

Effect of fertilization and inoculation with native rhizobial strains on growth of *Prosopis alba* seedlings under nursery conditions

Carla S. Salto · Mariana Melchiorre · Gustavo Pedro Javier Oberschelp · Ezequiel Pozzi · Leonel Harrand

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Abstract *Prosopis alba* (algarrobo blanco) is able to fix N₂ symbiotically associated with nodule inducing rhizobacteria. However, there is no information related to the effects and interactions of inoculation and fertilization during the production of “algarrobo blanco” seedlings. The aim of this work was to evaluate, under nursery conditions, the combined effect of inoculation with specific rhizobacteria and nitrate (NO₃⁻) levels on growth and nodulation of *P. alba* seedlings from two localities and phytogeographical provenances: La Paz and Campo Durán from Espinal and Parque Chaqueño regions, respectively. Seeds were inoculated two days after sowing with a mix containing three specific rhizobacteria isolates and fertilized with three NO₃⁻ concentrations (0, 2.8 and 21.6 mM). Inoculation combined with 2.8 mM

NO₃⁻ increased leaf number and favored collar diameter, without a significant effect on total height. Fertilization with 21.6 mM NO₃⁻ stimulated seedling growth, yielding the highest seedlings with the highest node number with leaves and the lowest percentage of nodulated plants. Seedlings from La Paz showed better response in collar diameter and leaf number than those from Campo Durán.

Keywords “Algarrobo blanco” · Symbiosis · Mineral nutrition · Nitrate

Introduction

The genus *Prosopis* has a wide distribution in Argentina, ranging from lowland environments to regions at 3000 m a.s.l., as well as in areas with annual precipitations between 80 and 1400 mm. The highest number of species is distributed in the phytogeographic provinces of Monte, Espinal and Chaco; these arid and semiarid regions cover 70% of the country area (Roig 1993; Cony 1996). *Prosopis alba* Griseb. (locally known as algarrobo blanco) is the species of highest economic importance within the genus in Argentina, being widely distributed in the central-northern region. It is one of the native species with highest commercial value in the sawnwood industry; it can be the basis for structuring sustainable productive systems, contributing to recovery of degraded areas.

C. S. Salto (✉) · G. P. J. Oberschelp · L. Harrand
Estación Experimental Agropecuaria Concordia, Instituto Nacional de Tecnología Agropecuaria (INTA), Casilla de Correo 34, CP 3200 Concordia, Entre Ríos, Argentina
e-mail: salto.carla@inta.gob.ar

M. Melchiorre
Centro de Investigaciones Agropecuarias (IFRGV-CIAP), Instituto de Fisiología y Recursos Genéticos Vegetales, INTA, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina

M. Melchiorre · E. Pozzi
Facultad de Ciencias Exactas Físicas y Naturales (FCEfyN), Universidad Nacional de Córdoba, Córdoba, Argentina

The fruits of *P. alba* are an important source of food for humans and forage for wildlife and livestock (Giménez et al. 1998; López et al. 2001). As other species of the genus, *P. alba* can fix atmospheric nitrogen by establishing a symbiotic association with soil bacteria, collectively named rhizobia, which nodulate its roots. Biological nitrogen fixation (BNF), due to its capacity to incorporate N from the air mediated by rhizobacteria, is the main modulator in the sustainability of natural ecosystems and areas where *P. alba* grows (Allen and Allen 1981; Cesco et al. 2012).

In the last decades, the use of rhizobacteria to improve soil fertility, enhance plant nutrition and improve crop yields has been an alternative practice to reduce the use of inorganic fertilizers and their negative environmental impact (Parra and Cuevas 2001). Using N-fixing trees, understanding the diversity of diazotrophic bacteria able to establish symbiotic relationships with them adequately selecting efficient N₂ fixers are basic tools to improve sustainability, mainly in suboptimal environments. (Woldemskel et al. 2004; Makatiani and Odee 2007).

The establishment of symbiosis is the result of a finely regulated process that implies the mutual recognition of both partners. *Mesorhizobium chacoense* had been the first species described as capable of nodulating *P. alba* in the Arid Chaco of Argentina (Velázquez et al. 2001). Recently, Chávez Díaz et al. (2013) reported that in addition to *Mesorhizobium*, rhizobacteria belonging to genera *Sinorhizobium* (*Ensifer*) and *Bradyrhizobium*, isolated from algarrobo environments in the Parque Chaqueño were able to induce nitrogen-fixing nodules in *P. alba*. The interaction between specific rhizobacteria and *P. alba* not only allows the incorporation of nitrogen to the systems via BNF, but also induces a number of systemic responses in plants, thereby contributing to the generation of efficient adaptive responses, especially under limiting environmental conditions. Therefore, inoculation with rhizobacteria makes a favorable contribution to plant survival during establishment and development (Zamioudis and Pieterse 2012).

Nitrogen is an essential element, as demonstrated by the amounts required by plants and the frequency at which deficiencies are observed in the soil. Growth and development of plants largely depend on nitrogen availability both at the right moment and in an adequate form of supply (Urzúa 2005). The symbiotic

legume-rhizobium association contributes to sustainable nitrogen availability, playing a key role under soil nitrogen limiting conditions. By contrast, high soil nitrogen levels, generally provided exogenously in the form of nitrates (NO₃⁻), has inhibitory effects on the number of nodules and their biomass, as well as on BNF (Streeter 1988; Ohyama et al. 2011). Several physiological mechanisms may explain the negative impact of nitrogen excess on symbiosis, such as a decrease in photoassimilate supply from leaf to nodules, Nitrogenase inhibition related to increases in nitrate metabolism products (e.g., glutamine), the competition for reductive power between Nitrogenase and Nitrate reductase, decreases in oxygen diffusion into nodules and Leghemoglobin nitrosylation due to the formation of complexes with reactive nitrogen species. High nitrate and ammonium (> 3 mM) inhibit nodulation while limited provision can stimulate it (Ohyama et al. 2011; Luciński et al. 2002; Mohd-Radzman et al. 2013). Fertilization is one of the most important cultural practices for large-scale seedling production under nursery conditions. The adequate nutrient supply can reduce seedling production time and increase seedling quality. Therefore, nutrient balance in fertilization is a key issue, especially in production in containers, where limited volume restricts root growth and development (Landis 1989).

Despite the importance of this practice, there is scarce information about the nutrient requirements of native legumes in nurseries, and even less about the influence of these regimes on symbiosis establishment and maintenance (Goicoechea et al. 2004; Hahne and Schuch 2006). Consequently, information about nursery management conditions in a species of great economic and ecological potential, such as *P. alba*, will contribute to the improvement of its production and conservation.

The aim of this work was to evaluate the combined effect of inoculation with specific rhizobacteria and fertilization with different nitrate (NO₃⁻) concentrations on growth of *Prosopis alba* (algarrobo blanco) seedlings of two provenances under nursery conditions.

Materials and methods

The trial was conducted in the forest nursery of the Estación Experimental Agropecuaria Concordia belonging to the Instituto Nacional de Tecnología Agropecuaria, INTA, located in Concordia city (31°22'S and 58°07'W; 47 m a.s.l.), Entre Ríos province, Argentina. The climate in the region is warm-temperate without a dry season; mean annual temperature is 18.7 °C and mean annual rainfall is 1345 mm, with a maximum of 2193 mm and a minimum of 868 mm.

Prosopis alba seeds were harvested from trees from two phytogeographic provenances: La Paz, Entre Ríos (30°56'25"S, 59°36'18"W), belonging to the Espinal, and Campo Durán (22°12'01"S, 63°40'33"W), in the northern end of Salta province, belonging to the Parque Chaqueño (Cabrera 1976). Pre-germination treatment steps included seed mechanical scarification with sand, immersion in 100 °C water and finally cooling at air temperature and maintained in water for 24 h. Seeds were sown in 125-cm³ plastic containers (R125, Dassplastic del Mercosur®), using a mixture (2:1:1 v/v) of composted pine bark, perlite (Perlome® Grueso) and vermiculite (Intersum® Mediano F1) as substrate.

The inoculum was composed of three native strains of rhizobacteria of the genera *Mesorhizobium* sp., *Bradyrhizobium* sp. and *Sinorhizobium* sp. able to nodulate *P. alba*, previously identified by Chavez Díaz et al. (2013) based in comparison with 16S rDNA sequences from representative reference rhizobial strains. Additionally, these isolates were selected for their free-living osmotic stress tolerance, indolic-type compound production, and efficient nodulation and promotion of *P. alba* growth (Chávez Díaz et al. 2013). *Mesorhizobium* sp. and *Sinorhizobium* sp. were represented by the isolates collected in the site Bolsa Palomo, accession NCBI KC759691.1, (<https://www.ncbi.nlm.nih.gov/nuccore/471180990/>) and NCBI KC759698.1 (<https://www.ncbi.nlm.nih.gov/nuccore/KC759698>) respectively, *Bradyrhizobium* sp. represented by the isolate collected in Padre Lozano, accession NCBI KC759699.1 (<https://www.ncbi.nlm.nih.gov/nuccore/471180998/>). Three sites belong to Parque Chaqueño region (Chávez Díaz et al. 2013). Rhizobacteria were cultured individually in LMA medium (Vincent 1970) at 28 °C with agitation for 5 days. The inoculant was an equal proportion mixture

of each isolate with a final concentration of 5×10^8 cell mL⁻¹. Seeds were inoculated with 1 mL of solution containing the three isolates 2 days after sowing.

Treatments with different nitrate (NO₃⁻) concentrations consisted of 0 (no fertilization); 2.8 and 21.6 mM NO₃⁻, plus the macro and micronutrients indicated in Table 1.

To define the highest level of nitrate (21.6 mM), the nutritional requirements of *P. alba* seedlings for nursery production described by Salto et al. (2016) were considered. The lowest nitrate level (2.8 mM NO₃⁻) was defined using data from a previous assay (Pozzi et al. unpublished data), in which was determined the maximum nitrate concentration that allowed nodules development in inoculated *P. alba* seedlings.

Nutrient solutions were formulated using Hakaphos Amarillo® (1 g l⁻¹), Ca(NO₃)₂ (0.7 g l⁻¹), MgSO₄·7H₂O (0.3 g l⁻¹), H₃BO₃ (0.002 g l⁻¹), Basafer Plus® (0.05 g l⁻¹), H₂KO₄P (0.22 g l⁻¹). The level of 2.8 mM NO₃⁻ was obtained by reducing the amount of NO₃⁻, and maintaining similar concentrations of the remaining nutrients of the solution with Hakaphos Base® (0.15 g l⁻¹), K₂SO₄ (0.26 g l⁻¹), CaCl₂ (0.37 g l⁻¹), MgSO₄·7H₂O (0.25 g l⁻¹), H₃BO₃ (0.0025 g l⁻¹), Basafer Plus® (0.057 g l⁻¹), H₂KO₄P (0.26 g l⁻¹), CuSO₄·5H₂O (0.00065 g l⁻¹), MnSO₄·H₂O (0.0013 g l⁻¹), (NH₄)₆Mo₇O₂₄·4H₂O (0.00016 g l⁻¹), ZnSO₄·7H₂O (0.0007 g l⁻¹) (Table 1). These fertilization treatments were applied, twice a week, in growth period from day 15 to day 80 after sowing.

Hardening is a common practice in forest production, which consists of subjecting seedlings to nitrogen starvation a few days before being transferred to the field. Hardening stimulates root growth at the expense of aerial growth; thus, the obtained seedlings have an adequate aerial/root biomass balance that contributes to field survival (Landis 1989). After hardening the seedlings were ready for transfer to the field. The solution was formulated using Hakaphos Base® (0.3 g l⁻¹), KCl (0.2 g l⁻¹), MnSO₄·H₂O (0.2 g l⁻¹), Basafer Plus® (0.05 g l⁻¹), H₃BO₃ (0.002 g l⁻¹). In all cases, fertigation was performed twice a week by applying the solution manually, until substrate saturation. Control treatment (0 mM NO₃⁻) was not fertilized in either stage.

Table 1 Concentration (mM) of macro- and micronutrients for each fertigation solutions applied on *P. alba* seedlings from day 15 to day 90 after sowing

Nutrient	Growth (15–80 days)		Hardening (81–90 days)
	2.8	21.6	
N	2.79	21.62	1.50
P	2.17	2.33	0.52
K	4.04	4.05	4.78
Ca	3.32	3.32	0.00
Mg	1.06	1.76	2.91
S	2.61	3.59	3.24
B	0.04	0.04	0.12
Cu	0.003	0.003	0.001
Fe	0.06	0.06	0.06
Mn	0.009	0.009	0.003
Mo	0.0001	0.0001	0.00003
Zn	0.003	0.003	0.001

The variables measured at 90 days were collar diameter (CD), total height (TH) and number of nodes with leaves (NN). Presence of nodules was evaluated as percentage of seedlings with visible nodules. The experimental design applied was split-split-plot with completely randomized blocks, with four repetitions and 20 plants per plot, where the main plot was NO_3^- level (0; 2.8 and 21.6 mM), the sub-plot inoculation (inoculated and non-inoculated) and the sub-sub-plot was seed provenance (La Paz and Campo Durán). A linear mixed model was applied, as follows:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \chi_k + \delta_{ij} + \varphi_{ik} + \gamma_{jk} + \eta_{ijk} + b_l + p_{il} + sp_{jil} + \varepsilon_{ijkl}$$

where μ represents the general mean, α_i the i -th level of the factor associated with the principal plots, β_j j -th level of the factor associated with the subplots within the main plots, χ_k the k -th level of the factor associated with the sub-sub-plots (within the sub-plots) and δ_{ij} , φ_{ik} , γ_{jk} , η_{ijk} , the corresponding interactions. The random terms of this model correspond to the effects of blocks b_l , plots p_{il} , the effects of sub-plots sp_{jil} and the experimental error ε_{ijkl} .

Statistical analyses were performed using the MIXED procedure in SAS V.9.1 software (SAS Institute Inc.). Mean comparison tests were performed using the LSMEANS sentence option ADJUST = Tukey for main effects or for simple effects when interaction was significant ($P \leq 0.05$).

Results and discussion

The analysis of seed Provenance and Fertilization \times Inoculation showed significant differences for collar diameter (CD). The highest statistically significant mean CD value was observed in seedlings from La Paz (LP) (Table 2).

Inoculation with rhizobacteria had a positive effect on CD values only in interaction with fertilization, whereas at 0 (no fertilization) and/or 21.6 mM NO_3^- CD values did not vary due to inoculation. At 2.8 mM NO_3^- , CD of inoculated seedlings was significantly higher than that of non-inoculated ones (Table 2). As observed by Pozzi et al. (unpublished data) in *P. alba* seedlings inoculated with 2.8 mM NO_3^- , growth promotion would be attributable to symbionts, whereas higher NO_3^- concentrations, which are potential inhibitors of nodulation, such as 21.6 mM, induce a higher CD, regardless of inoculation (Table 2).

Provenances and Fertilization levels showed significant differences in total height (TH). As observed in CD, TH increased with higher NO_3^- concentrations; seedlings from Campo Durán were slightly higher than those from La Paz (Table 2).

In *Acacia koa* seedlings inoculated with *Bradyrhizobium*, the application of Osmocote plus[®] (15 N-9P-12 K) at rates above 2.3 kg m⁻³ of substrate increased seedling diameter, height and biomass, and reduced nodule number and biomass (Dumroese et al. 2009).

Table 2 Analysis of variance and multiple comparisons of means (\pm SE) for collar diameter (CD), total height (TH) and number of nodes with leaves (NN) in *P. alba* seedlings from La Paz (LP) and Campo Durán (CD) subjected to fertilization treatments at different NO_3^- levels in the presence of specific rhizobacteria inoculum

Treatment		CD (mm)	TH (cm)	NN
Fertilization (F) (mM NO_3^-)				
21.6		3.81 (0.06)	38.19 (0.90) a	20.05 (0.40)
2.8		3.05 (0.06)	27.23 (0.90) b	16.05 (0.40)
0		2.25 (0.06)	18.75 (0.90) c	12.65 (0.40)
Inoculation (I)				
Si		3.08 (0.05)	28.57 (0.86)	16.50 (0.37) a
No		3.00 (0.05)	27.54 (0.86)	16.00 (0.37) b
Provenance (P)				
La Paz (LP)		3.09 (0.04) a	27.29 (0.83) a	17.91 (0.37)
Campo Duran (CD)		2.99 (0.04) b	28.82 (0.83) b	14.59 (0.37)
Fertilization \times inoculation				
21.6	Yes	3.82 (0.07) A	38.68 (1.01)	20.14 (0.43)
21.6 ⁻	No	3.80 (0.07) A	37.70 (1.01)	19.96 (0.43)
2.8	Yes	3.17 (0.07) B	28.18 (1.01)	16.54 (0.43)
2.8	No	2.94 (0.07) C	26.28 (1.01)	15.57 (0.43)
0	Yes	2.25 (0.07) D	18.85 (1.01)	12.82 (0.43)
0	No	2.26 (0.07) D	18.66 (1.01)	12.47 (0.43)
Fertilization \times provenance				
21.6	LP	3.86 (0.06)	37.14 (0.94)	22.33 (0.42) A
21.6	CD	3.76 (0.06)	39.23 (0.94)	17.77 (0.42) B
2.8	LP	3.13 (0.06)	26.85 (0.94)	17.70 (0.42) B
2.8	CD	2.97 (0.06)	27.60 (0.94)	14.41 (0.42) C
0	LP	2.27 (0.06)	17.89 (0.94)	13.70 (0.42) C
0	CD	2.24 (0.06)	19.61 (0.94)	11.59 (0.42) D
Factors				
F		***	***	***
I		*	ns	*
P		**	***	***
F \times I		*	ns	ns
F \times P		ns	ns	***
I \times P		ns	ns	ns
F \times I \times P		ns	ns	ns

Within each column, different lowercase or uppercase letters indicate significant differences according to Tukey test ($P \leq 0.05$). When interaction was significant, Tukey test was not performed on main factors
 ns Non-significant
 * $P < 0.05$; ** $P < 0.01$;
 *** $P < 0.001$

Robinia pseudoacacia seedlings showed enhanced growth at a nitrate concentration up to 2 mM (Röhm and Werner 1991). In *Retama sphaerocarpa* seedlings, Valladares et al. (2002) found that a weekly fertilization with 2.9 mM N had a positive effect on growth, but inhibitory effects on nodule formation, and that when NO_3^- was reduced to 0.29 mM, plants developed nodules, reached higher height and increased production of dry matter.

In *Dalbergia sissoo* seedlings, the highest height values were observed when seedlings were inoculated

with *Rhizobium* spp. and grown under low nitrogen levels, followed by treatments only inoculated with *Rhizobium* (Kumar et al. 2013). In *Leucaena leucocephala*, the combined treatment of fertilization and *Rhizobium* spp. inoculation yielded higher TH and higher CD values than individual inoculation or fertilization (Mrema et al. 1997).

In *Prosopis chilensis* seedlings, the increase in nitrogen application promoted stem growth but not root growth (Hahne and Schuch 2006 and references therein). Similar results were reported by Hahne and

Schuch (2006) for *P. velutina*. This lack of sensitivity of *Prosopis* seedlings to respond to a wide range of increases in the amount of supplied nitrogen suggests that, once a minimum of nitrogen is supplied, plants use that base level for growth, but they can take additional nitrogen to increase their internal concentration; that additional nitrogen can be mobilized for growth in case of limiting nitrogen supply (Hahne and Schuch 2006).

Inoculation with specific rhizobacteria and Fertilization \times Provenance interaction had significant effects on the number of nodes with leaves (NN). Higher nitrate concentrations were associated with increases in NN in seedlings from both provenances. At 21.6 mM NO_3^- , treated seedlings exhibited the highest number of NN and La Paz had significantly higher NN than Campo Duran for all $\text{NO}_3^- \times$ Provenance interactions (Table 2).

Prosopis velutina exhibited increases in NN associated with rises in nitrogen content (Hahne and Schuch 2006). In *Dalbergia sissoo*, NN increased when seedlings were inoculated with *Rhizobium*, with those increases being even higher when inoculation was combined with low nitrogen rates (Kumar et al. 2013). In agreement with those reports, our results showed that fertilization combined with inoculation promoted development of leaves and nodes (NN) as well as of CD (Table 2).

In the treatment not inoculated with specific rhizobacteria, nodule formation was observed in plants of both provenances (Table 3). This cannot be considered an unexpected result, since the substrate composed of composted pine bark, perlite and vermiculite was not sterilized previously. Even though the proportion of nodulated plants did not differ between inoculated and non-inoculated treatments, in the former, nodules were large and red inside due to the presence of Leghemoglobin, suggesting their functionality, whereas nodules from non-inoculated plants were numerous, small, and greenish, indicating the occurrence of oxidative processes. Nodule development without inoculation was reported by Lesueur and Duponnois (2005) and Dumroese et al. (2009) in studies conducted in *Acacia crassicarpa* and *A. koa*. The spontaneous appearance of nodules in non-fertilized and non-inoculated treatments may be due to higher susceptibility of plants under nutrient stress to be colonized even by parasitic bacteria (Dumroese et al. 2009). In addition, growth conditions in this work

were in the open air; consequently the presence of rhizobia in non-inoculated treatments may be also due to deposition of soil particles by wind, transference in the nursery via workers, precipitation or irrigation events.

Seedlings from La Paz provenance showed higher proportion of nodulation than those from Campo Durán at all fertilization levels, with maximum values at 2.8 mM NO_3^- in both provenances. The percentage of reduction in nodule number as a consequence of an increase of NO_3^- (21.6 mM) or absence of fertilization (0 mM) with respect to the optimum 2.8 mM NO_3^- concentration is shown in Table 3. Regarding the 21.6:2.8 ratio, reduction was about 50%, whereas for the 0:2.8 ratio, reduction was about 10%, showing that nodulation was affected by excess or deprivation of NO_3^- .

Systematic inoculation of *Acacia* (*A. senegal* and *A. nilotica*) nursery seedlings with selected rhizobia strains capable to induce nodules and fix nitrogen is a recommended practice before planting. A strong relationship between the genetic origin of woody legumes and their capacity to grow, nodulate, and fix atmospheric nitrogen in symbiosis with rhizobia has been repeatedly reported; however, strong differences in growth rates have been observed among inoculated individuals from the same seedlot (Sarr et al. 2005 and references therein).

The number of nodules developed by a plant and the subsequent nitrogen fixation is genetically and environmentally regulated in a process known as autoregulation of nodulation, which involves the participation of systemic signals that balance the energy requirements of a plant (Caetano-Anollés and Gresshoff 1991; Ferguson et al. 2010). Nitrogen availability is an important environmental regulator of nodulation and the inhibitory effects of high nitrate contents on nodulation are complex and still not fully understood. A few hypotheses have been proposed to explain this process, such as deprivation of carbon skeletons in nodules (Streeter 1988), feedback inhibition by products of nitrate metabolism, (e.g., glutamine and/or asparagine), and reduced O_2 availability in nodules, restricting respiration of bacteroids (Saito et al. 2014).

Nodule formation and growth inhibition by high NO_3^- depends mainly on the host plant. This inhibitory process is not often mentioned as associated with nitrate from symbiont metabolism, even though

Table 3 Percentage of 90-day-old *P. alba* seedlings (N = 80) with visible nodules and percent reduction of nodulated seedlings under 2.8 mM NO₃⁻ with respect to 0 (0:2.8) and 21.6 mM NO₃⁻ (21.6:2.8)

Provenance	Inoculum	Fertilization (mM NO ₃ ⁻)			% Reduction	
		0 (%)	2.8 (%)	21.6 (%)	21.6:2.8	0:2.8
CD	No	23.75	25.00	11.25	55	5
	Yes	21.25	20.00	10.00	50	- 6
LP	No	42.50	53.75	20.00	63	21
	Yes	55.00	61.25	27.50	55	10

during flooding, drought or high levels of nitrates, bacteroids activate the denitrification pathway and, consequently, inactivate key nodular proteins, such as Leghemoglobin (Arrese-Igor et al. 1997; Ohyama et al. 2011).

Prosopis nigra and *P. affinis* inoculated with strains of *Rhizobium* and *Bradyrhizobium* and irrigated with Hoagland nutrient solution developed a low number of nodules. This result was attributed to the lack of host specificity, without taking into account that Hoagland solution contains 15 mM NO₃⁻ and that this may be the main cause of the scarce number of nodules (Frioni et al. 1998). Other authors indicate that when inoculation is followed by the addition of N, there is an increase in the number and size of nodules; nevertheless, this occurs up to certain level of fertilization, which depends on the plant species and their symbionts (Sivasupiramaniam et al. 1986; Razz et al. 1995).

Röhm and Werner (1991) and Goicoechea et al. (2004) stated that low nitrate content (between 0.25 and 2 mM) in the fertilizer contributes to nodule formation by nitrogen-fixing bacteria in *Robinia pseudoacacia* and *Anthyllis cytisoides* plants. Here, although the addition of 2.8 mM NO₃⁻ produced the highest number of nodulated seedlings, the highest CD, TH and NN values were obtained in inoculated seedlings fertilized with 21.6 mM NO₃⁻, showing a synergistic effect of the presence of specific rhizobacteria and fertilization (Table 2).

Conclusion

A key element in nursery production of forest seedlings is having a precise fertilization plan; for *P. alba*, such plan should be compatible with

maintenance of the fixing capacity of nodules induced by their specific rhizobacteria. While nitrogen uptake via BNF is adequate for growth promotion in the limited space of containers, the remaining essential minerals should be absorbed by roots and, therefore, adequately supplied in terms of timing, form and rates.

This work contributes with preliminary information to the knowledge of the positive effects of inoculation with specific rhizobacteria and their compatibility with NO₃⁻ supply in the promotion of *P. alba* growth.

The formulation of an inoculum containing three genera of specific rhizobacteria stimulated a higher number of nodes with leaves (NN) and, combined with 2.8 mM NO₃⁻, enhanced collar diameter (CD). Higher NO₃⁻ levels increased CD, TH and NN, at the expense of a lower proportion of nodulated seedlings.

The study of NO₃⁻ supply at different rates, the use of another type of formulations of nutrient solutions, and the quantification of N supply from BNF are key aspects that will contribute to the optimization of nursery production processes of *P. alba* seedlings; inoculation is an aspect that should be included and considered central from a sustainability perspective.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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