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Vegetative propagation by mini-cuttings of Brazil nut "*Bertholletia excelsa* Bonpl" with the aid of rhizobacteria mixes

Propagação vegetativa por miniestacas de Castanha-do-Brasil "Bertholletia excelsa Bonpl" com auxílio de mixes de rizobactérias

Propagación vegetativa por miniesquejes de nuez de Brasil "Bertholletia excelsa Bonpl" con la ayuda de mezclas de rizobacterias

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Júlio Cézar Delfino Ribeiro
ORCID: https://orcid.org/0009-0006-1219-5185
Universidade Federal do Amazonas, Brasil
E-mail: jubeiro@gmail.com
Rogério Eiji Hanada
ORCID: https://orcid.org/0000-0002-4544-4882
Instituto Nacional de Pesquisa da Amazônia, Brasil
E-mail: rhanada.inpa@gmail.com
Suely de Souza Costa
ORCID: https://orcid.org/0000-0006-2718-0016
Instituto Nacional de Pesquisa da Amazônia, Brasil
E-mail: sscosta@inpa.gov.br

Abstract
The aim of this work was to analyze the vegetative propagation of *Bertholletia excelsa* through the mini-cutting technique, with the aid of rhizobacteria mixes. The methodology used was based on the collection of roots of *B. excelsa*, from which rhizobacteria were bioprospected. From them we formed mixes, for inoculation of mini-cuttings in the plantings in tubes containing commercial substrate, after 90 days they were dug up to collect the data. The experiment was conducted in a completely randomized design, consisting of 6 treatments (5 mixes and controls) with 6 replications, with 1 mini-cutting for each replication. The data were made available in an excel table. The variables analyzed were: survival, callus formation, formation and length of mini-cutting roots. According to MINITAB, the survival of mini-cuttings treated with rhizobacteria x controls presented a significant difference by the KRUSKAL-WALLIS test (p-0.01), for the other variables there was no significant variation. The MAO5PB mix, consisting of MAOPB12A, 12K, 12L, 12J and 12C bacillus, through qualitative analysis, was selected as the most productive and the 5 bacteria that constitute it were molecularly identified: PB12A as *Bacillus wiedmannii*; 12K as *B. paramycoides*; 12L as *Lysinibacillus fusiformis*; 12J as *B. paramycoides* and 12C as *Lysinibacillus fusiformis*. This work allows to affirm that it is possible to propagate *B. Excelsa* through the minicutting technique helped by rhizobacteria and contribute to reduce the pressure in the forest, with recovery of degraded areas, increase commercial production and support small producers in the *B. Excelsa* wood and nut production chain.
Keywords: Prospecting; Inoculation; Brazil nut.

Resumo
O objetivo deste trabalho foi analisar a propagação vegetativa de *Bertholletia excelsa* através da técnica de miniestaquia, com auxílio de mixes de rizobactérias. A metodologia utilizada, se deu a partir da coleta de raízes da *B. excelsa*, destas foram bioprospectadas rizobactérias. Delas formamos os mixes, para inoculação de miniestacas nos plantios em tubetes contendo substrato comercial, depois de 90 dias foram desenterradas para se levantar os dados. O experimento foi conduzido em delineamento inteiramente casualizado, constituído de 6 tratamentos (5 mixes e testemunhas) com 6 repetições, sendo 1 miniestaca para cada repetição. Os dados foram disponibilizados em tabela no excel. As variáveis analisadas foram: sobrevivência, formação de calos, formação e comprimento das raízes das miniestacas. De acordo com o MINITAB, a sobrevivência das miniestacas tratadas com rizobactérias x testemunhas apresentaram diferença significativa pelo teste de KRUSKAL-WALLIS (p-0,01), nas outras variáveis não houve variação significativa. O mix MAO5PB, constituído pelos bacilos MAOPB12A, 12K, 12L, 12J e 12C, através de análise qualitativa, foi selecionado como o mais produtivo e as 5 bactérias que o constituem foram identificadas molecularmente: PB12A como *Bacillus wiedmannii*; 12K como *B. paramycoides*; 12L como *Lysinibacillus fusiformis*; 12J como *B. paramycoides* e 12C como *Lysinibacillus fusiformis*. Este trabalho permite afirmar que é possível propagar *B. Excelsa* através da técnica de miniestaquia auxiliada por rizobactérias e contribuir para diminuir a pressão na floresta, com a recuperação de áreas

degradas, aumentar a produção comercial e favorecer os pequenos produtores na cadeia de produção de madeira e castanha da *B. Excelsa*.
Palavras-chave: Prospecção; Inoculação; Castanha-do-Brasil.

Resumen
El objetivo de este trabajo fue analizar la propagación vegetativa de *Bertholletia excelsa* mediante la técnica de mini esquejes, con la ayuda de mezclas de rizobacterias. La metodología utilizada se basó en la colecta de raíces de *B. excelsa*, a partir de las cuales se bioprospeccionaron rizobacterias. A partir de ellos formamos mezclas, para la inoculación de mini esquejes en las siembras en tubos que contenían sustrato comercial, a los 90 días se desenterraron para recolectar los datos. El experimento se condujo en un diseño completamente al azar, que constó de 6 tratamientos (5 mezclas y testigos) con 6 repeticiones, con 1 mini esqueje por cada repetición. Los datos, en un universo de 36 unidades de investigación, fueron puestos a disposición en una tabla de excel. Las variables analizadas fueron: supervivencia, formación de callos, formación y longitud de raíces en mini esquejes. Según MINITAB, la supervivencia de mini esquejes tratados con rizobacterias x testigos mostró diferencia significativa por la prueba de KRUSKAL-WALLIS (p-0.01), para las demás variables no hubo variación significativa. La mezcla MAO5PB, compuesta por los bacilos MAOPB12A, 12K, 12L, 12J y 12C, mediante análisis cualitativo, se seleccionó como la más productiva y se identificaron molecularmente las 5 bacterias que la constituyen: PB12A como *Bacillus wiedmannii*; 12K como *B. paramycoides*; 12L como *Lysinibacillus fusiformis*; 12J como *B. paramycoides* y 12C como *Lysinibacillus fusiformis.* Este trabajo de investigación permite afirmar que es posible propagar *B. excelsa* a través de la técnica de mini esquejes ayudado por rizobacterias y, contribuir a reducir la presión en el bosque, con la recuperación de áreas degradadas, aumentar la producción comercial y favorecer pequeños productores en la cadena productiva de madera y frutos secos de *B. Excelsa*.
Palabras clave: Prospección; Inoculación; Nuez de Brasil.

1. Introduction

The Brazil nut tree, *Bertholletia excelsa*, is a tree that reach 60 m of height and trunk diameter with around 5 meters (Salomão, 2014). The Brazil nut tree may have as the center of origin the southeastern Amazon rainforest (Mori & Prance, 1990). Its fruit is of great importance, with the chestnut being the only internationally traded seed, native of natural forests (Clay, 1997). Called vegetal meat, the fruit of *B. excelsa* presents twice the protein level compared to a beef steak, and it has a high protein, calorie and mineral content Shanley et al. (2010). As for wood, it is a good source of cellulose, suitable for civil and naval construction, in addition to resisting attacks by xylophagous organisms (Carvalho, 2012).

B. excelsa is distributed throughout the Amazon region, with greater evidence in the Brazilian Amazon, in the states of Pará, Amazonas, Acre, Rondônia, Roraima, Tocantins, north of Goiás, north of Mato Grosso and outside Brazil, the species is found in Peru, Bolivia, Venezuela, Guyana and Colombia (Müller *et al*., 1995; Mori & Prance, 1990).

The chestnut production presents its highest concentration in the North Region, representing 95.9% of what Brazil produces, where we have Acre with 35.5%, Amazonas with 30.8% and Pará with 23.5%, it is important to note that today, only about 2% of national production comes from cultivated areas (Maués *et al*., 2015). Due to non-commercial production, Brazil has lost its hegemony in the sale of nuts to Bolivia, and there was no solution yet to the problem of supplying this product (Homma *et al*., 2014), as domestic prices satisfy extractivists and commercial producers. In this context, single or mixed inoculations of plants growth-promoting bacteria can stimulate production growth. (Canellas *et al*., 2015).

Vegetative propagation by mini-cuttings with the aid of rhizobacteria can stand out as an alternative in the propagation of this species. In previous years, studies led to the development of vegetative propagation techniques known as mini-cuttings – a technique that consists of collecting plant shoots from rooted cuttings, by traditional method of cuttings or seminal seedlings (Andrejow, 2006; Xavier & Silva, 2010). At the moment, studies show the importance of using rhizobacteria in vegetative propagation and indicate the use of bacilli as the most widespread. PGPRs (Plant Growth-Promoting Rhizobacteria) prove to be a technology that can increase the rooting rate, as well as growth and biological control. (Raasch *et al*, 2013). Research with bacteria of the genus *Bacillus* has recognized that this microorganism is important in the biological control of diseases, the species of *Bacillus* sp. as plant growth-promoting rhizobacteria has been quite interesting. (Araujo, 2012).

The rhizosphere is an area where interactions among soil, microorganisms and plants happen, therefore, the zone of roots influence (Mafia *et al*., 2007). Since, its delimitation goes from the surface, of the root, to a distance from 1 to 3 mm, and that this compartment receives, from the roots, exudates of low concentration, allowing microbial communities to keep there and be influenced by the abiotic and biotic conditions (Moreira & Siqueira, 2006).

The rooting index infers the economic viability of the seedling nursery, besides the mini-cutting depends on the following factors: temperature, humidity, chemical and physical composition of the substrate, fertilization, luminosity, photoperiod, youth of the shoots, the position of the shoot in the cuttings, diameter of cuttings, the presence of buds and/or leaves, effect of the period of collection of cuttings and treatment and/or conditioning of shoots and cuttings before cutting. However, these factors can be managed, taking into account the genetic material produced, specifically, and the need for management, aiming at maximizing the productivity (Fernandes *et al*., 2018).

So, the mini-cutting technique can be an alternative for the commercial production of forest seedlings, especially for those who have difficulties in rooting the adult material. In addition, it can be noted that mini-cuttings are advantageous in terms of reducing rooting time (Ferriani, 2010). According to Fernandes *et al*., (2018), the major concern regarding the production of seedlings is the rooting rate of mini-cuttings.

Plant Growth-Promoting Rhizobacteria (PGPR) naturally colonize the interior and exterior of plant organs, they are considered epiphytic or endophytic plants and are not phytopathogenic. They promote an important increase in the roots surface area, increasing a greater efficiency in the removal of water and micronutrients by the plants (Fan *et al*, 2011; Silveira, 2008).

The PGPRs are influenced by plants and soil type, depending on the exuded organic compounds, which determine the growth and development of plants, mainly in the fixation of atmospheric nitrogen; solubilization of mineral compounds such as phosphorus; production of siderophores (iron chelators); production of hormones that regulate plant growth, such as auxin, gibberellin, cytokinin and ethylene; and secondarily, through the antagonism to phytopathogenic organisms such as fungi, viruses and nematodes, the induction of systemic resistance against diseases (Moreira & Siqueira, 2006; Garcia *et al*., 2015).

The PGPRs produce phytohormones (plant hormones): auxins, especially in this group indole acetic acid (IAA), gibberellins and cytokinins in plant development. (Karadeniz *et al*., 2008; Ashrafuzzaman *et al*., 2009) assert that in the rhizosphere 80% of isolated bacteria are able to produce IAA. In this same context, Kuss *et al*. (2007) described that among plant growth substances produced by bacteria, auxins, the indoleacetic acid (IAA), are the most active and best characterized, while IAA produces increased cell elongation (fast response) and cell division and differentiation (slow response), bacteria associated with the root of plants are more efficient in the production of auxins than non-associated ones. Among auxins, IAA is the most studied and they are the most produced by the bacteria.

Thus, plant hormones are substances produced by plants that, in low concentrations, act in growth and development processes, and are produced as endogenous signals. Furthermore, hormonal regulation takes place through interactions and collective modulations between hormonal systems (Amaral, 2011), for example: depending on the relationship between cytokinin and auxin, there is budding from aerial part or root development. Cutting is the most common vegetative propagation technique, however it presents poor quality seedling development in its root system. PGPRs prove to be a technology that can increase the rooting rate, as well as growth and biological control (Raasch *et al*., 2013).

All in all, the objective of this work is to verify the viability of rooting of *B. excelsa* cuttings, using vegetative propagation, through the mini-cutting technique with the aid of rhizobacteria mix (bacilli), which will contribute to the production of seedlings for the recovery of degraded areas, chestnut production and wood in commercial production, reduce the pressure on the forest and promote the inclusion of small producers in the chestnut and wood production chain.

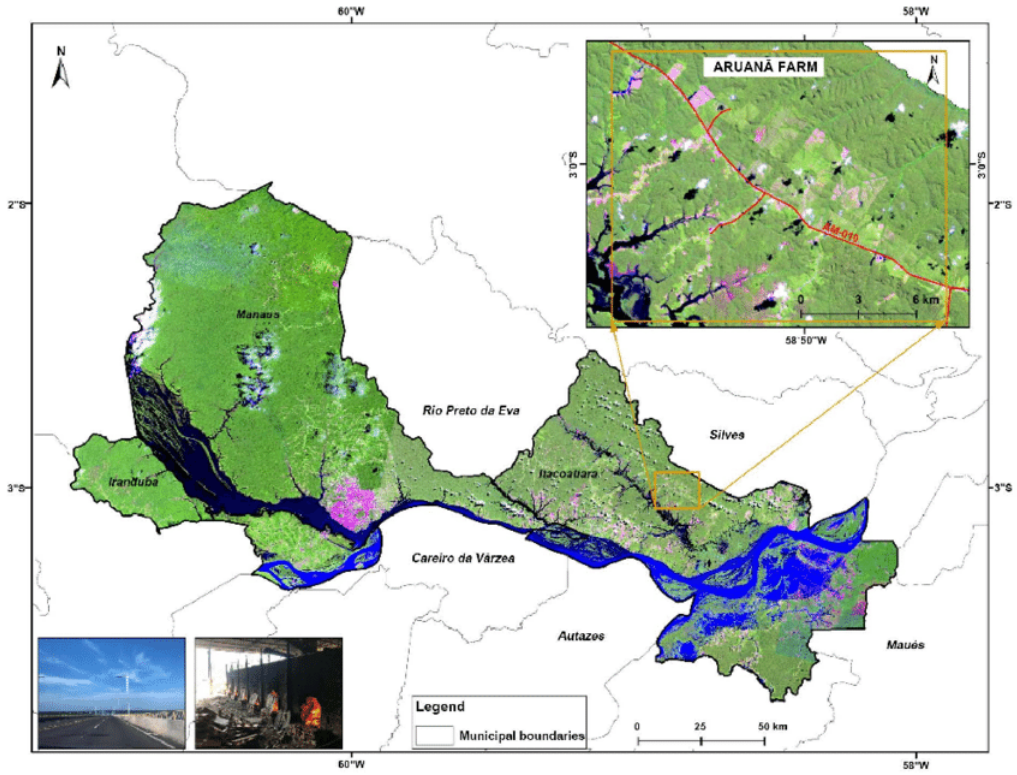
2. Methodology

Due to this work of vegetative propagation, of *B. excelsa*, through the mini-cutting technique with the aid of rhizobacteria mix, being the first, a descriptive research was carried out with a demonstrative experimental technical procedure and data collection, adapted from Gil, 2008, mainly taking into account root formation, according to Stork *et al*., 2000, demonstrative experiments serve to analyze the viability of a technical procedure without the need for statistical analysis, however, statistical analysis was also performed.

The isolation of *Bacillus* spp. was carried out in the phytopathology laboratory of the National Institute for Research in the Amazon (INPA), in Manaus, AM. The experiments with the plants were carried out in a greenhouse with 50% shading and in the Seedling Nursery of the Biodiversity Coordination (COBIO), of the National Institute for Research in the Amazon (INPA), Campus III (V8) in Manaus, AM (3° 08' S and 60° 01' W). The nursery was covered with a 70% shading screen; the floor was covered with pebble; and irrigation was done by intermittent nebulization controlled by an evaporation scale. Molecular identification of the bacteria was carried out in the molecular biology laboratory of Embrapa of Western Amazon.

Young specimens (seedlings) – 18 months old – of *B. excelsa*, for prospecting of rhizobacteria, were obtained in the Forest Garden of Manaus-AM City Hall, and the seedlings – 4 months old –, for the extraction of mini-cuttings, were obtained on the Aruana farm, located in the municipality of Itacoatiara-AM. Figure 1. All seedlings were initially kept in the greenhouse.

Figure 1 - Location of Aruana Farm situated on Itacoatiara – AM.



Source: Alves *et al. (2020).*

Aruanã Farm was established in 1970 at 213 KM of Manaus – Itacoatiara Highway (AM-010). Among other activities, the farm produces high quality Brazil nut seedlings.

2.1 Isolation of rhizobacteria

From the seedlings, obtained from the Forest Garden of Manaus-AM City Hall, ten of them had their roots extracted and taken to the laboratory where they were washed, and the soil adhered to the roots, in up to 3 mm, was maintained. They were fractionated and placed in an Erlenmeyer flask with 50 mL of sterilized saline solution (00.01M MgSO47H2O). The solution and the parts of the root with soil, inside the Erlenmeyer flask (250 mL), were subjected to 30 minutes of agitation in a mechanical shaker and the solutions were subjected to a thermal shock of 80ºC/20 minutes in an oven, to select the genus *Bacillus*. Samples of 1mL removed from the flask were submitted to serial dilutions in test tubes with 9 mL of sterile distilled water, which were carried out until 10^{-8}, and 100 µL of the last dilution were inoculated in Petri dishes (14 cm in diameter). The plating was carried out with 0.1 mL aliquots, collected in test tubes in NA medium (nutritive agar), with incubation of 96 hours, in an oven at 28º C. Colony-forming bacteria were considered belonging to the genus *Bacillus*. They were conserved in Castellani, until their use, at the time of inoculation, identification and conservation.

2.2 Collection of shoots and preparation of mini-cuttings

Part of the *Bertholletia excelsa* seedlings (36 seedlings) obtained, with 20 to 25 cm in height, had their apical shoots pruned to cause lateral shoots, from which the mini-cuttings were removed. Shootings were collected in the morning with pruning shears previously disinfected in 70% ethanol (v/v). The mini-cuttings were prepared using the apical part of the shoots, with dimensions between 4 and 8 cm, containing one, two or three leaves, adapted from (Titon *et al*., 2003).

Bacterial suspensions (rhizobacteria mix) were applied to the mini-cuttings, with immersion of the base of the mini-cuttings for a period of approximately one hour, for proper inoculation. These suspensions were prepared as follows: a) all the bacteria were grown on NA medium (Nutrient Agar); b) remained in B.O.D. for 48 hours; c) after 48 hours, scraping was performed in saline solution (0.85% NaCl) and placed in an Erlenmayer flask. One concentration was not diluted and it was measured with a spectrophotometer (2.52 Abs at 54nm), the other concentrations were diluted to 10^{-8}. Then they were placed under refrigeration (refrigerators) until the moment of use.

After the application of suspensions of bacterial isolates, the mini-cuttings were cut (2 cm deep) in 110 cm3 tubes containing commercial substrate VivattoSlim Pro 10 (a mixture of bio-stabilized pine bark, vermiculite, charcoal powder, phenolic foam and water), without adding fertilizer) was properly sterilized. The tubes and trays used were previously washed with neutral detergent and disinfected in hot water at 83°C for 30 seconds (Alfenas *et al*., 2009). The tubes were deposited in plastic trays and taken to a greenhouse regulated with 70% luminosity, with maintenance of humidity via intermittent nebulization, controlled by an evaporation scale. After 90 days, survival, rooting and callus formation were analyzed.

2.3 Molecular identification of bacteria

Molecular identification was performed according to protocols used in the molecular biology laboratory of EMBRAPA of WESTERN AMAZON in Manaus-AM. The bacteria, which were preserved in liquid medium (Castelani Method), were inoculated (0.1 mL) in LB liquid culture medium (5 mL) for identification.

Molecular identification was carried out according to the following steps: a) genomic DNA extraction (breaking of the cell membrane – lyse, separation of DNA from impurities, proteins, RNA), DNA quality and quantity analysis by NANODROP 1000, the quality was considered good, concentration (ng/µL with purity degree between 1,8 and 2,0); b) PCR for amplification of the 16S rDNA gene (conventional PCR, where the reactions of the gene amplification were executed in a thermocycler and the reactions of amplification, with the following primers: Primer 8F – 5` AGA GTT TGA TCC TGG CTC AG-3` (t) and Primer 1401R 5` CGG TGT GTA GGC GGA ACG-3`(f); c) purification of PCR samples (the amplified 16S gene fragments were purified using Kit, according to the protocol, soon after, they were evaluated by running them in an electric field on a 1.5%

agarose gel, stained with ethidium bromide for visualization and documentation; d) sequencing reaction (Sequencing performed by the -3500 sequencer – Genetic Analyzer (HITACHI); e) Molecular identification (done with the UGENE program and the BLAST tool).

3. Results and Discussion

3.1 Isolation of bacilli

From 50 plants, obtained from the forest garden of Manaus-AM City Hall, aged around 18 months and height of 1.5 meters (average), roots of 10 plants were extracted and taken to the laboratory where 68 colony forming bacteria were isolated and were previously considered to belong to the genus *Bacillus*.

In world agriculture, plant growth promoter microorganisms are widely used, however in the area of forest production this almost does not happen, nevertheless there are reports of success in the clonal production of hybrids of *E. urophylla x E. grandis* (Teixeira *et al*., 2007). However, the inoculation of rhizobacteria in forest species can provide considerable gains in plant development, in the rooting and growth of eucalyptus, two isolates of *Bacillus subtilis*, S1 and 3918, selected, were effective for rooting (Mafia *et al*., 2007)Also, in studies on the isolation and characterization of bacteria in Brazil nut rhizospheres in northern Brazil, it was concluded that among the 90 isolates of rhizobacteria, 22 of them showed favorable characteristics for promoting plant growth and can be used in production of Brazil nut seedlings (Chalita *et al*., 2019), the results of this work were isolated from 68 bacteria considered bacilli, in comparison the similarity of results can be extended. Also, in 10 locations in the western region of the State of São Paulo, a group of 127 bacterial isolates belonging to the genus *Bacillus* were prospected from the eucalyptus rhizosphere, all of which were considered positive for auxin production and 15 presented potential to promote growth (Moreira & Araújo, 2013), yet, in studies to verify the possibility of rhizobacteria acting as growth promoters of citrus plants, in Paraná, 30 rhizobacteria were prospected in seedling nurseries and in the field, 13 of which were of the genus *Bacillus*, concluded that bacterial isolates, including *Bacillus*, can act as growth promoters of citrus plants (Freitas & Aguilar Vildoso, 2004). Also, they bioprospected bacteria of the genus *Bacillus* in the rhizosphere of *Brachiaria brizantha* and concluded that they can promote plant growth when previously inoculated in seeds (Araujo *et al*., 2012), interesting data, which are similar to the data found in this work.

In other experiments (Rodrigues, 2018), 81 bacteria were isolated from sugarcane rhizosphere under organic management in nitrogen-free culture media, with the genus *Bacillus* being the second most found, these cellulase-producing microorganisms were widely distributed in the sugarcane rhizosphere. The data observed during this study demonstrate that many bacilli inhabit the rhizosphere of *Bertholletia excelsa*.

In some studies, bacterial isolates (bacilli) evaluated were considered positive for auxin production (Moreira & Araújo, 2013), information that reinforces the purpose of this work. In the same way (Rodrigues, 2018), the potential for inhibiting pathogens and the production of cellulases in rhizobacteria have important role in the penetration of these organisms into the host plant during colonization.

3.2 Collection of shoots and preparation of mini-cuttings

The decapitation of the apical shoots caused a break in dominance and production of lateral shoots. Dominance is the control exercised by the apical shoots over the lower axillary buds (Cline, 1991), the axillary buds of the ministumps assume dominance over the lower ones (Mantovani, 2017). In this experiment with *B. excelsa*, each pruned plant put forth a sprout.

At 15 days of decapitation, all the plants started sprouting, after 15 to 17 days, the shoots had dimensions from 5 to 8 cm and were cut.

3.3 Application of bacterial isolates and cutting

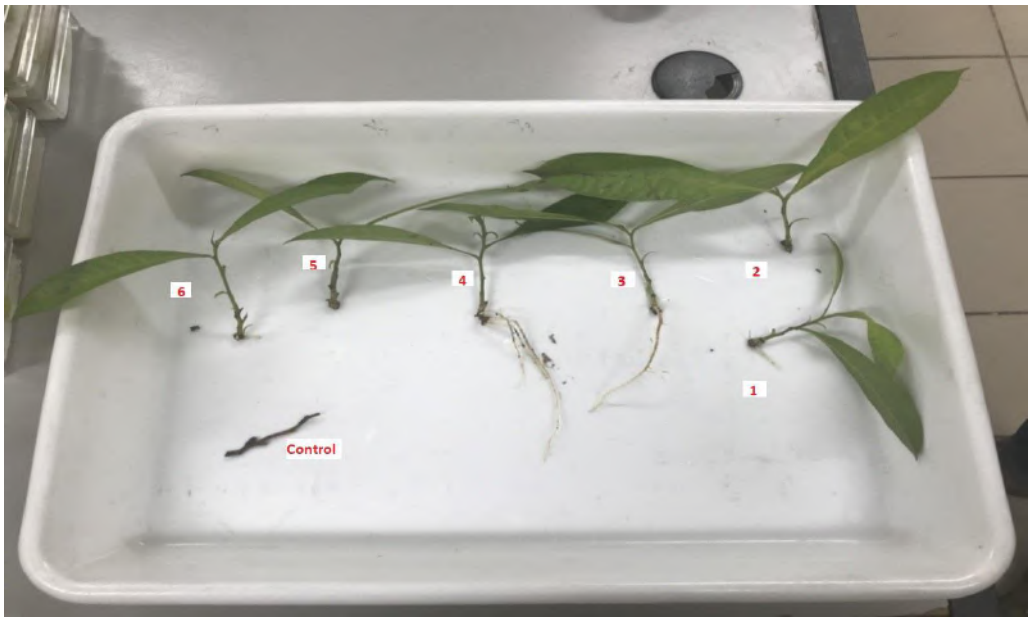
The bacilli mixes were inoculated into mini-cuttings to verify rooting, callus formation and survival. In the rooting verification, the root length was obtained by measuring with a ruler. The results are shown in Table 1. Qualitatively, the MAO5PB mix was considered the most productive mix in Figure 2.

Table 1 - Data obtained from the treatment with rhizobacteria mixes inoculated in mini-cuttings of *B. excelsa* in Manaus-AM in the year 2020.

Mix	Nº of bacteria	SB(%)	RF(%)	N/LR(cm)	CF(%)
MAO2PB	12	100	50,0	1/3 1/13-4 1/17	100
MAO5PB	05	100	83,3	1/1 1/0,5 1/8-9-15* 1/11 1/1,5-1-3	100
MAO3PB	19	100	16.6	1/8* 1/6	100
MAO22PB	11	100	83,3	1/1 -1 1/2-1,5 1/1,5-2 1/3,5 1/14,5*-2,5-3	100
MAO4PB	6	100	66,6	½ 1/2-1,5 1/3-3-1-2-2 ¼ - 4,5 - 2	100

*With root primordia – SB: Survival of mini-cuttings – RF: Root formation – N/LR: Number of roots/root length – CF: Callus formation. Source: Authors (2020).

Figure 2 - MAO5PB mix chosen among the 5 treated with rhizobacteria mixes.



Source: Authors (2020).

This photo illustrates the six cuttings that were treated with MIX MAO5PB and the control. About rooting, 05 cuttings have formed roots, where, from right to left: Cutting 1 had a root of 1 cm, Cutting 2 had a root of 0.5 cm, Cutting 3 had 3 roots,

one with 8 cm, another with 9 cm and one with 15 cm, all with root hairs, Cutting 4 had a root with 11 cm, with root hairs, Cutting 5 had no root, but had callus, Cutting 6 had 3 roots, one with 1.5 cm, another with 1 cm and the other with 3 cm. About callus formation, all of them presented callus, with exception of control cutting. In terms of survival, all cuttings survived, except control cutting. The control cutting (in the center of the image) did not form roots or calluses, it did not survive (necrized).

The present work has showed the survival of all (100%) mini-cuttings treated with rhizobacteria; studies on ipe-roxo (*Handroanthus heptaphyllus*), the survival of treated mini-cuttings was greater than 84% (Oliveira *et al*., 2016); in other experiments it was 100% (Mantovani *et al*., (2017); and in studies with *Eucalyptu sglobulus*, it was 91% (Borges *et al*., (2011),

All mini-cuttings in this study formed calluses. They are formed as a result of the cuttings, the plant responds to the stress it has suffered, initiating a regeneration through disordered meristematic cells. The tissue formed would be the scar tissue, which formation is independent of roots formation and which serves as a barrier against microorganisms, (Fachinello *et al*., 2005).

Of the 30 mini-cuttings inoculated with a rhizobacteria mix, in a commercial substrate, the relative rates of root formation ranged from 16.6 to 83.3%, bearing in mind that the substrate did not contain fertilizer, unlike the fertilized experiments, which reached rates from 18.8 to 95.8% root development of mini-cuttings of *Anadenanthera macrocarpa* (Angico-vermelho) (Dias *et al*., 2015); rooting experiments of guarana tree cuttings, without fertilization, measured a percentage of rooting, from 6.25 to 73.75% (Albertino *et al*., 2012). In studies on the rooting of cuttings using fertilizer in *Eucalyptus globulus*, root formation rates ranged from 25.0 to 100.0% (Borges et al., 2011). controls), In this study, reported here, with regard to the mini-cuttings (controls), which were treated with distilled water, they did not survive, they became necrotic.

3.4 Molecular identification

In the molecular identification of the most productive mix – MAO5PB, the following rhizobacteria were found: MAO5PB12-A – *Bacillus wiedmannii*;12K – *Bacillus paramycoides*;12L – *Lysinibacillus fusiformis*; 12J – *Bacillus paramycoides* and 12C – *Lysinibacillus fusiformis*.

About *Bacillus wiedmannii*, it was suggested the inclusion of this bacterium, through the strain (spnov), in the important group of *Bacillus cereus* (Miller *et al*. 2016). Epiphytic isolates of *B. cereus* (C210) showed antibiosis against *Xanthomonas campestrispv*. Campestres (Luna *et al*., 2002); they reduced disease severity in the field by 78% in 'Midori' cabbage plants (Assis *et al*., 1997).

In studies on the mitigation of water stress in wheat, the bacilli *B. Paramycoides* and *B. paranthrais* played a crucial role in improving plant growth during stress and, simultaneously, ensuring soil fertility and health, these microorganisms were bioprospected from plant rizosphere in a state of water stress (Yadav *et al*., 2022). As for the rhizobacterium *Lysinibacillus fusiformis*, it was efficient in the degradation of aflatoxin B1, reaching aflatoxin detoxification rates of 61.3% (Adebo *et al*., 2017). Aflatoxin can attack plantations in their pre-harvest and post-harvest stages, what may cause economic losses (Patriarca & Fernández, 2017). In rhizosphere soils of halophytic plants, from saline environments, in three regions of Rio de Janeiro, the rhizobacterium *L. Fusiformis* was identified (Xavier, 2021), it is a soil bacterium that stands out.

4. Conclusion

The following results were obtained in this work: 100% mini-cutting survival, 100% callus formation and 16.6 to 83.3% root formation. Therefore, it is concluded that it is possible to vegetatively propagate *B. excelsa*, through the mini-cutting technique with the aid of mixes of rhizobacteria.

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