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Article

Rooting Conditions for Production of Guarana Clonal Seedlings

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Abstract: The guaraná tree is a species known for the stimulating properties found in its seeds, leading to increased consumption and promising production. Cultivation is primarily carried out in family farming systems, and one of the challenges in the production process is the acquisition of seedlings. The conventional nursery system, which employs intermittent misting for the production of guaraná clones, raises the cost of seedlings, necessitating the exploration of alternatives to this approach. The aim of this study was to evaluate different conditions as alternatives to the conventional nursery system for rooting guaraná cuttings. The experimental design was entirely randomized, featuring three guaraná genotypes, BRS Maués, BRS CG 611, and BRS Luzeia, alongside two rooting conditions, the conventional nursery and a humid chamber with nebulization, with four repetitions. The rooting of guaraná clones was significantly influenced by the interaction between conditions and genotypes. The humid chamber with nebulization demonstrated superior root system characteristics among the tested conditions, with a rooting rate of 56.66% for BRS Luzeia. This method proved to be efficient and accessible for small producers, making it a satisfactory option for rooting guaraná trees.

Keywords: *Paullinia cupana*; adventitious roots; seedlings; nebulization; cuttings

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1. Introduction

The guaraná tree (*Paullinia cupana* var. *sorbilis* (Mart.) Ducke) is native to the Amazon and has high economic potential due to the significant caffeine content in its seeds, which possess energizing properties [1–3]. Brazil is the only commercial producer of guaraná globally, meeting both national and international demand [4], with an annual production of 2732 tons of dry seeds and an average productivity of 271 kg ha^{-1} [5]. Guaraná is certified by the Biodynamic Institute Certification Association (IBD), adhering to international standards and gaining acceptance in American, European, and Asian markets, with a growth rate of approximately 20% per year [6]. Currently, 300 to 500 tons of guaraná are exported each year, representing about USD 15 million in revenue [7]. The crop is also socioeconomically important, as it is cultivated on small properties and is a typical family farming activity in the state of Amazonas.

The guaraná tree originated in the study region and is cultivated in 24 of the 62 municipalities in Amazonas by both small and large producers [5]. While Amazonas was once the world's largest producer of guaraná, issues related to management and seedling production have hindered the region's production potential. The increasing demand for guaraná and the labor involved in its production present opportunities for developing the state's agricultural and industrial sectors, especially in the municipality of Maués, where guaraná cultivation is the main agricultural activity, involving over 500 families directly. Most guaraná-producing communities in Amazonas can only be reached by river, complicating access to certified nurseries and increasing transportation costs, which also has an environmental impact.

Despite the importance of the guaraná crop, research on the management and propagation of the species is still limited, particularly regarding seedling production. Generally, the technologies and management strategies developed by relevant institutions are targeted primarily at producers with greater financial resources, leaving small farmers without access to new technologies and products generated by these institutions. For seedling propagation to be effective, the quality of the root system and subsequent plant development are critical factors in the success or failure of the guaraná seedling formation process. Although some research has been conducted on these factors [8–11], studies focusing on simple, accessible, and low-cost technologies for rooting conditions are scarce.

To produce quality clonal seedlings, Embrapa [12] recommends using a screen that provides 70% shading and intermittent misting, along with indole-3-butyric acid (IBA) at a dosage of 2000 ppm, hardwood or treated wood, pillars made from PVC pipes filled with concrete, oval galvanized steel wire, and an artesian well with a submerged pump. However, this system is costly due to the materials used and is incompatible with the realities of small producers in the Amazon, making its implementation challenging.

Therefore, research into more economical and efficient seedling production methods is necessary.

A simple system that provides quality water, called a rooting humidity chamber, has been tested and approved for various species [13]. The rooting humidity chamber with nebulization employs a cover to protect against sunlight, a wooden structure covered with five layers of blue anti-static plastic, and a misting system consisting of a motor pump and hoses with ten misting nozzles connected to a water tank and PVC channels. The materials required for this system are more economical to obtain, making it an efficient alternative for producing guaraná cuttings in Amazonas.

Implementing the humid chamber with nebulization for rooting guaraná cuttings will benefit farmers, who currently spend significant amounts on purchasing cuttings. The advantages extend beyond economic factors; producing their own cuttings enhances farmers' connection with their products, fostering a more profound relationship with nature in a sustainable and ecologically responsible manner.

The aim of this research is to evaluate the production of cuttings from three guaraná genotypes under controlled nursery and humid chamber conditions with nebulization, quantifying the rooting percentage of cuttings in different rooting conditions and relating the carbohydrate content in the roots of the cuttings to the rooting characteristics of the guaraná genotypes.

2. Materials and Methods

2.1. Area of Study

The experiment was conducted in the experimental field of Embrapa Amazônia Ocidental, km 29 of the AM-010 highway, latitude 02°55′ S and longitude 59°59′ W, in the municipality of Manaus, AM. The region's climate, according to the Köppen classification, is of the Af type: high average air temperature, with a monthly average of over 18 °C and high rainfall.

2.2. Experimental Delineation

The experimental delineation used was entirely randomized, in a 3 × 2 factorial arrangement, consisting of three guarana genotypes (BRS Maués, BRS Luzeia and BRS-CG 611) and two rooting environments (humid chamber with nebulizer and conventional nursery), with four replications. The experimental unit consisted of 15 herbaceous cuttings, totaling 360 cuttings, with 180 cuttings per rooting environment.

The criterion used to choose the genotypes was their rooting ability, according to previous exploratory research carried out into the production of guaranazeiro seedlings. BRS Maués is considered easy to root and is one of the two genotypes planted in 90% of the state. BRS-CG611 is classified as having medium-difficulty rooting and BRS Luzeia is considered difficult to root. These materials have already been evaluated, and are highly productive, resistant to pests and diseases, and therefore recommended for commercial planting in the state of Amazonas by Em-brapa Amazônia Ocidental.

2.3. Collection and Preparation of Cuttings

The material used and the following steps followed the recommendations of the Guarana Production System [12]. Black polyethylene bags measuring 23 × 18 cm, which were 0.15 mm thick and had 24 holes 5 mm in diameter to drain excess water, were used as a container for planting the cuttings. The substrate for filling the bags was composed of a mixture of forest soil and sand, in a 4:1 ratio. For every 1 cubic meter of the mixture, 3 kg of simple superphosphate was added. After filling the bags, a thin layer of sand (1 to 2 cm thick) was placed on the surface to prevent crusting and reduce the incidence of invasive plants [12]. The branches collected for the cuttings were in the vegetative stage, the period of intense growth of the guaranazeiro cuttings' donor mother plants, with no symptoms of nutritional deficiencies and/or incidence of pests and diseases, from Embrapa's clonal gardens. The branches were collected in the early hours of the morning in order to reduce water loss from the material to be propagated. After cutting, the branches were moistened and packed for transportation to the nursery, where the cuttings were made according to [12]. Each cutting had a pair of leaflets cut in half to reduce transpiration. Using a round, pointed piece of wood with a diameter similar to that of the cuttings, a central hole was made in the substrate to facilitate planting.

2.4. Conduction in a Humid Chamber with Nebulization

The humidity chamber consisted of a wooden structure, covered with blue plastic in five layers, antistatic, with protection against UVa/UVb rays, 120 microns thick, PAR light transmission: 88% and PAR light diffusion: 58% (Manufacturer: AGROESTUFA®, Birigui, São Paulo, Brazil. It contained a misting system made up of a motor pump and a hose with ten misting nozzles connected to a water tank. To prevent the temperature inside from exceeding 35 °C, the timer was activated by the motor pump and the misting irrigation promoted a mist inside the chamber, similar to the nursery conventional system. And inside the chamber, PVC pipers filled with water were set up, to which more water was added to keep the relative humidity constant inside the chamber as it was lost through evaporation.

2.5. Conduction in Conventional Nursery

In the conventional nursery, the cuttings were kept at room temperature, with irradiance reduced by 70% and under intermittent misting, controlled by an evaporation scale, where the surface of the leaflet leaflets was protected by a thin layer of water. The misting system worked in sync with the transpiration rate to avoid dehydration of the tissues, providing the necessary humidity to ensure the physiological processes of rooting the cuttings.

The cuttings were kept in different environments, and the temperature and humidity were recorded daily using a digital thermo-hygrometer.

2.6. Biometric Evaluations

After 90 days, the chamber was opened to assess rooting, and the cuttings in the nursery were also assessed at the same time. The cuttings were separated from the substrate by dispersing them in running water and shaking them by hand. The intact root system was obtained to assess the number of cuttings with callus (live cuttings with an undifferentiated cell mass at the base and no roots); the number of rooted cuttings (cuttings with at least one adventitious root formed); the number of dead cuttings (cuttings with necrotic tissues); the number of roots formed; root length; root volume; and weight of root dry matter.

All the roots were cut at the point of insertion and then counted and measured. The length was measured using a micrometer ruler.

The volume of the roots was measured by the displacement of water caused by inserting the roots into a measuring cylinder.

The weight of the dry matter was obtained by drying the roots in an oven at 70 °C until constant weight and then weighing them on a precision digital scale.

2.7. Laboratory Analysis

For the extraction of total soluble sugars, the root samples were ground, and 0.1 g of them were macerated with a pistil, adding 10 mL of a mixture of methanol–chloroform–water, in a ratio of 120:50:30, heated to 60 °C. The product of the maceration was quantitatively transferred to plastic tubes and centrifuged at 2000× *g* for 15 min. The maceration product was quantitatively transferred to plastic tubes and centrifuged at 2000× *g* for 15 min. The volume of the supernatants was recorded and they were stored under refrigeration. They were then partitioned in a separating funnel with 15 mL of chloroform to clarify the extract and remove the lipids.

Total soluble sugars were quantified using the anthrone reaction, according to the methodology recommended by [14]. Then, 0.1 mL aliquots were placed in screw-top test tubes, and the volume was topped up to 1 mL with distilled water. The tubes were kept in an ice bath while 5 mL of the anthrone reagent was added, then they were capped and placed in boiling water for 10 min. The reaction was stopped by immersion in an ice bath. Once the color had stabilized, the absorbance at a wavelength of 625 nm was determined using a Micronal model B580 spectrophotometer.

To extract the starch, the residue from the alcoholic extractions was treated with 10 mL of 35% perchloric acid, left to react for 20 min and then centrifuged at 2000× *g* for 10 min. Aliquots of 0.1 mL were placed in screw-top test tubes for the quantification of starch by the reaction with anthrone [14], as described for the quantification of total soluble sugars.

2.8. Statistical Analysis

The data were submitted to an analysis of variance, and the means were compared using the Tukey test at a 5% probability. The software used was the R program, version 4.1.1.

3. Results

The results of the analysis of variance indicated that for the condition factor, in terms of the percentages evaluated, only the variable dead cuttings was significant, with the conventional nursery condition having a high mortality rate, with 52.22% of dead cuttings, while the variables rooted cuttings and cuttings with callus did not differ. As for the quality of the root system of the cuttings, the results were significant for three of the four variables evaluated: root length, number of roots per cutting and root dry mass, with only volume showing no difference (Table 1).

Table 1. Averages for the percentage of dead guaranazeiro cuttings (%), root length (cm), number of roots per cutting and root dry mass (g) in different conditions for rooting guaranazeiro cuttings. Manaus, AM, 2023.

Conditions	Dead (%)	Length (cm)	Number/Cutting	Dry Matter Weight (g)
Conventional nursery	52.22 a	5.15 b	3.15 b	0.08 b
Humid chamber with nebulization	21.11 b	9.87 a	8.61 a	0.19 a

Averages followed by the same letters in the column do not differ according to Tukey's test at a 5% probability level.

As for the genotype factor, there was significance only for the percentage of rooted cuttings and cuttings with callus. When the factors were compared separately, the guarana genotypes showed statistically similar averages (Table 2), with 23.33% for BRS Maués and 31.66% for BRS Luzeia, while for BRS CG 611, only 9.16% of the cuttings rooted. As for the percentage of callus, the BRS Maués genotype obtained 47.5%, followed by 46.66% for BRS-CG 611 and 12.5% for the BRS Luzéia genotype.

Table 2. Averages of the guarana genotypes for the variables percentage of rooted cuttings (%) and percentage of cuttings with callus (%), regardless of the rooting conditions studied. Manaus, AM, 2023.

Genotype	Rooted (%)	With Callus (%)
BRS Maués	23.33 ab	47.5 a
BRS CG 611	9.16 b	46.66 a
BRS Luzeia	31.66 a	12.5 b

Averages followed by the same letters in the column do not differ according to Tukey's test at a 5% probability level.

As for the interaction between condition and genotype, there was significance only for the percentage of rooted cuttings and for the qualitative variables length, number and weight of roots; only volume was not significant. Therefore, by breaking down the condition × genotype interaction, there was a variation in rooting within these factors (Table 3), so there was a relationship depending on the genotype and the condition tested.

Table 3. Breakdown of the interaction between genotype and conditions for the variable percentage of rooted guaranazeiro cuttings. Manaus, AM, 2023.

Genotype	Conditions		
	Conventional Nursery	Humid Chamber with Nebulization	Genotype Average
BRS Maués	31.66 Aa	15.00 Ab	23.33 a
BRS CG 611	6.66 Aa	11.66 Ab	12.49 a
BRS Luzeia	6.66 Ba	56.66 Aa	31.66 a
Average of conditions	14.99 A	27.77 A	22.49

Averages followed by the same uppercase letters horizontally and lowercase letters vertically do not differ according to Tukey's test at a 5% probability level.

Therefore, the condition tested has a direct influence on the number of roots produced by each genotype evaluated, as can be seen in Figure 1.

As for the quality of the root system, there was also a significant interaction between environments and genotypes for the variables root length (Table 4), number of roots (Table 5) and weight of root dry mass (Table 6); only the variable root volume was not significant.

Table 4. Breakdown of the interaction between guaranazeiro genotypes and conditions for variable root length characteristic (cm). Manaus, AM, 2023.

Genotype	Conditions		
	Conventional Nursery	Humid Chamber with Nebulization	Genotype Average
BRS Maués	9.30 Aa	3.81 Ab	6.55 a
BRS CG 611	3.28 Ba	10.43 Aab	6.85 a
BRS Luzéia	2.87 Ba	15.36 Aa	9.11 a
Average of conditions	5.15 B	9.86 A	7.50

Averages followed by the same uppercase letters horizontally and lowercase letters vertically do not differ according to Tukey's test at a 5% probability level.

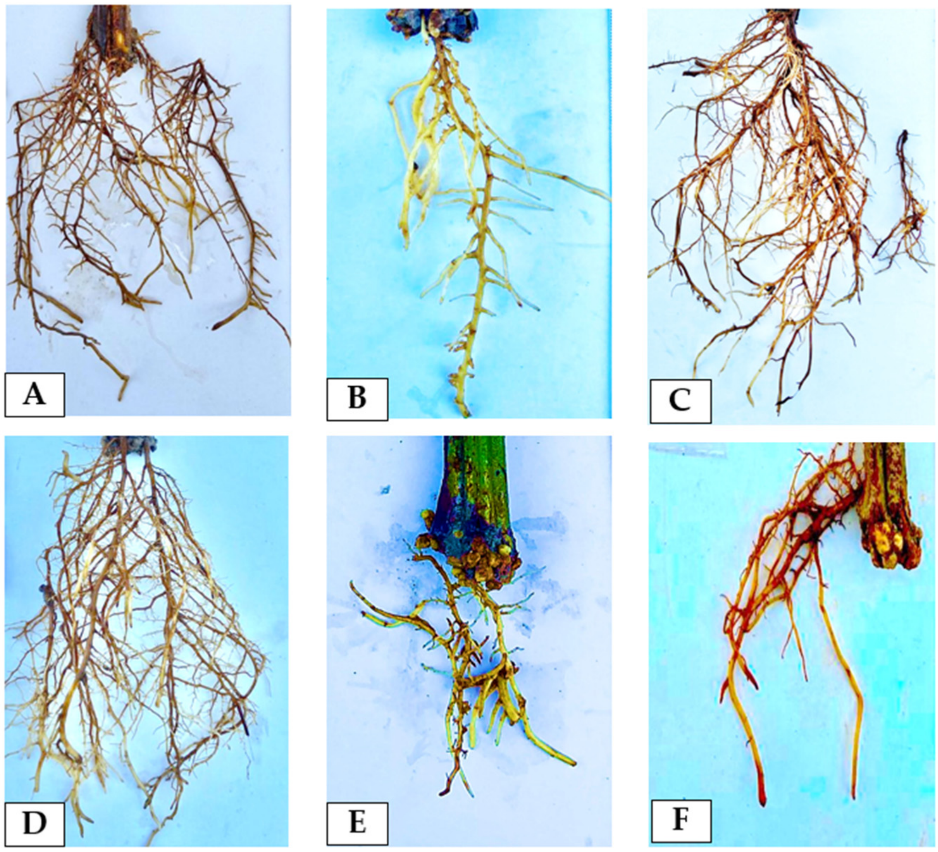

Figure 1. Genotypes in humid chamber conditions with nebulization: (**A**) BRS Maués, (**B**) Clone 611 and (**C**) BRS Luzeia. Genotypes in conventional nursery conditions: (**D**) BRS Maués, (**E**) Clone 611 and (**F**) BRS Luzeia. Manaus, AM, 2023.

Table 5. Breakdown of the interaction between guaranazeiro genotypes and conditions for variable number of roots by cuttings. Manaus, AM, 2023.

Genotype	Conditions		
	Conventional Nursery	Humid Chamber	Genotype Average
BRS Maués	6.09 Aa	2.40 Ab	4.24 a
BRS CG 611	2.12 Ba	11.37 Aa	6.74 a
BRS Luzeia	1.25 Ba	12.08 Aa	6.66 a
Average of conditions	3.15 B	8.61 A	5.88

Averages followed by the same uppercase letters horizontally and lowercase letters vertically do not differ according to Tukey's test at a 5% probability level.

Table 6. Breakdown of the interaction between guaranazeiro genotypes and conditions for variable weight of root dry matter (g) (cm). Manaus, AM, 2023.

Genotype	Conditions		
	Conventional Nursery	Humid Chamber with Nebulization	Genotype Average
BRS Maués	0.11 Aa	0.23 Aa	0.17 a
BRS CG 611	0.13 Aa	0.15 Aa	0.14 a
BRS Luzeia	0.13 Ba	0.33 Aa	0.23 a
Average of conditions	0.12 B	0.23 A	0.18

Averages followed by the same uppercase letters horizontally and lowercase letters vertically do not differ according to Tukey's test at a 5% probability level.

As for the chemical analyses, the results showed that there were no significant differences in the starch content between the conditions evaluated, but as for the total soluble sugar content, the conventional nursery condition was superior, with a content of 20.80%, while in the humid chamber rooting condition with nebulization, it was 18.58% (Table 7).

Table 7. Soluble total sugar content (%) and starch content (%) in different conditions for rooting guaranazeiro cuttings. Manaus, AM, 2023.

Conditions	Soluble Totals Sugar Content (%)	Starch Content (%)
Conventional nursery	20.80 a	23.38 a
Humid chamber with nebulization	18.58 b	22.90 a

Averages followed by the same letters in the column do not differ according to Tukey's test at a 5% probability level.

For the genotypes used, there were significant differences in total soluble sugar content and starch content, with BRS Luzeia having the highest values, with a 20.23% soluble total sugar content and 24.14% starch content (Table 8).

Table 8. Soluble total sugar content (%) and starch content (%), independent of the conditions studied for the rooting of guaranazeiro cuttings. Manaus, AM, 2023.

Genotype	Soluble Totals Sugar Content (%)	Starch Content (%)
BRS Maués	17.48 b	23.52 a
BRS CG 611	21.35 a	20.90 b
BRS Luzeia	20.23 ab	24.14 a

Averages followed by the same letters in the column do not differ according to Tukey's test at a 5% probability level.

4. Discussion

According to Table 1, the mortality of the cuttings was influenced by the rooting environment. The conventional nursery had the highest mortality rate and, consequently, lower-quality roots.

Temperature is an important factor in root formation. The ideal interval of temperature for rooting cuttings of most species is between 21.1 °C and 26 °C [15]. However, the conditions in this experiment often exceeded this range, which may have increased the mortality of the cuttings. Excessively high temperatures during rooting are not recommended, as they increase transpiration and water loss through the leaves, causing tissue necrosis. In addition, they can stimulate the development of lateral buds before root formation or reduce physiological activity, negatively affecting rooting [15]. The qualitative characteristics of the root system, such as length, number and dry weight of roots, were higher in the humid chamber, resulting in more physiologically active roots and a greater surface area. This increases the volume of soil explored, allowing for greater absorption of water and nutrients, promoting faster growth and greater plant vigor [16].

The guaranazeiro genotypes, analyzed separately, showed statistically similar averages (Table 2), highlighting that the genetic effect is crucial for successful rooting, as it influences the rooting capacity of different genotypes. This can determine the viability of large-scale reproduction, even if the material has high productive potential [17]. In genetic improvement, the characterization and evaluation of germplasm are essential procedures for estimating genetic diversity in a germplasm bank. Embrapa Amazônia Ocidental's Breeding Program maintains an Active Germplasm Bank (BAG) with 270 guaranazeiro clones collected from different locations. As a result of these studies, the genetic variability of the guaranazeiro indicates valuable genetic resources [17]. There is research that supports the condition × genotype interaction; if there was no genotype × condition interaction, a single genotype would prevail throughout the world and a single experiment would suffice for genotype evaluation. The results show the expression of the phenotype, a set of observable characteristics of an individual, such as physical, morphological or physiological.

There was a difference in rooting between the genotypes evaluated, a trend already predicted by previous research [9,18]. Rooting rates in guava cuttings can vary from 14.3% to 100% [19]. In all treatments, the cuttings showed callus, which was expected.

There was a difference in rooting between the genotypes evaluated, with BRS Maués reaching 75.7% and BRS-CG 611, 58.57%, a trend already predicted by previous research [9,18]. Rooting rates in guarana cuttings can vary from 14.3% to 100% [19]. In all treatments, the cuttings showed callus, which was to be expected, as guarana roots begin with the formation of callus tissue, which progressively differentiates into a root [20]. In many species, the formation of adventitious roots occurs from callus, but not all callus at the base of the cutting turns into roots [21]. It is known that in many species, adventitious root formation occurs from callus, but callus that develops at the base of the cutting may or may not become a root [15]. Root formation in guarana cuttings begins with the swelling of the basal end of the cutting with the formation of callus tissue. The cells of this tissue undergo progressive differentiation and form the root, and subsequently, the root system of the cutting [20]. In research already carried out on guarana, cuttings that showed callus did not necessarily root, indicating that the formation of callus is not an indication of root formation.

The potential for a cutting to form roots varies depending on the species, genotype, parent plant, age of the cutting and other natural characteristics [22]. However, in this research, the donor plants were under the same conditions and received the same cultural treatments, directing the results towards the interaction between environment and genetic material.

Analysis of this interaction showed variation in rooting between the factors. In the conventional nursery, there was no difference in rooting percentage between the genotypes. However, in the humid chamber with nebulization, BRS Luzeia stood out with 56.66% rooting, compared to only 6.66% in the nursery (Table 3). BRS Maués performed better in the conventional nursery, with 31.66%, compared to 15% in the humid chamber with misting. BRS CG 611 had low rooting rates in both conditions, with 6.66% in the nursery and 11.66% in the humid chamber with misting. The genetic factor contributes greatly to the rooting results of guarana cuttings; there is a strong genetic component in relation to the capacity and/or ability for rooting between the different genotypes [17]. The potential for a cutting to form roots varies with the species, and with the genotype, the age of the cutting and the nature of it also influence the potential for rooting, as well as where the roots will form [22]. When comparing the conditions evaluated, BRS Maués is superior in the conventional nursery and BRS Luzeia in the humid chamber with nebulization; these results show that the condition directly influences how much the root of a given genotype will grow, and that the root system changes according to the species, varieties within the species and other factors that affect the root system.

As for the BRS-CG 611 genotype, it has been studied in other research, and although it is tolerant to anthracnose and has a high productivity of around 1.39 kg/plant/year, it is a genotype that has difficulties in rooting, as it has been evaluated in different conditions and still expresses a low capacity to emit roots. This may be related to its genotype [17], its genetic composition, the set of genes it has inherited for this characteristic and the fact that the genotype is a non-visual characteristic that rarely undergoes changes.

Genetic variability for the rooting percentage trait has already been observed in several studies on guarana. For example, ref. [18] found that the rooting rate of eleven guaranazeiro clones varied from 16.6% to 85.2%; ref. [17] evaluated twelve genotypes and found a variation of 15% to 88.1%; ref. [9] observed a variation of 28.75% to 73.75% in six guaranazeiro genotypes.

The results of this study indicate that adaptations to the conventional propagation system can increase the rooting rate, depending on the genotype, highlighting the genetic effect as a determining factor for successful rooting and the establishment of clones [18]. BRS

Luzeia has good agronomic characteristics, such as high productivity, resistance to common guarana diseases and a high caffeine content (4.6%) [23]. However, it faces difficulties in propagation via cuttings in conventional nurseries, limiting its use in commercial crops. Rooting in a humid chamber with misting was satisfactory for BRS Luzeia, especially with the blue plastic cover, which favors the passage of the light wavelengths needed to stimulate rooting and sprouting.

The relative humidity of the air varied between 82% and 95%, within the ideal interval of 80% to 100%, which helps to maintain tissue turgidity. Automated irrigation in the humid chamber, unlike conventional nurseries where nebulization is controlled by an evaporation scale, better adjusts to the transpiration rate [12,15]. The humid chamber method by nebulization offers a low cost in the initial management of seedlings but shows great variation in the percentage of rooting and survival. In the case of BRS Maués, only 15% of the cuttings rooted, while BRS CG 611 reached 11.66% and BRS Luzeia 56.66%. Similar results were observed in cocoa, with clone CCN 51 achieving more than 80% rooting and clone TSH 1188 only 30% [24].

This system also improves seedling production, especially for genotypes with rooting difficulties, such as BRS Luzeia, due to the strict internal environmental control of the chamber. There was a significant interaction between conditions and genotypes in the variables length, number and weight of root dry mass (Tables 4–6). These results confirm that the condition directly influences root development, depending on the species, variety and other factors, such as nutrient levels, soil preparation, type, and humidity, as well as infestation by diseases and pests [25].

Rooting and the growth of several roots are equally important for seedling production. Thus, in addition to the percentage of rooted cuttings, the number and length of roots formed are crucial variables [26]. Long roots are the result of the condition as well as the genetic factor; possibly, earlier emergence of roots provides a longer period of growth, so the length of the roots defines growth and root density [27].

Irrigation on a smaller scale in the humid chamber with misting may have favored root growth, because in situations of water stress, the root system competes more effectively with the aerial part, limiting its growth and promoting greater root development, increasing the absorption area [28]. In the humid chamber condition with nebulization, the BRS Luzeia genotype had an average of 12.08 roots, compared to just 1.25 roots in the conventional nursery, representing just 10% of the other condition. A greater number of roots means a larger volume of soil is explored, allowing for greater absorption of nutrients and, consequently, greater productivity, improving plant adaptation to adverse conditions [29].

The survival of seedlings is directly linked to the number of roots. The greater the number of roots, the greater the capacity to absorb water and nutrients, favoring plant development. Roots that are too long are more susceptible to damage during transplanting [30]. In addition to the number, the volume and mass of roots also influence the vigor of the plant. Environmental factors such as temperature, light and water, and the type of substrate [31,32] directly affect root development. The results can be explained by the different substrate conditions in the two conditions. In the humid chamber with nebulization, the substrate, protected from the weather, allowed for greater aeration of the roots and consequently greater root development.

The high porosity of the substrate allowed for good aeration of the roots, avoiding the occurrence of anaerobic conditions, which can cause physiological and anatomical damage to root cells, as described in [33]. The absence of water and thermal stress in the humid chamber with nebulization may have reduced the need for soluble sugars to accumulate in the plants, as suggested in [34]. The genotypes showed different levels of sugars and starch, with BRS Luzeia standing out (Table 8). The amount of carbohydrates directly influences the rooting of cuttings, as demonstrated in [35]. BRS Luzeia, with its higher starch content, had the highest rooting rate. Similar results were found in [36], which also associated high carbohydrate content with greater success in rooting other guarana genotypes.

These results show the expression of the phenotype, a set of observable characteristics of an individual, such as physical, morphological or physiological. The phenotype is the result of the interaction between the genotype and the environment. To support this, we present an analysis of carbohydrates. In guarana, the content of carbohydrates present in the cuttings varies according to the genotype studied; there is an amount of reserve present, but the relationship is direct: the higher the content of total soluble sugars and starch, the greater the rooting.

A humid chamber with nebulization could be a promising alternative for propagating genotypes that are difficult to root, such as BRS Luzeia. Research with other guaraná genotypes that perform well in the field, but that produce cuttings using the conventional system inefficiently, needs to be carried out in order to diversify the genotypes in the plantations. Thus, the humid chamber with nebulization can be widely recommended. In these studies, it is still necessary to collect quantitative variables (% of rooted cuttings, % of cuttings with callus and % of dead cuttings) and qualitative variables (length of roots, number of roots per cutting, volume of roots and dry weight of root dry matter) of the root system.

5. Conclusions

The humid chamber with nebulization was efficient for guaranazeiro genotypes with rooting difficulties, such as BRS Luzeia, which had a higher carbohydrate content and better root formation compared to the conventional system.

This technique is a viable and economical alternative for small producers, and allows for the diversification of plantations with high-performance genotypes in the field that are currently underutilized due to the inefficiency of conventional seedling production methods.

The BRS-Maués genotype did not root well in the humid chamber with nebulization, but it is a genotype that is easy to root in the conventional system.

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References

1. Henman, A.R. Guaraná (*Paullinia cupana* var. sorbilis): Ecological and social perspectives on an economic plant of the central Amazon basin. *J. Ethnopharmacol.* **1982**, *6*, 311–338. [CrossRef] [PubMed]
2. Marques, L.L.M.; Panizzon, G.P.; Aguiar, B.A.A.; Simionato, A.S.; Cardozo-Filho, L.; Andrade, G.; Oliveira, A.G.; Guedes, T.A.; de Mello, J.C.P. Guarana (*Paullinia cupana*) seeds: Selective supercritical extraction of phenolic compounds. *Food Chem.* **2016**, *212*, 703–711. [CrossRef] [PubMed]
3. Torres, E.A.; Pinaffi-Langley, A.C.D.C.; Figueira, M.D.S.; Cordeiro, K.S.; Negrão, L.D.; Soares, M.D.; da Silva, C.P.; Alfino, M.C.Z.; Sampaio, G.R.; de Camargo, A.C. Effects of the consumption of guarana on human health: A narrative review. *Compr. Rev. Food Sci. Food Saf.* **2012**, *21*, 272–295. [CrossRef] [PubMed]
4. Atroch, A.L.; do Nascimento Filho, F.J. Guarana—*Paullinia cupana* Kunth var. sorbilis (Mart.) Ducke. In *Exotic Fruits, Sueli Rodrigues, Ebenezer de Oliveira Silva and Edy Sousa de Brito*; Academic Press: Cambridge, MA, USA, 2018; pp. 225–236. [CrossRef]
5. Brazilian Institute of Geography and Statistics—IBGE. Survey of National Guarana Production, Harvest Season, Safra 2021. 2022. Available online: https://www.ibge.gov.br/explica/producao-agropecuaria/guarana/br (accessed on 1 September 2024).
6. Brazilian Micro and Small Business Support Service—SEBRAE. Guarana Market Information, Consultant: Graciela Montero Cunha, Interactive Library SEBRAE. 2019. Available online: http://www.sebrae.com.br (accessed on 8 July 2024).
7. Agrospice Brasil—Guarana Newsletter. Available online: http://www.agrospice.com.br/ver_produto.asp?cod=47 (accessed on 5 July 2024).
8. Atroch, A.L.; Cravo, M.S.D.A.; Santos, J.A. Enraizamento de clones de guaranazeiro tratados com ácido Indol-3-Butírico (AIB). Rooting of guarana clones treated with indole-3-butyric acid (AIB). *J. Agric. Environ. Sci.* **2007**, *47*, 103–111. Available online: https://ajaes.ufra.edu.br/index.php/ajaes/article/view/198/88 (accessed on 4 July 2024).
9. Albertino, S.M.F.; Nascimento Filho, F.J.D.; Silva, J.F.D.; Atroch, A.L.; Galvão, A.K.D.L. Enraizamento de estacas de cultivares de guaranazeiro com adubação de plantas matrizes. Rooting of guarana cultivar cuttings with fertilization of matrix plants. *Amaz. J. Agric. Environ. Sci.* **2012**, *47*, 1449–1454. [CrossRef]
10. Gama, L.A.D.; Pinto, K.G.D.; Leite, B.N.; Gonçalves, G.S.; Pereira, J.O.; Silva, J.F.D.; Albertino, S.M.F. Rizobacteria isolated in the Amazon and its influence on the growth of guarana seedlings. *J. Agric. Sci.* **2019**, *11*, 428–435. [CrossRef]
11. Pinto, K.G.D.; Albertino, S.M.F.; Leite, B.N.; Soares, D.O.P.; de Castro, F.M.; da Gama, L.A.; Atroch, A.L. Indole-3-butyric acid improves root system quality in guaraná cuttings. *HortScience* **2020**, *55*, 1670–1675. [CrossRef]
12. Pereira, J.C.R. (Ed.) *Sistemas de Produção: Cultura do Guaranazeiro no Amazonas*, 4th ed.; Embrapa Amazônia Ocidental: Manaus, Brazil, 2005; 40p.
13. Bergo, C.L.; de Menezes, W.P. Propagação vegetativa do cafeeiro (*Coffea canephora)* por meio de enraizamento de estacas nas condições climáticas de Rio Branco-Acre. Vegetative propagation of the coffee tree (*Coffea canephora*) through rooting cuttings in the climatic conditions of Rio Branco-Acre. *Embrapa-Acre Tech. Commun.* **1999**, *105*, 1–4.
14. McCready, R.M.; Guggolz, J.; Silveira, V.; Owens, H.S. Determination of starch and amylose in vegetables: Applications to peas. *J. Anal. Chem.* **1950**, *22*, 1156–1158. [CrossRef]
15. Hartmann, H.T.; Kester, D.; Davies, F.; Geneve, R.; Wilson, S. *Plant Propagation: Principles and Practices*; Pearson: New York, NY, USA, 2018.
16. dos Santos, R.J.N.; Pereira, R.Y.F.; de Sousa, L.A.M.; Silva, B.; Araujo, M.B.F.; dos Santos Reis, I.; da Silva-Matos, R.R.S. Propagation of Polyscias guilfoylei in substrate based on decomposed babassu stem and carbonized rice husk. *Res. Soc. Dev.* **2020**, *9*, e635997889. [CrossRef]
17. Atroch, A.L.; do Nascimento Filho, F.J. Classificação do coeficiente de variação na cultura do guaranazeiro. Classification of the coefficient of variation in the guarana crop. *Amaz. J. Agric. Environ. Sci.* **2005**, *43*, 43–48.
18. Arruda, M.R.D.; Pereira, J.C.R.; Moreira, A.; Teixeira, W.G. Survival rate of guarana herbaceous cuttings in different substrates. *Ciência e Agrotecnologia* **2007**, *31*, 236–241. [CrossRef]
19. Correa, M.P.F.; Stolberg, W. Propagação vegetativa do guaranazeiro (*Paullinia cupana)* var. *Sorbilis* (Mart. Ducke). Vegetative propagation of guarana (*Paullinia cupana*) var. Sorbilis (Mart. Ducke). In Proceedings of the 1st Brazilian Guaraná Symposium, Manaus, AM, Brasil, 24–28 October 1981.
20. Mendonça, M.S.D.E. Sistema Radicular do Guaraná *(Paullinia cupana* var. Sorbilis (Mart.) Ducke): Origem, Estrutura e Desenvolvimento. Root System of Guaraná (*Paullinia cupana* var. Sorbilis (Mart.) Ducke): Origin, Structure and Development. Ph.D. Thesis, PhD in Biological Sciences—Instituto Nacional de Pesquisas da Amazônia—INPA, Manaus, Brazil, 1991; 129p.
21. Foladori-InvernizzI, S.; Maggioni, R.A.; Zuffellato, K.C.R. Estaquia caulinar herbácea e semilenhosa de *Aegiphila brachiata* Vell.(Lamiaceae). Herbaceous and semi-woody stem cuttings of *Aegiphila brachiata* Vell. (Lamiaceae). *Mesoam. Mag. For. Mesoam. Kurú* **2021**, *18*, 71–78. [CrossRef]
22. Altmann, T.; Szutkoski, J.; Fioravanço, G.P.; Souza, P.V.D.D. Enraizamento adventício e desenvolvimento vegetativo de citrandarineiros (*Citrus sunki* hort. ex Tan. × *Poncirus trifoliata* (L.) Raf.) e progenitores propagados por estaquia. *Delos Desarro. Local Sosten.* **2023**, *16*, 2200–2220. Available online: https://lume.ufrgs.br/handle/10183/267275 (accessed on 1 September 2024). [CrossRef]
23. do Nascimento Filho, F.J.; Atroch, A.; Pereira, J.; de ARAÚJO, J.C.A. BRS Luzéia: Nova cultivar para o agronegócio do guaraná no Estado do Amazonas. Luzéia: New cultivar for guaraná agribusiness in the State of Amazonas. *Tech. Commun. Embrapa Amaz. Ocident.* **2007**, *57*, 2.

24. Sodré, G.A.; Marrocos, P.C.L. Manual da produção vegetativa de mudas de cacaueiro. In *Manual for the Vegetative Production of Cocoa Seedlings*; Universidade Estadual de Santa Cruz: Ilhéus, Brazil, 2009; 46p.
25. Ferriani, A.P.; Mayer, J.L.S.; Ribas, K.C.Z.; Bona, C.; Koehler, H.S.; Deschamps, C.; de Cacia, O.M. Estaquia e anatomia de vassourão-branco. White broom cuttings and anatomy. *Sci. Agrar.* **2008**, *9*, 159–166.
26. Ribeiro, M.M.A.; Antunes, A.; Vieira, S. Enraizamento de estacas de azevinho (*Ilex aquifolium* L.): Uma metodologia possível para a produção de plantas. Rooting holly (*Ilex aquifolium* L.) cuttings: A possible methodology for plant production. *Agroforum Mag. Esc. Super. Agrar. Castelo Branco* **1996**, *19*, 9–14.
27. Benedetti, E.L.; Santin, D.; de Barros, N.F.; Pereira, G.L.; Martinez, H.P.; Neves, J.C.L. Alumínio estimula o crescimento radicular de erva-mate? Does Aluminum stimulate mate root growth? *Braz. For. Res.* **2017**, *37*, 139–147. [CrossRef]
28. de Sousa, B.T.; Zucareli, V.; Martins, A.P.C. Quantificação de açúcares e auxina e desenvolvimento inicial de mini-toletes de cana-de-açúcar. Total sugars and auxin quantification in the initial development of sugarcane mini-stalks. In Proceedings of the Colloquium Agrariae, São Paulo, Brazil, 9 June 2020; Volume 16, pp. 94–103. Available online: https://revistas.unoeste.br/index.php/ca/article/view/3130 (accessed on 31 August 2024).
29. Borcioni, E.; Mógor, Á.F.; Pinto, F. Aplicação de ácido fúlvico em mudas influenciando o crescimento radicular e produtividade de alface americana. Influence of the application of fulvic acid on seedling root growth and yield in lettuce. *Agric. Sci. Mag.* **2016**, *47*, 509–515. [CrossRef]
30. Lima, C.C.; Ohashi, S.T.; Silveira, A.S. Efeito de diferentes concentrações de AIB e procedências geográficas no enraizamento de estacas de paricá. Effect of concentration of different iba and origins geographical in parica stakes of rooting. *For. Sci.* **2018**, *28*, 1282–1292. [CrossRef]
31. da Guirra Bernardo, F.; de Freitas Silva, P.A.; Oliveira, G.C.B.; de Sousa, Â.A.; de Omena Serafim, E.; de Oliveira, N.P. Biometry and dry matter of ant stick seedlings when submitted to different substrates. *Braz. J. Dev.* **2021**, *7*, 79487–79498. [CrossRef]
32. de Melo Almeida, D.; da Silva, B.R.F.; Ucella Filho, J.G.M.; de Sousa, A.N.; da Costa, T.L.N.; da Silva Santana, J.A. Effect of different substrates on the initial development of *Handroanthus impetiginosus* (Mart. ex DC) seedlings. *Braz. J. Dev.* **2020**, *6*, 24619–24631. [CrossRef]
33. Aguilar, E.A.; Turner, D.W.; Sivasithamparam, K. Mecanismos propuestos acerca de la predisposición de los bananos Cavendish al marchitamiento por Fusarium durante la hypoxia. Proposed mechanisms on how Cavendish bananas are predisposed to Fusarium wilt during hypoxia. *Infomusa* **2000**, *9*, 9–13.
34. Pimentel, N.; Lencina, K.H.; Kielse, P.; Rodrigues, M.B.; Somavilla, T.M.; Bisognin, D.A. Produtividade de minicepas e enraizamento de miniestacas de clones de erva-mate (*Ilex paraguariensis* A. St.-Hil.). Productivity of mini-stumps and rooting of mini-cuttings of erva mate (*Ilex paraguariensis* A. St.-Hil.) clones. *For. Sci.* **2019**, *29*, 559–570. [CrossRef]
35. de Aquino Arantes, C.R.; Correa, A.R.; da Silva, A.M.P.; Spiller, C.; Camili, E.C.; Coelho, M.D.F.B. Vegetative propagation of *Talinum triangulare* at different harvest periods, types of cuttings and substrate. *Mag. Agribus. Environ.* **2021**, *14*, 1–17. [CrossRef]
36. Albertino, S.M.F. Adubação, Níveis Crescentes de Irradiância Nas Plantas Matrizes e uso do AIB Nas Estacas Para o Enraizamento de Cultivares de Guaranazeiro (*Paullinia cupana*, var. *Sorbilis*, (Mart.) Ducke). Fertilization, Increasing Levels of Irradiance in Parent Plants and Use of IBA in Cuttings for Rooting Guarana Cultivars (*Paullinia cupana*, var. *Sorbilis*, (Mart.) Ducke). Ph.D. Thesis, PhD in Tropical Agronomy-Universidade Federal do Amazonas, Manaus, Brazil, 2011; 95p.