Fast Growing Nitrogen-Fixing Bacteria Engineered for Seedling Production Applied at Post-Mining Restoration Areas

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Abstract

Ecosystem restoration following bauxite mining is hampered globally by soil degradation that limits vegetation reestablishment. Our study investigated innovative strategies to promote reforestation by applying of nitrogen-fixing bacteria in seedling cultivation. These bacteria engage in symbiosis with plants, enhancing soil fertility and viability in degraded environments. The research was conducted using the seedling of *Clitoria fairchildiana*, known for its rapid growth. We examined its response under three conditions: (1) control with no inoculation, (2) inoculated with nitrogen-fixing bacteria, and (3) inoculated with bacteria supplemented with sugar. The addition of sugar aimed to provide an immediate energy source to support the symbiotic process. Seedling growth was measured in height and stem diameter. The bacteria inoculation combined with sugar resulted in the tallest seedlings, with a maximum height of 85 cm, compared to 80 and 60 cm for inoculation without sugar and the control group. Moreover, seedlings from the sugar-enhanced inoculation group exhibited a six-fold higher number in maximum stem diameter, peaking at 6 cm versus 1 cm in the control group. These growth parameters are indicators of a seedling's potential for survival and stability, suggesting successful adaptation and nutrient utilization. The pronounced improvements with bacterial inoculation, particularly with added sugar, point out to be a potential method for accelerating the restoration of vegetation on bauxite-mined lands. This research underscores the advantages of integrating nitrogen-fixing bacteria in reforestation, presenting a sustainable process to improve the rehabilitation. These findings contribute critical insights to the field of sustainable land restoration and ecological recovery.

Keywords: Sustainable bauxite mining, Nitrogen-fixing bacteria, Reforestation, Rehabilitation, Seedling production.

1. Introduction

Open-pit mining can considerably alter the environment since it requires the removal of the vegetation along with the most fertile topsoil and the subsurface layers above the minerals [1]. For bauxite extraction, this disruption can be enhanced since the mining progresses horizontally throughout the terrain.

After bauxite extraction and soil reshaping, the land surface undergoes a dramatic transformation, leaving behind a mixture of soil from the original B and C horizons, known as overburden. This soil is deficient in nutrients and organic matter [2]. These physical changes and nutrient deficiencies can severely hinder vegetation reestablishment and growth [3–5]. Therefore, it is imperative to invest in research and develop strategies to restore vegetation on post-mining soils, ensuring effective and cost-efficient forest restoration.

In Brazil, Embrapa, the state-owned agricultural research company, has been studying the symbiotic relationship between forest species and nitrogen-fixing bacteria (NFB) for over 30 years [6,7]. This approach offers several advantages, including reduced planting costs and improved seedling survival in low-fertility and drought conditions [8, 9]. It also enhances water and nutrient absorption, particularly phosphorus [10], boosts disease resistance [11], and facilitates biological nitrogen fixation, thereby reducing the reliance on chemical nitrogen fertilizers [12].

In this context, a partnership between Hydro Paragominas and Embrapa conducted a study to evaluate the growth of seedlings after nitrogen-fixing bacteria inoculation.

2. Materials and Methods

The study was conducted in two locations. The laboratory experiments were conducted at Embrapa Agrobiology, located at Seropédica, State of Rio de Janeiro, Brazil. Nursery experiments were conducted at Hydro Paragominas, located at Paragominas, State of Pará, Brazil. Hydro's nursery is located inside the mining site, and it can produce over 180 000 seedlings per year from more than one hundred native species. The region's climate is classified as "Aw" according to the Köppen-Geiger classification, characterized as hot and humid with well-defined rainy and dry seasons. The average annual temperature is 26.3 ºC, with an average annual relative humidity of around 81 % [13].

2.1 Selection of the Seedling Species

First, Embrapa conducted a floristic survey in the conserved forest fragments, areas in rehabilitation, and the seedling nursery of Hydro Paragominas to identify species capable of associating with nitrogen-fixing bacteria. In Paragominas, 37 floristic samples were collected, and nodulation was observed in ten species: *Bowdichia nitida, Campsiandra laurifolia, Clitoria fairchildiana, Enterolobium maximum, Inga edulis, Inga laurina, Inga capitata, Inga splendens, Ormosia paraensis* and *Samanea tubulosa*. The collected nodules were brought to Embrapa's laboratory in Rio de Janeiro, Brazil, where the bacteria were isolated. Along with the collections, geographic coordinates were recorded, and the parent plants were marked for seed collection and subsequent seedling production in the nursery (Figure 1).

Clitoria fairchildiana from the Fabaceae family was the first species to be chosen for a nursery experiment. *Clitoria fairchildiana* is a fast-growing tree [14], commonly known as sombreiro, palheteira and butterfly pea. It is found in the states of Amazonas, Pará, Maranhão and Tocantins, in Brazil's Amazon rainforest. *C. fairchildiana* is used for reforestation [14], as it can grow in substrates consisting solely of Yellow Latosol [15]. Additionally, *Clitoria fairchildiana* was identified as a key species for seedling planting at the Hydro Paragominas site due to its high incidence in areas under rehabilitation [16].

was lower than the treatments without inoculation and with bacterial inoculation without sugar. This result can be explained by the high genetic variation found in forest tree species; an intrinsic trait that serves as raw material for adaptation to the ever changing and variable natural environments in which these species reproduce [23].

At Hydro Paragominas alone, there are more than fifteen *Clitoria fairchildiana* parent trees from which the seeds used in this experiment were collected. Therefore, it may be essential that, in addition to the use of nitrogen-fixing bacteria, a genetic material selection process occur to meet the reforestation objectives.

4. Conclusion

The pronounced improvements in seedling growth with bacterial inoculation, particularly with added sugar, make it a potential method for accelerating vegetation restoration on bauxite-mined lands. This research underscores the advantages of integrating nitrogen-fixing bacteria in reforestation efforts, presenting a sustainable method to improve rehabilitation in post-mined lands. These findings contribute critical insights to sustainable land restoration and ecological recovery with gains in productivity and cost reduction.

5. References

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