



Influence of fluorescent *Pseudomonas* on the growth of *Mimosa scabrella* seedlings

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Abstract - Bracantiga (*Mimosa scabrella* Benth) is a native leguminous specie from the south of Brazil, with great potential for commercial use. It can be used to recover degraded areas due to their fast growth, high adaptability and root nodule formation capacity. One alternative to reduce fertilization costs of seedlings production, are the plant growth promoting rhizobacteria. The fluorescent group of *Pseudomonas* is common at several plant rhizosphere. The objective of this work was to assess the growth stimulus of fluorescent *Pseudomonas* isolates at bracatinga seedlings. The seeds were inoculated with three bacterial isolates (CBSAL14, CBSAL18 and CBSAL05), genetically identified as *Pseudomonas* sp. They were previously grown in 50 mL of Luria Bertani medium for 48 h and then the seeds were submerged for 30 min in the suspensions. Subsequently, they were sown in tubes containing sterilized commercial substrate and kept in greenhouse for five months. From 61 days after sowing, seedling height and stem diameter were measured monthly. The CBSAL14 and CBSAL18 isolates promoted increase in growth of seedlings height of inoculated individuals compared to control, suggesting their potential use as plant growth promoters.

Influência de *Pseudomonas* fluorescentes no desenvolvimento de mudas de *Mimosa scabrella*



Resumo - A bracantiga (*Mimosa scabrella* Benth) é uma leguminosa nativa do sul do Brasil, com grande potencial para uso comercial. Pode ser usada para a recuperação de áreas degradadas, devido ao seu rápido crescimento, alta adaptabilidade e capacidade de formar nódulos. Uma alternativa para reduzir custos de fertilização na produção de mudas, é o uso de rizobactérias promotoras de crescimento de plantas. O grupo fluorescente de *Pseudomonas* é comum nas rizosferas de diversas espécies de plantas. Neste trabalho, o objetivo foi avaliar o estímulo de isolados de *Pseudomonas* fluorescentes nas plântulas de bracatinga. As sementes foram inoculadas com três isolados bacterianos (CBSAL14, CBSAL18 e CBSAL05), geneticamente identificados como *Pseudomonas* sp. Foram, previamente, cultivados em 50 mL de meio Luria Bertani por 48 h, submergindo as sementes, na sequência, por 30 min nas suspensões. Posteriormente, foram semeadas em tubetes contendo substrato comercial esterilizado e mantidas em casa-de-vegetação por cinco meses. A partir dos 61 dias após a semeadura, a altura da muda e o diâmetro do coleto foram mensurados mensalmente. Os isolados CBSAL14 e CBSAL18 promoveram aumento do incremento em altura das plântulas de indivíduos inoculados em relação ao controle, sugerindo seu potencial de uso como promotores do crescimento das plantas.

Introduction

Mimosa scabrella Benth known as bracinga is a native tree of southern Brazil, belonging to the Fabaceae family. It is a wood species, perennial, and has low demands on physical and chemical soil features (Steenbock et al., 2011; Menegatti et al., 2019). The growth starts fast, enabling the tree to reach 20 and 30 cm at 1.30 m above ground level (DBH) and an average height of 15 m. However, when under adverse soil conditions and topography, its height can be jeopardized, reaching only approximately 3 m (Mazuchowski et al., 2014).

According to Guareschi et al. (2015), forestry of native tree species are not properly handled for commercial purposes. However, they are demanded for environmental reforestation projects and recovery of degraded areas or landscape. For these reasons, these authors mention that it is important to know and control their seeds and seedlings quality.

Mimosa scabrella is a perennial plant and a facilitating species. It contributes in recovering degraded areas, minimizing in a few months the effects of weathering, avoiding the leaching and erosion processes and contributing to the incorporation of high levels of nitrogen and phosphorus (Saturno & Andrade, 2015; Silva et al., 2019).

In degraded areas projects, it is necessary a very high number of plants and it is important to reduce costs. In addition, chemical fertilizers can cause environmental impacts (Santos et al., 2018). Therefore, it is necessary to find alternatives that enable the seedlings production. The use of plant growth promoting microorganisms is a promising and low-impact option to reduce and to optimize chemical fertilizer use, ensuring high yield and lower cost-benefit ratio (Spolaor et al., 2016; Santos et al., 2018).

Several authors have described that plant growth promoting rhizobacteria (PGPR) represents a variety of soil bacteria that may associate to plants, stimulating their growth. They are biofertilization, bioremediation, biopesticides and phytostimulators (Pereira et al., 2008; Marques & Uesugi, 2013; Graças et al., 2015; Souza et al., 2015; Cardoso & Andreote, 2016; Santos et al., 2018; Qessaoui et al., 2019).

Among several genera described, *Pseudomonas* and *Bacillus* are the most studied (Das et al., 2013; Santos et al., 2018; Ferreira et al., 2019). The fluorescent group

has the highest number of species within the genus *Pseudomonas* (Botelho & Mendonça-Hagler, 2006; Marques & Uesugi, 2013; Sonawane et al., 2014). The species stand out for their nutritional versatility and ability to grow at diverse environments and substrates. In addition, they have the ability to improve growth, plant health and biocontrol, due to their production of secondary metabolites (Méliani et al., 2017; Turatto et al., 2018).

Several parameters may be evaluated to demonstrate the influence on seedlings quality, estimating PGPR contribution to the early development of forest species. Among them, the most used are plant height and stem diameter. These parameters are easily measured and they can provide reliable data on seedlings strength and functionality (Nicoletti et al., 2015; Santos et al., 2018). The stem diameter is the most analyzed feature to indicate the seedlings survival capacity in the field (D'Avila et al., 2011). However, they should not be considered individually when determining seedlings quality, due to their close relatedness. The evaluation of both parameters can help to determine higher quality seedlings.

In this work, the objective was to evaluate the influence of fluorescent *Pseudomonas* on the development of *M. scabrella* seedlings.

Material and methods

The present study was carried out at Federal University of Santa Catarina in Curitiba, Santa Catarina State. We used four rhizobacteria isolates from garlic rhizosphere (Botelho et al., 2019), from the microorganisms collection of the laboratory of plant growth.

For isolates identification, DNA extraction, Polymerase Chain Reaction (PCR) amplification and PCR fragments sequencing were performed according to the following methods: isolates were grown in Luria Bertani medium (LB) for 24 h at 28 °C under stirring at 120 rpm. Then, 2 mL of cell suspension were centrifuged (12,000 × g) and the total genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega, USA), following the manufacturer's instructions. Amplification was performed at a volume of 50 µL, containing approximately 50 ng template DNA, 1x buffer, 1.75 mM MgCl₂, 0.25 mM each dNTP, 0.20 mM both, 27F primer and 1492R primer (sequence of primer

and reference) and Taq polymerase 1.5 U. 16S RNAr sequencing was carried out at Embrapa Agrobiologia (Botelho et al., 2019), using sense and antisense primers on the Applied Biosystems 3500 equipment. Contigs assembly was performed using the BioNumerics 7.0 program.

Mimosa scabrella seeds were collected from ten mother plants in the surrounding *campus* area in 2016. They were manually benefited by sifting, to discard immature, deteriorated or damaged seeds.

Before isolates inoculation, the seeds were immersed in water at 80 °C for 24 h (Brasil, 2013). Then, they were disinfested by immersion in 95° alcohol for 5 min and in sodium hypochlorite 3% for 2 min. They were washed six times with sterilized distilled water and dried in laminar flow.

The four rhizobacteria isolates (treatments – T) were CBSAL02 - T2, CBSAL14 - T3, CBSAL05 - T4 and CBSAL18 - T5. They were inoculated into flasks containing 50 mL of liquid LB medium, for 48 h at 27 °C, as well as the control, without inoculation (T1). Sixteen seeds in each bacterial suspension and the medium without inoculation (control) were dried for 30 min under laminar flow in sterile plates. Subsequently, tubes (volume = 290 cm³) containing previously sterilized commercial substrate of each treatment received three seeds. They were placed in greenhouse, at ± 25 °C and 2 min irrigation every hour.

Seedlings thinning was carried out when they reached 5 cm, leaving only one per tube. They stayed in the greenhouse for 5 months. The analyzed parameters were height (H) and stem diameter (CD). Height was measured by centimeter-graduated ruler and stem diameter by an electronic caliper. The first assessment was carried out at 61 days after sowing and following

monthly, along five months. The experimental design was completely randomized with 5 treatments and 19 replicates. The Analysis of variance (ANOVA) and Scott-Knott mean test were performed using the statistical package DEX/UFLA Sisvar 5.7 (Build 91).

Results

At King B medium, the isolates emitted fluorescence that are typical of the fluorescent group of *Pseudomonas*. According to 16S RNAr sequencing, the four isolates tested belong to the genus *Pseudomonas* (Table 1).

Table 1. Isolates identification by 16S RNAr gene sequencing for *Pseudomonas* sp. 99% similarity and E value = 0.0

Isolates	ID/NCBI
CBSAL02	KT321658.1
CBSAL05	LT629702.1
CBSAL14	LT629702.1
CBSAL18	KT321658.1

The seedlings height and stem diameter for each treatment over time is presented in Figure 1. Seedlings showed bigger heights when inoculated with CBSAL14 and CBSAL18 isolates (25.02 cm and 24.27 cm, respectively). Other treatments presented average height of 20 cm, at 150 days. CBSAL14 and CBSAL18 inoculated seedlings also showed higher increase in height (0.19 cm and 0.18 cm, respectively) over time, when compared to the control (0.16 cm) and the other treatments (0.15 cm).

It was possible to verify that the initial growth height of seedlings inoculated with CBSAL05 (7.1 cm), CBSAL14 (7.12 cm) and CBSAL18 (6.95 cm) isolates had better performance than the control (5.97 cm) and CBSAL02 isolate (5.97 cm).

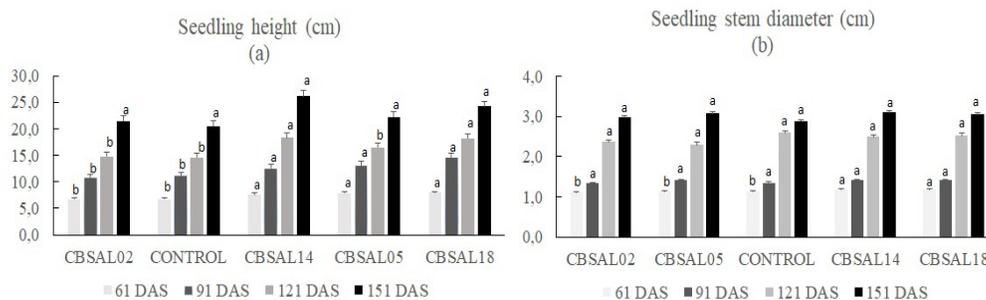


Figure 1. Height (a) and stem diameter (b) of *Mimosa scabrella* seedlings inoculated with fluorescent *Pseudomonas* sp. DAS - days after sowing. Treatments followed by the same letters did not differ statistically by Scott-Knott average test at 5%.

At 121 DAS, height increase was observed only at CBSAL14 and CBSAL18, although they showed no significant difference among treatments and control at 151 DAS. The CBSAL02 isolate stimulus did not differ statistically from the control over time (Figure 1).

The final stem diameter showed no difference among treatments. However, the increase in the diameters of plants inoculated with CBSAL14, CBSAL18 and CBSAL05 isolates was significant over time.

Discussion

Previous results indicated that the isolates used in this research promoted plant development. CBSAL02, CBSAL05, CBSAL14 and CBSAL18 stimulated the size of the fourth leaf of garlic, that is related to its bulbification and production (Botelho et al., 2019).

These isolates demonstrated ability to solubilize calcium phosphate and produce IAA (Indole Acetic Acid) *in vitro* (Botelho et al., 2019), suggesting their potential to plant growth induction. It is important to emphasize that fluorescent *Pseudomonas* are common in the rhizosphere of different plant species (Ferreira et al., 2009; Méliani et al., 2017; Lopes et al., 2018), indicating their ability to adapt and metabolize different plant exudates.

For seedling production, techniques that aim at short-term production and high-quality plants to successfully establish themselves at the field are extremely important. They result in high percentage of survival and replanting reduction (Felker et al., 2015). It is common to perform the inoculation of beneficial microorganisms in plants in order to improve their development, as well as to increase tolerance to diseases and enable the establishment under stress conditions such as salinity and drought (Niu et al. 2018; Ferreira et al., 2019). Raasch et al. (2013) observed that microorganisms influenced *Eucalyptus* seedlings, decreasing, decreasing their period in the nursery and enhancing their establishment in the field.

It was possible to verify that the seedlings inoculated with CBSAL05, CBSAL14 and CBSAL18 isolates showed a better performance of initial height growth than the control and CBSAL02. These results are similar to those at Raasch et al. (2013), that observed increase in the height of inoculated *Eucalyptus* minicuttings. Brunetta et al. (2010), evaluating rhizobacterial isolates in *Pinus taeda*, observed that four isolates significantly induced growth of seedlings aerial part. This initial growth can

be important for faster seedling's development, reducing their nursery time.

According to Raasch et al. (2013), height should not be the only parameter to represent the quality of seedlings, especially when there is no balance between the shoot and the root system.

This parameter is influenced by environmental conditions, population density and nutritional status. When evaluated in the field, the stem diameter is considered a reliable feature, since it is easy to be measured and has high correlation to the seedling survival, thus helping to determine the quality of seedlings (Nicoletti et al., 2015). Rudek et al. (2013) and Nicoletti et al. (2015) studying *Eucalyptus* sp., observed that the combination of leaf area and stem diameter was a reliable parameter when determining seedling quality.

According to Araujo et al. (2014), within the same species, the larger diameter provides higher plant survival, since it indicates its ability to produce more roots. The authors also mentioned that the stem diameter and height are essential for evaluating survival potential and post-planting seedlings of forest species growth.

In general, the isolates CBSAL14 and CBSAL18 promoted the highest increase in plant height and diameter over the 150 day, differing from the control and others isolates. The CBSAL02 isolate showed good results when tested with (Botelho et al., 2019), suggesting different forms of interaction, plant growth stimulation and adjustment in rhizobacteria communities among different plant species.

Conclusions

Two *Pseudomonas* isolates from fluorescent group (CBSAL14 and CBSAL18) increased the height of *Mimosa scabrella* seedlings, indicating their potential to be used as plant growth inducers in nurseries.

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