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Inoculation with *Azospirillum brasilense* enhances the quality of mesquite *Prosopis juliflora* seedlings

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Abstract

Inoculation of mycorrhizal fungi and rhizobacteria in plants can improve their growth and physiological status, which could be particularly important for agricultural and forestry plants used for the revegetation of arid areas. *Prosopis juliflora* is a forest pioneer species that is drought resistant and has multiple uses (fodder, shade and shelter for livestock; timber and firewood, live fences and windbreaks in agroforestry systems). *Azospirillum brasilense* is a rhizobacterium that improves the growth of many agricultural crops. The hypothesis of this study was that *P. juliflora* seedlings produced in the nursery can respond positively to inoculation with *A. brasilense CECT 590.* Five months after inoculation, we examined the growth, water relations (osmotic potential at full turgor, osmotic potential at zero turgor, and the modulus of elasticity at full turgor), and concentration and content of macronutrients (N, P, K, Ca and Mg) in the seedlings. Subsequently, a trial was conducted to analyse root growth potential. *A. brasilense CECT 590* inoculation caused an osmotic adjustment in *P. juliflora* seedlings but decreased the elasticity of the cell walls. Inoculation with *A. brasilense CECT 590* significantly improved plant growth due in part to an increase of N concentration in the seedlings. *A. brasilense CECT 590* inoculation also caused an increase in the root growth potential. The increased growth of *P. juliflora* seedlings inoculated with *A. brasilense* was probably caused by more than one mechanism. Inoculation with *A. brasilense* at the nursery may be a suitable technique for producing improved seedling material for restoration purposes.

Key words: PGPR; osmotic adjustment; elastic adjustment; mineral nutrition; nursery; root growth potential.

Resumen

La inoculación de *Azospirillum brasilense* mejora la calidad de las plántulas de un mezquite (*Prosopis juliflora*)

La inoculación de hongos de micorrizacion y rizobacterias en las plantas puede mejorar su crecimiento y calidad fisiológica, especialmente en plantas agrícolas y forestales empleadas para la regeneración de zonas áridas. *Prosopis juliflora* es una especie forestal pionera, resistente a la sequía, y de usos múltiples (forraje, sombra y cobijo para el ganado, madera y leña, cercas vivas y cortinas cortavientos en sistemas agroforestales). *Azospirillum brasilense* es una rizobacteria que mejora el crecimiento de muchos cultivos agrícolas. La hipótesis de este estudio fue que las plántulas de *P. juliflora* producidas en vivero pueden responder positivamente a la inoculación con *A. brasilense CECT 590*. Cinco meses después desde la inoculación, se analizó el crecimiento, relaciones hídricas (potencial osmótico en saturación, potencial osmótico en el punto de pérdida de turgencia, y módulo de elasticidad a total turgor), concentración y contenido de macronutrientes (N, P, K, Ca y Mg) en las plántulas. Posteriormente se realizó un ensayo para analizar el Potencial de Crecimiento Radical. La inoculación de *A. brasilense CECT 590* causó un ajuste osmótico de las plántulas de *P. juliflora*, pero disminuyó la elasticidad de las paredes celulares. *A. brasilense CECT 590* mejoró significativamente el crecimiento de la planta, debido en parte al incremento de la concentración de N en las plántulas. La inoculación con *A. brasilense CECT 590* también causó un incremento del potencial de crecimiento radical. El incremento del crecimiento en las plántulas de *P. juliflora* inoculadas con *A. brasilense* fue probablemente debido a más de

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un mecanismo. La inoculación con *A. brasilense* en vivero puede ser una técnica adecuada para la producción de material de plántulas mejorado con fines de restauración.

Palabras clave: PGPR (Rizobacterias promotoras del crecimiento de plantas); ajuste osmótico; ajuste elástico; nutrición mineral; vivero; potencial de crecimiento radical.

Introduction

In semiarid Mediterranean ecosystems characterised by summer drought, water and soil nutrient availability are the main constraints on plant productivity and the diversity and abundance of the microflora associated with the roots of plants (Marulanda *et al.*, 2006).

Prosopis juliflora is a pioneer forest species from Mexico that easily adapts to arid and semiarid ecosystems. *P. juliflora* has multiple uses. Its leaves and fruits are used for fodder, shade and shelter for livestock; and the wood is harvested for timber and firewood as well as for live fences and windbreaks in agroforestry systems. *P. juliflora* is often selected for the restoration of arid and semiarid areas because of its fire resistance and its tolerance to extremely poor soils, strong winds, temporary flooding, saline soils (Bradbury & Ahmad, 1990; Bhojvaid and Timmer, 1998), and drought (Clinch *et al.*, 1993). *Prosopis* trees also facilitate the establishment and regeneration of other plant species by fixing atmospheric nitrogen through the formation of root nodules of *Rhizobium* (Carrillo-Garcia *et al.*, 1999). *Prosopis* trees can be intercropped or not with other annual crops in various conditions, such as hedges, bush fallows and long-term rotations. *Prosopis juliflora* has been introduced for use in reforestation in the Mediterranean basin.

Numerous studies on the reforestation of Mediterranean areas have been conducted with the aim of improving the quality of seedlings produced in nurseries (Caravaca *et al.*, 2005; Rincon *et al.*, 2006). Among the cultural practices, inoculation with ectomycorrhizal fungi and plant growth promoting rhizobacteria (PGPR) has shown promise in improving the quality of seedlings and increasing their survival in plantations, especially in soils with low microbial activity (Chanway, 1997; Probanza *et al.*, 2001; Duponnois *et al.*, 2005).

Azospirillum brasilense (Tarrand *et al.*, 1978) is a PGPR. The ability of *A. brasilense* to stimulate plant growth has been extensively demonstrated. The stimulation of growth is usually due to the production of phytohormones such as auxins, cytokinins and gibberellins (Tien *et al.*, 1979; Horemans *et al.*, 1986; Fallik *et al.*, 1989; Omay *et al.*, 1993). Other mechanisms responsible for growth stimulation have been linked to *A. brasilense* activity (Zimmer *et al.*, 1988), such as the improvement of water and nutrient absorption or N fixation (Bashan, 1999; Bashan and Gonzalez, 1999; Bashan *et al.*, 2004; Bashan and de-Bashan, 2010).

Although there are numerous studies on the effects of *A. brasilense* in improving the performance of agricultural crops (Okon and Lavandera-Gonzalez, 1994; Bashan *et al.* 2004), there is a lack of knowledge regarding its effect on forest plants (Bashan *et al.*, 1999; Puente *et al.*, 1999; Bacilio *et al.*, 2006; Leyva and Bashan, 2008; Bashan *et al.*, 2009) or its application in agroforestry systems.

In this study, we hypothesised that inoculation with *A. brasilense CECT 590* on *P. juliflora* seedlings in the nursery would increase the vigour and performance of the seedlings, thus producing good material for restoration purposes. For this purpose, seedling growth, water relations and nutrient concentrations were studied. A possible change in water parameters due to any of the inoculations could indicate the reconditioning of plants to withstand drought; the effect of *A. brasilense CECT 590* on the root growth potentials of the seedlings was also investigated.

Materials and methods

Plant production

The study was conducted at the IFAPA (Center for Agricultural Research and Training) La Mojonera, Almeria, Spain. *P. juliflora* seeds were collected in 2008 and remained in closed polyethylene bags at 4°C. We used Forest Pot 300® containers (conical pots: 300 ml, 4.6 × 4.8 cm in the top section and 1.9 × 1.9 cm in the bottom section, 19 cm depth). For the growth medium (substrate), a mixture of light and dark peat for the organic component (*Sphagnum* moss at pH 6) and vermiculite for the inorganic component in a 3:1 peat/vermiculite mixture was used. The peat was sterilised by autoclaving at 120°C for 2 h.

Before sowing, the seeds were submerged in distilled water for 24 h. Then, all the surfaces of the seeds were disinfected by immersion in 30% hydrogen peroxide (H_2O_2) for 15 min followed by several rinses with distilled water. In mid-April 2008, the *P. juliflora* seeds were sown in 300 cells (6 containers, 50 cells per container). Two to three seeds per cell were planted, and a single seedling was allowed to germinate in each cell. The plantings were conducted in an IFAPA shade house, and the plants were watered daily to saturation at temperatures ranging from 6 to 45°C (28°C mean) until the inoculations were performed.

Bacterial cultivation

A lyophilised pre-culture of *A. brasilense CECT 590* (obtained from CECT, Spanish Type Culture Collection, University of Valencia) was stored at 10-15°C until inoculation, and it was subjected to 3 successive incubations. A lyophilised pre-culture vial was first suspended in 0.3 ml of nutritive medium (1 g l^{-1} beef extract, 5 g l^{-1} peptone, 5 g l^{-1} NaCl and 1 l distilled water, pH 7.2). A drop (0.02 ml) of that suspension was added to 5 ml of nutritive medium and incubated at 30°C for 12 h; then, it was transferred to 75 ml of nutritive medium and incubated on an orbital shaker (200 rpm) at 30°C for 12 h. After the incubation period, this 80 ml was added to 720 ml of nutritive medium and incubated again as before. This final preparation of medium was used as the inoculum. A plate count was performed on a growing solid medium (1 g l^{-1} beef extract, 5 g l^{-1} peptone, 5 g l^{-1} NaCl , 15 g l^{-1} agar and 1 l distilled water, pH 7.2); 0.10 ml of inoculum was seeded on Petri dishes with this nutritive solid medium, at 30°C for 24 h; 2 replicates × 4 subreplicates × 4 dilutions were performed, revealed an average of 1.86×10^8 C.F.U.s ml^{-1}.

Experimental Design and Bio-inoculations

A two-level (*A. brasilense* inoculation and control) and two factorial (inoculation and block) design was performed, and three blocks were randomly distributed with 50 plants per block .

The inoculum was applied two times, separated by a period of 15 days (late June and early July). The inoculum was injected in the substrate via a syringe of 5 ml (without needle), to depth of the nozzle of the syringe (8 mm), as close to the each seedling. A total of 10 ml per plant (5 ml per injection) of liquid bacterial inoculum (total of 1.86×10^{10} C.F.U.s per plant) of *A. brasilense CECT 590* (50% of seedlings) was inoculated. As a control, 50% of the seedlings were left uninoculated. No fertiliser was added to any of the seedlings.

Measurements

Pressure-volume analysis. Plant water measurements

To study the influence of inoculation on plant water relations, the pressure-volume curves of the plants were calculated (Tyree and Hammel, 1972; Robichaux, 1984) in November 2008. These curves were determined by shoot xylem pressure potentials (shoot water potentials) measured in a pressure chamber (Scholander *et al.*, 1965). The water-relation parameters were obtained from these graphs: the osmotic potential at full turgor ($\Psi\pi_{full}$) and at zero turgor ($\Psi\pi_0$); and the modulus of elasticity near full turgor (E_{max}) (Cheung *et al.*, 1975; Jones and Turner, 1980; Tyree and Jarvis, 1982; Bowman and Roberts, 1985). Nine seedlings per treatment (three per block) were analysed.

Growth

In November 2008, nine plants per treatment (three per block) were randomly chosen. The shoot heights and basal stem diameters were recorded. The rooting substrate of each plant was immersed in water, and most of the growth substrate in which the seedlings had been grown was removed. Then, the total root tip numbers per plant were analysed using a stereoscopic microscope (× 40). After the samples had been dried at a temperature between 65°C and 70°C for 48 h, the dry weights of shoots and the total root mass were measured.

Nutrient Analysis

In November, a sample of 10 seedlings per block (n = 3) was randomly selected and pooled, and the whole plant was finely ground and homogenised. The concentrations of P, K, Ca and Mg were determined using an inductively coupled plasma atomic-emission

spectrometer (Perkin-Elmer Plasma 400 Sequential ICP-OES) after subsample digestion in a microwave with a closed system using HNO_3 concentrate; nitrogen was determined using a LECO CHN-600 autoanalyser.

Root Growth Potential

In February 2009, nine new plants per treatment (three per block) were randomly chosen. The height and basal diameter of each plant were measured. Subsequently, each plant was carefully transplanted to a 3-litre prismatic pot and filled with white perlite. The pots were placed randomly in the greenhouse for 21 days under optimal environmental conditions to facilitate their growth (Burdett, 1987; Simpson and Ritchie, 1997). Irrigation was applied daily for root growth, and the air temperature was maintained between 16 and 22°C. The relative humidity remained at approximately 95%. After 21 days (March), each plant was carefully removed, the number of new roots (distinguishable by their greater thickness and colour differentiation) that were greater than 0.5 cm was recorded, and the total length of new roots was measured.

Data analysis

Analysis of variance (two-way ANOVA) was performed, and the means of all study parameters were calculated, and Tukey's multiple-range test at the 0.05 confidence level was performed. In cases where the variance was nonhomogenous, a nonparametric Kruskal-Wallis test was performed. All the statistical analyses were performed using the Statgraphics Plus computer software package (StatPoint Technologies, Inc. Warrenton, VA). For statistical analysis of the root growth potential, the height was selected as the covariate for the parameters analysed.

Results

There was no interaction between factors for any of the parameters analyzed; in addition, the block factor did not affect any of the analyzed parameters.

Inoculation with *A. brasilense CECT 590* significantly (ANOVA, Tukey tests, $\alpha = 0.05$) increased all growth parameters. The inoculation caused a significant decrease in the number (mean ± standard error) of total root tips measured in terms of root dry weight (764 ± 52 per g dw in inoculated plants vs. 2,068 ± 208 per g dw in control; table 1 & 2), but the total number of root tips per plant was significantly higher in the inoculated plants (134 ± 9 in inoculated plants vs. 91 ± 9 in control; Table 1 and 2). The inoculation also significantly ($p < 0.05$) increased the total length of new roots per plant (8.61 ± 1.15 cm in inoculated plants vs. 3.94 ± 1.15 cm in control; Table 1 and 2), demonstrating improve root growth potential.

A. brasilense CECT 590 inoculation was significantly (ANOVA, Tukey tests, $\alpha = 0.05$) related to an osmotic adjustment (mean ± standard error) of both $\Psi\pi_{full}$ (–1.75 ± 0.09 MPa in inoculated plants vs. –0.99 ± 0.09 Mpa in control) and $\Psi\pi_0$ (–2.99 ± 0.16 MPa in inoculated plants vs. –2.23 ± 0.19 Mpa in control); by contrast, the inoculation was related to a significant decrease in cell elasticity, ie, an increase in the modulus of elasticity E_{max} (11.13 ± 2.11 MPa of in inoculated plants vs. 2.48 ± 0.62 Mpa in control; Table 1 and 2).

The inoculation significantly (ANOVA, Tukey tests, $\alpha = 0.05$) increased the N concentration (12.78 ± 0.33 mg g^{-1} in inoculated plants vs. 11.4 ± 0.19 mg g^{-1} in control) and the content of N (4.08 ± 0.11 mg $plant^{-1}$ in inoculated plants vs. 0.83 ± 0.01 mg $plant^{-1}$ in control), P (0.1 ± 0 mg $plant^{-1}$ in inoculated plants vs. 0.04 ± 0.01 mg $plant^{-1}$ in control), K (2.57 ± 0.1 mg $plant^{-1}$ in inoculated plants vs. 0.86 ± 0.14 mg $plant^{-1}$ in control), Ca (1.94 ± 0.07 mg $plant^{-1}$ in inoculated plants vs. 0.61 ± 0.06 mg $plant^{-1}$ in control) and Mg (1.47 ± 0.29 mg $plant^{-1}$ in inoculated plants vs. 0.25 ± 0.06 mg $plant^{-1}$ in control) (Table 1 and 2).

Discussion

In the scientific literature, *A. brasilense* inoculation caused the highest plant yields under suboptimal environmental conditions for plant development, such as nutrient deficiencies or water stress, usually in arid or semiarid areas (Bashan and Holguin, 1997). Other studies have also shown that under water stress conditions, the activity of *A. brasilense* can improve growth and stabilise the maintenance of physiological conditions in the host plants (Alvarez *et al.* 1996; Bashan *et al.*, 2009). However, this trial was conducted under non-restrictive water conditions suitable for plant growth in a forest nursery, under which the promotion of seedling growth, by different mechanisms, was observed.

Table 1. Statistical effects

	Block			*Azospirillum* treatment			KW statistic[+]	P value[+]
	Df[++]	F[++]	P[++]	Df[++]	F[++]	P[++]		
Water-Relations parameters								
$\Psi\pi_{full}$[1]	2	0.82	0.461	1	42.74	0.000*		
$\Psi\pi_0$	2	2.74	0.099	1	14.85	0.002*		
E_{max}							12.79	0.000*
Growth								
Height	2	2.84	0.092	1	202.03	0.000*		
Basal Diameter							8.51	0.004*
Shoot							12.8	0.000*
Root							12.8	0.000*
Total root tips[2]							11.57	0.001*
Nº Total plant^{-1}	2	1.31	0.302	1	14.59	0.002*		
Nutrients concentration								
N				1	13.14	0.022*		
P							3.23	0.072
K							2.33	0.127
Ca				1	6.78	0.060		
Mg				1	1	0.374		
Nutrients Content								
N							3.86	0.049*
P							3.97	0.046*
K				1	99.84	0.001*		
Ca				1	206.44	0.000*		
Mg				1	17.69	0.014*		
Root Growth Potential								
Nº Roots[3]	2	0.05	0.948	1	0.81	0.385		
Length[3]	2	0.74	0.496	1	4.97	0.044*		

[+] Kruskal-Wallis test: ANOVA one-way (*Azospirillum* Inoculation Factor); K-W statistic and corresponding P value. [++] ANOVA two-way (*Azospirillum* Inoculation and block Factors; no interaction between factors). * Indicate significant differences ($p < 0.05$). [1] $\Psi\pi_{full}$: osmotic potential at full turgor, $\Psi\pi_0$: osmotic potential at zero turgor and E_{max}: modulus of elasticity near full turgor. [2] Total root tips = number of total root tips, is referred to as grams of root dry weight. [3] Number and total length of new roots per plant; covariate using the height parameter.

The ability of *A. brasilense* to promote root development has been extensively demonstrated (Levanony and Bashan, 1988; Burdman *et al.*, 2000; Itzigsohn *et al.*, 2000), and previous studies have also revealed nutritional and water benefits, with an increase in the area of root exploration in the soil. In this study, *A. brasilense CECT 590* inoculation promoted significant development of the roots, but proportionally, the increase was greater in weight than the total number of root tips. Moreover, there were promising results from the analysis of root growth potential. This indicates that *A. brasilense CECT 590* inoculation may be a promising cultivation technique in reforestation programs in semiarid Mediterranean areas.

The capacity to adjust osmotic potential and increase the elasticity of the cell wall (elastic adjustment) is traditionally associated with the increased ability of plants to withstand water stress. Through both mechanisms, plants are able to maintain turgor potential, the capacity for growth and photosynthesis, and the ability to tolerate greater negative water potential and lower water availability (Villar-Salvador *et al.*, 1997). In this study, under non-limiting irrigation conditions, *A. brasilense CECT 590* inoculation caused an osmotic adjustment (to both $\Psi\pi_{full}$ and $\Psi\pi_0$), perhaps improving tolerance to water stress. However, inoculation reduced the elasticity of the cell walls, causing a loss of responsiveness in the seedlings to possible changes to their water status. Osmotic adjustment, as a mechanism of

Table 2. Mean values of water-relations parameters, growth parameters, nutrients concentration and contents, and root growth potential in control and *Azospirillum brasilense* inoculated *Prosopis juliflora* seedlings

Treatment	Control	Azospirillum
Water-Relations parameters		
$\Psi\pi_{full}$[1] (MPa)	–0.99 (± 0.09)	–1.75 (± 0.09)
$\Psi\pi_0$ (MPa)	–2.23 (± 0.19)	–2.99 (± 0.16)
E_{max} (MPa)	2.48 (± 0.62)	11.13 (± 2.11)
Growth		
Height (cm)	5.71 (± 0.34)	13.14 (± 0.56)
Basal Diameter (mm)	1.28 (± 0.05)	1.76 (± 0.11)
Shoot (g)	0.029 (± 0.003)	0.144 (± 0.016)
Root (g)	0.044 (± 0.004)	0.175 (± 0.018)
Total root tips[2] (Nº g^{-1} dw)	2,068 (± 208)	764 (± 52)
Nº Total $plant^{-1}$	91 (± 9)	134 (± 9)
Nutrients concentration		
N (mg g^{-1})	11.4 (± 0.19)	12.78 (± 0.33)
P (mg g^{-1})	0.55 (± 0.13)	0.3 (± 0)
K (mg g^{-1})	11.84 (± 1.95)	8.07 (± 0.3)
Ca (mg g^{-1})	8.32 (± 0.83)	6.08 (± 0.22)
Mg (mg g^{-1})	3.42 (± 0.79)	4.61 (± 0.89)
Nutrients content		
N (mg $plant^{-1}$)	0.83 (± 0.01)	4.08 (± 0.11)
P (mg $plant^{-1}$)	0.04 (± 0.01)	0.1 (± 0)
K (mg $plant^{-1}$)	0.86 (± 0.14)	2.57 (± 0.1)
Ca (mg $plant^{-1}$)	0.61 (± 0.06)	1.94 (± 0.07)
Mg (mg $plant^{-1}$)	0.25 (± 0.06)	1.47 (± 0.29)
Root Growth Potential		
Nº Roots[3]	4 (± 1)	7 (± 1)
Length[3] (cm)	3.94 (± 1.15)	8.61 (± 1.15)

[1] $\Psi\pi_{full}$: osmotic potential at full turgor, $\Psi\pi_0$: osmotic potential at zero turgor and E_{max}: modulus of elasticity near full turgor. [2] Total root tips = number of total root tips, is referred to as grams of root dry weight. [3] Number and total length of new roots per plant; covariate using the height parameter.

Values in parentheses represent the standard error. N = 9 (Water-Relations, Growth, and Root Growth Potential parameters); N = 3 (Nutrients parameters).

drought tolerance, can be caused by the intracellular accumulation of organic solutes or osmolytes such as glycine, proline, glutamate, and some types of glucans (Bashan and Holguin, 1997). These compounds, which can be assimilated by the bacteria directly from the ecosystem or produced by *A. brasilense,* have been isolated from *A. brasilense* in arid ecosystems (Riou *et al.*, 1991; Altabe *et al.*, 1994). Potassium is an important solute associated with the regulation of cell turgor and stomatal opening (Benlloch-González *et al.*, 2008). Thus, according to the results of this study, there was a relationship between the regulation of cellular osmotic potential and the amount of K absorbed by the plants.

In the present study, *A. brasilense CECT 590* inoculation caused an increase of total N concentration in plants. Others authors have observed increases in the total N content of *A. brasilense* inoculated plants (Cohen *et al.*, 1980; Schank *et al.*, 1981; Baldani *et al.*, 1983; Smith *et al.*, 1984; Kapulnik *et al.*, 1985; Pacovsky *et al.*, 1985; Murthy and Ladha, 1988) and the incorporation of the ^{15}N isotope (Rennie and Thomas, 1987). However, most of these studies showed no significant variation in N concentration or in protein content, so biological N fixation may not be the principal mechanism responsible for the observed effects. However, *A. brasilense* is able to fix N (Christiansen-Weniger, 1994) and transform it into NH_4^+ (which is assimilated by the plant) through the activity of nitrogenase. *A. brasilense* can also change and improve the N permeability of soil for plants. Additionally, *A. bra-*

silense does not form atmospheric N-fixing nodules, but previous research has shown that *A. brasilense* colonisation can improve subsequent atmospheric N fixation by *Rhizobium* sp. Therefore, more than one mechanism, and possibly cumulative mechanisms (Bashan and de Bashan, 2010), may be involved in improved N uptake (and other nutrients) by plants.

We found that *A. brasilense CECT 590* inoculation increased all morphological parameters, highlighting the stimulatory effect in the root system (Leyva and Bashan, 2008). This increased growth might be caused by phytohormone activity, N fixation, various small molecules and enzymes, enhanced membrane activity, proliferation of the root system, enhanced water and mineral uptake, mobilisation of minerals, mitigation of environmental stressors of plants, and direct and indirect biological control of numerous phytopathogens (Bashan and Holguin, 1997; Bashan and Gonzalez, 1999; Bashan and de Bashan, 2010). In this study, there was low availability of nutrients in the substrate. Therefore, the increase in growth is likely due partly to the increased concentration of N; however, the evidence presented here indicates that growth promotion is probably due to more than one mechanism, or a "multiple mechanisms theory" (Bashan and de Bashan, 2010).

We conclude that inoculation with *A. brasilense CECT 590* caused an osmotic adjustment in *P. juliflora* seedlings but decreased the elasticity of the cell walls. Plant growth significantly improved because of *A. brasilense CECT 590* inoculation, particularly stimulating root growth and increasing the macronutrient content, especially the N contribution. These results indicate that treatment with *A. brasilense* in the nursery may be a suitable technique for producing improved seedling material for restoration purposes.

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