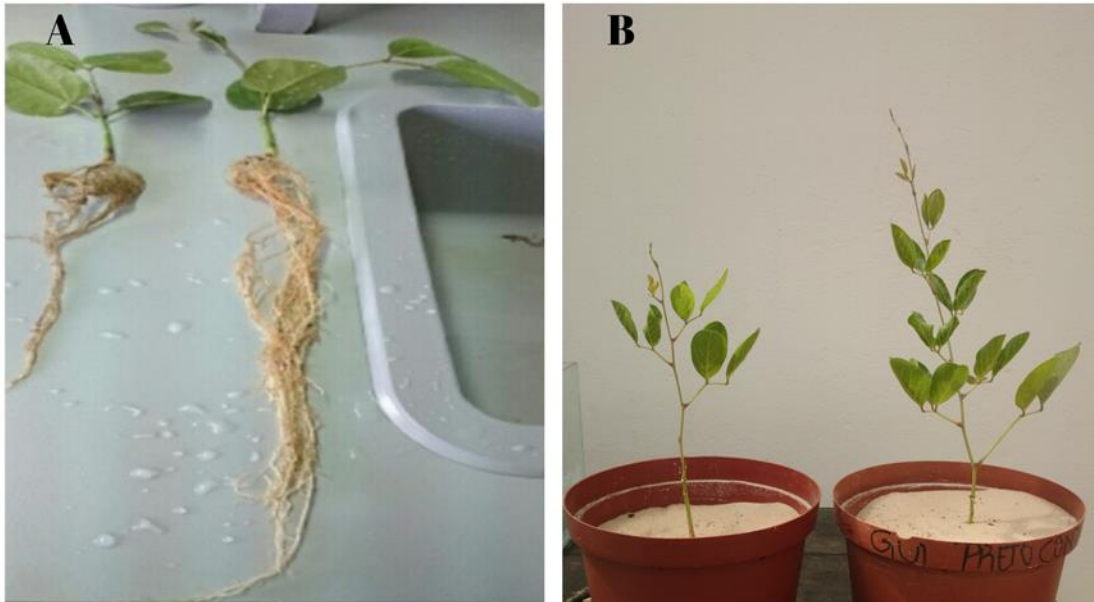
In the first phase, the sowing was carried out in 1kg pots containing autoclaved soil, and these were divided into two treatments: 1) control (16 pots without inoculum) and 2) inoculum (16 pots with *Glomus intraradices*). In the "control" treatment, the seeds were placed in opened clumps in the pots, and in the "inoculum" treatment, a 9 g of inoculum was added per pot, followed by the seed. Irrigation was done with 20 ml of distilled water, in other days for five weeks. After five weeks, all the seedlings (control and inoculum) were transplanted to 5 kg pots, where watering per pot was done with 100 ml of running water every two days. In the third and fifth week after transplantation, the eight and tenth weeks of growth after sowing, the plants were harvested in both treatments. The plants were separated into roots, stems and leaves for determination of growth parameters (dry weight, root length, plant height, number of leaves and leaf area) and percentage of roots colonized by AMF. Dry weight was obtained after drying for 72 h in a 65°C oven, and weighed on a root, stem and leaf electronic scale. The maximum root length and plant height were measured with a ruler. The number of leaves was determined by manual counting, and the leaf area was measured by using a leaf area meter (Model 3100 LI-Cor Inc., Lincoln, NE, USA). The percentage of colonized root was determined by the Locatelli and Lovato (2002) root staining method.

### Data analysis

The data were analyzed using the statistical package SPSS Statistics. The non-parametric Mann-Whitney test was used to compare growth averages between inoculated and control plants, and it was considered significant at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

The *C. rosea* (Sw.) DC associated with mycorrhizal fungi responded positively to the mycorrhizal inoculation, and presented 60% higher dry weight production ( $p \leq 0.05$ ) compared to the non-inoculated bean (Figures 1B and 3A). This corroborates with the results of De Oliveira et al. (2009), in a greenhouse experiment using Brazilian dunar native soil with AMF who found an increase of 75.93% of aerial biomass and 76.79% of radicular biomass in *Tabebuia roseo-alba* (Ridl.) Sandw. and 88.34% increment of aerial biomass and 87.17% of



**Figure 1.** Effect of inoculation with arbuscular mycorrhizal fungi on plant root length (A), and plant height (B) in *C. rosea* (Sw.) DC. On the left, non-inoculated plant, and on the right, inoculated plant.

radicular biomass in *Tocoyena selloana* Schum. The biomass increase may be a consequence of the improvement of the nutritional status due to the AMF inoculation as elucidated in a review by Chen et al. (2018). This fact was confirmed by Wang *et al.* (2019) who found an increase in phosphorus, potassium and magnesium uptake in a study with *Zelkova serrata* seedlings inoculated with *Funneliformis mosseae*.

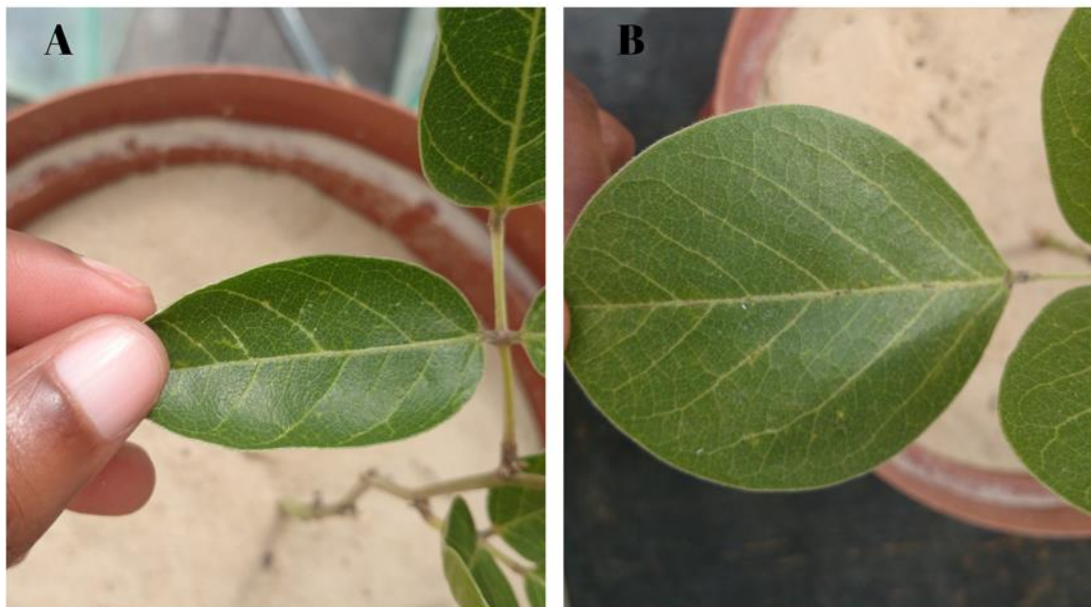
The mycorrhizal inoculation resulted in a higher root growth, reflecting the increase by 28% of the maximum length of the *C. rosea* root compared to the non-inoculated ( $p \leq 0.05$ ) (Figures 1A and 3B). A higher growth of inoculated plant roots was expected, since several studies reported the benefits of mycorrhizal association for root growth (Carneiro et al., 2004; Júnior and Da Silva, 2006; Balota et al., 2011; Hidalgo, 2015; Sharma et al., 2017).

According to Smith and Read (2008), the response is related to the scarcity of water and nutrients in the soil, causing AMF stimulus of root branching and better development and consequently giving the host plant a greater capacity to absorb water and nutrients. Similar results to those verified in the present study were also observed by Little and Maun (1996) and Feagin et al. (2008) when studying the efficiency of the mycorrhizal association in pioneer dune species, *Ammophila breviligulata* and *Uniola paniculata* for the restoration of degraded dunes in the United States. Pérez-de-Luque et al. (2017) also showed the AMF stimulation on root growth.

An increase of 20% in height was also observed in inoculated bean compared to non-inoculated, although it

was a statistically non-significant difference ( $p \geq 0.05$ ) (Figure 1B and Figure 3C). This is according to Peña-Becerril et al. (2016) who observed an increase 3 times greater in *Mimosa biuncifera* Benth. height when inoculated with native AMF compared to non-inoculated. The increase in height is a positive point because combining the positive responses obtained during the experiment in the other parameters showed the effect of the AMF on the plant and how this higher uptake of nutrients resulted in increased plant growth (Marschner and Dell, 1994; Lanfranco et al., 2018; Begum et al., 2019). The fact that it has not differentiated from non inoculated plants in the study by Brandon and Shelton (1993), who reported that there was a latency phase between mycorrhizal inoculation and the time when its effects were manifested in the plant and this fact was also corroborated by Lanfranco et al. (2018). Even having a height above the control may not be significant until 45 days, the difference being significant from 60 days until the end of the experiment.

The results of the experiment by Oliveira et al. (2009) are in agreement with those of previous authors, since the native Brazilian dune plant, *Tabebuia roseo-alba*, used in the study grew to a higher height than non-inoculated plants; however, the superiority was shown to be significant only at 75th day. This suggested that the same effect probably would happen in this experiment by extending the plant observation time and the differences would become significant. Besides the experiment of Oliveira et al. (2009), similar results were verified by Dos Santos et al. (2016), studying AMF effects on a leguminous species called *Albizia polycephala*, where



**Figure 2.** Effect of inoculation with arbuscular mycorrhizal fungi on leaf area in *C. rosea* (Sw.) DC. **A:** Non-inoculated plant; **B:** Inoculated plant.

they found that *Acaulospora colombiana* (AMF species) had increased positively and significantly the neck diameter, leaf size and dry matter. On the other hand, it hadn't increased significantly the height at 135 days of experiment, although it had shown an increase in height of 20% more than non-inoculated plants. This suggested that these criteria probably would not change even if the experiment had continued for an extended period.

In contrast, Lu et al. (2015) found different results studying AMF effects of *Glomus intraradices* and *Glomus mosseae* on the growth of *Morus alba* L. There were significant increases in dry matter, number of roots, as well as the height of the plant compared to non-inoculated plants. Since the same species (*G. intraradices*) was used in the present study, but in plant belonging to other family, this result suggested that it is not correct to assume that the same fungus species can contribute in the same way to all hosts, the same species can contribute in a different way on the growth of different hosts.

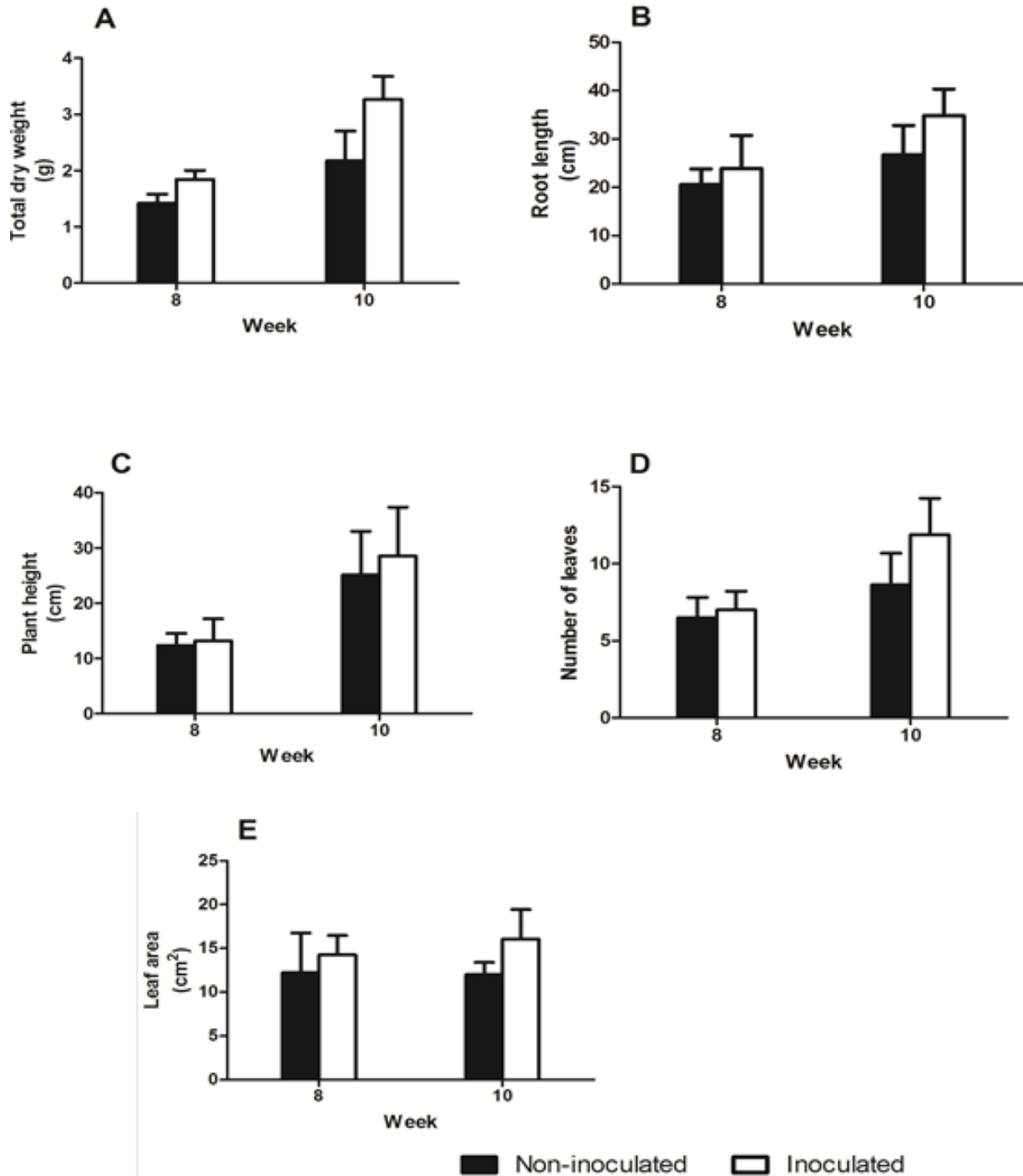
At leaf level, mycorrhizal inoculation resulted in the production of a larger number of leaves and a larger leaf area compared to the non inoculated bean, although this was statistically significant only at the tenth week of growth ( $p \leq 0.05$ ) with increases of 56 and 36%, respectively (Figures 2 and 3D and E). The same result was verified by Lu et al. (2015) studying AMF effects of *G. intraradices* on the growth of *M. alba* L. Shi et al. (2016) observed in all tested plant growth parameters, including aboveground criteria, an increase of 31 to 121% in AMF inoculated mulberry plants.

For Oliveira et al. (2005), the processes of faster leaf

growth and the production of greater numbers of leaves and leaf areas may be related to greater energy reserving (phosphorus) in inoculated plants. As a result of this, AMF stimulate these plants to increase their exploitation of solar irradiation and their potential for photosynthesis (because their leaves have greater chlorophyll a+b), consequently an increase in the biomass accumulation occurs (Oliveira et al., 2005; Tristão et al., 2016). This is corroborated by Gao et al. (2020) in a study with *Gossypium hirsutum* L. demonstrating the positive effect of AMF in phosphorus absorption and consequent enhance of plant growth. The observed productivity is a consequence of functional efficiency as a response to the higher uptake of water and nutrients verified in AMF inoculated plants (Romero, 2012), which allows greater development of leaves than non-inoculated plants (Atwell et al., 1999; Peña-Becerril et al., 2016; Wang et al., 2019).

The colonization of the bean roots by AMF was verified from the eighth week of growth, evidenced by the presence of hyphae and vesicles in the cortex of the root segments. Root colonization aroused probably as a result of the germination of AMF spores present in the soil associated with low nutrient availability in the soil (Avila, 2004; Silva-Flores et al., 2019) and other soil factors such as water availability, temperature and relative humidity (Dell-Santo, 2011; Kilpeläinen et al., 2020).

The spore germination starts when the plant (host) under low phosphate or nitrogen conditions exudes or excretes strigolactones from its roots into the rhizosphere, and these are recognized by AMF, inducing the spore germination and then enhancing fungal

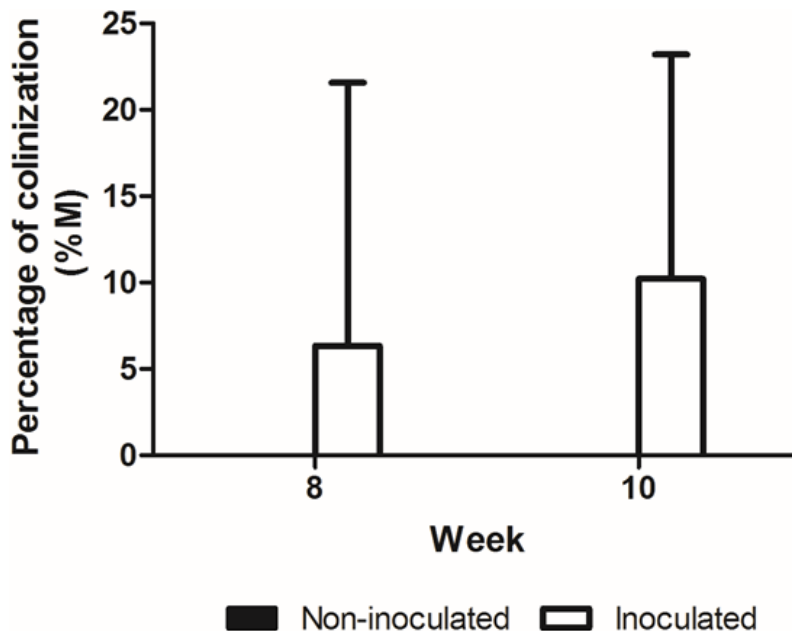


**Figure 3.** Effect of inoculation with arbuscular mycorrhizal fungi on plant dry weight (A), root length (B), plant height (C), number of leaves (D) and leaf area (E), in the eighth and tenth week of growth in *C. rosea* (Sw.) DC. Each bar represents an average of eight plants. The vertical lines indicate the standard deviation. In each week, the Mann-Whitney Test was performed for comparison at 5% probability.

metabolic activity, hyphal growth and branching, and exudation of a short chain of chitin oligosaccharides (Pimprikar and Gutjahr, 2018).

The justification presented above may be applicable to

the present work, since dune soil, characteristically known for low organic matter decomposition and low soil fertility (Camprubíet et al., 2010; Oliveira and Landim, 2020), the same characteristics were presented by the



**Figure 4.** Colonization percentage (% M) in *C. rosea* (Sw.) DC. roots in the eighth and tenth week of growth. Each bar represents an average of eight plants. The vertical lines indicate the standard deviation.

substrate used, and as suggested by Smith and Read (2008) and Nayak et al. (2019), the AMF were probably stimulated in soils with low fertility.

The percentage of root colonization (% M) by AMF increased from 6.4 to 10.2% throughout the experiment (Figure 4). Similar percentages were also found by Santos et al. (2004) in *Ammophila arenaria* (L.) plants associated to AMF, having observed values of 5.9 and 1.9% in dune systems of two different regions, and by Mocuba (2005) in *Carpobrotus dimidiata* (L.) L. Bolus, a dune plant, having observed 3.5 and 17.5% of colonization throughout the experiment. However, the percentages over 50% of root colonization were observed by Shi et al. (2016) in mulberry seedlings with *Acaulospora scrobiculata*, *Funneliformis mosseae*, and *Glomus intraradices* and by Peña-Becerril et al. (2016) in a study with *Mimosa biuncifera* (Benth.) inoculated with native AMF.

Simpson and Daft (1990) reported that the development of AMF colonization in the roots initially showed a period of colonization delay and low intensity due to the need for perfect symbiotic integration between the mycorrhizae and plant (fungi and roots), followed by a growing phase involving colonization and stabilization therefore over the growing time. This fact was also referred by Lanfranco et al. (2018) explaining the different stages of the communication between fungi and plant roots in AMF symbiosis.

The results of the present study corroborated these observations, since the percentage of colonization was low, with an increase over the experiment, suggesting

that the percentage of colonization probably followed the trend reported by Simpson and Daft (1990) and could increase over time to a stabilization phase. A different pattern of colonization was found by Juntahum et al. (2019), in a study with sugarcane inoculated with AMF decreasing the colonization percentage over the time. These results showed once again the dynamic complexity of AMF symbiosis in field conditions.

## Conclusion

From this study, it was found that *C. rosea* (Sw.) DC. have association with *G. intraradices* and the mycorrhizal fungi have a positive effect on *C. rosea* (Sw.) DC. growth and the levels of root colonization were 6.4 and 10.2% with a tendency to increase along its growth.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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