

Effects of biofertilization with plant growth promoting bacteria in micropropagation of *Jacaranda mimosifolia*

Ariel Horacio Salotti

Departamento de Ciencias Básicas, Universidad Nacional de Luján

Mauro Enrique Yarte

Departamento de Ciencias Básicas, Universidad Nacional de Luján

Ezequiel Enrique Larraburu (✉ ezequiel.e.larraburu@gmail.com)

Departamento de Ciencias Básicas, Universidad Nacional de Luján

Research Article

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Abstract

Jacaranda mimosifolia (Bignoniaceae) is an important economic, ecological and ornamental tree native to South America. It is cultured in squares, parks and gardens throughout America, Europe, South Africa and Asia. During plant-microbe interactions,

plant growth promoting bacteria (PGPB) could optimize plant production by mitigating undesirable features which trouble *ex vitro* transferring. So, the aim of this work was to evaluate the effect of biofertilization with *A. brasilense* Az39 (as reference strain), *Methylobacterium* sp. L10 and *Stenotrophomonas* sp. L20 on micropropagation of *Jacaranda mimosifolia*. Woody Plant Medium (WPM) was used for seeds germination whereas Murashige and Skoog salts with Gamborg vitamins (MSG) supplemented with 6-benzylaminopurine (BA) were used in multiplication stage and supplemented with indole butyric acid (IBA) were used in rooting stage. All stages were inoculated or not with Az39, L10 or L20. Culture system, bacteria inoculation in each micropropagation stage and their interaction were significant. *Methylobacterium* sp. L10 and *Stenotrophomonas* sp. L20 decreased fungal contamination at initial establishment by seeds. In addition, *Methylobacterium* sp. L10 inoculation increased the multiplication rate by 94% relative to non-inoculated shoots. *Stenotrophomonas* sp. L20 inoculation in combination with a pulse with 30 μ M IBA increased *in vitro* and *ex vitro* rooting percentage. In the acclimatization stage, 95% of *ex vitro*-rooted plants inoculated with *Stenotrophomonas* sp. L20 survived. The strains used at this work could act as biological control agents and could be used for biofertilizers development.

Introduction

Jacaranda mimosifolia (Bignoniaceae) is an important economic and ecological tree distributed throughout northern Argentina, Bolivia, Brazil and northwestern Paraguay. Its abundant blue-violet flowering promotes its plantation as ornamental in squares, parks and gardens throughout America, southern Europe, South Africa and Asia (Fauerharmel et al. 2020; Xie et al. 2021). Compounds extracted from its bark, leaves, roots or flowers such as jacaranda acid, jacaranone, coumaric acid, among others, are capable of inhibiting venereal, digestive, liver and skin diseases. In addition, it is excellent as a timber resource because of its light, malleable and attractive wood (Mostafa et al. 2014).

During beneficial interactions between plants and microorganisms, plants provide exudates rich in carbohydrates from their roots, which are the main source of carbon for microorganisms. Microbes, including plant growth promoting bacteria (PGPB) generate responses, actions and mechanisms that help directly or indirectly to plant growth (Afzal et al. 2019; Glick 2020). Bacteria could facilitate the acquisition of resources such as nitrogen, phosphorus, and other essential minerals through nitrogen fixation, phosphorus solubilization, and iron sequestration by siderophores secretion. Also, PGPB synthesizes phytohormones such as auxins, gibberellins, cytokines and nitric oxide (Afzal et al. 2019; Glick 2020). Among indirect mechanisms, it has been described the reduction or elimination of phytopathogens by antibiotics, lytic enzymes or volatile organic compounds production as well as by induced systemic response (ISR) activation (Afzal et al. 2019; Glick 2020).

Plant tissue *in vitro* culture produces plants with reduced photosynthetic activity, lack of cuticle development, presence of non-functional stomata and roots with decreased root hair density. These feature difficult transfer to *ex vitro* conditions by reducing considerably the survival rate (Soumare et al. 2021). In this sense, *in vitro* culture represents an adequate experimental system to study the plant-bacteria interaction since possibilities totally or partially replace an organic compound or other substance by bacteria inoculation in order to analyze their effect on plant growth (Larraburu & Llorente, 2015). Since Nowak (1998), biotization has been carried out by various researchers as a valuable tool that can be applied at different stages of plant tissue culture in order to optimize plant production (Kanani et al. 2020; Soumare et al. 2021). *Azospirillum brasilense* is able to improve micropropagation of woody species such as pink lapacho (*Handroanthus impetiginosus*) and yellow lapacho (*Handroanthus ochraceus*) (Llorente et al. 2016). In addition, *Methylobacterium* sp. increases significantly root and shoot number in pine (*Pinus sylvestri*) seedlings under *in vitro* conditions (Pohjanen et al. 2014). Also *Stenotrophomonas* is a plant growth promoting rhizobacteria endophytic from poplar (*Populus* sp.) (Ulrich et al., 2021). Therefore, the aim of our work was to evaluate the effect of biofertilization with *A. brasilense* Az39, *Methylobacterium* sp. or *Stenotrophomonas* sp. on micropropagation of *Jacaranda mimosifolia*.

Materials And Methods

Bacterial strains and culture conditions

A. brasilense Az39, *Methylobacterium* sp. L10 and *Stenotrophomonas* sp. L20 (locally isolated) were used for the experiments. Bacterial inocula were grown in 250 ml Erlenmeyer flasks with 150 ml of medium for *A. brasilense* (Okon et al. 1977) and 150 ml of Luria-Bertani medium (LB) for the other strains. Strains were incubated at $32 \pm 1^\circ\text{C}$ on an orbital SontecTM shaker (140 rpm) for 72 h. Viable cell count was evaluated by dilution plate counts on agarized RC (Rodríguez-Cáceres, 1982) or LB medium for *A. brasilense* Az39 and the other stains, respectively.

In vitro germination

J. mimosifolia seeds were obtained from adult trees from Luján, General Rodríguez and Rosario cities, in Central-eastern Argentina. Seeds were washed in running water and two drops of aqueous solution of non-ionic detergent Tween 20 at 0.1% for 30 min, disinfected with sodium hypochlorite solution (1.1% active chlorine) with a drop of detergent Tween 20 at 0.1% stirring at 140 rpm with an eccentricity of 2.5 cm for 30 minutes. Finally, seeds were rinsed 3 times (1 min each) with sterile distilled water. Each axenic seed was grown in a flat-bottom glass tube (55 mL) with 15 mL of Woody Plant Medium (WPM) (Lloyd & McCown, 1980) supplemented with 5 g L⁻¹ activated charcoal, 100 mg L⁻¹ myo-inositol, 20 g L⁻¹ sucrose and 6 g L⁻¹ agar, at pH 5.8, and inoculated with 100 μ L (approximately 10⁷ cfu) *A. brasilense* Az39, *Methylobacterium* sp. L10 or *Stenotrophomonas* sp. L20 inoculum (Fig. 1). Control treatment was non-inoculated seeds. Germination (radicle elongation > 5mm) was recorded weekly during 30 d. At the end of the experiment, germination and fungal contamination percentage were recorded as well as epicotyl and hypocotyl lengths of the obtained seedlings.

Multiplication of shoots

Epicotyls from successful germination and non-inoculated seeds were cut (1–1.5 cm each) and placed in Murashige and Skoog (1962) salts supplemented with Gamborg et al. (1968) vitamins, 3% sucrose, 100 g L⁻¹myo-inositol and 6 g L⁻¹agar (MSG) with 5 µM 6-benzylaminopurine (BA). Five shoots per glass flask (350 mL) were cultured in 60 mL of medium and inoculated with 100 µL (approximately 10⁷ cfu) *Methylobacterium* sp. L10, *Stenotrophomonas* sp. L20 or *A. brasilense* Az39 inoculum at the base (Fig. 1). In all treatments, the average number of shoots per initial shoot (multiplication rate, MR), length of shoots, shoot number (initial plus adventitious shoots) per explant were recorded after 30 d.

In vitro rooting and acclimatization

Shoots (2–3 cm) derived from the multiplication stage were cultured on half-strength MSG (½MSG). Shoots were induced with 0 or 30 µM IBA for 3 days and then were transferred to auxin-free ½MSG medium and inoculated with 100 µL (approximately 10⁷ cfu) *A. brasilense* Az39, *Methylobacterium* sp. L10 or *Stenotrophomonas* sp. L20 inoculum. Control treatment was non-inoculated plants. Tubes were maintained in a growth chamber at 29 ± 2°C, with a photoperiod of 16 h day⁻¹ and a light intensity of 30–40 µmol m⁻²s⁻¹, for 30 days. The rooting criterion was the presence of at least one 10 mm root. after 30 days of culture (Fig. 1). Rooting percentage was determined and rooted shoots induced with 30 µM IBA were sampled for acclimatization studies. For the acclimatization stage, each rooted shoot was removed carefully from the culture medium and rinsed off the agar by washing roots with tap water without injuring them. Plants were placed in a plug tray (3 cm diameter multialveoli) containing a mix of soil:peat:perlite (1:1:1) inside a transparent and sealed polyvinyl chloride container (25 cm × 20 cm × 15 cm). Substrate was previously autoclaved at 120°C (1 atm) for 45 min. Plants were cultured in a growth chamber and gradually exposed from 100–50% humidity and 115 ± 20 µmol m⁻²s⁻¹light intensity for 24 days, according to Larraburu et al. (2016) (Fig. 1).

Ex vitro rooting-acclimatization

Shoots were induced with 30 µM IBA in ½MSG for 3 days and then were transferred to a plug tray using the same procedure described above. Shoots were inoculated with 100 µL (approximately 10⁷ cfu) *A. brasilense* Az39, *Methylobacterium* sp. L10, *Stenotrophomonas* sp. L20 inoculum. Non inoculated shoots were controls. Rooting percentage was determined at 30 days and survival rate was evaluated at 60 days.

Statistical analysis

A completely randomized design with three replicates per experiment and 10–15 repetitions per treatment was conducted. A factorial design to analyze the effect of rooting system (in vitro and substrate) and bacterization (non-inoculated and inoculated with endophytic strains) was used. Data were square root or natural log transformed as necessary to satisfy conditions of normality. In addition, paired contrasts of the rooting percentages were performed. Means were compared by Tukey’s test. Statistical analyses were performed using SPSS v 21 software. To analyze global effects on rooting for factors and their interactions, an index was constructed using all the biometric parameters (biometric parameter index (BPI)) (Larraburu & Llorente, 2015). It was calculated as follows:

BPI(t) = ∑_{i=1}^8 [(CM(i,t)-M(i)) / SG(i)]

- CM(i,t): Cell mean for biometric parameter i in treatment t
- MG(i): Grand mean for biometric parameter i
- SG(i): Overall standard deviation for biometric parameter i

Results

Methylobacterium sp. L10 inoculation significantly increased the length of the epicotyl while *Stenotrophomonas* sp. L20 significantly reduced the length of the hypocotyl (Table 1). In addition, biofertilization with *Methylobacterium* sp. L10 and *Stenotrophomonas* sp. L20 significantly decreased (p ≤0.05 and p ≤0.10 respectively) fungal contamination by 69% and 53% respectively compared to controls (Figure 2).

Table 1. Growth parameters of *J. mimosifolia* seedlings derived from *in vitro* germination in WPM medium with activated charcoal inoculated or not with *Methylobacterium* sp. L10, *Stenotrophomonas* sp. L20 or *Azospirillum brasilense* Az39 after 30 days of culture.

Bacterization	Germination (%)				Hypocotyl length (mm)				Epicotil length (mm)				Leaf number			
Control	70.0	±	17.3	a	20.7	±	2.2	a	9.7	±	2.7	b	5.4	±	0.9	a
<i>Methylobacterium</i> sp. L10	73.3	±	12.0	a	17.6	±	2.7	ab	19.1	±	4.7	a	6.2	±	1.6	a
<i>Stenotrophomonas</i> sp. L20	73.3	±	14.5	a	12.8	±	2.7	b	10.8	±	3.1	ab	5.3	±	1.0	a
<i>A. brasilense</i> Az39	73.3	±	14.5	a	16.30	±	3.5	ab	13.3	±	4.1	ab	5.9	±	1.3	a

Comparison of means by Tukey's test. Different letters indicate significant differences between treatments (p ≤0.05).

At the multiplication stage, shoots exhibited expanded green leaves and root formation was not observed. After 60 days of culture, *Stenotrophomonas* sp. L20 increased shoot length ($p \leq 0.05$) by 43% relative to non-inoculated shoots whereas *Methylobacterium* sp. L10 significantly increased ($p \leq 0.05$) shoot number between 92-120% with respect to the other treatments. *Methylobacterium* sp. L10 inoculated shoots reached the highest MR value with significant increases ($p \leq 0.05$) with respect to non-inoculated shoots (94%), although no significant differences were observed compared to *Stenotrophomonas* sp. L20 treatment (Table 2).

Rooting experiments were carried out under in vitro or substrate conditions. Non-induced shoots did not root in any culture systems after 30 days of culture, regardless of bacteria inoculation. Among auxin-induced shoots, factor analysis revealed that the rooting percentage was significantly affected ($p \leq 0.05$) by the culture system, bacterization and the interaction of factors. Root number and shoot length were significantly affected ($p \leq 0.05$) by the bacterization whereas main root length was significantly affected ($p \leq 0.05$) by culture system and bacterization (Table 3).

Table 2. *In vitro* and substrate rooting of *J. mimosifolia*. Effect of bacterization after three days of induction in ½ MSG supplemented with 30 µM IBA at 30 days of culture.

Culture system	Bacterization	Rooting (%)				Root number				Main root length (mm)				Shoot length (mm)				Leaf number				
Substrate	Control	13.3	±	1.9	c	4.5	±	0.3	a	12.5	±	1.1	ab	15.0	±	0.7	a	5.3	±	0.6		
	<i>Methylobacterium</i> sp. L10	4.4	±	2.8	c	3.0	±	0.1	ab	9.5	±	2.7	ab	15.0	±	0.7	a	4.0	±	0.2		
	<i>Stenotrophomonas</i> sp. L20	48.9	±	6.7	b	5.7	±	0.6	a	20.6	±	1.1	a	25.3	±	2.0	a	7.0	±	1		
	<i>A. brasilense</i> Az39	15.6	±	1.9	c	2.7	±	0.9	ab	9.0	±	0.8	ab	20.0	±	1.0	a	5.0	±	0.3		
<i>In vitro</i>	Control	45.0	±	8.7	b	3.7	±	0.3	ab	4.7	±	0.4	b	18.6	±	1.7	a	6.0	±	0.7		
	<i>Methylobacterium</i> sp. L10	6.7	±	2.1	c	1.4	±	1.4	b	1.6	±	1.6	c	20.0	±	10.0	a	4.0	±	2.0		
	<i>Stenotrophomonas</i> sp. L20	80.0	±	5.8	a	4.5	±	0.4	a	6.6	±	0.4	ab	21.5	±	2.6	a	6.2	±	0.8		
	<i>A. brasilense</i> Az39	75.0	±	2.9	a	2.9	±	0.4	ab	4.2	±	0.6	bc	14.6	±	0.9	a	4.4	±	0.7		
Source		fd	F																			
Culture system (S)		1	91.1		**		1.5			16.7			**		0.0			0.5				
Bacterization (B)		3	65.3		**		5.6			**		4.9			**		2.9		*		1.7	
S x B		3	14.4		**		0.5			2.2			1.4			0.2						

Comparison of means by Tukey's test. Different letters indicate significant differences between treatments ($p \leq 0.05$). * indicates $p \leq 0.05$. ** indicates $p \leq 0.01$.

Table 3. *In vitro* and substrate rooting of *J. mimosifolia*. Effect of bacterization after three days of induction in ½ MSG supplemented with 30 µM IBA at 30 days of culture.

Culture system	Bacterization	Rooting (%)				Root number				Main root length (mm)				Shoot length (mm)				Leaf number					
Substrate	Control	13.3	±	1.9	c	4.5	±	0.3	a	12.5	±	1.0	ab	15.0	±	0.8	a	5.3	±	0.6			
	<i>Methylobacterium</i> sp. L10	4.4	±	2.8	c	3.0	±	0.1	ab	9.5	±	2.7	ab	15.0	±	0.8	a	4.0	±	0.2			
	<i>Stenotrophomonas</i> sp. L20	48.9	±	6.7	b	5.7	±	0.6	a	20.6	±	1.1	a	25.3	±	2.0	a	7.0	±	0.9			
	<i>A. brasilense</i> Az39	15.6	±	1.9	c	2.7	±	0.9	ab	9.0	±	0.8	ab	20.0	±	1.0	a	5.0	±	0.3			
<i>In vitro</i>	Control	45.0	±	8.7	b	3.7	±	0.3	ab	4.7	±	0.4	b	18.6	±	1.7	a	5.9	±	0.7			
	<i>Methylobacterium</i> sp. L10	6.7	±	2.1	c	1.4	±	1.4	b	16	±	1.6	c	20.0	±	10.0	a	4.0	±	2.0			
	<i>Stenotrophomonas</i> sp. L20	80.0	±	5.8	a	4.5	±	0.4	a	6.6	±	0.4	ab	21.5	±	2.6	a	6.2	±	0.8			
	<i>A. brasilense</i> Az39	75.0	±	2.9	a	2.9	±	0.4	ab	4.2	±	0.6	bc	14.6	±	0.9	a	4.4	±	0.7			
Source		fd																					
		F																					
Culture system (S)		1	91.1	**		1.5				16.7				**		0.3				0.5			
Bacterization (B)		3	65.3	**		5.6				**		4.9		**		2.9		*		1.7			
S x B		3	14.4	**		0.5				2.2				1.4				0.2					

Comparison of means by Tukey's test. Different letters indicate significant differences between treatments ($p \leq 0.05$), * indicates $p \leq 0.05$ ** indicates $p \leq 0.01$.

Significantly higher rooting percentages ($p \leq 0.05$) were observed in *in vitro* shoots inoculated with *A. brasilense* Az39 and *Stenotrophomonas* sp. L20 with respect to the other treatments (Table 3). *Stenotrophomonas* sp. L20 inoculation increased rooting percentage compared to in vitro or substrate non-inoculated plants (77% and 500%, respectively). In contrast, plant inoculation with *Methylobacterium* sp. L10 decreased rooting percentages regardless of culture system (Table 3). Inoculation with *Stenotrophomonas* sp. L20 in both culture systems also produced higher ($p \leq 0.05$) root number with respect to the in vitro and *Methylobacterium* sp. L10 treatment. Plants inoculation with *Stenotrophomonas* sp. L20 in substrate exhibited the highest number of roots, although differences from non-inoculated plants were not significant (Table 3). *Stenotrophomonas* sp. L20 inoculation increased significantly ($p \leq 0.05$) main root length in substrate relative to all *in vitro* treatments. There were not significant differences in aerial biometric parameters between treatments although plants inoculated with *Stenotrophomonas* sp. L20 in substrate exhibited the longest shoot length (25.33 mm) and the highest leaf number (7 leaves) (Table 3). When analyzing the biometric parameters index (BPI), a significant increase was observed in the plants inoculated with *Stenotrophomonas* sp. L20 compared to the rest of the treatments. The highest BPI value was observed in *Stenotrophomonas* sp. L20 inoculated plants. A higher BPI value indicates a better global state of the plants (analyzing all the parameters measured simultaneously) compared to the rest of the treatments (Figure 3).

The rooted plants from in vitro and substrate rooting experiments advanced to acclimatization stage. Acclimatized plants exhibited green and expanded leaves (Figure 1). Acclimatization percentages were significantly affected by the culture system, bacterization and their interaction. *Stenotrophomonas* sp. L20 and substrate rooted plants reached the highest acclimatization percentage (95%). Among *in vitro* rooted plants, *Stenotrophomonas* sp. L20 inoculation also was the treatment with the highest acclimatization percentage (46.60%) although differences were not significant with respect to non-inoculated plants (Figure 4).

Discussion

During initial establishment, it is essential to eliminate pathogens that negatively affect plants development by reducing multiplication rate, inhibiting rooting or leading to plant death. Sodium hypochlorite solution is recommended to disinfect seeds of Bignoniaceae species *Handroanthus impetiginosus*, *Handroanthus ochraceus* and *Handroanthus lapacho* (Apóstolo et al. 2016; Larraburu et al. 2012). This methodology as a disinfection method for *J. mimosifolia* seeds had similar results to previous reports for those species. Inoculation of *J. mimosifolia* seeds with *Methylobacterium* sp. L10 and *Stenotrophomonas* sp. L20 was highly effective in pathogens biocontrol. Similar results were reported in *Beta vulgaris*, *Solanum tuberosum*, *Gossypium* sp., *Oryza sativa* and *Solanum lycopersicum* (Ghosh et al. 2020) as a consequence of *Stenotrophomonas* sp. inoculation. This bacteria genus is known to act as an antagonist in plant pathogenic fungi through the generation of broad spectrum antibiotics, siderophores, volatile organic compounds (VOC), secretion of hydrolytic enzymes such as proteases, chitinases, glucanases, lipases that play an important role in the destruction of pathogenic cells and the activation of plant defense mechanisms (Singh & Jha, 2017; Ghosh et al. 2020). Also *Methylobacterium* sp. acts as a biocontrol agent in *Citrus* spp. (Lacava et al. 2004) and *Arachis hypogaea* (Madhaiyan et al. 2006). Increases of enzyme activities and induced systemic resistance (ISR) activation are proposed as possible mechanisms of action of methylotrophic bacteria (Madhaiyan et al. 2006).

Methylobacterium sp. L10 increased over 120% shoot number compared to the control and more than 102% compared to the other strains used in our studies. Multiplication rate was also increased as a consequence of *Methylobacterium* sp. L10 inoculation (94%, 45% and 165% relative to non-inoculated plants, *Stenotrophomonas* sp. L20 and *A. brasilense* Az39 treatments, respectively). These findings could be explained by the fact that *Methylobacterium* spp. are

capable of using compounds of mono carbons and polycarbonates secreted by symbiotic plants to synthesize high concentrations of cytokinins of the zeatin type (Senthilkumar & Krishnamoorthy, 2017).

At the rooting stage, development of adventitious roots is stimulated in order to regenerate complete plants. This procedure can be performed both under *in vitro* and *ex vitro* conditions (Larraburu et al. 2016). Usually, elevated levels of auxin are required to promote *in vitro* rooting. Since continuous exposure to auxins generally induces basal callus formation with disruption of the stem-root vascular connection and can also repress the later stages of rhizogenesis (Naija et al. 2008), we applied IBA pulses and subsequent transplantation to hormone-free medium to stimulate root expression in *J. mimosifolia* shoots, following the protocol developed for *H. impetiginosus* (Larraburu & Llorente, 2015). Non-induced shoots did not root as observed in *Morus indica* (Gogoi et al. 2017), *Bauhinia racemosa* (Sharma et al. 2017) and *Eucalyptus* spp. (Vilasboa et al. 2018). In contrast, *H. impetiginosus* non-induced shoots reach a 25% rooting percentage (Larraburu & Llorente, 2015). In addition, *A. brasilense* Cd inoculation improves this parameter (40%). Likewise, *in vitro* biofertilization of *H. ochraceus* with *A. brasilense* Az39 produced high rooting percentages (65%) in shoots grown in auxin-free medium (Llorente et al. 2016). However, bacterial inoculation did not affect rooting in non-induced shoots in our work. This result demonstrates that requirements in rhizogenesis are variable, even in the same family and depend on the genotype (Llorente et al. 2016).

Auxin-induced *J. mimosifolia* micro-cuttings inoculated with *Azospirillum brasilense* Az39 reached 75% rooting at 30 days of culture. This result coincides with that described by Larraburu & Llorente (2015) for *H. impetiginosus* where *A. brasilense* Az39 in combination with 30 μ M IBA generates 60% rooting. Similar effects were observed in the *in vitro* rooting of jojoba and photinia (Larraburu et al. 2007; Larraburu et al. 2016). Differences between studies can be explained by variations in clone genotypes and by the differential production of growth regulators among the bacterial strains of *A. brasilense* (Perrig et al. 2007).

Strains belonging to *Methylobacterium* and *Stenotrophomonas* genera are capable of producing auxins, particularly indole-3-acetic acid (IAA), which could modify hormonal balance in plants and induce root development (Madhaiyan et al. 2006; Berg et al. 2010; Ghosh et al. 2020). *Methylobacterium* strains increase root number and length which led to a better absorption of nutrients in jojoba (*Simmondsia chinensis*) and pine *in vitro* plants (*Pinus sylvestris* L.) (Perez-Rosales et al. 2018; Pohjanen et al. 2014). Particularly, L10 strain increases significantly rooting percentages in non-induced shoots of *H. impetiginosus* at *in vitro* rooting and enhances BPI value in auxin-induced shoots (Yarte et al. 2022b). Similar results are reported for peanut (*Arachis hypogaea*) plants as a consequence of *Stenotrophomonas* strains inoculation (Alexander et al. 2019). In this sense, in our study, *J. mimosifolia* plants inoculated with *Stenotrophomonas* sp. L20 increased root number by 17%-21% and root length by 29%- 40% compared to controls in *in vitro* and substrate culture, respectively. Also, *Stenotrophomonas* sp. L20 treatments reached the highest rooting values in each culture system. In addition, a significant increase was observed in BPI which could be translated into plants with a better global state and better prepared for the acclimatization stage (Larraburu & Llorente, 2015). In contrast, *Methylobacterium* sp. L10 inoculation produced the lowest rooting percentages in both culture systems and did not improve any biometric parameter. The observed differences may be due to plant growth promoting characteristics of the strains used in our work. In previous studies carried out in our laboratory, it was determined that *Methylobacterium* sp. L10 strains produce an IAA concentration of 4.37 μ g mL⁻¹ (Yarte et al. 2022a) whereas *Stenotrophomonas* sp. L20 secretes a 10-fold higher concentration of IAA (40.63 μ g mL⁻¹) (data not published). This ability would explain the best overall performance observed for L20 strain at the rhizogenesis stage.

Acclimatization is one of the most difficult stages of an *in vitro* propagation system. Plants with thin cuticles, reduced metabolism, abnormal functioning of the stomata and poorly developed roots must be transferred to *ex vitro* conditions that are physiologically stressful. These morphological, anatomical and physiological alterations, occurring as a result of the *in vitro* environment, make plants unable for controlling the loss of water during the first weeks of acclimatization which causes them a sudden increase in transpiration and a reduction in photosynthetic activity (Indacochea-Ganchozo et al. 2017). It is necessary to control environmental factors and simulate environmental conditions *in vitro* until plants achieve adequate development of their stomata and cuticle, thus allowing, gradually adapting to new environmental conditions (Larraburu & Llorente, 2015). In general, micropropagated woody species exhibited low survival rates. For example, only 50% of total *H. impetiginosus* micropropagated plants survive acclimatization (Larraburu & Llorente, 2015). Variable results are reported for *Eucalyptus urophylla* (85%), *Eucalyptus grandis* (75%) (Espinoza et al. 2005) and *Swietenia macrophylla* (27%) (Azofeifa, et al. 2009). Species such as *Tabebuia chrysantha* and *Tabebuia billbergii* did not survive in the acclimatization phase (Indacochea-Ganchozo et al. 2017). Maruyama et al. (1993) reported acclimatization in *J. mimosifolia* micropropagated plants but did not indicate the survival percentage or other parameters of the acclimatized plants. In our work, the survival percentage of non-inoculated *in vitro* plants was 33.33% and around 40% in those derived from *ex vitro* rooting. Plant growth-promoting bacteria could facilitate the acclimatization process in woody species (Soumare et al. 2021). In our work, inoculated *J. mimosifolia* plants from substrate rooting increased the survival percentage with respect to controls by 87.5%, 137.5% and 115% (*Methylobacterium* sp. L10, *Stenotrophomonas* sp. L20 and *A. brasilense* Az39, respectively). *In vitro* rooted and L20 inoculated plants increased by 40% survival percentage compared to the control.

Conclusions

J. mimosifolia micropropagation was implemented using plant growth promoting bacteria. *Methylobacterium* sp. L10 and *Stenotrophomonas* sp. L20 decreased fungal contamination at initial establishment by seeds. Biofertilization also improved shoot multiplication and development. *Methylobacterium* sp. L10 increased shoot number and multiplication rate with respect to non-inoculated shoots. *Stenotrophomonas* sp. L20 inoculation increased rooting percentage, root number and length in both *in vitro* and *ex vitro* rooting. Consequently, increases in survival percentages of the inoculated plants were observed during acclimatization. The strains used in our studies could act as biological control agents and be used for biofertilizers development.

Declarations

FUNDING

DATA AVAILABILITY

Data available on request.

COMPETING INTERESTS

The authors have no relevant financial interests to disclose.

AUTHOR CONTRIBUTIONS

All authors contributed to the experimental design. Material preparation, data collection and analysis were performed by Mauro Yarte and Ariel Salotti. Ezequiel Larraburu, Mauro Yarte and Ariel Salotti analysed data.. The first draft of the manuscript was written by Ezequiel Larraburu and Mauro Yarte. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Afzal, I., Shinwari, Z. K., Sikandar, S., & Shahzad, S. 2019. Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. *Microbiological research*, 221, 36-49. <https://doi.org/10.1016/j.micres.2019.02.001>
2. Alexander, A., Singh, V. K., Mishra, A., & Jha, B. 2019. Plant growth promoting rhizobacterium *Stenotrophomonas maltophilia* BJ01 augments endurance against N2 starvation by modulating physiology and biochemical activities of *Arachis hypogaea*. *Plos one*, 14(9), e0222405. <https://doi.org/10.1371/journal.pone.0222405>
3. Apóstolo, N. M., Larraburu, E. E., Gil, M. N., Zapater, M. A., & Llorente, B. E. 2016. In vitro and ex vitro germination of three *Handroanthus* species (Bignoniaceae). *Bonplandia*, 25(1), 5-16.
4. Azofeifa, J. B., Rojas-Vargas, A., & Hine-Gómez, A. 2009. Optimización del proceso de enraizamiento y aclimatación de vitroplantas de *Swietenia macrophylla* King (Orden: Meliaceae). *Revista Tecnología en Marcha*, 22(3), ág-34.
5. Berg, G., Egamberdieva, D., Lugtenberg, B., & Hagemann, M. 2010. Symbiotic plant-microbe interactions: stress protection, plant growth promotion, and biocontrol by *Stenotrophomonas*. In *Symbioses and Stress* (pp. 445-460). Springer, Dordrecht. https://doi.org/10.1007/978-90-481-9449-0_22
6. Espinosa, M. G., Alcalá, V. M. C., Rivero, H. S. A., De La, J. L. R., & Ruiz, R. M. 2005. Aclimatación de plantas obtenidas in vitro *Eucalyptus urophylla* ST Blake *Eucalyptus grandis* Hill Ex Maiden. *Ra Ximhai: revista científica de sociedad, cultura y desarrollo sostenible*, 1(3), 591-597.
7. Fauerharmel, M., Bisognin, D. A., Lencina, K. H., da Silva Tonetto, T., Meltzer, J. M., & Maggioni, J. H. 2020. Production of jacaranda plantlets by minicutting. *Semina: Ciências Agrárias*, 41(5supl1), 1951-1962. <https://doi.org/10.5433/1679-0359.2020v41n5Supl1p1951>
8. Gamborg, O. L., Miller, R., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental cell research*, 50(1), 151-158.
9. Ghosh, R., Chatterjee, S., & Mandal, N. C. 2020. *Stenotrophomonas*. In *Beneficial Microbes in Agro-Ecology* (pp. 427-442). Academic Press. <https://doi.org/10.1016/B978-0-12-823414-3.00020-4>
10. Glick, B. R. 2020. Introduction to plant growth-promoting bacteria. In *Beneficial plant-bacterial interactions* (pp. 1-37). Springer, Cham. https://doi.org/10.1007/978-3-030-44368-9_1
11. Gogoi, G., Borua, P. K., & Al-Khayri, J. M. 2017. Improved micropropagation and in vitro fruiting of *Morus indica* L.(K-2 cultivar). *Journal of Genetic Engineering and Biotechnology*, 15(1), 249-256. <https://doi.org/10.1016/j.jgeb.2017.02.005>
12. Gonzalez, A. J., Larraburu, E. E., & Llorente, B. E. 2021. *Azospirillum brasilense* mitigates anatomical alterations produced by salt stress in jojoba in vitro plants. *Vegetos*, 1-13. <https://doi.org/10.1007/s42535-021-00275-1>
13. Indacochea-Ganchoso, B., Parrales-Villacreses, J., Castro-Piguave, C., Vera-Tumbaco, M., & Gabriel-Ortega, J. 2017. Aclimatación in vitro de especies forestales nativas del Sur de Manabí en peligro de extinción. *Journal of the Selva Andina Research Society*, 8(2), 124-134.
14. Irum, N., & Asghari, B. 2012. Assessment of phytohormones producing capacity of *Stenotrophomonas maltophilia* SSA and its interaction with *Zea mays* L. *Pakistan Journal of Botany*, 44(1), 465-469.
15. Kanani, P., Arpan, M. & Kumar, A. 2020. Biotización de endofitos in micropropagation: a helpful enemy. *Microbial Endophytes*, 357-379. <https://doi.org/10.1016/B978-0-12-818734-0.00015-2>
16. Lacava, P. T., Araújo, W. L., Marcon, J., Maccheroni Jr, W., & Azevedo, J. L. 2004. Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus-variegated chlorosis. *Letters in applied microbiology*, 39(1), 55-59. <https://doi.org/10.1111/j.1472-765X.2004.01543.x>
17. Larraburu, E. E., & Llorente, B. E. 2015. *Azospirillum brasilense* enhances in vitro rhizogenesis of *Handroanthus impetiginosus* (pink lapacho) in different culture media. *Annals of forest science*, 72(2), 219-229. <https://doi.org/10.1007/s13595-014-0418-9>
18. Larraburu, E. E., Apóstolo, N. M., & Llorente, B. E. 2012. In vitro propagation of pink lapacho: response surface methodology and factorial analysis for optimisation of medium components. *International Journal of Forestry Research*, 2012. <https://doi.org/10.1155/2012/318258>
19. Larraburu, E. E., Bususovich, A. C., & Llorente, B. E. 2016. *Azospirillum brasilense* improves in vitro and ex vitro rooting-acclimatization of jojoba. *Scientia Horticulturae*, 209, 139-147. <https://doi.org/10.1016/j.scienta.2016.06.016>

20. Llorente, B. E., Alasia, M. A., & Larraburu, E. E. 2016. Biofertilization with *Azospirillum brasilense* improves in vitro culture of *Handroanthus ochraceus*, a forestry, ornamental and medicinal plant. *New biotechnology*, 33(1), 32-40. <https://doi.org/10.1016/j.nbt.2015.07.006>
21. Lloyd, G., & McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Combined Proceedings-International Plant Propagators' Society (USA)*.
22. Madhaiyan, M., Reddy, B. S., Anandham, R., Senthilkumar, M., Poonguzhali, S., Sundaram, S. P., & Sa, T. (2006). Plant growth-promoting *Methylobacterium* induces defense responses in groundnut (*Arachis hypogaea* L.) compared with rot pathogens. *Current microbiology*, 53(4), 270-276. <https://doi.org/10.1007/s00284-005-0452-9>
23. Maruyama, E., Ishii, K., Saito, A., & Ohba, K. (1993). Micropropagation of jacaranda (*Jacaranda mimosaeifolia* D. Don) by shoot-tip culture. *Journal of the Japanese Forestry Society*, 75(4), 346-349. https://doi.org/10.11519/jjfs1953.75.4_346
24. Morgado González, A., Espinosa Victoria, D., & Gómez Merino, F. C. (2015). Efficiency of plant growth promoting rhizobacteria (PGPR) in sugarcane. *Terra Latinoamericana*, 33(4), 321-330.
25. Mostafa, N. M., Eldahshan, O. A., & Singab, A. N. B. (2014). The genus *Jacaranda* (Bignoniaceae): an updated review. *Pharmacognosy Communications*, 4(3), 31-39.
26. Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, 15(3), 473-497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
27. Naija, S., Elloumi, N., Jbir, N., Ammar, S., & Kevers, C. (2008). Anatomical and biochemical changes during adventitious rooting of apple rootstocks MM 106 cultured in vitro. *Comptes rendus biologies*, 331(7), 518-525. <https://doi.org/10.1016/j.crv.2008.04.002>
28. Nevita, T., Sharma, G. D., & Pandey, P. 2018. Composting of rice-residues using lignocellulolytic plant-probiotic *Stenotrophomonas maltophilia*, and its evaluation for growth enhancement of *Oryza sativa* L. *Environmental Sustainability*, 1(2), 185-196.
29. <https://doi.org/10.1007/s42398-018-0017-z>
30. Okon, Y., Albrecht, S. L., & Burris, R. H. 1977. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Applied and Environmental Microbiology*, 33(1), 85-88. <https://doi.org/10.1128/aem.33.1.85-88.1977>
31. Perez-Rosales, E., Alcaraz-Meléndez, L., Puente, M. E., Vázquez-Juárez, R., Zenteno-Savín, T., & Morales-Bojórquez, E. 2018. Endophytic bacteria isolated from wild jojoba [*Simmondsia chinensis* L.(Schneider)] roots improve in vitro propagation. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 135(3), 515-522. <https://doi.org/10.1007/s11240-018-1483-9>
32. Perrig, D., Boiero, M. L., Masciarelli, O. A., Penna, C., Ruiz, O. A., Cassán, F. D., & Luna, M. V. 2007. Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. *Applied microbiology and biotechnology*, 75(5), 1143-1150. <https://doi.org/10.1007/s00253-007-0909-9>
33. Pohjanen, J., Koskimäki, J. J., Sutela, S., Ardanov, P., Suorsa, M., Niemi, K., ... & Pirttilä, A. M. 2014. Interaction with ectomycorrhizal fungi and endophytic *Methylobacterium* affects nutrient uptake and growth of pine seedlings in vitro. *Tree Physiology*, 34(9), 993-1005. <https://doi.org/10.1093/treephys/tpu062>
34. Rodríguez Cáceres, E. 1982. Improved medium for isolation of *Azospirillum spp.* *Applied and Environmental Microbiology*, 44, 990-991.
35. Santos, M. P., Martínez, S. J., Yarte, M. E., Carletti, S. M., & Larraburu, E. E. 2022. Effect of *Azospirillum brasilense* on the in vitro germination of *Eustoma grandiflorum* (Raf.) Schinn.(Gentianaceae). *Scientia Horticulturae*, 299, 111041. <https://doi.org/10.1016/j.scienta.2022.111041>
36. Senthilkumar, M., & Krishnamoorthy, R. 2017. Isolation and characterization of tomato leaf phyllosphere *Methylobacterium* and their effect on plant growth. *Int J Curr Microbiol App Sci*, 6(11), 2121-2136. <https://doi.org/10.20546/ijcmas>
37. Sharma, U., Kataria, V., & Shekhawat, N. S. 2017. In vitro propagation, ex vitro rooting and leaf micromorphology of *Bauhinia racemosa* Lam.: a leguminous tree with medicinal values. *Physiology and Molecular Biology of Plants*, 23(4), 969-977. <https://doi.org/10.1007/s12298-017-0459-2>
38. Singh, R. P., & Jha, P. N. 2017. The PGPR *Stenotrophomonas maltophilia* SBP-9 augments resistance against biotic and abiotic stress in wheat plants. *Frontiers in microbiology*, 8, 1945. <https://doi.org/10.3389/fmicb.2017.01945>
39. Soumare, A., Diédhiou, A. G., Arora, N. K., Al-Ani, L. K. T., Ngom, M., Fall, S., ... & Sy, M. O. 2021. Potential Role and Utilization of Plant Growth Promoting Microbes in Plant Tissue Culture. *Frontiers in Microbiology*, 12. <https://doi.org/10.3389/fmicb.2021.649878>
40. Ulrich, K., Kube, M., Becker, R., Schneck, V., & Ulrich, A. 2021. Genomic analysis of the endophytic *Stenotrophomonas* strain 169 reveals features related to plant-growth promotion and stress tolerance. *Frontiers in microbiology*, 12, 1542. <https://doi.org/10.3389/fmicb.2021.687463>
41. Vilasboa, J., Da Costa, C. T., & Fett-Neto, A. G. 2019. Rooting of eucalypt cuttings as a problem-solving oriented model in plant biology. *Progress in biophysics and molecular biology*, 146, 85-97. <https://doi.org/10.1016/j.pbiomolbio.2018.12.007>
42. Xie, C., Zhang, G., Jim, C., Liu, X., Zhang, P., Qiu, J., & Liu, D. 2021. Bioclimatic Suitability of Actual and Potential Cultivation Areas for *Jacaranda mimosifolia* in Chinese Cities. *Forests*, 12(7), 951. <https://doi.org/10.3390/f12070951>
43. Yarte, M. E., Gismondi, M. I., Llorente, B. E., & Larraburu, E. E. 2022a. Isolation of endophytic bacteria from the medicinal, forestal and ornamental tree *Handroanthus impetiginosus*. *Environmental Technology*, 1-11. <https://doi.org/10.1080/09593330.2020.1818833>

Figures

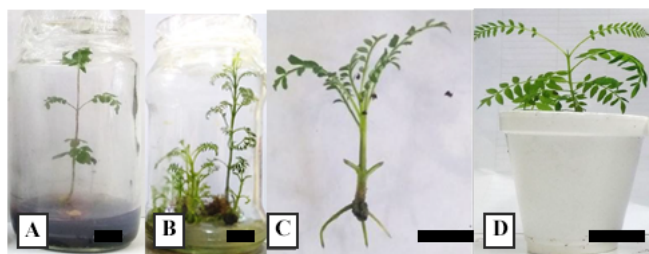


Figure 1
Micropropagation of *Jacaranda mimosifolia*. A. In vitro germination (30 days of culture). B. Shoot multiplication (60 days of culture). C. In vitro rooted plant (30 days of culture). D. Acclimatization stage (60 days). Scale bar: 2 cm

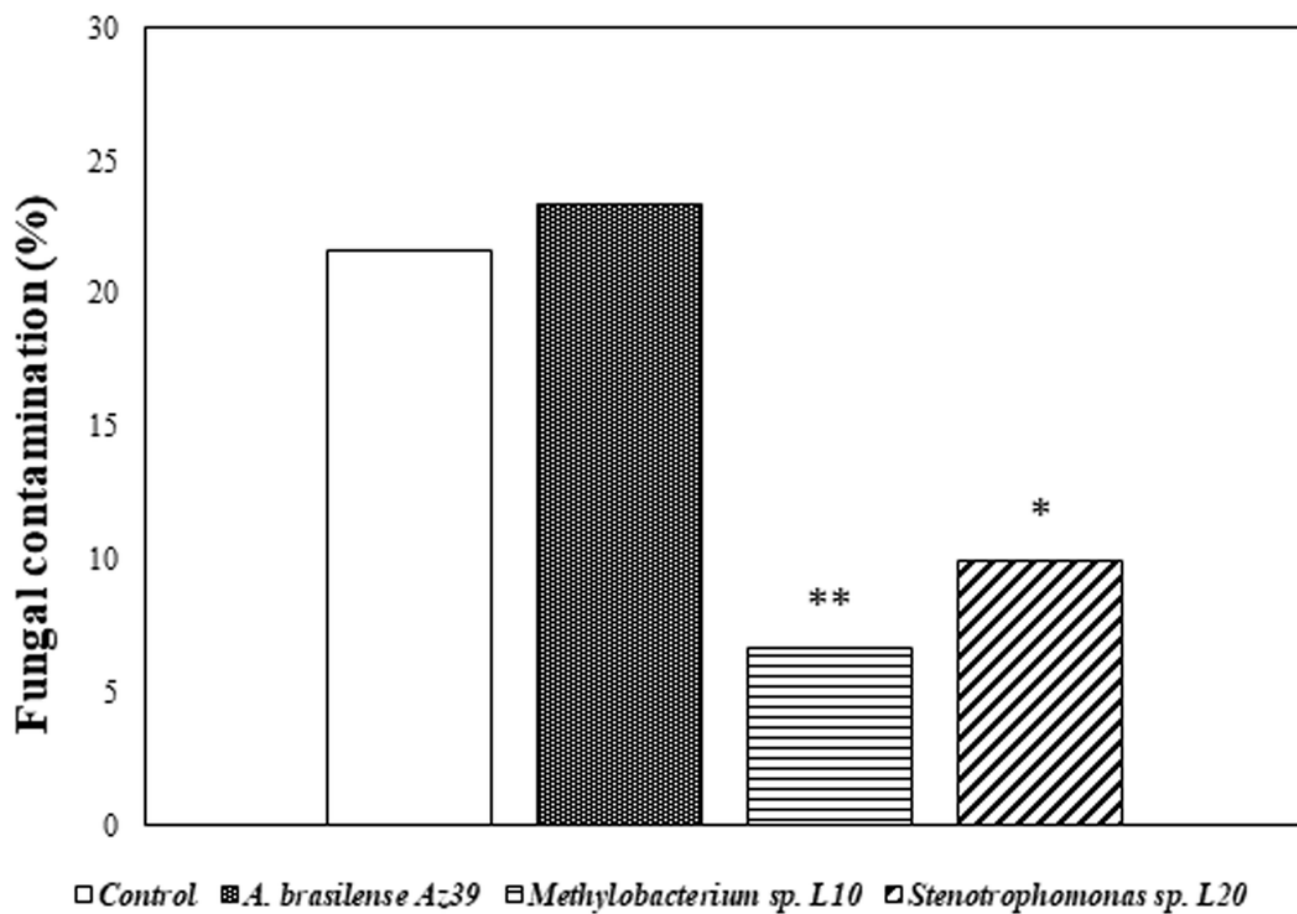


Figure 2
Fungal contamination percentages of *Jacaranda mimosifolia* after 30 days post-inoculation. * indicates significant differences ($p < 0.05$) and ** indicates significant differences ($p < 0.01$) relative to non-inoculated seeds.

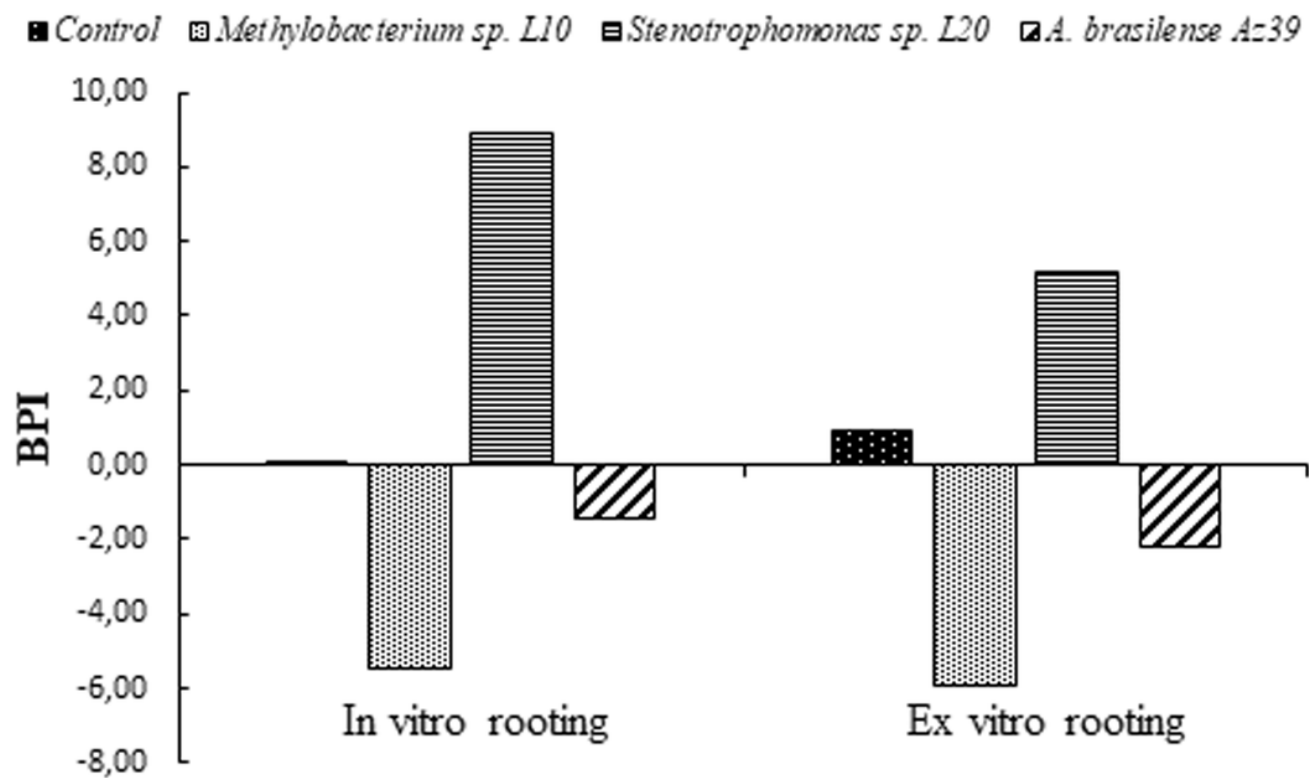


Figure 3

Biometric parameters index (BPI) of *Jacaranda mimosifolia* plants induced with 30 μ M IBA and inoculated or not with PGPB.

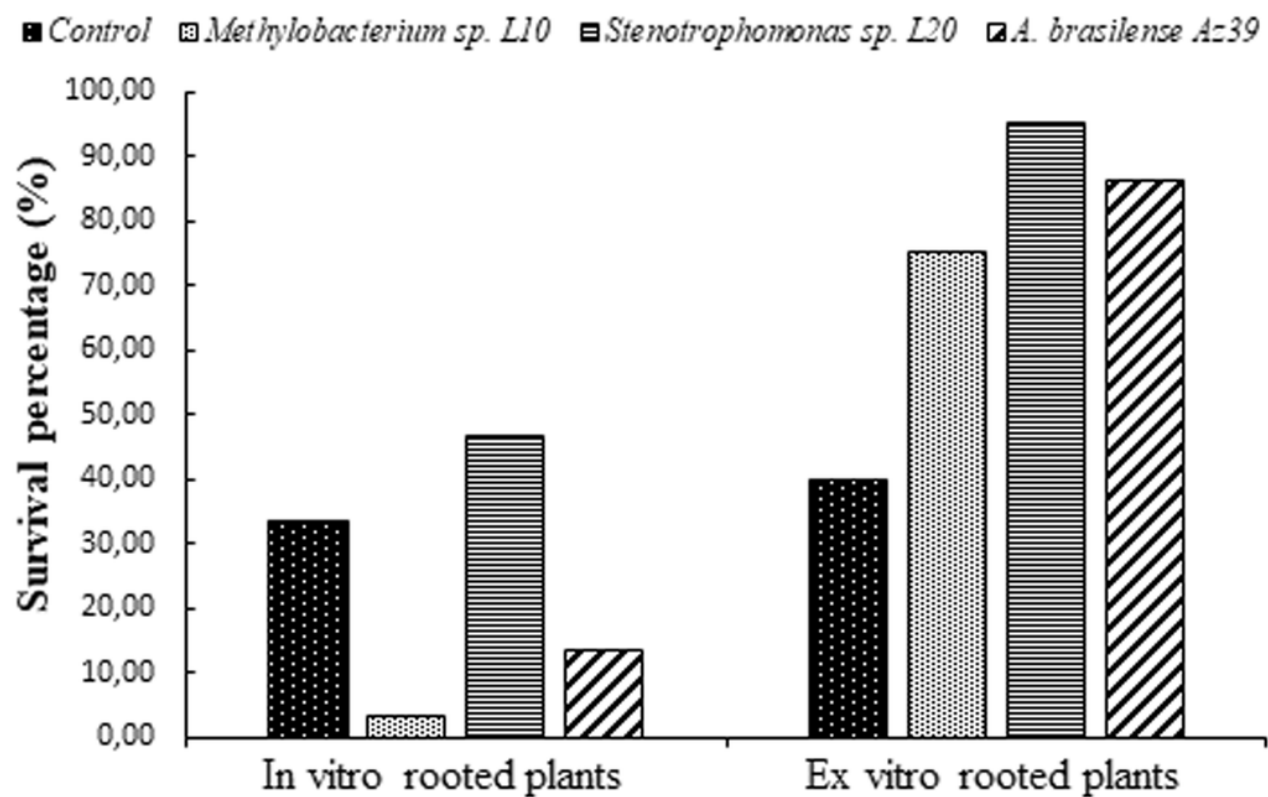


Figure 4

Survival percentages of *Jacaranda mimosifolia* plants after acclimatization for 60 days.