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Original Article

# Plant growth-promoting bacteria enhance chlorophyll *a* fluorescence, biomass, and acclimatization rate of *Stryphnodendron adstringens* grown *in vitro*

## Bactérias promotoras de crescimento vegetal melhoram a fluorescência da clorofila *a*, biomassa e a taxa de aclimatização de *Stryphnodendron adstringens* cultivado *in vitro*

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**Abstract**

A significant knowledge gap exists regarding how beneficial microorganisms affect *in vitro* plant cultivation. Although aseptic conditions are essential, they exclude microorganisms that could enhance plant growth and acclimatization. This study aimed to evaluate the effects of inoculating *Bacillus methylotrophicus* and *Serratia marcescens* on the *in vitro* growth and development of *Stryphnodendron adstringens*, a tree with high medicinal value. We grew *S. adstringens* plants *in vitro* with four treatments: control, *B. methylotrophicus*, *S. marcescens*, and a combination of both. After 45 days, we measured chlorophyll *a* fluorescence, growth (shoot length, biomass, and leaf area), and acclimatization rate. Then, plants were transferred to *ex vitro* conditions and monitored for 20 days. Plants inoculated with *B. methylotrophicus* and *S. marcescens* showed higher chlorophyll *a* fluorescence and significantly longer shoots (57% and 35%, respectively) compared to the control. *S. marcescens* also promoted the highest root biomass, with increments of 158% more fresh mass and 180% more dry mass than the control. Most strikingly, plants treated with both bacteria had a 100% survival rate during acclimatization, representing a 40% increase over the control. These findings show that plant growth-promoting bacteria, particularly *S. marcescens*, can enhance *in vitro* growth and improve acclimatization. This research supports more efficient micropropagation and advances the biotechnology of native tree species.

**Keywords:** *Bacillus methylotrophicus*, *Serratia marcescens*, photosystem II efficiency, *ex vitro* survival, root development.

**Resumo**

Há uma lacuna significativa de conhecimento sobre o papel de microrganismos benéficos no cultivo *in vitro* de plantas. Condições assépticas, embora essenciais, excluem microrganismos que poderiam melhorar o crescimento e a aclimatização das plantas. Este estudo teve como objetivo avaliar o efeito da inoculação de *Bacillus methylotrophicus* e *Serratia marcescens* no crescimento e desenvolvimento *in vitro* de *Stryphnodendron adstringens*, uma árvore de alto valor medicinal. O experimento avaliou plantas de *S. adstringens in vitro* com quatro tratamentos: controle, *B. methylotrophicus*, *S. marcescens* e uma combinação de ambos. Após 45 dias de cultivo, parâmetros de fluorescência da clorofila *a*, crescimento (comprimento do caule, biomassa, área foliar) e taxa de aclimatização foram avaliados. Em seguida, as plantas foram transferidas para condições *ex vitro*, onde a taxa de sobrevivência foi monitorada por 20 dias. As plantas de *S. adstringens* cultivadas *in vitro* e inoculadas com *B. methylotrophicus* e *S. marcescens* apresentaram melhoria na fluorescência da clorofila *a*, acompanhada de um aumento significativo no comprimento da parte aérea (57% e 35%, respectivamente) em comparação ao controle. Além disso, *S. marcescens* também promoveu o maior aumento na massa fresca e seca da raiz, com incrementos de 158% e 180%, respectivamente, em relação ao controle. Mais impressionantemente, as plantas tratadas com ambas as bactérias alcançaram uma taxa de sobrevivência de 100% durante a aclimatização, representando uma melhoria de 40% em relação ao controle. Esses achados destacam o papel poderoso de bactérias promotoras do crescimento de plantas, particularmente *S. marcescens*, na otimização do crescimento *in vitro* de plantas e na garantia de aclimatização bem-sucedida. Esta pesquisa fornece uma base para estratégias de micropropagação mais eficientes, contribuindo para o avanço biotecnológico de espécies nativas de árvores.

**Palavras-chave:** *Bacillus methylotrophicus*, *Serratia marcescens*, eficiência do fotossistema II, sobrevivência *ex vitro*, desenvolvimento radicular.

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## 1. Introduction

Plant tissue culture is a broad biotechnological platform used to cultivate and propagate whole plants, tissues, organs, and cells under controlled laboratory conditions. It supports large-scale clonal production, genetic modifications, secondary metabolite synthesis, conservation of endangered species, plant breeding, and other applications (Aslam et al., 2023). Traditionally, tissue culture involves growing in aseptic conditions and nutrient media supplemented with essential hormones and nutrients (Phillips and Garda, 2019; Khezri et al., 2024). However, in some cases, introducing microorganisms into the culture system has improved plant growth and development *in vitro* (Orlikowska et al., 2017; Soumare et al., 2021; Xiang et al., 2023) or altered secondary metabolism (Ahmad et al., 2019; Bhaskar et al., 2022; Fazili et al., 2022).

Aseptic cultivation in plant tissue culture creates a biological vacuum, excluding microorganisms that could benefit plant growth. This exclusion can limit *in vitro* development and reduce *ex vitro* survival during acclimatization (Soumare et al., 2021). In recent years, the targeted introduction of beneficial microorganisms such as plant growth-promoting bacteria (PGPB) has gained importance in *in vitro* cultivation systems (Cantabella et al., 2022; Salotti et al., 2023; Ram et al., 2024).

PGPB are a diverse group of epiphytic or endophytic bacteria that enhance plant growth, development, and health even under *in vitro* conditions (Gunjal and Glick, 2024). These benefits are attributed to a wide range of mechanisms involved in plant-bacteria interactions, including alterations in endogenous phytohormone levels (e.g., auxins, ethylene regulated by ACC deaminase, cytokinins, and gibberellins), increased nitrogen fixation, phosphate solubilization, and iron uptake (Gunjal and Glick, 2024). Together, these mechanisms highlight the significant role of PGPB in enhancing plant physiology, making them promising candidates for *in vitro* cultivation (Mirza et al., 2001). Among the many PGPB species studied, *Bacillus methylotrophicus* and *Serratia marcescens* have emerged as two notable examples (Radhakrishnan and Lee, 2016; Niu et al., 2022).

*Bacillus methylotrophicus* is a Gram-positive bacterium from the Bacillaceae family, while *S. marcescens* is a Gram-negative bacterium from the Enterobacteriaceae family. Both species are recognized for their high potential in agriculture as plant growth promoters. They can fix nitrogen, solubilize phosphorus, and produce phytohormones (e.g., indole-3-acetic acid - IAA), thereby enhancing plant growth and development (Radhakrishnan and Lee, 2016; Niu et al., 2022). Nevertheless, the mode of action varies depending on the PGPB species and its specificity with the host plant (Pardo-Díaz et al., 2021; Pellegrini et al., 2023; Gunjal and Glick, 2024). This variability highlights the need for studies that characterize plant–microbe relationships, particularly in the *in vitro* propagation of tree species with high medicinal value, such as *Stryphnodendron adstringens* (Mart.) Coville (Fabaceae family).

*Stryphnodendron adstringens*, commonly known as "barbatimão" in Brazil, is a small tree that typically reaches up to 5 m in height. It has a leafy crown, a small to medium trunk (up to 30 cm in diameter at 30 cm above ground level), and rough, grayish-brown bark (Felfili et al., 1999; Lima et al., 2024).Its wood has a medium density of 0.80 g $cm^{-3}$, thick texture, and good resistance suitable for veneer production (Vale et al., 2001). Despite its potential for timber production, this species is primarily exploited for its medicinal properties (see Ricardo et al., 2018; Ribeiro et al., 2022; Reis et al., 2023; Xavier-Silva et al., 2024).

Given the relevance of *S. adstringens* and the promising characteristics of PGPB, we hypothesize that inoculating *B. methylotrophicus* and *S. marcescens* during *in vitro* cultivation will improve plant growth and development, along with increased survival rate during the acclimatization phase. To test this hypothesis, we evaluated the effects of individual and combined inoculations of these bacteria on chlorophyll *a* fluorescence, growth, biomass, and acclimatization rate of *S. adstringens* grown *in vitro*.

## 2. Materials and Methods

### *2.1. Plant material and in vitro germination*

Seeds of *S. adstringens* were collected from mother trees in Estreito, Maranhão, Brazil (5° 46' 60" S and 43° 15' 0" W). After harvesting, seeds were sent to the Tissue Culture Laboratory at the State University of Maranhão in São Luís, to conduct the experiment.

During *in vitro* germination, seed dormancy was broken by making a small cut in the seed coat opposite the hilum using pliers. In a laminar flow chamber, seeds were then surface disinfected by immersion in 70% ethanol for 1 minute (v/v), followed by immersion in a 2.5% active chlorine (m/v) solution of commercial sodium hypochlorite (NaClO; Ypê® Ltda, Química Amparo Ltda, Passos, Minas Gerais, Brazil) two drops of Tween−20 (Isofar® Ltda, Duque de Caxias, RJ, Brazil) per 100 mL. Seeds were agitated in this solution for 15 minutes, then rinsed three times with autoclaved distilled water for 3 minutes each.

Afterward, seeds were inoculated in glass test tubes (25 × 150 mm) containing 10 mL of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with half-strength salts and vitamins (2.22 g $L^{-1}$; PhytoTechnology®, Lenexa, KS, USA). The medium was supplemented with 15 g $L^{-1}$ of sucrose (Isofar® Ltda, Duque de Caxias, Rio de Janeiro, Brazil), 100 mg $L^{-1}$ of myo−inositol (Sigma-Aldrich®, St. Louis, MO, USA), and solidified with 3.25 g $L^{-1}$ of agar (Agargel® Ltda, São Paulo, SP, Brazil). The pH was adjusted to 5.7 ± 0.1, and the medium was autoclaved at 121 °C and 108 kPa for 15 minutes.

The tubes containing the seeds were kept in the dark at 25 ± 2°C for 7 days. After this period, they were maintained at the same temperature under a 16-hour photoperiod with an irradiance of 40 µmol $m^{-2}$ $s^{-1}$ provided by two light-emitting diode lamps (T8 LED tube, 10 W, Avant, São Paulo, SP, Brazil) until germination stabilized (radicle ≥ 3 mm) and initial seedling growth occurred (shoot length up to ~2 cm).

### *2.2. Preparation of B. methylotrophicus and S. marcescens inoculant*

The experiment used *B. methylotrophicus* from the culture collection of the Phytopathology Laboratory at UEMA and *S. marcescens* from the fungal collection of the Microbiology Laboratory at CEUMA University (UNICEUMA).

Bacterial cultures were grown on disposable Petri dishes (90 × 15 mm) (Firstlab, Weissópolis Pinhais, PR, Brazil) containing 20 mL of 10% Tryptic Soy Agar medium (TSA; Sigma-Aldrich®, St. Louis, MO, USA) and incubated at 30°C for 24 hours in a Biochemical Oxygen Demand (BOD) chamber (SPlabor, Presidente Prudente, SP, Brazil). Based on preliminary tests, we used bacterial suspensions at a $10^{-8}$ dilution in 0.85% saline solution.

### *2.3. Determination of indole-3-acetic acid (IAA) concentration in B. methylotrophicus and S. marcescens*

IAA synthesis was evaluated using the colorimetric method described by Gordon and Weber (1951), with modifications. In a laminar flow chamber, bacterial isolates were incubated in glass test tubes (25 × 150 mm) containing 10 mL of 10% TSA medium enriched with tryptophan (Sigma-Aldrich®, St. Louis, MO, USA) for 48 hours at 28°C under constant agitation at 175 rpm in a rotary shaker (Laborglas, LGI-SK-O330, São Paulo, SP, Brazil). After incubation, the cultures were transferred to new test tubes and centrifuged at 10,000 rpm for 10 minutes.

In triplicate, the supernatant from each isolate was mixed with Salkowski reagent [0.45% $FeCl_3$ (w/v) in 10.2 M $H_2SO_4$ (Sigma-Aldrich®, St. Louis, MO, USA)] in a 2:1 (v/v) ratio. The mixtures were kept in the dark at room temperature (25 ± 2°C) for 30 minutes.

IAA quantification was performed by measuring absorbance at 540 nm using a digital spectrophotometer (Biospectro, SP220, Curitiba, PR, Brazil). A calibration curve was prepared with synthetic IAA standards (0–100 µg $mL^{-1}$; Sigma-Aldrich®, St. Louis, MO, USA), as proposed by Radwan et al. (2005).

### *2.4. Experimental design and cultivation conditions*

Plants with approximately 2 cm of shoot length were transferred from test tubes to transparent glass jars (350 mL capacity) containing 70 mL of liquid half-strength MS medium (2.22 g $L^{-1}$; PhytoTechnology®, Lenexa, KS, USA) supplemented with 15 g $L^{-1}$ of sucrose (Isofar® Ltda, Duque de Caxias, RJ, Brazil), 100 mg $L^{-1}$ of myo-inositol (Sigma-Aldrich®, St. Louis, MO, USA), and 100 $cm^3$ of vermiculite as a support. The jars were sealed with transparent polypropylene lids with two 10 mm diameter holes, covered by a membrane composed of two layers of microporous tape (Missner® Ltda, Blumenau, Santa Catarina, Brazil) and polytetrafluoroethylene (PTFE) (Poly® Ltda, São Paulo, SP, Brazil) with a thickness of 0.05 ± 0.01 mm, following the method proposed by Saldanha et al. (2012).

During *in vitro* cultivation, plants received treatments with PGPBs at 15 and 30 days: control (no PGPBs), *B. methylotrophicus*, *S. marcescens*, and *B. methylotrophicus + S. marcescens*. Bacterial inoculation followed a protocol adapted from Belincanta et al. (2022), consisting of the application of 50 µL of bacterial suspension ($10^{-8}$ dilution) to the basal region and 50 µL to the roots of each plant. In the control treatment, only autoclaved distilled water was applied. The jars were maintained in a growth room for 45 days under controlled conditions: temperature of 25 ± 2°C, a 16–h photoperiod, and light irradiance of 60 µmol $m^{-2}$ $s^{-1}$ provided by two white LED lamps (T8 LED tube, 10 W, Avant, São Paulo, SP, Brazil).

The experiment followed a completely randomized design with four treatments (control, *B. methylotrophicus*, *S. marcescens*, and *B. methylotrophicus + S. marcescens*), with 16 replicates. Each experimental unit consisted of one plant per jar.

### *2.5. Growth analyses*

After 45 days of *in vitro* cultivation of *S. adstringens* with and without PGPBs, the following parameters were measured: shoot length (cm), root length (cm), number of leaves, leaf area ($cm^2$), shoot fresh mass (g), root fresh mass (g), shoot dry mass (g), and root dry mass (g). Leaf area was measured using ImageJ® software, while biomass was determined after drying the plants in a forced-air oven at 70°C for 48 hours.

### *2.6. Determination of chlorophyll a fluorescence*

Chlorophyll *a* fluorescence was measured on the second fully expanded leaf below the apex. The following parameters were assessed: PSII maximum quantum yield (Fv/Fm), total number of active reaction centers per absorption (RC/ABS), and performance index. Measurements were taken using a portable non-modulated fluorimeter (Pocket PEA, Hansatech Instrument Ltd., Norfolk, UK). Leaves were dark-adapted for 30 minutes using a leaf-clip system (Hansatech Instrument Ltd., Norfolk, UK) before the measurements.

### *2.7. Ex vitro acclimatization*

At 45 days of *in vitro* cultivation, five randomly selected plants from each treatment were transferred to a greenhouse for acclimatization. The plants were removed from the jars, and their roots were gently washed with water to remove all traces of culture medium. They were then transplanted into polyethylene tubes (10 cm height x 5 cm diameter) containing 250 g of autoclaved commercial substrate (Carolina Soil® Ltda, Santa Cruz do Sul, Rio Grande do Sul, Brazil).

During acclimatization, plants were irrigated by micro-sprinklers every 4 hours for 5 minutes during the day between 6:00 AM and 6:00 PM (local time). The average temperature was ~30 ± 2°C, with 75% relative humidity, under natural light conditions (~1,002 µmol photons $m^{-2}$ $s^{-1}$) and a 12–h photoperiod. After 20 days, plant survival rates were assessed.

### *2.8. Statistical analyses*

Data were tested for normality using the Shapiro–Wilk test, followed by analysis of variance (ANOVA). Treatment means were compared using the Tukey test ($P \leq 0.05$). All analyses were performed with Sisvar software (Ferreira, 2011).

## 3. Results

As expected, both bacterial isolates synthesized IAA, with concentrations of 175.3 µg $mL^{-1}$ for *B. methylotrophicus* and 118.81 µg $mL^{-1}$ for *S. marcescens*.

*Stryphnodendron adstringens* plants inoculated with *B. methylotrophicus*, *S. marcescens*, or both showed enhanced growth and development during *in vitro* cultivation compared to the control (Figures 1A–H). All treatments supported complete organ formation (leaf, stem, and root), and no visible disease symptoms, such as chlorosis or wilting, were observed in the plants (Figures 1A–H).

The analysis of chlorophyll *a* fluorescence showed that *S. adstringens* plants inoculated with *B. methylotrophicus* and *S. marcescens*, or both exhibited higher performance compared to the control during *in vitro* cultivation. The maximum quantum yield of PS II was 8%, 11%, and 7% higher in plants treated with *B. methylotrophicus*, *Serratia marcescens*, and the combined treatment, respectively, compared to the control (Figure 2A). Additionally, plants inoculated with *S. marcescens* showed a performance index 5.8 times higher than that of the control (higher) (Figure 2B).

Plants inoculated individually with *B. methylotrophicus* and *S. marcescens* showed the highest values for results regarding the total number of active reaction centers per absorption (RC/ABS) compared to other treatments. Notably, these values were 46% and 77% higher than the control for *B. methylotrophicus* and *S. marcescens*, respectively (Figure 2C).

Overall, *S. adstringens* plants inoculated with *S. marcescens* and *B. methylotrophicus* showed significant increases (by 57% and 35%, respectively) in length of aerial part compared to the control (Figure 3A). Additionally, plants treated with *B. methylotrophicus* had 26% more leaves and 44% greater leaf area than the control (Figures 3C and 3D).

Concerning root biomass, *S. marcescens* inoculation resulted in the highest fresh and dry root mass values. Both parameters increased significantly compared to all other treatments, with gains of 158% and 180%, respectively, relative to the control (Figure 3F and 3H).

The remaining parameters were not significantly affected by the treatments. *S. adstringens* plants showed no differences in root length (5.56 - 8.91 cm), fresh mass of aerial part (0.25 - 0.42 g), and dry mass of aerial part (0.06 - 0.11 g) across treatments (Figures 3B, 3E and 3G, respectively).

*Stryphnodendron adstringens* plants inoculated with *B. methylotrophicus*, *S. marcescens*, or both showed a 100% survival rate after acclimatization. This represents a 40% increase compared to the control (Figure 4).

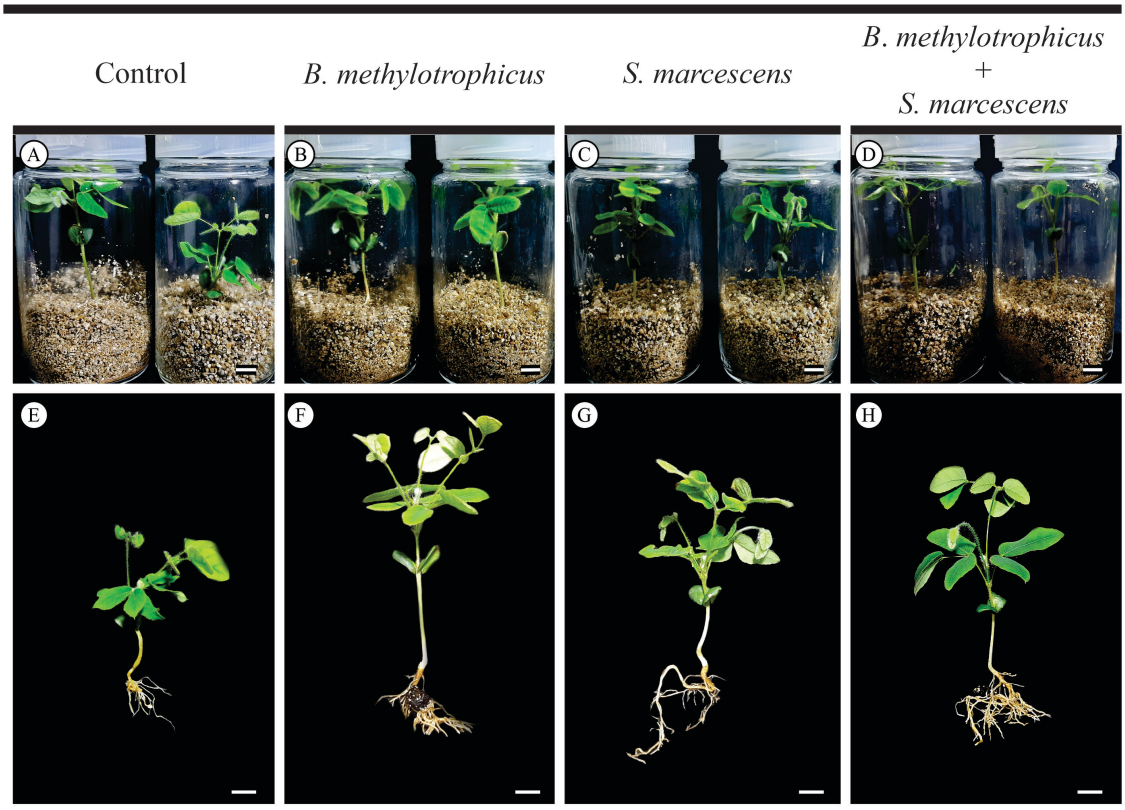


**Figure 1.** *Stryphnodendron adstringens* plants after 45 days of *in vitro* cultivation with or without inoculation of plant growth-promoting bacteria. (A, E) Control, (B, F) *Bacillus methylotrophicus*, (C, G)*Serratia marcescens*, (D, H) *Bacillus methylotrophicus* + *Serratia marcescens*. Bars = 1 cm.

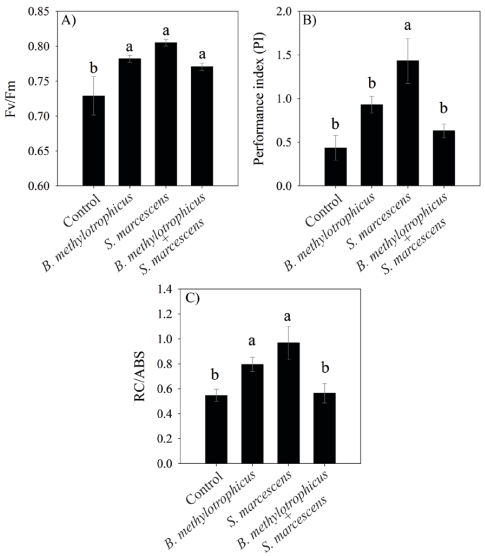


**Figure 2.** Chlorophyll *a* fluorescence of *Stryphnodendron adstringens* plants inoculated with plant growth-promoting bacteria after 45 days of *in vitro* cultivation. (A) Maximum quantum yield of PS II (Fv/Fm), (B) Performance index (PI), (C) Total number of active reaction centers per absorption (RC/ABS). Means followed by the same letter do not differ significantly according to Tukey's test at 5% significance. Error bars represent the standard error of the mean (n = 6).

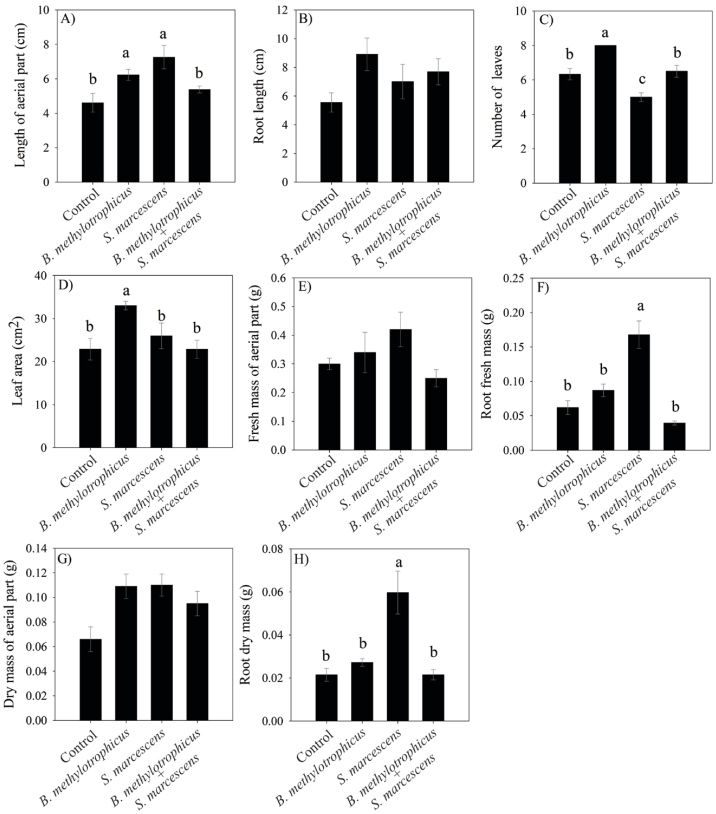


**Figure 3.** Growth of *Stryphnodendron adstringens* plants inoculated with plant growth-promoting bacteria after 45 days of *in vitro* cultivation. (A) Shoot length, (B) Root length, (C) Number of leaves, (D) Leaf area (D), (E) Shoot fresh mass, (F) Root fresh mass, (G) Shoot dry mass, (H) Root dry mass. Means followed by the same letter do not differ significantly according to Tukey's test at 5% significance. Error bars represent the standard error of the mean (n = 6).

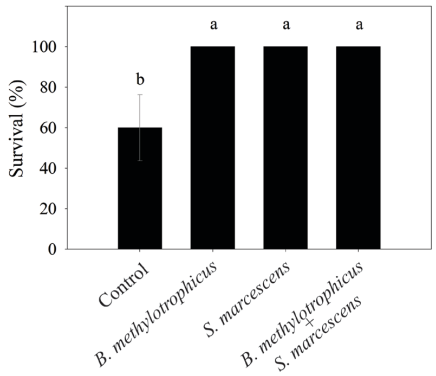


**Figure 4.** Survival percentage during the acclimatization phase of *Stryphnodendron adstringens* plants inoculated with plant growth-promoting bacteria during *in vitro* cultivation. Means followed by the same letter do not differ significantly according to Tukey's test at 5% significance. Error bars represent the standard error of the mean (n = 10).

## 4. Discussion

Native tree species propagated *in vitro* often face serious challenges during the acclimatization phase, particularly due to poor root development, which reduces survival rates (Oakes et al., 2020). This issue is a global concern, as it hinders genetic conservation, clonal propagation, and plant improvement through tissue culture techniques (Negi et al, 2024). Here, our hypothesis was confirmed: the inoculation of *B. methylotrophicus* and *S. marcescens* during the *in vitro* cultivation of *S. adstringens* enhanced plant growth and development, as well as *ex vitro* survival rates.

*Bacillus methylotrophicus* and *S. marcescens* improved chlorophyll *a* fluorescence performance in *S. adstringens* plants grown *in vitro*. In particular, *S. marcescens* significantly increased the performance index, a parameter that integrates light energy absorption, excitation energy trapping, and the conversion of excitation energy into electron flow (Thach et al., 2007). A higher performance index suggests more efficient PSII function and greater potential for energy conversion during photosynthesis (Thach et al., 2007). Although most evidence comes from *ex vitro* conditions, it is well-documented that PGPBs can enhance PSII efficiency. For example, *Capsicum chinense* plants inoculated with *Bacillus* spp. Showed improved PSII performance (Samaniego-Gámez et al., 2016), supporting our findings under *in vitro* conditions.

Two key factors may explain the observed increase in PSII efficiency. First, PGPBs enhance nutrient availability and absorption, particularly of nitrogen, phosphorus, and iron (Singh et al., 2019; Gunjal and Glick, 2024). Here, we believe that although nutrients were readily available in the culture medium, we suggest that the interaction between the bacteria and *S. adstringens* improved the plant's nutritional balance, contributing to enhanced PSII efficiency. Nitrogen plays a role in chlorophyll synthesis and PSII-associated proteins, while iron is essential for electron transfer within the photosystems (Saito et al., 2021).

Another important factor to consider is the positive regulation of genes involved in chlorophyll *a* fluorescence. In *Oryza sativa* cv. Nipponbare plants inoculated with *Azospirillum brasilense*, there was an upregulation of two genes involved in plastocyanin synthesis (e.g., LOC_Os02g43660 and LOC_Os03g02400) were upregulated (Cook et al., 2022). Plastocyanin is a key protein in the electron transport chain. This finding indicates that PGPBs can influence the expression of genes that enhance photosynthetic performance. The photochemical responses observed in *S. adstringens* plants inoculated with *B. methylotrophicus* and *S. marcescens* suggest that photosynthesis was improved during *in vitro* cultivation. This enhancement likely contributed to increased carbon fixation for growth, energy storage, or biomass accumulation.

Interestingly, *B. methylotrophicus* promoted an increase in leaf area in line with greater shoot biomass in *S. adstringens*. In contrast, *S. marcescens* enhanced root biomass. This gain in root biomass is essential for the acclimatization phase, as a well-developed root system improves water and nutrient uptake under *ex vitro* conditions. One likely explanation is the ability of PGPBs to synthesize IAA, an auxin precursor that triggers intracellular signaling cascades. These cascades regulate the expression of genes such as auxin response factors (ARFs) and those involved in polar auxin transport (PAT), which promote lateral and adventitious root formation and contribute to increased root system biomass (Duca and Glick, 2020).

The *in vitro* cultivation of *S. adstringens* inoculated with *B. methylotrophicus* and *S. marcescens* also improved plant performance during the acclimatization phase. This is an important result, as high mortality often occurs at this stage due to the atypical conditions of *in vitro* culture, such as high relative humidity, limited gas exchanges due to jar sealing, low irradiance, low $CO_2$ concentration, and elevated carbohydrate levels (Martins et al., 2015; Fortini et al., 2021). These conditions lead to the development of leaves with large intercellular spaces in the mesophyll, poorly developed vascular tissues, and non-functional stomata (Fortini et al., 2021; Manokari et al., 2023). Collectively, these morphological and physiological limitations compromise successful *ex vitro* acclimatization.

Although no morphophysiological analyses were conducted to elucidate the mechanisms underlying the improved acclimatization of *S. adstringens*, the role of PGPBs in enhancing plant adaptation to abiotic stress is well established (see Kumar et al., 2022; Al-Turki et al., 2023; Karnwal et al., 2024). It is important to note that the acclimatization phase itself is a stressful condition for plants derived from *in vitro* cultivation.

We highlight the potential of incorporating beneficial microorganisms into *in vitro* cultivation as a powerful strategy to optimize micropropagation of tree species. Additionally, research on *S. adstringens* is essential as a foundation for advancing knowledge needed to domesticate the species through *in vitro* genetic conservation. Such efforts will support its morphophysiological characterization, breeding, and large-scale micropropagation.

## 5. Conclusion

Our findings demonstrate that inoculating *Stryphnodendron adstringens* plants with *B. methylotrophicus* and *S. marcescens* during *in vitro* cultivation improves chlorophyll *a* fluorescence, biomass accumulation, and acclimatization success . Most importantly, *S. marcescens* significantly increases root biomass, making it a promising tool for improving plant quality. These results support the application of beneficial bacteria in the micropropagation of *S. adstringens* and contribute to advancing plant biotechnology for native tree species.

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## Data Availability Statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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