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Could biostimulants with plant active compounds improve the tolerance to oxidative stress in *Prosopis alba*(Griseb.)?†

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Abstract: The aim of this work was to explore the potential of four phytoextracts derived from species with recognized antioxidant activity and/or rich in polysaccharides as plant biostimulants of seedlings of *Prosopis alba.* Malondialdehyde (MDA), an oxidative stress biomarker, was measured at 21 days after the acclimation phase beginning as preliminary results showed a significant accumulation of MDA in rustified seedlings compared with control seedlings at this time. As main results, rustified seedlings sprayed with *I. paraguariensis* and *L. divaricata* extracts showed lower MDA concentration than rustified seedlings without any phytoextract application. Both extracts were characterized by their antioxidant activity. These results suggest that natural extracts of *I. paraguariensis* and *L. divaricata* could be considered plant biostimulants, reducing oxidative stress biomarkers in *P. alba.*

Keywords: Biostimulants; phytoextracts; plant active compounds; oxidative stress; antioxidant activity

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1. Introduction

Biostimulants are natural extracts or microorganisms that applied to plants are able to act on the physiology of the plant to improve their stress tolerance and could enhance their crop quality traits and yield [1]. However, the activity of biostimulants obtained from plants is less known compared with other kind of biostimulants applied to plants. Phytoextracts are rich sources of bioactive compounds. These products are characterized by improving the nutritional efficiency and the abiotic stress tolerance of plants [2]. Indeed, there are numerous studies or reports that highlight the protective effect of biostimulants against environmental stress conditions as drought and salinity [3]. Application of these phytoextracts as agriproducts (i) reduces production costs, (ii) provides environmental respect and (iii) added-value to crops and / or plantations [1].

Algarrobo blanco (*Prosopis alba*) is a woody species of great forest interest both for its commercial value and for its adaptability to different environmental conditions. For this reason, it is used for restoration in arid and semi-arid areas [4]. This species is considered for sites with a limited capacity for other productive activities due to its tolerance to saline soil [5]. However, there is still insufficient evidence regarding its tolerance to abiotic stress

in nursery, acclimation, and open field. Furthermore, the effects on the stress tolerance mechanisms of biostimulant applications in *P. alba* are unknown.

Although the use of biostimulants is more associated with the production of organic or agroecological crops devoted to food uses, it is important to extend this form of plant management also to other productions such as forestry [3]. The aim of this work was to explore the potential of four different phytoextracts derived from species with recognized antioxidant activity and/or rich in polysaccharides as plant biostimulants of seedlings of *P. alba*. We proposed that natural extracts of native species could reduce oxidative stress biomarkers in *P. alba* which could be used as potential biostimulants of this species.

2. Material and methods

2.1. Plant Material and experimental conditions

The experiment was carried out in the Experimental station "Fernández" in Santiago del Estero, Argentina, (– 27°56'S, 65° 52.5' W). An experiment from December 2020 to January 2021 was conducted to evaluate the effect of biostimulants in the *P. alba* production, during nursery stage. Seedlings were produced in trays with individual cells. Until January 8th, 250 plants were cultivated in a nursery with 50% of shading under natural light conditions. After that, plants were divided in two groups, 5 treatments were kept inside nursery with 50% of shading (control plants), and 5 treatments established outside nursery (rustified plants), where seedlings were exposed to full sunlight in the acclimation phase.

2.2. Evaluation of the oxidative stress condition

To evaluate the condition of oxidative stress, the concentration of malondialdehyde (MDA), a recognized oxidative-stress biomarker, was measured in control and rustified seedlings in different days of the acclimation stage to identify the day on which the oxidative stress condition takes place. Samples of 1 g of fresh material per plant were analyzed according to the methodology described in Yonny et al. (2018)[6].

2.3. Phytoextracts Application

To evaluate the effect of the biostimulant application on the growth of *P. alba* seedlings, fifty individual plots of 5 plants were randomly established. For the assay, ten treatments were established giving a total of 25 plants per treatment.

The selection of the species to obtain the biostimulants was performed considering their antioxidant activity and/or their polysaccharide contents [7]. The treatments are detailed in Table 1. Every phytoextract was applied twice as foliar spray since the beginning of the acclimation phase. Based on preliminary experiments, MDA was measured at 21 days after the acclimation phase beginning. Additionally, stem neck-diameter (Dac), shoot height (Ht) and number of rachis (NR) were recorded.

Table 1. Experiment details, treatments and growth condition.

Treatments	Site	Biostimulants	Condition
CA	Acclimation	Control (water)	seedlings were exposure to full sunlight
JA		*Larrea divaricata* (2% w/v)	
AA		*Aloe barbadensis* (0.1% w/v).	
YMA		*Ilex paraguariensis* (2% w/v)	
QCA		*Schinopsis lorentzii*(2% w/v)	
CN	Inside nursery	Control (water)	50% of shading under natural light conditions
JN		*Larrea divaricata* (2% w/v)	

AN	*Aloe barbadensis* (0.1% w/v).
YMN	*Ilex paraguariensis* (2% w/v)
QCN	*Schinopsis lorentzii* (2% w/v)

2.4. Statistical Analysis

Data were analyzed through a mixed linear model (MM), using treatment (phytoextract or water) and site (nursery or acclimation area) as fixed effects. A principal components analysis (PCA) was performed to determine the plant's response to the biostimulant application. The statistical software used was Infostat/2017 (InfoStat Group V.2017, Córdoba, Argentina) with $\alpha = 0.05$.

3. Results and discussion

3.1. Evaluation of the oxidative stress condition

The first assay showed a significant accumulation of MDA in rustified seedlings (in acclimation area) as compared with control seedlings (remaining in nursery) at 21 days after the beginning of the acclimation stage. After that, significant changes in MDA concentration were not observed. Our results suggest that after 21 days, seedlings in the acclimation area effectively suffer a noticeable oxidative cell damage due to the environmental conditions (reduced irrigation and higher sun exposure) [5,6]. Based on these results, this lapse of experimental time was selected to evaluate the effect of phytoextracts in preventing the oxidative stress of seedlings during the acclimation stage.

3.2. Phytoextract application

3.2.1. Exploratory analysis

The first component (CP1) separated MDA levels from other variables considered in the analysis. As expected, it is associated with acclimation treatments, especially *Aloe barbadensis*, *Schinopsis lorentzii* and control in acclimation stage (Fig 1). According to the formed angles between the variables, stem neck-diameter has little or no correlation with the other variables. While the morphological parameters (stem neck-diameter, shoot height and number of rachis) were positively associated with nursery treatments, which is expected according to the non-stressing conditions in this area. Both components account for 86% of the total variation.

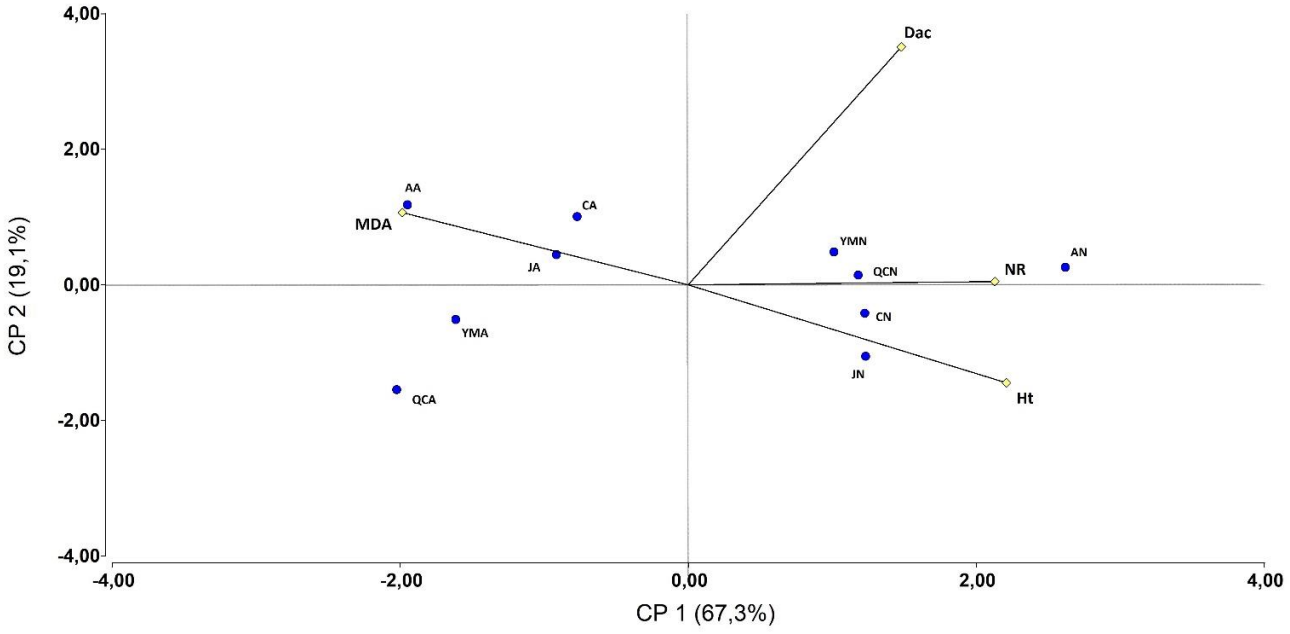

Figure 1. Principal component analysis (PCA) of the seedling response to the phytoextract application. The ordination was based on the biostimulants applicattion and the evaluated parameters: malondialdehyde level (MDA), stem neck-diameter (Dac), shoot height (Ht) and number of rachis (NR), in seedlings in both nursery and acclimation area.

3.2.2. Oxidative stress condition monitoring

As results, rustified seedlings (in acclimation area) with applications of *I. paraguariensis* and *L. divaricata* showed lower MDA concentration than rustified seedlings without any extract application. Both extracts are characterized by their antioxidant activity according to previous assays. In contrast, foliar applications of *Aloe barbadensis* and *Schinopsis lorentzii* may induce an oxidative stress as the biomarker accumulation in the seedlings of *P. alba*, according to the higher MDA values showed by these plants as comparing to control seedlings. Thus, in biostimulated seedlings with *I. paraguariensis* and *L. divaricata*, the ROS increase rate seemed to be lower than its clearance rate by endogenous antioxidants [7].

3.2.3. Morphological parameters

Results of diameter, height and number of rachis measurements showed a significant interaction between biostimulants and site (nursery or acclimation). The diameter of the rustified seedlings sprayed with *Schinopsis lorentzii* (QCA) showed that this parameter was significantly thinner than the other seedlings. The shoot height of QCA were significantly increased by the biostimulant application compared to the other treatments in the acclimation phase. However, previous studies showed that the plant quality improves as the diameter increases, rather than the plant height [8]. No significant effects were detected in the number of rachis between any treatments. This could indicate that foliar applications of *S. lorentzii* may not be the most appropriate option to be used as plant biostimulants.

4. Conclusion

Natural extracts of *I. paraguariensis* and *L. divaricata* could be considered potential plant biostimulants which reduce oxidative stress biomarkers in *P. alba.* Biostimulant application could contribute to mitigating stress consequences in *P. Alba* growth during the acclimation phase. This phase is an essential process during the production of seedlings in nursery. These results could lead a more environmentally friendly management of *P. alba*, as this is one of the most important species considered for forest restoration in Argentina. In addition, it is necessary to evaluate the phytoextract dose-effect relation to estimating the optimal concentration of biostimulant to apply to the seedlings and, thus, deepen in the mechanism knowledge to improve this action.

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