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Could the interaction between arbuscular mycorrhizal fungi and biostimulants improve the plant physiological status of *Prosopis alba* seedlings? †

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Abstract: This study aimed to evaluate the synergy between arbuscular mycorrhizal fungi (AMF) and foliar biostimulant applications (phytoextracts) on an important Argentinian forest native species *(Prosopis alba)* during the nursery stage. We tested biochemical parameters (MDA, malondialdehyde, an oxidative stress biomarker, and photosynthetic pigments) on *P. alba* seedlings sprayed with three different phytoextracts and inoculated with mycorrhizal strains of different local origins. Considering that the statistical model was not significant at the preliminary level, we did not observe synergism between the different forms of bioinsumes evaluated by analysing biochemical characteristics. However, regardless of AMF inoculation, plants with foliar applications of *Larrea divaricata* at 3% w/v showed a lower accumulation of the oxidative stress biomarker, MDA and a lower total carotenoid content ($p>0.1$). Although there were no significant differences, trends indicate positive relationships between neck diameter and mycorrhizal response in plants treated with M1 with foliar applications of *Larrea divaricata* at 3% w/v. However, height does not consistently respond to mycorrhizae interacting with biostimulants. Consequently, deeper analysis is needed to understand the effect of the interaction between AMF with biostimulants for improving the plant's physiological status. Deepening research in this regard will result in significant benefits for restoration activities.

Keywords: Biostimulants, Biostimulants, Chaco, Mycorrhizae, Plant physiological status. AMF

1. Introduction

Mycorrhizae and biostimulants are among the most innovative biological techniques used in large-scale food production. Positive effects on plant growth have been reported. However, the use of these "biofertilizers" in afforestation is limited. Currently, there is growing market interest in bioinsumes for agribusiness. This is due to the need to promote sustainable production techniques that efficiently use their resources [1,2]. Bioinsumes are natural extracts or microorganisms that could act on plant physiology, improving their tolerance to stress, nutritional efficiency and/or quality characteristics [1,3]. An example of these products is *biocontrol* agents. These products are mainly derived from beneficial fungi, which, through symbiotic relationships, protect plants against pathogen attack [4,5]. Another class of bioinsumes are *biostimulants*. These products promote germination, growth, flowering and/or fruit development, despite not being nutrients, soil improvers or pesticides. These kinds of products are characterized by improving tolerance to plant abiotic stress [1,6]. Natural extracts have great potential as biostimulants; however, their activity is still little known [1]. Interestingly, numerous studies highlight the protective effect of bio-inputs against stress factors such as drought, salinity and pathogen attack, among others [3,7,8]. Deepening research about the interaction between different bioinsumes could represent an effective strategy to enhance plant responses to oxidative stress.

2. Methodology

2.1 Plant material and experimental conditions

We conducted an experiment from November 2021 to February, 2022 in the Experimental Station “Fernández" (Agreement Catholic University of Santiago del Estero-Province of Santiago del Estero) in Santiago del Estero, Argentina (−27◦560 S, 65◦ 52.50 W). 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.

2.2 Isolation, multiplication and application of AMF inocula, and Preparation and application of Biostimulants

We selected mixed inocula of native AMF from *P. alba* stands located in the Argentine Chaco Region, with two different locations: Padre Lozano (M1) in the Western Chaco Domain, and Colonia Benítez (M2) in the Eastern Chaco Domain, Chaco province, according to the methodology proposed by Sagadin et al. (2018) [9]. M1: *Claroideoglomus claroideum, Claroideoglomus etunicatum; Diversispora spurca, Funneliformis mosseae and Rhizophagus intraradices*; M2: *Claroideoglomus claroideum, Claroideoglomus etunicatum, Funneliformis constrictum, Funneliformis mosseae, Rhizophagus clarus*. SM: Non-inoculated. The inoculation was performed at sowing by applying 10 g of AMF inoculum per container for each inoculum in the planting hole Salto et al. (2020) [10].

Biostimulants were prepared and applied according to the methodology described in Santacruz-García et al. (2022)[11]. J1Y1 biostimulant is a mixture of both species (*Larrea divaricata* and *Ilex paraguariensis*, 1% w/v: 1%w/v). Foliar applications were made twice during the acclimation stage (on days 7st and 14th). The measurements were made on the 21st day of the acclimation stage.

2.3 Plant Biochemical Responses

In this study, (i) malondialdehyde (MDA), an oxidative stress biomarker, and (ii) photosynthetic pigments: total contents of chlorophylls and carotenoids were evaluated according to methodology followed by Santacruz-Garcia et al. (2022) [11]. Leaf samples were collected by triplicate, evaluating three plants per treatment.

2.4 Plant Morphological Responses

For this assay, we considered the following morphological characteristics: (i) stem neck diameter (SND) and (ii) shoot height (SH), according to standard methodologies proposed by Santacruz-Garcia et al. (2022) [11]. Measurements were made on ten plants per treatment.

2.5 Mycorrhizal Response

Mycorrhizal Response (MR) was calculated for (i) stem neck diameter (SND) and (ii) shoot height (SH) according to the relationship described by Cavagnaro et al. (2003) [12]:

MR= [(M -mean SM) /mean SM) x 100]

M corresponds to the morphological characteristic evaluated, and mean SM corresponds to the non-inoculated plants

2.5 Statistical analysis

For assessments of biochemical responses to the biostimulant application, data were analysed through a mixed linear model (MM), using factors (biostimulant, AMF inoculum, and their interactions) as fixed effects. The individual plant was considered a random effect. For the analysis of the morphological variables (SND and SH), a mixed linear model was used using as a random effect the repetition by plot (AMF inoculum_Bioestimulant). While the mycorrhizal response (MR) of SND and SH was analyzed with a mixed general model using heteroscedasticity of variances in repetition per plot. The statistical software used was Infostat/2017 (InfoStat Group V.2017, Cordoba, Argentina) with an $\alpha = 0.05$.

3. Results and Discussion

3.1 Plant Biochemical Responses

We did not observe synergism between the different forms of bioinsumes evaluated considering biochemical characteristics. According to the statistical analysis, the interactions between treatments significantly did not affect the plant's biochemical responses (Table 1). These results could be explained considering that the colonization efficiency of AMF is highly related to the environmental conditions and plant genotypes [13]. Probably, the interaction between the selected biostimulants and the AMF strains used in this study did not enhance plant growth and nutrition. It is necessary to explore different AMF species to evaluate their synergist potential with biostimulants to enhance the production of *P. alba* seedlings [14].

Table 1. Mean and standard deviation for the biochemical variables: total contents of chlorophylls (μg g^{-1} FW), carotenoids (μg g^{-1} FW) and malondialdehyde (MDA, nmol g^{-1} FW). Treatments are combination of inoculation (SM: Non inoculated, M1: M1: *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*; *Diversispora spurca*, *Funneliformis mosseae* and *Rhizophagus intraradices*; M2: *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, *Funneliformis constrictum*, *Funneliformis mosseae*, *Rhizophagus* clarus) and biostimulant foliar applications (SB: Seedlings sprayed with only

water, J3: seedlings sprayed with *Larrea divaricata* (3 % w/v), Y2: seedlings sprayed with *Ilex paraguariensis* (2 % w/v), and J1Y1: mixture of both biostimulants (1 % w/v: 1 % w/v). Stars indicate the significance level. Significance levels: *** <0.001; ** <0.01; * <0.05; <0.1.

Treatment	Chlorophylls	Carotenoids	MDA
SMSB	2151.90 ± 160.16	372.13 ± 12.46	28.84 ± 1.53
SMJ3	1880.24 ± 129.30	326.79 ± 29.06	26.20 ± 0.99
SMY2	2014.71 ± 63.37	349.14 ± 4.64	29.07 ± 3.30
SMJ1Y1	2140.50 ± 139.36	373.87 ± 26.33	29.18 ± 3.50
M1SB	2161.63 ± 122.16	366.99 ± 20.26	28.10 ± 2.79
M1J3	2018.92 ± 179.92	344.04 ± 6.95	26.85 ± 3.63
M1Y2	2070.84 ± 368.22	361.34 ± 59.37	28.65 ± 4.83
M1J1Y1	2375.28 ± 195.11	396.44 ± 80.80	29.41 ± 1.15
M2SB	2070.59 ± 104.51	366.92 ± 1.75	30.83 ± 2.37
M2J3	2078.85 ± 555.13	348.94 ± 97.71	25.09 ± 2.63
M2Y2	2125.39 ± 253.52	379.42 ± 33.70	26.96 ± 1.21
M2J1Y1	2375.28 ± 195.11	412.21 ± 25.35	27.99 ± 5.85

However, foliar applications of biostimulants showed a slightly significant effect on the total content of carotenoids and MDA ($p<0.1$, Table 2). Seedlings sprayed with *Larrea divaricata* (3% w/v) exhibited lower values in both biochemical characteristics, which could be related to the antioxidant effect of this biostimulant. These results could be associated with the preventive action of this biostimulant application against the oxidative stress that affects different physiological processes [7]. It is interesting to highlight that our results confirmed the observed effects of *L. divaricata* (3% w/v) as a potential biostimulant of *P. alba* during the acclimation stage [11].

Table 2. Mean and standard deviation for the biochemical variables: total contents of chlorophylls ($\mu g\ g^{-1}$ FW), carotenoids ($\mu g\ g^{-1}$ FW) and malondialdehyde (MDA, nmol g^{-1} FW). Treatments are biostimulant foliar applications (SB: Seedlings sprayed with only water, J3: seedlings sprayed with *Larrea divaricata* (3 % w/v), Y2: seedlings sprayed with *Ilex paraguariensis* (2 % w/v), and J1Y1: mixture of both biostimulants (1 % w/v: 1 % w/v). Different letters indicate significant differences, according to LSD Fisher pairwise comparison procedure with α: 0.1.Stars indicate the significance level. Significance levels: **** <0.001; *** <0.01; ** <0.05; *<0.1.

Treatment	Chlorophylls	Carotenoids*	MDA*
SB	2128.04 ± 121.44 *a*	369.68 ± 12.14 *ab*	29.26 ± 2.33 *b*
J3	1992.67 ± 311.59 *a*	339.92 ± 52.07 *a*	26.09 ± 2.42 *a*
Y2	2070.31 ± 230.72 *a*	363.30 ± 36.67 *ab*	28.23 ± 3.14 *ab*
J1Y1	2244.57 ± 297.77 *a*	394.17 ± 47.38 *b*	28.86 ± 3.52 *ab*

3.2 Plant Morphological Responses

The diameter and height of the seedlings did not show significant differences with the application of mycorrhizae and biostimulants in interaction, nor with the fixed effects. But there are trends in favour of M1 and J3. However, there are positive trends between the SND with the application of J3 and M1, while for the variable height is not so evident this trend in the interaction between AMF inoculum and biostimulant applications. (Table

3). This may be related to the fact that the diameter is a conduction tissue and the inoculum M1 comes from an arid site could favour this seedling feature [15].

Table 3. Mean and standard error of Stem neck diametre (SND) and Stem height (SH) of *Prosopis alba s*eedlings Interaction are combination of inoculation (SM: Non inoculated, M1: *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*; *Diversispora spurca*, *Funneliformis mosseae* and *Rhizophagus intraradices*; M2: *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, *Funneliformis constrictum*, *Funneliformis mosseae*, *Rhizophagus* clarus) and biostimulant foliar applications (SB: Seedlings sprayed with only water, J3: seedlings sprayed with *Larrea divaricata* (3 % w/v), Y2: seedlings sprayed with *Ilex paraguariensis* (2 % w/v), and J1Y1: mixture of both biostimulants (1 % w/v: 1 % w/v).

Treatment	SND (mm)	SH (cm)
M1 J3	3,60 ± 0,1	38,62±1,39
M1 SB	3,54 ± 0,13	38,69±1,37
SM SB	3,53 ± 0,07	36,15±1,34
SM J1Y1	3,52 ± 0,07	37,92±1,36
M2 Y2	3,47 ± 0,09	37,1±1,43
M2 J3	3,46 ± 0,09	37,69±1,39
SM J3	3,44 ± 0,06	38,69±1,33
M1 J1Y1	3,42 ± 0,09	38,26±1,36
M2 J1Y1	3,41 ± 0,07	38,03±1,31
M1 Y2	3,40 ± 0,07	37,14±1,33
M2 SB	3,32 ± 0,07	35,87±1,41
SM Y2	3,27 ±0,11	38,91±1,34

There are no significant differences in MR on SND and SH of *Prosopis alba* seedlings. However, similar trends to allometric variables (SND and SH) are verified. Thus, the diameter MR shows positive trends with respect to M1 and J3. While the MR of the height of the plantin shows positive trend with respect to M1 and the combination of biostimulant J1 Y1.

4. Conclusions

Our results did not show a clear synergism between the different forms of bioinsumes evaluated considering biochemical and morphological characteristics or by evaluating the mycorrhizal response. Regarding the biostimulants use (regardless of AMF inoculation), foliar application of L. divaricata 3% w/v exhibited the lowest values of MDA and total contents of carotenoids. These results confirmed our previous study [11] related to the potential of this biostimulant in enhancing the abiotic stress tolerance of *P. alba*. There were no significant differences, trends indicate positive relationships between neck diameter and mycorrhizal response in *P. alba* seedling treated with M1 with foliar applications of *L. divaricata* at 3% w/v. However, height does not consistently respond to mycorrhizae interacting with biostimulants. Consequently, deeper analysis is needed to understand the effect of the interaction between AMF with biostimulants for improving the plant's physiological status.

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