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How does mycorrhiza interact with different levels of fertilization on *Prosopis alba*? †

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Abstract: This study assessed how the interactions between chemical fertilization levels and arbuscular mycorrhizal fungus species (AMF) affected the seedling's morphology and biochemical traits in *Prosopis alba*. Subsequently, the seedlings were inoculated or not with a mixture of native AMF from two origins of contrasting sites in the Chaco Region. Preliminarily, we observed a positive mycorrhizal response to the AMF in interaction with chemical fertilization (at 60% fertilizer concentration). These results were reflected mainly over some morphological parameters than over biochemical parameters. The lack of a clear answer is probably since the benefits of the symbiosis on the host plant could be observed in the establishment phase in the field.

Keywords: Arbuscular mycorrhizal fungus; bio-fertilizers; biochemical parameters; mycorrhizal response; morphological parameters; quality seedlings

1. Introduction

The production and application of microbial fertilizers are gaining global increase owing to the negative impacts of overuse or improper usage of chemical-based fertilizer and the increased awareness about the association between rhizosphere microorganisms and plants [1];[2];[3];[4]. AMF has even shown a significant potential to be used as a biofertilizer to improve the initial growth and establishment of tree seedlings in

restoration projects[5]. Inoculated plants could show higher growth rates, lower resource needs, and may be more tolerant to transplanting stress, critical features in restoring degraded areas[6]. In this sense, the study species *Prosopis alba* has considerable potential as native species in restoring areas in the Chaco Semi-arid Region [7]. AMF are soil-borne fungi that could significantly improve plant nutrient uptake and resistance to several abiotic stress factors [8];[5]. AMF hyphae exclusively colonize the root cortex and form highly branched structures inside the cells,i.e., arbuscules, which are considered the functional site of nutrient exchange [9]. Therefore, we aimed to evaluate the effect of the interactions between the levels of chemical fertilization and the inoculum of arbuscular mycorrhizal fungi (AMF) on the production of *P. alba* during the nursery stage.

2. Materials and Methods

2.1. Plant Material and Experimental conditions

The experiment was carried out at the Experimental Station "Fernández" (Agreement Catholic University of Sgo del Estero-Province of Santiago del Estero) in Santiago del Estero, Argentina (−27◦560 S, 65◦ 52.50 W). An experiment was conducted from October 28, 2021, to January 28, 2022. Seedlings were produced in trays of individual cells in a nursery with 50% of shading under natural light conditions for 45 days. After that, the plants were exposed to full sun in the acclimation phase until completing 90 days. The levels of chemical fertilization applied according to concentration were: F0%, F30%, F60% and F100%, varying from no fertilizer application to maximum concentration. The foliar fertilizer was applied by spraying once a week in the first hours of the day. In the nursery phase, "YOGUEN N° 3" fertilizer (NPK 25-14-8) was used in a proportion of 5g/L (F100). In the acclimation phase, "HAKAPHOS Base" fertilizer (NPK 7-12-40) was used in a proportion of 2g/L (F100). A control situation without application of mycorrhizae (SM) was compared with two inoculum of arbuscular mycorrhizal fungi (M1 and M2) associated with two different rainfall patterns (explained in 2.2).

2.2. Mycorrhizal: isolation, multiplication and application of AMF inocula

Mixed inocula of native AMF were selected from *P. alba* stands located in the Argentine Chaco Region, with two different rainfall regimes: Padre Lozano (PL=M1) in the Western Chaco Domain, Salta province, with 650 mm annual precipitation and Colonia Benítez (CB=M2) in the Eastern Chaco Domain, Chaco province, with annual precipitation of 1300 mm[10]. Six soil samples per tree were collected at a depth of 20 cm. The AMF inoculum is composed of mycorrhizal roots, spores and hyphae of alfalfa (*Medicago sativa*) and sorghum(*Sorghum sp.*) plants. As trap plants, this inoculum was initially used with "Algarrobo blanco" (*Prosopis alba*). M1: *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*; *Diversispora spurca*, *Funneliformis mosseae* and *Rhizophagus intraradices*; M2: *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, *Funneliformis constrictum*, *Funneliformis mosseae*, *Rhizophagus clarus* [11]. Inoculation was performed at sowing by applying 20 g of AMF inoculum (consisting of a mixture of substrate, spores, mycelium and fragments of mycorrhizal roots of trap plants) in the planting hole per container for inoculum (M), and a control treatment was not inoculated (NI=SM)[12].

2.3. Morphological Characteristics

Morphological traits were measured to evaluate the effects of the treatments on plants. Ten plants per treatment were selected and registered (i) stem neck diameter (SND), (ii) shoot height (SH), and (iii) slenderness index (SI) according to standard

protocols. The slenderness index was determined considering the diameter and shoot height ratio. For biomass evaluation, three plants were randomly selected and each plant was divided into shoots and roots. Root-balls were carefully washed to remove substrate l and weighed immediately. The material was dried to constant weight in an oven at 72°C (±5°C) for 48 h to determine dry weight. Data were processed and registered the aerial dry weight (ADW), and root dry weight (RDW).

2.4.Mycorrhizal response

The Mycorrhizal Response (MR) was calculated for the diameter (SND) according to the relationship described by Cavagnaro et al. (2003) [13]:

MR= [(M -media NM) /media NM) x 100]

M corresponds to the diameter parameter of the inoculated plants, and the mean NM corresponds to the non-inoculated plants.

2.5. Biochemical Characteristics

For this assay, chlorophylls and carotenoids contents were evaluated according to methodology followed by Santacruz-Garcia et al. (2022) [14]. Leaf samples from three plants per treatment were collected for this assay.

2.6. Experimental design and Statistical analysis

The experiment was performed using a factorial design consisting of two factors, the AMF inoculum with three levels (two different AMF inoculation strains and control) and the chemical fertilizer with four levels (three different concentrations and a control). The treatments were distributed entirely randomized. For assessments of biochemical and morphological responses to the treatment application, data were analyzed by an ANOVA using a factorial model. The variable MR was evaluated for the test non-parametric Kruskal Wallis. The statistical software used was Infostat/2017 (with an α = 0.05.)

3. Results and Discussion

3.1. Evaluation of the interaction effect of AMF with chemical fertilizer

3.1.1. Morphological response

The growth of SND showed an interaction between factors ($p< 0.0002$). However, SH was not significantly affected by fertilizer and mycorrhiza factors nor the interaction between factors. The SND was significantly lower in SMF60 treatment (value 3.07 mm) than in all other seedlings. However, M1F60 and M1F30 showed the best performance of SND (3.46 mm), stimulating plant growth compared to M1F0 and M1F100 (3.1 to 3.2mm). No significant differences were detected between M2 and SM, except SMF100 with SND similar to M1F60 or M1F30. It could be said that M1 inoculum in determined concentrations had a positive effect. No significant differences between the slenderness index and ADW were detected among treatments. RDW showed no significant effects between interaction or fertilizer. However, the mycorrhiza factor showed significant differences in RDW ($p< 0.02$). This M2 treatment showed the lowest values (0.73g) compared to SM (1.13g) (Table 1). The inoculation with AMF has not always resulted in an enhancement for the plants. Thus diverse authors reported a growth depression attributed to different reasons [15];[16]. Often this behaviour is attributed to AMF parasitism, where carbon demand from the fungus exceeds the benefits of increased nutrient uptake [6];[17]. The application of the inoculum showed a more pronounced effect on the growth of diameter than the development of roots.

Table 1. Mean and standard deviation for the morphological variables: slenderness index (SI), root dry weight (RDW, g), aerial dry weight (ADW, g). Stars indicate the significance level. Significance levels: *** <0.001; ** <0.01; * <0.05; <0.1. Different letters indicate significant differences, according to Kruskal Wallis test with α: 0.05

Treatments	SM	M1	M2
Morphological variables			
SI	9.4±0.3A	9.1±0.3A	8.9±0.3A
RDW*	1.13±0.1A	0.84±0.1AB	0.73±0.1B
ADW	1.71±0.1A	1.71±0.1A	1.75±0.1A

SM: without mycorrhizal treatment, M1: Padre Lozano inoculum, M2: Colonia Benitez inoculum

3.1.2. Mycorrhizal response

The mycorrhizal response (MR) was evaluated. Results suggest that there were differences between inoculum (AMF). For example, this response on stem neck diameter was significantly higher ($p<0.0001$) in the treatment inoculated with M1 and 60% fertilizer concentration (M1F60) followed by M1F30, M2F60 and M2F30. We observed a positive MR in this concentration (60%). However, a negative response at 0% and 100% fertilizer concentrations for both inoculum were detected (Figure 1). This coincides with Malusa et al., (2007)[15], which considered low-to-moderate substrate fertility favors AMF formation and increases the supply of mineral nutrients, particularly from minimally soluble sources.

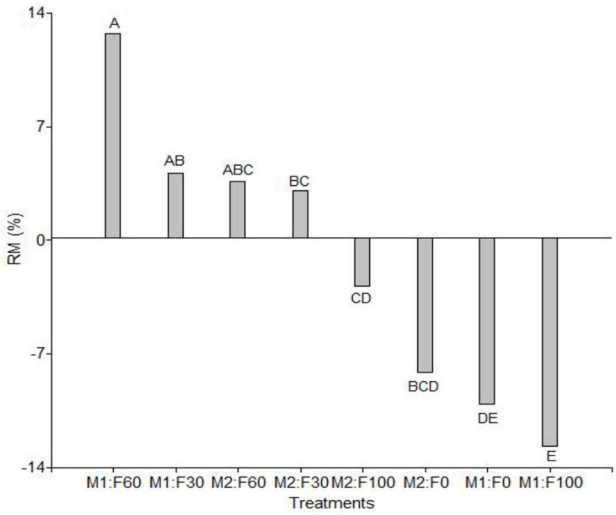

Figure 1. Mycorrhizal response of stem neck-diameter for AMF and level chemical fertilizer. Different letters indicate significant differences according to the Kruskal Wallis test

3.1.3. Biochemical response

Although no positive effect was found in the interaction of AMF and chemical fertilizer on photosynthetic pigments, the biochemical response was significantly affected by the mycorrhizal factor. The SM treatment showed significantly higher contents of photosynthetic pigments chlorophylls ($p<0.01$), carotenoids and xanthophylls ($p<0.0002$) (Table 2). These results could be related to higher physiological stability of the seedlings of the SM treatment compared to other treatments. Photosynthetic pigments could be considered an indicator of the stability of plant regulatory functions [18]. Although previous studies have reported that the effect of AM fungi improved chlorophyll content in leaves under drought or salt stress conditions[19], the mycorrhizas did not induce a clear biochemical response in this study.

Table 2. Mean and standard deviation for the biochemical variables: total contents of chlorophylls (μg/ml), carotenoids and xanthophylls (μg/gMF). Stars indicate the significance level. Significance levels: *** <0.001; ** <0.01; * <0.05; <0.1. Different letters indicate significant differences, according to Kruskal Wallis test with α: 0.05

Treatments	SM	M1	M2
Biochemical variables			
Carotenoids and xanthophylls ***	228.67±6.8A	188.28±6.8B	186.04±6.8B
Chlorophylls **	1258.85±51.2A	1065.32±51.2B	1054.34±51.2B

SM: without mycorrhizal treatment, M1: Padre Lozano inoculum, M2: Colonia Benitez inoculum

4. Conclusions

Our results suggest reducing the doses of chemical fertilisers by incorporating biofertilisers in the production of *Prosopis alba* nursery plants is possible.

Although the AMF did not influence specific morphological and biochemical parameters as expected concerning the plants without mycorrhizae, maybe it is necessary to advance in isolating species of native AMF compatible with the site and the *Prosopis alba* species. For this reason, deepen studies of the ecological complexity of mycorrhizal systems.

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