

Review Article

# Guarana propagation strategies: a review

## Estratégias de propagação do guaranazeiro: uma revisão

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### Abstract

Guarana [*Paullinia cupana* var. *sorbilis* (Mart.) Ducke] is a species of great economic and social important in Brazil, as it is the only commercial guarana producer in the world. The vegetative propagation method indicated for the culture is stem cuttings, which aims at productivity, tolerance, and uniformity of clonal cultivars, because reproduction by seeds has slow germination and high genetic variability, which in traditional varieties is an undesirable factor. Genetic factors can interfere with the rooting capacity of the crop. Studies seek alternatives that can improve this condition and enhance the production system. Use of growth regulators, microorganisms that promote plant growth, variation of substrates and fertilization, have been strategies used. Preliminary tests on the rate of stem rooting and seed germination with the use of exogenous phytohormone did not demonstrate in relation to the non-application of these inducers. The use of rhizobacteria, which presents itself as a promising activity in many cultures, has not yet been demonstrated in the culture of guarana. On the other hand, the influence of different substrates on rooting has already shown consistent results as a function of rooting rate. Fertilizing the mother plants as recommended by the production system for the crop has proven to be an efficient procedure. There are still few studies aimed at improving the spread of guarana, demonstrating that new protocols need to be explored, or that the protocols already used are reviewed from another perspective.

**Keywords:** *Paullinia cupana*, cutting, rooting, growth inducers.

### Resumo

O guaranazeiro [*Paullinia cupana* var. *sorbilis* (Mart.) Ducke] é uma espécie de grande importância econômica e social no Brasil, pois é o único produtor comercial de guaraná no mundo. O método de propagação vegetativa indicado para a cultura é a estaquia que visa produtividade, tolerância e uniformidade das cultivares clonais, isso porque a reprodução por sementes possui lenta germinação e alta variabilidade genética, o que nas variedades tradicionais é um fator indesejado. Fatores genéticos podem interferir na capacidade de enraizamento da cultura. Estudos buscam alternativas que possam melhorar essa condição e potencializar o sistema produtivo. Uso de reguladores de crescimento, microrganismos promotores do crescimento vegetal, variação de substratos e adubações, tem sido estratégias utilizadas. Ensaios preliminares sobre a taxa de enraizamento caular e germinação de sementes com uso de fitormônio exógenos não demonstraram efeitos expressivos em relação à não aplicação desses indutores. O uso de rizobactérias, apresenta-se como atividade promissora em muitas culturas, ainda não foi demonstrado na cultura do guaraná. Por outro lado, a influência de diferentes substratos sobre o enraizamento, já demonstrou resultados significativos em função da taxa de enraizamento. A adubação das plantas matrizes conforme recomendação do sistema de produção para a cultura, comprovadamente é um procedimento eficiente. Constata-se que ainda são escassos estudos voltados à melhoria da propagação do guaranazeiro, demonstrando que novos protocolos precisam ser explorados, ou que os protocolos já utilizados sejam revistos sob outra perspectiva.

**Palavras-chave:** *Paullinia cupana*, estaquia, enraizamento, indutores de crescimento.

## 1. Introduction

Some species present conditions that limit seed propagation, for presenting dormancy, for being recalcitrant, with little germination capacity, with little germination uniformity and low plant growth (Moura, 2022). Being

the cutting an alternative method of propagation, whose result depends on endogenous and/or exogenous factors that influence the method, such as physiological state of the mother plant, species, proportion of branches, types

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and doses of hormones and environmental conditions such as light, temperature and humidity (Souza et al., 2020).

The guaranazeiro can be propagated by two ways: sexually (seeds) and asexually (cuttings) (Atroch et al., 2007). The production of seedlings by seeds is not recommended, due to the great genetic variability, producing uneven plants of variable productivity, if the genetic material is not improved. In asexual propagation, on the other hand, the plants are obtained from a propagule taken from the parent plant, consisting only of somatic cells, allowing the characteristics of the parent plant to be maintained (Hartmann et al., 2002; Pereira, 2005).

The variables that interfere in the rooting of cuttings are diverse, and their effects can occur alone or in interaction with others. All these factors must be studied since the simple modification of one or more conditions can allow the vegetative propagation of species of difficult rooting, as in some varieties of guarana (Pinto, 2019). Studies show that despite the selection of matrices to produce high-quality seedlings, guarana cultivars have different rooting capacities. Atroch et al. (2007) portrayed that there is a strong genetic component in relation to the capacity and/or ability for rooting among the different cultivars of guaranazeiro, which can make the multiplication of a certain material unfeasible on a large scale, even if it has good productive potential.

The Brazilian Agricultural Research Corporation – Embrapa is the institution responsible for the conservation of genetic resources of guaranazeiro [*Paullinia cupana* var. *sorbilis* (Mart.) Ducke] in Brazil and, through the selection of parent plants, developed cultivars with productive potential and resistance to anthracnose (Escobar, 1986; Nascimento Filho, 2003; Atroch, 2009). A disease caused by the fungus *Colletotrichum guaranicola* which can destroy up to 80% of cultivated areas (Muniz et al., 2011). Embrapa recommends the technique of vegetative propagation by rooting cuttings, aiming at large-scale seedling production with short time intervals, which provides homogeneous crops in productivity and quality, precocity, and tolerance to phytopathogens (Pereira, 2005; Hartmann et al., 2018).

The objective of this review was to survey the different strategies employed to date to improve the production system of the guaranazeiro crop.

## 2. The Guarana Tree

The guaranazeiro is native to the Amazon, whose fruit is the guarana. It belongs to the family Sapindaceae, with about 140 genera and 2,000 species distributed in three subfamilies (Buerki et al., 2012). It is a shrubby and climbing plant species, of which 27 genera and 419 species occur in Brazil (Somner et al., 2014).

The genus *Paullinia* has about 200 species, is restricted to the Amazon region with a few exceptions in tropical and subtropical America. The species *P. cupana* has two botanical varieties, the *sorbilis* variety found only in Brazil, and therefore known as “Brazilian guarana”, and *P. cupana* var. *typica*, the guarana found in Venezuela and Colombia (Schimpl et al., 2013; Tricaud; Pinton; Pereira, 2016).

Known as “guarana from the Amazon”, “guarana”, “uarana” or “narana”, it is a spherical, shiny black, capsule-shaped fruit with one to three leaflets containing only one seed. When ripe, it has mostly red and orange peels, partially opening to reveal the seeds. The pericarp is dark brown and partially covered by a white substance (aril) and is used for fruit dispersal (Kuskoski et al., 2005; Schimpl et al., 2013).

The development of guaraná is related to the rainfall regime in the state of Amazonas, considering that guaraná planting occurs in the rainy period (January to March) and flowering in the dry period (July to September), with fruiting of two or three months later (October to December), depending on the ripeness of the fruit, as guaraná has uneven fruiting (Embrapa, 1998; Santos et al., 2021).

The soil and climatic conditions for the crop are hot and humid, with an average annual temperature between 23 and 28 °C, relative humidity of 80%, and precipitation of 1,500 to 3,000 mm/year. The soils must be deep and well-drained, without stones, and the terrain can be flat (drained) or inclined (Pereira, 2005).

The guarana was domesticated by the Sateré-Mawé indigenous peoples between the Madeira and Tapajós rivers, on the border of the states of Amazonas and Pará (Pereira, 1954). They transformed a wild vine into a cultivated shrub, creating the complex process of processing guarana (Lorenz, 1992). It is an allogamous plant, pollinated mainly by bees and with high genetic variability. The *sorbilis* variety is distinguished from the other species of the genus *Paullinia* by presenting morphological and genetic characteristics, which we now know to be caused by polyploidy of the species, containing  $2n=210$  chromosomes, species of the same genus presented  $2n=24$  (Freitas et al., 2007).

Guaraniculture is economically and socially important in Brazil, as it is the only commercial guarana producer in the world. Production meets national and international needs. It is cultivated in the states of Bahia, Amazonas, Rondônia, Mato Grosso, Pará and Acre for commercial purposes (Atroch and Nascimento Filho, 2018) due to its high potential because of its medicinal characteristics with stimulating, aphrodisiac, and healing properties (Marques et al., 2016).

Guarana has become an important raw material, accounting for about 70% of the manufacture of soft drinks and energy drinks (Suframa, 2013; Machado et al., 2018). It constitutes a fruit with high levels of caffeine, widely used in the pharmaceutical, cosmetic, and food industries, and especially in the beverage industry, where the concentrated extract is responsible for the color, aroma, and flavor of soft drinks (Schimpl et al., 2013; Marques et al., 2019).

The seeds can be sold in four different forms: raw guarana (roasted grain); guarana sticks (paste of roasted grain, crushed, ground, and mixed with water in the form of a stick); guarana powder (roasted and ground grain); syrups and essences, the latter being the most available form on the market (Majhenic; Skerget; Zeliko, 2007).

## 3. Vegetative Propagation

Vegetative propagation is used to obtain a plant that is genetically identical to the parent plant. The asexual route

consists of using part of the segments of a plant, and is based on the premise of inducing adventitious rooting, in which, it is intended to obtain the formation of a new individual identical to the original plant (Lafetá et al., 2016). Besides preserving the genetic characteristics of the mother plant (matrix), it allows obtaining many seedlings from a single plant, in less time, when compared to sexual reproduction (Bernardo et al., 2020).

The advantages range from obtaining many plants from a single parent plant, improved propagation materials capable of resisting diseases, and higher survival rate in the field. The time for seedling formation is shorter (seven months), the start of production is earlier (two years after definitive planting), there is rapid stability of production (three years) and relatively high productivity (1.5 kg dry seeds. plant<sup>-1</sup>) which are also considered advantages (Atroch and Nascimento Filho, 2005).

Plants can be classified according to their rooting capacity into three groups: 1. Easy rooting plants: the plant tissues possess endogenous substances necessary for root initiation and the application of exogenous auxins is not necessary for cuttings to form adventitious roots; 2. Moderately easy-to-root plants: plant tissues have necessary cofactors but do not have sufficient auxins, and exogenous auxins must be applied in order for cuttings to succeed in the formation of adventitious roots; 3. Plants of difficult rooting: plant tissues do not have one or more cofactors, regardless of the amount of endogenous auxins, so, only the application of exogenous substances is not sufficient for rooting cuttings (Hartmann et al., 2002).

The propagation of guaraná by cutting still meets Embrapa's recommendations. Cuttings must be made from mother plants with good vegetative vigor, new branches, and non-woody, expanded leaves. Cut into a bevel and containing a pair of leaflets cut in half. The substrate used for seedling production is a combination of earth and sand, in a 4:1 (v/v) ratio, with the addition of simple superphosphate per cubic meter (Pereira, 2005).

The cuttings should be kept under greenhouse conditions of seven to nine months, with 50% shading and nebulization made through evaporation balance so that the water is evenly distributed on the surface of the leaflets. Fertilization should be done directly on the substrate every 30 days, with 200 g of urea and 200 g of potassium chloride diluted in water and prepared before use (Pereira, 2005).

For the formation of adventitious roots in a stake, after cutting at the base of the stake, through a histological reaction of healing of the outer cells, a necrotic plate is formed, which is sealed with a cork material (suberin). This plate prevents desiccation and the entry of pathogens into the stake. The living cells behind this plate begin to divide, and a layer of cells from the parenchyma forms an irregular mass called the "callus" (Hartmann et al., 2002). This tissue is poorly differentiated, originating from the vascular cambium, the cortex or the medulla, whose formation represents the beginning of the regeneration process (Fachinello et al., 2005).

The cells that become meristematic and have begun to divide by mitosis, present in the vicinity of the vascular cambium and phloem, initiate the formation of adventitious

roots. The formation of these roots takes place in two phases, the first of which is initiation, characterized by cell division and then comes the differentiation phase of cells in a root primordium, which results in the growth of the adventitious root (Fachinello et al., 2005).

The success of the cuttings depends on endogenous and/or exogenous factors that interfere with the method. For example, the physiological state of the mother plant, the cultivar, the portion of the branch cut, substrate, rooting facility, type and concentrations of growth regulators, and environmental conditions, such as light, temperature, and humidity (Rios et al., 2012; Stuepp et al., 2015; Souza et al., 2020). In addition to proper phytosanitary care, such as cleaning of pruning shears and elimination of materials with symptoms of diseases and pests (Broch et al., 2021).

The cutting is considered a technique of great economic viability for the establishment of clonal plantings, since initially well-developed plants with rapid establishment can reduce costs and maximize the production system, besides the vegetatively propagated individuals are also able to produce early and show greater survival in the initial establishment (Silva et al., 2018).

#### 4. Seed Propagation

Sexual reproduction (seed germination) is the main method of reproduction in higher plants and the only method available for some species. It is considered the safest way, because it produces more vigorous plants, with a rich and deep root system and, therefore, with greater longevity (Fachinello et al., 2005).

The propagation of guarana by seeds has not been very recommended since it presents cross-fertilization (allogamous) (mainly by bees) that induces the appearance of segregating individuals, or due to self-fertilization (Escobar et al., 1984; Gondim, 1978).

Guarana has recalcitrant seeds that quickly lose their viability. Furthermore, this type of propagation results in high variability in qualitative and quantitative characteristics, diversity in size, shape, and coloration of leaves, fruits, and seeds (150 g of dry seed. plant<sup>-1</sup>), variations in resistance to disease, and low survival rates in the field (Atroch et al., 2007; Albertino et al., 2012).

To take advantage of their characteristics, the seeds need to be germinated after harvest, but germination only starts sixty days after stratification, and it can take up to ninety days for the process to begin. In this way the seedlings will grow until they are ready for planting, which occurs, 12 months after initial germination, needing to be vigorous to withstand transplanting into bags and planting in the definitive location (Carvalho et al., 1982).

Regarding the germination characteristics, the literature is quite contradictory. According to Calzavara (1979), seeds germinate slowly and unevenly, taking 60 to 80 days to germinate, and may last up to 180 days, contributing to low seedling production, since there is a high rate of discarding seedlings for repotting and seedlings at different stages of development in nurseries (Frazão et al., 1981). Cardoso (1944) cited that germination time can also vary depending on the substrate, reporting that germination time is 90 to

150 days when seeds are sown in sand substrate, and 40 to 70 days in sawdust substrate.

Carvalho et al. (1980) when evaluating the germination of guarana seed from different times of the year, verified that the germination and vigor of the seeds were not affected by the harvest times, since they presented high germination power, due to the when sown immediately after harvest, starting its germination at 67 days of sowing, and ending at 177 days.

The prolonged period for the seed to start germinating may be because the guaranazeiro does not present an externally differentiated radicle, requiring some time for this structure to differentiate (Milanez, 1958).

Several attempts to standardize the planting using guaranazeiro seeds have been made to obtain fast and uniform germination, such as using seeds from different harvests, different sizes, and submitted to thermal and chemical treatments. However, none of the methods proved efficient in seedling production (Conceição et al., 1999).

At the end of 2021, Embrapa launched the first guaranazeiro cultivar to be reproduced by seeds called BRS-Noçoquém. The development of this cultivar seeks to reduce the propagation of the culture by clonal cultivars, indicating that vegetative propagation is considered an expensive method for the producer, since it requires more care. Despite all the attributes (anthracnose resistance, high productivity, and high genetic variability) the time for seedling formation is still slow, being 12 months (Souza, 2021).

## 5. Vegetative Propagation Treated with Indol-3-Butyric Acid (IBA)

Woody cuttings may have difficulty rooting, since they are far from the production zone of rooting promoters and thus may have low levels of endogenous auxin (Cardoso, 2022).

Regarding phytohormones or plant regulators, auxins, gibberellins, cytokinins and growth inhibitors stand out. They are organic compounds that act at low concentrations as chemical messengers that interact at specific sites with receptor proteins linked to signal transduction routes. In plants, hormones help in obtaining abilities in response to factors such as light or stresses present in the environment where the plant is. Generally, these hormones are synthesized in a specific organ of the plant, and act in other adjacent tissues regulating plant development (Taiz and Zeiger, 2013; Sivasakthi et al., 2014).

The auxin used more frequently and the first plant hormone to be discovered, was identified as indole-3-acetic acid (IAA), is the most studied and synthesized phytohormone naturally. However, there are synthetic auxins such as indole-3-butyric acid (IBA) and acetic naphthalene acid (NAA) that have been used commercially in agriculture for more than 50 years for various purposes such as fruit and leaf abscission, promoting flowering, fruit induction and rooting of cuttings for vegetative propagation (Taiz and Zeiger, 2013). These synthetic compounds, which are not produced by plants, are applied exogenously,

and can produce effects like groups of plant hormones (Vieira et al., 2010).

According to Fachinello et al. (2005) studies have stated that IBA can activate root initiation, promote root formation in cuttings, stimulate the increase in the number and quality of the root system, to standardize the rooting of seedlings.

The use of IBA in staking has been consecrated a fundamental practice for rooting because it is considered photostable, of localized action and little sensitive to biological degradation, responsible for the mechanisms of cell expansion, being a class synthesized from tryptophan, whose characteristic relates to the ability to induce cell elongation (Câmara et al., 2017).

A factor that can influence the formation of adventitious roots in cuttings is the concentration of growth regulator used (Almeida et al., 2007), being of utmost importance the correct use of the concentrations of phytohormones to be applied at the base of the cuttings (Dias et al., 2012).

Rodrigues and Lucchesi (1987), evaluating the vegetative propagation of induced guarana cuttings (capped) and with IBA at 50 ppm, demonstrated that the induced cuttings obtained a rooting percentage of 91.70% when compared to the cuttings treated with IBA, that the percentage fell 30%, however, despite having a lower percentage of rooting, when the cuttings were treated with IBA, they had a higher number of roots.

Rios (1995) evaluated the asexual propagation of herbaceous cuttings of guarana, with application of different levels of concentration of indole-3-butyric acid (IBA) (0, 2,000, 4,000, 6,000 and 8,000 ppm) in three varieties named CS1, CS2 and CP1. The results showed a positive response at the 4,000 ppm concentration presenting a higher percentage of number of cuttings with callus and number of cuttings with roots. The concentration of 6,000 ppm showed an adequate level for the formation of a greater number of roots per cutting, as well as better longitudinal development of the roots. The concentration of 8,000 ppm proved to be toxic to all three varieties. Different responses were also observed among the guarana varieties, with the variety CS1 showing the highest levels of response, surpassing CP1 and CS2, which were less sensitive to AIB treatments.

García et al. (1999) in technical circulars recommended the application of IBA of guaranazeiro by two routes: dry (powder) or liquid. In the dry method, the dosage was 6,000 ppm while the liquid method was 4,000 ppm. However, by updating the production system for guaranazeiro culture, Pereira (2005) decreased the concentration of the phytormone to 2,000 ppm, and now its treatment is done only by dry route with the help of inert talc.

Atroch et al. (2007) evaluated the rooting performance of 11 clones of guaranazeiro through various concentrations of IBA (0, 2,000, 4,000, 6,000, 8,000 and 10,000 ppm), and observed that there is genetic variability regarding the rooting percentage, and that the clones behaved in the same way, regardless of the level of IBA used, establishing that the interaction between clones and doses of IBA were not significant, being these clones in classes (CMU619 and BRS-Amazonas - class 1 (easy rooting); BRS-CG611, CIR196,



BRS-CG608, CMU723 and CMU375 - class 2 (median rooting); CMU606, BRS-CG505 and CMA514 - class 3 (difficult rooting); CIR203 - class 4 (poor rooting). It was also possible to affirm that the clones did not respond to the increase of the IBA dosage, and that clones of easy rooting do not need to make use of phytormon.

According to Albertino et al. (2012) evaluating the rooting of guarana cuttings with and without exogenous addition of IBA, it was found that regardless of the cultivar, it was obtained a higher percentage of rooted cuttings, cuttings with callus, and lower mortality rate of cuttings without IBA.

Pinto (2019) in his study proposed to estimate the rooting capacity of herbaceous cuttings of three guaranazeiro cultivars (BRS-Amazonas, BRS-CG372 and BRS-CG611), submitted to five concentrations of IBA (0, 1,000, 2,000, 3,000 and 4,000 ppm). The cultivar BRS-Amazonas showed rooting potential (75%) with the highest concentration of phytormon (4000 ppm), while BRS-CG372 showed good rooting index (60%) without use of IBA, and the phytormon may have expressed certain toxicity, considering that the higher dose caused a reduction in its rooting percentage. However, it presented promising results regarding the quality of the root system, since when submitted to IBA doses, biometric variables such as length, root volume and root dry mass increased significantly.

Lemos (2020) evaluated the effect of rooting solutions, including IBA in 15 clones of guarana and one cultivar, at a concentration of 2,000 mg L<sup>-1</sup>, and once again it was demonstrated that the use of regulators does not influence the rooting of cuttings, only favors the improvement of the quality of the root system of the cuttings, highlighting the variables such as volume, fresh mass and dry mass of roots, generating expressive results.

IBA is considered one of the least plant-toxic regulators (Hartmann et al., 2002), favoring rooting in many species. However, as described above, the studies related to the culture of guarana obtained not very promising results with its use or with the increase of its concentration.

## 6. Propagation by Seeds Treated with Gibberellic Acid (GA3) and Cytokines

Gibberellic acid stimulates the fractionation of seed reserves, releasing and directing energy to embryo development sites. Thus, there is the stimulation of elongation and cell division, causing the disruption of the seed coat by the root, accelerating the speed of emergence of seeds with uniformity (Stenzel et al., 2003; Silva et al., 2014). Cytokines can lead to cell division and activate embryo growth, leading to germination (Miransari and Smith, 2014).

According to Atroch et al. (2020) when evaluating the germination and vigor of guaranazeiro seedlings using different concentrations of growth regulators such as gibberellin and the cytokine BAP (0.0 mg/L (control), 10 mg/L, 15 mg/L and 20 mg/L), they found that the use of gibberellin had an effect on germination and increased vigor of seedlings according to the variables: mean germination, height and number of leaves per seedling, compared to

treatments with BAP. It also presented results like those observed in vegetative propagation using IBA, due to the increase in the dosages of regulators, demonstrating inhibitory effect in relation to the witness. The hypothesis presented for this effect was the possibility of the seeds to present morphological dormancy or morphophysiological, which leads to new studies.

## 7. Propagation by Seeds Induced by Plant Growth Promoters Rhizobacteria

Rhizobacteria form a vast group of rhizospheric microorganisms that inhabit the soil and surround the roots, attracted by root exudates (Ahemad and Kibret, 2014). These microorganisms act to promote plant growth and are called plant growth promoting rhizobacteria (GGRP) or multifunctional rhizobacteria, and may be of different genera, used alone (inoculation) or in combination (coinoculation) and contributing beneficially to the development of one or more plant species, through different mechanisms of action (Compant et al., 2005; Freitas, 2007).

According to Gray and Smith (2005), rhizobacteria can be classified into intracellular and extracellular. The intracellular ones live inside root cells in specialized nodule structures, highlighting those that fix nitrogen symbiotically in leguminous plants, such as bacteria of the *Bradyrhizobium* and *Rhizobium* genera (Bhattacharyya and Jha, 2012). The extracellular bacteria inhabit the rhizosphere or the spaces between the cells of the root cortex, including the free-living rhizobacteria and endophytes, especially the genera *Azospirillum* sp., *Bacillus* sp., *Burkholderia* sp., *Pseudomonas* sp. and *Serratia* sp.

The promotion of plant growth arising from the use of biological inputs is directly related to the production and exudation of phytohormones, such as auxins, which when interacting with plants can promote cell growth and elongation, reflecting in the growth of the root system and the aerial part of the plant (Paulino et al., 2018).

The changes due to this association result in improvements in water absorption, mineral salts, and tolerance to abiotic stresses (Araújo et al., 2020; Aboelmagd, 2021), as well as induction of resistance to pathogens (Atakan and Ozkaya, 2021). The use in crops of interest can be beneficial, especially under conditions where the soil is of low fertility. This is because, from the synthesis of plant hormones the root growth increases, which allows greater exploration for water and nutrients, as well as direct mechanisms linked to greater availability of nitrogen, phosphorus, and potassium (Hungria et al., 2016).

Another well-known benefit of using microorganisms is the control of plant diseases. The mechanisms that help explain it is the antibiosis process, in which metabolites with fungicidal, antibiotic and nematocides properties are produced, these being able to degrade the cell membrane of the pathogen, causing its death (Ferreira, 2019). In addition to these, there is parasitism, the competition process, predation, and resistance induction (Wu et al., 2015; Ferreira, 2019).

The increase in root biomass is a beneficial factor for the plant in acclimatization and rooting processes, thus

reducing losses in these processes. The use of rhizobacteria for this purpose has been reported by several authors, due to the increase in root biomass and greater area of nutrient uptake (Ovando-Medina et al., 2007).

When evaluating the inoculation of rhizobacteria such as *Bacillus* sp. and *Burkholderia ambifaria* on the development of seed propagated guaranazeiro seedlings, observed that regardless of the type of interaction, the rhizobacteria did not show beneficial effects on the variables analyzed as aerial dry matter, root dry matter, total dry matter, root diameter and leaf area (Gama, 2015).

The strain of the genus *Bacillus* sp. described as RZ2MS9, used in the work of Gama (2015), was previously isolated from the rhizosphere of Amazonian guaranazeiro plants, and showed potential as a growth promoter of crops of commercial interest such as corn and soybean, with the production of indole-3-acetic acid (IAA) being noted for its growth-promoting effect (Batista, 2012).

The group of bacteria of the genus *Bacillus* sp. stands out as growth promoters, and its mechanisms of action have been observed through the rapid germination of seeds, emergence of seedlings until the growth/development of plants, which made the plant reach the adult stage faster, staying less time in the field and favoring the escape against pests and diseases (Ribeiro; Sei; Leite, 2011).

Despite the beneficial effects of rhizobacteria such as the genus *Bacillus* sp. described and reported in some research, there is still a great variability of responses with the use of inoculation and coinoculation of multifunctional microorganisms, that is, some results show the promotion and others the inhibition of plant growth (Silva et al., 2022).

## 8. Vegetative Propagation Through the Influence of Different Substrates

Among the factors that influence root formation in cuttings, is the substrate and the genetic potential for rooting (Fachinello et al., 1995). The growing substrate implies a preponderant factor in the development and greater vigor of seedlings, especially, when it incorporates physical and chemical characteristics suitable for production (Silva et al., 2017; Marques et al., 2019).

The quality of the substrate is related to the presence of characteristics such as the absence of pathogens, essential nutrients, texture, structure and adequate pH, water retention, porosity, available water, salinity, and organic matter composition (Silva et al., 2022).

According to Klein and Agne (2012) and Xavier et al. (2013) the substrate performs functions such as support at the time of adventitious root formation, in addition to keeping its base moist, dark, and aerated for rhizogenesis to occur, providing seedlings with superior characteristics.

In nurseries, the use of nutrient-poor or nutritionally unbalanced substrate is common, causing low quality seedlings, compromising their performance in the field, making it necessary to use mineral fertilization, especially of macronutrients, which are required in larger quantities by the plants (Silva et al., 2015).

It is not easy to find a product that has all the necessary growing conditions for different species, including forest

species (Siqueira et al., 2018). The best gains regarding seedling production and/or rooting of cuttings have been achieved with the use of substrates formed by plant remains or organic composts (Mourão Filho et al., 1998; Salvador and Moreira, 1999; Bezerra; Rosa, 2002).

When using organic waste to compose substrates, this waste as a raw material can result in economic benefit, in addition to reducing chemical inputs and increasing the availability of nutrients to plants (Ferreira et al., 2015). In the past, the formulation of substrates was the soil itself, combined with bovine manure, mainly to produce seedlings in plastic bags (Trazzi et al., 2012). However, with the advancement of technology and research in seedling production, other types of alternative constituents have emerged and started to be widely used, such as rice husk, coconut fiber, coffee husk, vermiculite, peat, sugarcane bagasse, agro-industrial by-products, among others (Kratz and Wendling, 2016).

Rodrigues and Lucchesi (1987) evaluated the rooting of the guaranazeiro using a substrate containing only washed sand sterilized with methyl bromide. Hartmann et al. (2002) stated that the adequate substrate is the one that favors rooting and should contain enough space to allow gas exchange to take place, considering that the greater availability of oxygen at the base of the cuttings improves cell activity during the process of callus formation and consequently the emission of roots. Sand in this sense is composed of inert mineral particles with low water retention (Wendling et al., 2002), high density, fast and efficient drainage, giving ideal aeration (Kämpf, 2000). Since the guaranazeiro needs to receive water continuously in small quantities during rooting, and the cuttings should not be soaked, the use of sand as a substrate is justified.

Embrapa, through the Production System for guarana (1998), proposed the use of a substrate composed of a mixture of forest soil and sand, in a proportion of 4:1, in addition to the supplementation in one cubic meter of the mixture, with the addition of 1 kg of simple superphosphate.

After seven years, the Production System for guarana was updated, and the current substrate recommendation until today is formed by a mixture of forest soil and sand, in the proportion of 4:1, in addition to the supplementation in one cubic meter of the mixture, with the addition of 3 kg of simple superphosphate (Pereira, 2005). The increase in supplementation is justified since phosphorus often limits plant development; besides this fertilization has other elements such as calcium that stimulates root development and sulfur that actively participates in the formation of organic molecules such as amino acids, proteins, and enzymes that are involved in the plant life cycle. According to Wendling et al. (2002) generally, the greater the amount of organic matter in the substrate, the lower the drainage of it and the greater its water retention capacity.

Since the percentage of rooting of several cultivars is still relatively low, Arruda et al. (2007) sought to evaluate the percentage of rooting of 12 clones of guarana in three different substrates. The substrates tested were a mixture based on volume of 50% soil + 50% chicken manure (v/v); 50% chicken manure + 50% ground coal passed in a 10 mm (v/v) sieve and commercial substrate for vegetables (Plantmax®). In general, considering the average of all clones, the commercial substrate

and manure of chicken with coal provided the best results 55.9 and 49.3% of settling among clones, respectively. The substrate formed by soil and poultry manure proved to be inefficient, as it resulted in a dense substrate, which retained a lot of water and made it impossible to oxygenate the tissues responsible for the development of the root system.

Thus, the selection of substrates that can prevent dehydration of the base of the cutting, and that have spaces that facilitate the supply of oxygen (Sommer et al., 2022) is essential for seedling formation, since low oxygenation causes the paralysis of root growth (Wendling et al., 2002).

**Table 1.** Nutrient requirements during the adventitious rooting process for eucalyptus.

Function/Activity	Required nutrient(s)
----- Induction phase -----	
Protein synthesis	N, K, Mg, S, Zn, Mo
Synthesis of nucleic acids	N, P, Mg, Zn, B, Mo
Carbohydrate metabolism	N, P, K, S, Mn, B
Hormone metabolism	N, Ca, Zn, B, Fe, Mn, Cu
Osmoregulation	K
Cell division	Ca, B
Peroxidases metabolism	Ca, Cu, Fe, Mn, B
Phenol metabolism	Cu, B, Zn
Breathing	N, P, K, S, Mg
----- Formation Phase -----	
Cell wall formation	Ca, Fe, Cu, B
Lignification	Cu, Fe, Mn, B
Cell elongation	Ca, Fe, Mn, Zn, B

Source: Cunha et al. (2009).

## 9. Vegetative Propagation with Fertilizing Influence

The clone garden, where the matrix plants are cultivated, is an important component of the structure of rural enterprises to produce woody species seedlings. Normally, these areas are composed of matrix plants selected according to characteristics of interest such as productivity, resistance to diseases, water deficit and have the objective of producing vegetative propagules to produce seedlings (Carvalho and Silva, 2012).

Among the stages of seedling production that directly interfere with plant growth, even when in field conditions, is fertilization (Rosário et al., 2022).

The sequential removal of vegetative parts to produce cuttings is responsible for the export of essential nutrients to the plant, and therefore the replacement of these nutrients is necessary (Bazoni et al., 2020). The nutritional management of clonal garden plants should be carried out to meet the physiological needs for the growth and development of matrices and propagules production (Kolln, 2020).

The vigor of the parent plant, combined with balanced nutrition, can positively induce the rhizogenesis of the cuttings, defining the concentration of carbohydrates, nitrogenous substances, amino acids, auxins, phenolic compounds, among other rooting promoting substances (López-Bucio et al., 2002; Cunha et al., 2009).

Cunha et al. (2009) describe in Table 1 the macro and micronutrient demands in *Eucalyptus globulus* during root formation of woody species.

The fertilization of the guaranaizeiro should be done in three different periods throughout the year, and in proportions that vary over the years. The recommendation described by Pereira (2005) is shown in Table 2.

As knowledge about the culture is still somewhat scarce, and has not been updated since 2005, Albertino et al. (2012) sought to evaluate the effect of fertilization of mother plants (BRS-Amazonas, BRS-Maués, BRS-Mundurucânia, BRS-CG611,

**Table 2.** Recommendation of fertilization for the guaranaizeiro in the state of Amazonas.

Age	Parceling	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Mg	B	Zn
1 <sup>st</sup> Year*	At planting	-	25	-	-	-	-
	3 months after planting	8	-	24	5	1	1
Total Fertilizer of the year		8	16	24	5	1	1
2 <sup>nd</sup> Year*	1 <sup>st</sup> Application	8	50	-	5	-	-
	2 <sup>nd</sup> Application	8	-	24	-	1	1
	3 <sup>rd</sup> Application	8	-	24	-	-	-
Total Fertilizer of the year		24	50	48	10	1	1
3 <sup>rd</sup> Year*	1 <sup>st</sup> Application	18	50	-	10	-	-
	2 <sup>nd</sup> Application	18	-	24	-	1	1
	3 <sup>rd</sup> Application	36	-	48	-	-	-
Total Fertilizer of the year		72	50	72	10	1	1

1<sup>st</sup>Application: End of the productive period, right after cleaning pruning (January); 2<sup>nd</sup>Application: Right after fruiting pruning, new branches are laid down (April); 3<sup>rd</sup>Application: Right before beginning of flowering (May). \*This fertilization should always be done by May, even if the three months are up.

Source: Pereira (2005).

BRS-CG882 and the genotype CMU 381) on the rooting of guaranazeiro cuttings, following the recommendations described by Pereira (2005) for plants aged three years. Thus, it was observed that fertilization of guarana seedlings, compared to plants that did not receive fertilization, increased the percentage of rooting, and reduced the mortality of the cuttings, significantly. In the joint analysis of the percentage of rooted and dead cuttings in the two years of the experiment, the rooting values were higher than 60% in the cuttings taken from fertilized matrices.

These data prove that the nutritional condition of the parent plant is essential not only regarding the appearance of its vegetative vigor and the development of sprouts, but also regarding the amount and concentration of minerals in the vegetative propagules, significantly increasing rooting rates and speed of rhizogenesis (Mondenezi, 2019).

It was also found that the biometric parameters increased with fertilization (number, volume, and dry matter mass of the guaranazeiro roots). The only parameter that was not influenced by fertilization was root length. These characteristics show that certain cultivars of guaranazeiro are dependent on the initial levels of nutrients within the portion of the cuttings where the roots are formed (Albertino et al., 2012).

## 10. Conclusions

The propagation method recommended in the production systems for the guaranazeiro is by the technique of vegetative propagation.

The propagation via cutting, despite promoting relevant characteristics for the propagation of the culture, still faces some obstacles regarding rooting by some clonal cultivars. Studies with different approaches tested methodologies that tried to overcome this obstacle. The treatment of cuttings with different concentrations of IBA did not reveal significant results with its use or with the increase of its concentration. At certain concentrations the different clonal cultivars showed only an increase in some biometric variables. The use of gibberellins and cytokines in the germination and vigor of seeds also showed no efficiency, even the gibberellin showed significant difference compared to cytokine.

The use of rhizobacteria in guarana seeds, despite having shown promising results in other crops of commercial interest such as corn and soybeans, did not show significant results for the variables analyzed. The commercial substrate Plantmax®, along with poultry manure and charcoal provided the best rooting results among the clones. It was verified that the fertilization of the mother plants is essential for the rooting of the guaranazeiro, since for root formation, the cuttings depend on the nutrients that are stored in the cuttings provided by the mother plant.

It is observed with this survey the scarcity of works directed to the improvement of the methods of propagation of the culture of the guaranazeiro. New protocols need to be established to favor seed germination and vegetative propagation techniques. One of the options would be to explore the use of rhizobacteria, both in the germination of seeds, making use of different concentrations of the

culture, as the inoculation of the culture at the base of the cuttings, favoring its entry, considering that it produces IAA, the same phytohormone used by the plant, stimulating the production of adventitious roots.

It is recommended that further studies be carried out regarding the use of different substrates in contrast to those recommended by Embrapa, so that they can interfere in the production of seeds with better reserves, which will influence the percentage of germination and preparation of cuttings. Studies that go deeper into the chemical and physical composition of the substrates, taking into consideration that most of the production is carried out by small family groups, should pay attention to the raw materials most used by them, to obtain a quality substrate, which is economically viable, available in the region, free of pathogens and with adequate nutrients for seedling growth.

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