

ARTICLE

Biopriming of Begonia seedlings with endophytic fungal isolates *Beauveria bassiana* led to significant increase in growth characteristic

Biopriming para promoção do crescimento vegetativo em mudas de Begonia rex com isolados endofíticos de *Beauveria bassiana*

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Abstract

The ornamental species *Begonia rex* has a high commercial demand in the flower industry, including plant tissue culture techniques. To develop this approach for mass production of begonia, the disadvantages of post-transplant stress need to be mitigated. For this purpose, the effect of inoculation with entomopathogenic endophyte *Beauveria bassiana* conidia in *B. rex* seedlings obtained from *in vitro* culture was investigated. Throughout the course of the experiment, treated specimens exhibited a consistently high level of fungal invasion, with colonization rates ranging from 60 % to 80 %. During this study, seedlings were not fertilized at any stage so as not to disturb the mutualistic plant–fungus relationship. Inoculated by the “substrate treatment” method, *B. bassiana* had positive effects on root and leaves area of seedlings: total leaf blade area increased by 50%, while the root system increased threefold. *B. bassiana* also promoted water-holding root capacity of *B. rex* seedlings. Under transplantation stress conditions, the fresh weight and relative water content of *in vitro* produced begonia plants increased significantly (2–2.5-fold) after colonization with *B. bassiana*. It can be assumed that under conditions of nutrient deficiency, the fungus, which promotes both the transport and absorption of nutrients, can also enhance the growth of begonia seedlings by changing the level of phytohormones. Thus, it was found that inoculation with *B. bassiana*, which helps *B. rex* to tolerate water and nutrient deficiency during *ex vitro* transplantation, could be beneficial for the production of high-quality begonia plants.

Keywords: *Begonia rex*, easy post-transplantation, entomopathogenic fungi (EPF), fungal colonization.

Resumo

A espécie ornamental Begônia rex apresenta elevada demanda comercial no setor florícola, destacando-se pelo potencial de propagação via cultura de tecidos. Entretanto, o estresse associado ao processo de aclimatização *ex vitro* constitui uma limitação para a produção em larga escala. Nesse contexto, avaliou-se o efeito da inoculação com o fungo entomopatogênico endofítico *Beauveria bassiana* em mudas de Begônia rex provenientes de cultivo *in vitro*. As plantas tratadas apresentaram colonização fúngica consistente, com taxas variando entre 60% e 80%. Durante o ensaio, não foi realizada adubação em nenhuma fase, a fim de preservar a interação mutualística planta–microrganismo. A aplicação de *B. bassiana* pelo método de tratamento do substrato resultou em efeitos positivos no desenvolvimento das mudas, com incremento de 50% na área foliar e triplicação do volume do sistema radicular. Além disso, observou-se aumento da capacidade de retenção hídrica das raízes. Sob condições de estresse no momento do transplante, a colonização por *B. bassiana* promoveu aumento significativo da massa fresca e do teor relativo de água das plantas, com incrementos entre 2 e 2,5 vezes em comparação ao controle. Esses resultados sugerem que, em condições de limitação nutricional, o fungo pode favorecer tanto o transporte quanto a absorção de nutrientes, além de modular os níveis de fitohormônios, contribuindo para o crescimento e a tolerância a stress das mudas. Conclui-se que a inoculação com *B. bassiana* representa uma estratégia promissora para atenuar o estresse hídrico e nutricional durante a aclimatização *ex vitro*, possibilitando a obtenção de mudas de *B. rex* de maior qualidade.

Palavras-chave: aclimatização, *Begonia rex*, colonização endofítica, fungos entomopatogênicos.

Introduction

The genus *Begonia* L. comprises, according to various authors, from 2100 to 2500 species (Tian et al., 2020; Borah et al., 2025), most of which mainly occur in the tropical regions of the Earth. Representatives of this family are popular throughout the world as ornamental plants (Menegaes et al., 2015).

Since ancient times, many species of begonias have been considered as medicinal plants in alternative medicine in tropical countries (Borah et al., 2025). A thorough study of the biochemical properties of the species of this family revealed the presence of a wide range of secondary metabolic products - alkaloids, isoprenoids, phenolic compounds (Bashilov, 2021), as well as anthocyanins (Karpova et al., 2021), which determines the practical use of begonia plants in the pharmaceutical industry. One of the well-known decorative species of this family is *Begonia rex* Putz., exhibiting colorful, variegated foliage; the species was first described by J.A.A.H. Putzen in 1857. The species occurred from northeast India, to southeast China at the level of 200 - 1250 m. (Gu et al., 2007). Extensive studies of the morphology and reproductive characteristics of this species,

including its developmental rhythms, have been carried out at the Central Siberian Botanical Garden (Fershalova and Baikova, 2013). *B. rex* has been included as a model species in the CSBG's studies on the introduction of subtropical plants into protected ground conditions.

In addition to ongoing work to optimize the reproduction of begonia species (Aswathy and Murugan, 2017; Hosseinabadi et al., 2022), biotechnological approaches have been introduced as an effective alternative to traditional propagation methods (Nabieva and Fershalova, 2023; Rezapour et al., 2025) to produce genetically uniform and highly ornamental begonia plants.

However, a transfer from *in vitro* conditions is often the most challenging stage of *ex vitro* adaptation technology. To solve this problem, the practice of using various groups of endophytes has been increasingly employed during the recent years, including both mycorrhizal fungi (AMF) (Furtado et al., 2023) and facultatively endophytic fungi, primarily used in pest management and belonging to entomopathogenic fungi (EPF) (Zheng et al., 2023). As far as we know, studies considering the use of endophytes in growing begonias are not numerous. Thus, inoculation with *Rhizophagus irregularis* (AMF fungi) resulted in accelerated growth

*Corresponding author: sibflower05@gmail.com | <https://doi.org/10.1590/2447-536X.v31.e312976> | Editor: Gilmar Schafer, Universidade Federal do Rio Grande do Sul, Brasil | Received: July 08, 2025 | Accepted: Sep 10, 2025 | Available online: Sep 28, 2025. Licensed by CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

and improved ornamental qualities of *Begonia* × *semperflorens-cultorum* when grown in industrial floriculture (Sabatino et al., 2019).

It has been confirmed that fungi of the genus *Beauveria* are capable of developing symbiotic relationships with terrestrial plants (Yerukala et al., 2022; Hu and Bidochka, 2021), including agricultural crops, such as wheat (Kramski et al., 2023), potato (Tomilova et al., 2023). One of the promising non-specific endophytes capable of beneficially affecting plants is the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. This easy-to-cultivate fungus is widespread in all climatic zones. By colonizing the rhizosphere and above-ground parts of plants, in particular stems, leaves, flowers and seeds, both naturally and through artificial inoculation, endophytic fungi exerted systemic effects including promoting plant growth and enhancing resistance to phytopathogens and abiotic stresses (Guo et al., 2024; Tang et al., 2025). Previously no similar effects associated with the use of entomopathogenic endophytes have been recorded for the plants of the Begoniaceae family and information on the endophytic activity of *B. bassiana* on *B. rex* growth is lacking. Thus, a completely new and productive approach will be efficient to evaluate the influence of endophytic colonization of *B. bassiana* on the growth characteristics of begonia plants. The aim of this study was to investigate the effect of inoculation with *B. bassiana* conidia on overcoming post-transplantation shortcomings in *B. rex* seedlings obtained from *in vitro* culture.

Material and Methods

Indoor experiments

B. rex was introduced into the CSBG stock collection as leaf cuttings obtained from the Kunming Botanical Garden, China in 2000. *B. rex* mother plants were grown in a polycarbonate-covered greenhouse in individual pots, separately from other collection specimens, to avoid cross-pollination. The greenhouse temperature was daily fluctuating within 20 - 28 °C during the day and 16 - 20 °C at night. A shading net of acrylic covering material, density - 50 g m⁻², was placed above the plants to provide 50% - 55% illumination level, which was 3000 - 5000 Lx. Air humidity varied from 30% to 60%. When replanting *B. rex* plants, a well-draining and slightly acidic potting mixture was used, containing birch leaf mold: high moor peat: coarse sand (1: 1: 1), pH = 5.1 - 5.5. The seeds for the experiment were obtained by hand pollination. Pollen was transferred, using a brush, from the male flowers of five independent genets to the female flowers of other individuals. Dry, ripened seeds, without a storage period, were used for sowing in the *in vitro* culture.

In vitro seed germination and initial seedlings growth

Seed surface sterilization procedure

Seeds were surface sterilized as follows: submerging in 70% ethanol for 1 min was followed by 5% NaOCl, where one drop of Tween-20 was added with 20 min constant agitation. Seeds were rinsed three times with sterile water to remove the sterilant. Then at least 100 seeds were sown in sterile plastic Petri dishes with a diameter of 90 mm, wrapped in a double layer of transparent stretch film, on half-strength modified MS (Murashige and Skoog, 1962) medium supplemented with 40 mg·L⁻¹ adenine sulfate and 100 mg L⁻¹ myo-inositol and 20 g L⁻¹ sucrose. The seedlings cultures were maintained at 22 ± 2 °C under 12/12 h light/dark photoperiod provided by cool-white fluorescent tubes (Phillips F20T12/CW; Poland) at 30 mmol m⁻² s⁻¹ (PAR).

The whole period of seed germination was 3 - 4 weeks. After one month, all germinated seeds were transferred on the same medium, where seedlings were grown for the next two months.

Pot experiments

Only seedlings at the four leaves-bearing stage were taken in the pot experiments. The plantlets were removed from tissue culture into 200 mL plastic pots with sterile perlite (0.2 - 0.4 mm fraction) and maintained in plastic containers under the same physical conditions as during seed germination. Seedlings were watered at three days intervals with sterilized water. Seedlings were not fertilized at any point during this study because there is evidence that fertilizers may be harmful or change plant-fungus relationship, resulting in a potentially confounding factor for this type of research (Zale et al., 2022).

Fungi inoculation

Fungal inoculation was carried out five days after transplantation of *B. rex* seedlings *ex vitro*. The strain of the entomopathogenic fungus *B. bassiana* (Sar-31) was obtained from the collection of microorganisms at the Institute of Systematics and Ecology of Animals. The *B. bassiana* (Sar-31) (GenBank accession number MZ564259) fungal culture was grown on a modified artificial nutrient medium 1/4 SDAY, consisting of one-quarter strength Sabouraud dextrose agar supplemented with 0.25% peptone, 0.25% yeast extract, 1% glucose, and 2% agar, according to a SDAY protocol commonly used for entomopathogenic fungi. Following 14-day incubation at 25 °C in Petri dishes, fungal samples were suspended in a 0.003% aqueous Tween 80 solution, and the conidial concentration was quantified using a Neubauer hemocytometer (Fristaden Lab, Chicago, IL, USA).

The fungal inoculation was performed by applying 2 mL of a *B. bassiana* conidial suspension (1 × 10⁸ conidia mL⁻¹) as a drench of substrate to pots containing the seedlings.

In the whole, begonia seedlings were grown in the presence of fungal conidia for 30 days.

Plant Colonization Assay

The frequency of plant colonization by entomopathogenic fungus was assessed by plating surface sterilized leaf tissues. Leaves were sterilized in sodium hypochlorite and ethanol (Posada et al., 2007), imprinted onto modified Sabouraud agar (10 g L⁻¹ peptone, 40 g L⁻¹ anhydrous D glucose, 20 g L⁻¹ agar, 1 g L⁻¹ yeast extract, 0.35 g L⁻¹ cetyltrimethylammonium bromide, 0.05 g L⁻¹ cycloheximide, 0.05 g L⁻¹ tetracycline, and 0.6 g L⁻¹ streptomycin) in 90 mm Petri dishes (one plant per dish), and incubated in total darkness at 24 °C for 10 days. Imprints yielding any fungal growth were omitted from further analysis.

Colonies obtained from PDA plates were counted 5 days later and were observed under optical microscopy for morphological identification of the fungal species.

Plant growth measurements

To assess the effect of *B. bassiana* inoculated on the growth of begonias, measurements were carried out throughout the experiment at 10-day intervals, and the results were collected on days 10, 20, and 30 after inoculation.

Three response variables were assessed in pot experiments: mean leaf area per plant; mean total area of all leaves per plant; mean total root area per plant. The variables were expressed in square centimeters.

Living material was scanned and the plant images were processed in the program SIAMS Photolab (<http://www.siams.com>). After measuring the growth parameters of begonia seedlings, it was possible to plant them in the same substrate without damaging the plants.

The gravimetric method was utilized for measuring fresh and dry biomass of the plant material. To estimate the fresh plant weight (FW), plants were dried in advance with filter paper, and FW was determined to within 1 mg. Dry weight (DW) was determined after the material was dried at 90 °C until constant weight. To calculate the water content (WC), the following formula was used: $WC = [(FW - DW)/FW] \times 100$.

Statistical analysis

The pot experiments were set up based on a completely randomized design comprising three treatments, with three replicates (n = 30), including control.

Before analysis, data were subjected to normality and homogeneity tests of variance using the Shapiro-Wilk Normality test (at 0.05 significance level). Data were subjected to one-way analysis of variance in Statistica, version 10.0 (StatSoft Inc., Tulsa, OK). The Duncan's test was used to make multiple comparisons of the means ($p \leq 0.05$).

Results

After 7 days of plant growth, colonization of *B. bassiana* was detected, whereas no fungal growth was observed among control plants (Fig. 1A and 1B).

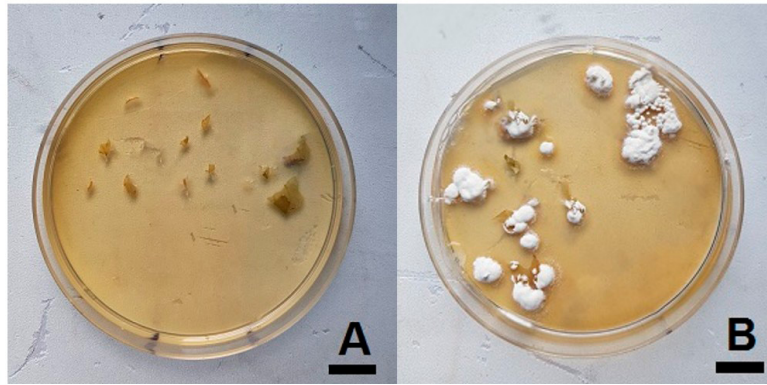


Fig. 1. Colonization of *Begonia rex* leaves by *Beauveria bassiana*: Control treatment (A) and Fungus inoculation (B). Note: The colonization was estimated on 20 plants in each treatment group. The scale bar is 1 cm.

Throughout the course of the experiment, treated specimens exhibited a consistently high level of fungal invasion, with colonization rates ranging from 60 % to 80 %. In the control plants, the growth of the fungus was not detectable.

After the 20th day from inoculation, the growth of begonia plants inoculated with *B. bassiana* intensified (Fig. 2A and 2B).

Inoculated plants showed a significant increase in leaf surface area by 50% and total roots area by 60% (Fig. 2A and 2B, 3A and 3B). Root squares under the Bb treatment were significantly greater than those in the control treatment assessed at 10-, 20-, and 30-days post-inoculation.

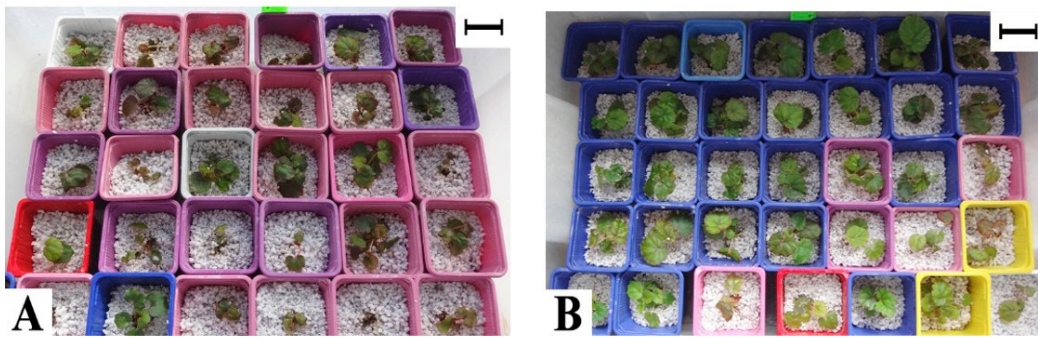


Fig. 2. Overall view of *Begonia rex* plantlets acclimatized on the sterile substrate for 25 days: Control treatment (A) and Treatment with *Beauveria bassiana* blastospore suspension (1×10^8 conidia mL⁻¹ conidia) (B). The scale bar is 3 cm.

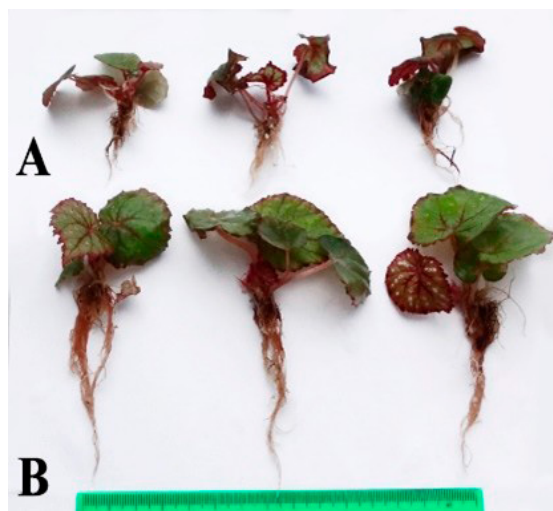


Fig. 3. Effect of inoculation with *Beauveria bassiana* on *Begonia rex* plantlets growth at 25-day post inoculation: The untreated control plants (A) and the plantlets treated with *Beauveria bassiana* (B). The scale bar is presented in millimeters.

It is worth noting that the growth of leaves inoculated with *B. bassiana* was much faster, and by the end of the experiment the surface area of leaves was 2.5 times larger than that of roots. In control samples, leaves also developed faster than roots (Fig. 3A and 3B, 4A, 4B, 4C and 4D). Therefore, the fact that leaf blade formation proceeds faster than root growth can be attributed to the species peculiarity of *B. rex*. It should be

noted that the plants treated with the fungus showed not only increased leaf growth, but also the most saturated leaf color, and the pattern characteristic of this species appeared at an earlier stage (Fig. 4A, 4B, 4C and 4D).

The results of the experiment showed that the development of plants inoculated with *B. bassiana* occurred at a significantly faster rate than in the control (Fig. 5).



Fig. 4. The promotion effect of *Beauveria bassiana* inoculation on the leaves size and color development of *Begonia rex* plantlets: leaves isolated from control plants at 25 days of experiment (A); from treated plantlets at 25 days of experiment (B); control immature plant at 90 days of experiment (C); treated immature plant at 90 days of experiment (D). The scale bar is presented in millimeters (A, B); 1 cm (C, D).

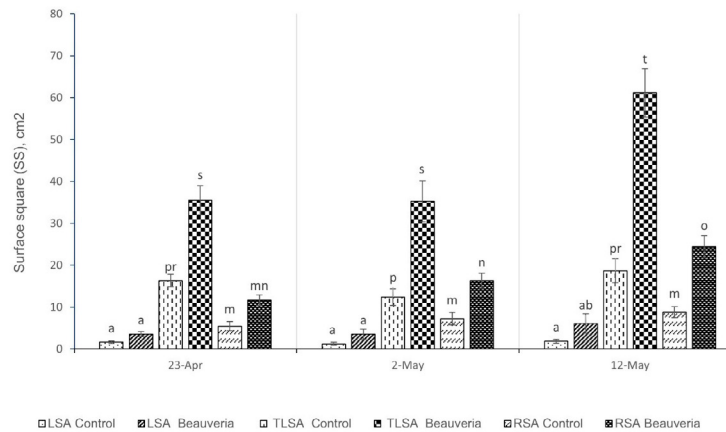


Fig. 5. Effect of *Beauveria bassiana* treatment on the growth-related parameters of *in vitro*-derived begonia plants at *ex vitro* acclimatization. Designations: LSA - leaf surface area per plant; TLSA - total surface area of all leaves per plant; TRA - total root area per plant. Data are mean \pm standard error (SE) of three replicates ($n = 30$). Mean values followed by different letters indicated significant difference at $p \leq 0.05$ according to Duncan's test.

Moreover, the growth rates of roots and leaf mass were not the same: significant differences in the growth of leaf mass were noted already on the 10th day until the end of the experiment, while the root system of inoculated plants began to grow rapidly starting from the 20th day.

The area of all leaves on one plant in the experiment was 50% larger than the area of leaves in the control. The root system of the experimental

plants also developed more vigorously than in the control and by the end of the experiment had increased threefold.

Under transplantation stress conditions, the fresh weight and relative water content increased significantly after *B. bassiana* colonization via root irrigation (Fig. 6).

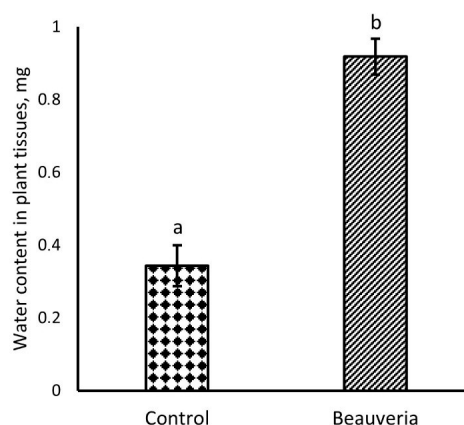


Fig. 6. The water absorption capacity of begonia seedlings during acclimatization *ex vitro* in control and with *Beauveria bassiana* treatments. Values are means \pm standard error (SE). Different letters above bars indicate significant differences between two treatments according to Duncan's test ($p \leq 0.05$).

It has been shown that *B. bassiana* can improve water uptake by begonia seedlings: inoculated plants contained 2-2.5 times more water at 30 day post-treatment (Fig. 6).

Discussion

In this study, the fungal colonization observed could be described as high throughout the experiment (up to 80%). *B. bassiana* had successfully penetrated and established within all plant organs, including roots, leaves and stem. Importantly, these colonization levels showed minimal fluctuation over the 30 days assessment period, indicating a stable, systemic association between the entomopathogenic fungus and plant. Thus, we suppose that the colonization of begonia plants was successful due to the concentration of fungal conidia and immature age of the plantlets.

Inoculated by the "substrate treatment" method, *B. bassiana* had positive effects on root and leaves area of seedlings, which is consistent with the findings of other studies showing that EPF could endophytically colonize and promote plant growth, both in monocots and in dicots (Mantzoukas and Eliopoulos, 2020). What is unique about this study is that while *B. bassiana* promoted begonia growth under nutrient-deficient conditions, typically the fungus treatment could enhance plant growth under nutrient-rich conditions. Thus, water absorption capacity of tomato plants which were placed in Hoagland nutrient solution was significantly higher than that of the control (Guo et al., 2024). A similar effect of *B. bassiana* was also observed on cotton seedlings where the water-holding capacity of the potting medium and the root length appeared to be positively impacted by the fungus inoculation (Mantzoukas et al., 2023).

An increase in leaf growth rate in Bb treatment leading to leaf area expansion obviously improved the transpiration rate in inoculated plants, and this effect was observed prior to increased root formation. Better plant growth during the early stages may result in an increase in overall plant biomass (Sui et al., 2023).

Previous studies have shown that *B. bassiana* can also act as an endophyte forming symbiosis with plants, subsequently transferring nutrients during habitation (Quesada-Moraga et al., 2023), significantly improving iron availability, chlorophyll content and water-holding root capacity of plants involved in both greenhouse and field experiments.

The water status of begonia plantlets during transplantation depends on the water loss by transpiration from the shoot and on water uptake by the root system of seedlings. It was remarkably higher ($p \leq 0.05$) in the fungus-treated plants than in the control plants, which obviously contributed to better overcoming of post-transplantation stress.

It is known that endophytic fungi can act as bio-stimulants, facilitating both the movement and absorption of nutrients, enhanced plant growth by modifying plant-host phytohormones, or producing their own ones, such as cytokinins, indoleacetic acid (Macuphe et al., 2021; Shah et al., 2022). Furthermore, Chen et al. (2020) reported that increased concentration of nutrient elements in leaves and roots is one of the possible mechanisms by

which endophytic fungus *Epichloë festucae* var. *lolii* enhances survival of *Lolium perenne* in low-fertility soil.

Thus, endophytic colonization by *B. bassiana* promotes growth-related parameters of *in vitro*-derived begonia plants at *ex vitro* acclimatization and can play a critical role in *Begonia* production. However, the underlying mechanisms of the positive effects in plants are largely unknown and require further elucidation.

Our results agree with the results of Liu et al. (2025) and, Thepbandit and Athinuwat (2024), supporting the proposal that endophytic fungus enhanced the capacity of plants to tolerate stressful environmental conditions, namely water and nutrient deficiencies along the transplantation *ex vitro* stage.

Conclusions

B. bassiana in this study successfully colonized the begonia plantlets inoculated with its conidia during *ex vitro* transplantation and improved both growth characteristics and plantlets adaptation. Also, it was noted that the greatest decorative qualities are exhibited by plants inoculated with *B. bassiana*.

Overall, our results clearly demonstrated that inoculation with *B. bassiana* during *B. rex* *ex vitro* acclimatization is beneficial for growers wishing to produce high quality plants.

Acknowledgments

The work was carried out with the financial support of the government-funded projects of the Central Siberian Botanical Garden (CSBG) No AAAA-A21-121011290025-2 and AAAA-A21-121011290027-6 within the framework of the State Assignment. In this study USU 440534 "Collections of living plants indoors and outdoors" was used.

Author Contribution

TF: Conceptualization, Resources, Visualization, Investigation, Statistical Analysis, Writing - Original Draft. **AN:** Conceptualization, Methodology, Visualization, Investigation, Statistical Analysis. **MT:** Conceptualization, Methodology, Visualization, Investigation, Writing - Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All the research data is contained in the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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