

Article

# Morphometric Characterization of Fruits, Seeds, Seedlings, and Emergence of Guaranazeiro (*Paullinia Cupana* Var. *Sorbilis* (Mart.) Ducke) in Different Types of Substrates

Josué Pinheiro Machado<sup>1</sup> , Emerson Dechechi Chambó<sup>2</sup> , Maria Iraildes de Almeida Silva Matias<sup>3</sup> , Narjara Walessa Nogueira de Freitas<sup>4</sup> , Rômulo Magno Oliveira de Freitas<sup>5</sup>

<sup>1</sup> PhD candidate in Agricultural Sciences. Federal University of Recôncavo da Bahia. ORCID: 0000-0002-4618-666X. Email: josueagro95@gmail.com

<sup>2</sup> PhD in Animal Science. Federal University of Recôncavo da Bahia. ORCID: 0000-0003-3507-3106. Email: chamboed@ufrb.edu.br

<sup>3</sup> PhD in Geology. Federal Institute of Education, Science, and Technology of Bahia, Valença Campus. ORCID: 0000-0003-0287-4919. Email: maria.matias@ifbaiano.edu.br

<sup>4</sup> PhD in Plant Science. Federal Rural University of the Semi-Arid Region. ORCID: 0000-0002-6242-463X. Email: narjara.nogueira@ufersa.edu.br

<sup>5</sup> PhD in Plant Science. Federal Institute of Education, Science and Technology of Rio Grande do Norte. ORCID: 0000-0002-2422-0118. Email: romulo.freitas@ifrn.edu.br

## ABSTRACT

The Southern Bahia Lowlands are Brazil's largest producer of guarana, with an emphasis on organic production. Sexual propagation may be an alternative for greater genetic diversity of the species, thus increasing the resistance of the crop in the field. The objective of the research was to perform the morphometric characterization of fruits, seeds, and seedlings of *Paullinia cupana* var. *Sorbilis*, and subsequently evaluate the best substrate for emergence and initial development of seedlings of this species. Measurements of length, width, thickness, and weight of the fruits and seeds of *Paullinia cupana* var. *Sorbilis* were performed. The experiment was conducted in a completely randomized design, and the treatments consisted of four substrates: commercial, sawdust, vermiculite, and topsoil. Emergence speed (ES), emergence index (EI), and cumulative emergence (%) were evaluated. The morphological characteristics of the seeds and fruits were photographed and analyzed using *Image Processing and Analysis in Java* (ImageJ®) software. The development of seedlings, primary and secondary roots, hypocotyl, metaplylls, and the external morphological characteristics of seeds and fruits were described. The fruits of *P. cupana* showed high biometric variations. The seeds showed low biometric variations. The commercial substrate and vermiculite provided the best emergence speed and emergence rates for *P. cupana* seedlings. The morphometry of seeds, fruits, and seedlings can aid in studies of germination, taxonomy, and the production of morphologically well-structured seedlings.

**Keywords:** Guarana cultivation; plant morphology; recalcitrant seeds.

## RESUMO

O Baixo Sul da Bahia é o maior produtor de guaraná do Brasil, com destaque para produção orgânica. A propagação sexuada pode ser uma alternativa para maior diversidade genética da espécie aumentando assim a resistência da cultura em campo. O objetivo da pesquisa foi realizar a caracterização morfométrica de frutos, sementes e plântulas da *Paullinia cupana*- var. *Sorbilis*, e sucessivamente avaliar qual o melhor substrato para emergência e desenvolvimento inicial de plântulas dessa espécie. Foram realizadas medidas de comprimento, largura, espessura, e peso dos frutos e sementes de *Paullinia cupana*- var. *Sorbilis*. O experimento foi conduzido no delineamento inteiramente casualizado e os tratamentos foram constituídos por quatro substratos: comercial, serragem, vermiculita e



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solo vegetal. Foram avaliados velocidade de emergência (VE), índice de emergência (IVE) e emergência acumulada (%). As características morfológicas das sementes e frutos foram fotografadas e analisadas utilizando o software *Image Processing and Analysis in Java* (ImageJ®). Foram descritas o desenvolvimento das plântulas, raízes principais, secundárias, hipocótilo, metáfilos, e as características morfológicas externa das sementes e frutos. Os frutos de *P. cupana* apresentaram altas variações biométricas. As sementes apresentaram baixa variações biométricas. O substrato comercial e vermiculita possibilitaram os melhores índice de velocidade de emergência e emergência de plântulas de *P. cupana*. A morfometria de sementes, frutos e plântulas podem auxiliar em estudos de germinação, taxonomia e produção de mudas bem estruturadas morfologicamente.

**Palavras-chave:** Guaranaicultura; morfologia vegetal; sementes recalcitrantes.

## Introduction

Native to the Amazon, the guarana tree has been gaining prominence in global exports and in the country's agricultural productivity. The Southern Bahia Lowlands contribute approximately 1,831 tons to production, generating an estimated income of 24,000 reais, which makes the production of this species an important source of income in the region (IBGE 2021).

The soft drink and juice industries are the largest consumers of the product, followed by the pharmaceutical and cosmetics industries, which use guarana as a source of caffeine (Silva et al., 2020), theobromine, and theophylline (Nina et al. 2021).

In response to the low genetic diversity of the species, caused by asexual reproduction methods, susceptibility to attack by various pathogens, hosts such as guaraná tree thrips or lacerdinha, which severely affect crop productivity, has been causing significant economic damage to local producers. This problem occurs due to herbivory of fruits, branches, and young flowers, as well as the spread of spores of the fungus *Fusarium decemcellulare* Brick and anthracnose (Gasparotto et al., 2020), which hinders organic crop management.

Sexual reproduction may be an alternative for obtaining quality guaraná seedlings, which may be more resistant to pathogen attack, in addition to being the simplest and most economical propagation method for producers.

In seed reproduction, gene recombination occurs when the parent plants are heterozygous, and the offspring may exhibit genetic variability different from the parent plants (Fachinello et al. 2005; Canhoto 2016), which may result in seedlings that are more resistant to biotic factors found in the field.

In addition to storage problems, another characteristic is the long emergence period, which can extend from 70 to 150 days after sowing. In this sense, a quality substrate plays a fundamental role in seedling production, as it can significantly influence the architecture of the root system and the nutritional status of plants (Antunes et al. 2014), in addition to contributing to the reduction of exposure time and costs in the nursery phase.

Biometrics and seed characteristics are of great importance for the study of a species, constituting a fundamental parameter for understanding the dispersion and establishment of seedlings (Pereira et al. 2018). In addition, the same authors point out that studies involving the morphological analysis of fruits and seeds can aid in understanding the processes of germination, vigor, storage, viability, and methods of propagation of species.

Guarana cultivation has great economic and social potential for the southern lowlands of Bahia, but there are few studies on the emergence and initial development of seedlings with different types of organic substrates. Therefore, it is of utmost importance to conduct research related to the initial growth of the guarana tree, as well as the morphometric characteristics of fruits, seeds, and seedlings, aiming at possible contributions to the reproduction and management of this recalcitrant species.

The objective of this study was to analyze the morphometric characterization of fruits, seeds, and seedlings of *Paullinia cupana* var. *Sorbilis* and, subsequently, to evaluate the best substrate for the emergence and initial growth of guaraná seedlings under the climatic conditions of the southern lowlands of Bahia.

## Materials and Methods

### Study site

The research was conducted in the seedling production nursery and in the Soil and Plant Nutrition Laboratory of the Federal Institute of Bahia, Valença Campus, BA, ( $13^{\circ} 22' 26''$  South,  $39^{\circ} 4' 3''$  West). According to the Köppen-Geiger classification (1928), the climate is classified as Af, with an average annual temperature of  $25^{\circ}\text{C}$ , average annual relative humidity of 80%, and average annual rainfall of 2,900 mm.

The guarana seeds (*Paullinia cupana*- var. *sorbilis*) used in this experiment were randomly collected from fruits from 30 matrices with approximately 20 years of cultivation, located in the rural area of the municipality of Valença-BA. Subsequently, healthy fruits without deformities were selected. The seeds remained in the fruits during the biometric evaluation process, as they are considered recalcitrant and cannot be stored outside the mother plant.

### Morphometry of *Paullinia cupana* fruits and seeds

After collection, 200 fruits were separated for biometric analysis. Measurements were taken of fruit length (mm), fruit width (mm), fruit thickness (mm), fruit weight (g), and number of seeds per fruit. Fruit length was measured from the apex to the base of the stalk, width was measured at the widest part of the fruit, and thickness was inferred at the thickest part of the fruit.

For seed biometrics, 200 seeds were characterized, and the following characteristics were measured: seed length (mm), seed width (mm), seed thickness (mm), and seed weight (g). For length, the apex of the seeds to the base was measured; thickness and width were considered the widest and thickest part of the seeds.

The length, width, and thickness of the seeds and fruits were measured using a digital caliper, and an analytical balance was used to measure weight.

### *Paullinia cupana* emergence test

The experiment was conducted in the seedling production nursery (50% sunlight) at the Federal Institute of Bahia, Valença Campus, in the city of Valença-BA.

The experimental design used was completely randomized (CRD), with four replicates, consisting of 50 seeds. The treatments consisted of four substrates: commercial (Plantmax), sawdust, vermiculite, and topsoil. The seeds were sown in 50 ml cell trays containing the substrates studied.

Watering was performed daily in the morning and, when necessary, in the afternoon, when the substrate was dry. All irrigation in the experiment was done manually.

The characteristics evaluated were: a) emergence speed (ES, days), determined using the formula:  $ES = [(N1 G1) + (N2 G2) + \dots + (Nn Gn)] / (G1 + G2 + \dots + Gn)$ , where: ES = emergence speed (days); G = number of emerged seedlings observed in each count; N = number of days from sowing to each count (Maguire, 1962). Counts were performed once a week until 170 DAS (days after sowing), when stand stability was observed. b) cumulative emergence (E%): given by the ratio between the number of emerged seedlings at 170 DAS and the total number of seeds.

### ***External morphology of seeds, fruits, and seedlings of Paullinia cupana***

Seeds, fruits, and seedlings of *P. cupana* were photographed and subsequently evaluated for different morphological structures. a) Seeds and fruits: fruit epicarp, protective envelope (teguments or testa and arilloid), position of the hilum and micropyle, shape, coloration, and texture; b) Seedlings: initial development of the epicotyl, tegument, and radicle; c) Seedlings: main roots, secondary roots, hypocotyl, metaphylls, and tegument). Seedlings were considered to be those in which the first leaf or first pair of leaves were fully expanded (Gurgel et al., 2006). *Image Processing and Analysis in Java* (ImageJ®) software (National Institutes of Health, 2004) was used to efficiently capture external images of seedlings, fruits, and seeds.

### **Data analysis**

The data from the quantitative variables were submitted to descriptive analysis, with calculations of the arithmetic mean, minimum and maximum values, range, standard deviation, and coefficient of variation, all obtained using Microsoft Excel 2013 software. For the emergence test, analysis of variance was performed and the means were compared using Tukey's test at a 5% probability level, with the aid of the statistical program System for Analysis of Variance - SISVAR (Ferreira, 2019).

### **Results and discussion**

#### ***Biometrics of fruits and seeds***

The descriptive analysis of the variables length, width, thickness, fruit weight, and number of seeds per fruit is presented in Table 1.

Table 1: Minimum, mean, maximum, range, standard deviation, and coefficient of variation values for the variables length, width, thickness, fruit weight, number of seeds per fruit, and length, width, thickness, and weight of seeds of *Paullinia cupana* var. *sorbilis*, collected in the municipality of Valença-BA.

|                | Minimum | Mean  | Maximum | Range | Standard deviation | Coefficient of variation |
|----------------|---------|-------|---------|-------|--------------------|--------------------------|
| <b>Fruits</b>  |         |       |         |       |                    |                          |
| Length (mm)    | 11.0    | 16.82 | 24.40   | 13.40 | 3.14               | 18.68                    |
| Width (mm)     | 10.9    | 17.77 | 33.00   | 22.10 | 3.63               | 20.43                    |
| Thickness (mm) | 12.2    | 19.62 | 32.80   | 20.60 | 3.80               | 19.38                    |
| Weight (g)     | 0.90    | 4.582 | 14.30   | 13.40 | 2.06               | 45.07                    |
| Seeds/fruit    | 1.00    | 1.921 | 3.00    | 2.00  | 0.77               | 40.08                    |
| <b>Seeds</b>   |         |       |         |       |                    |                          |
| Length (mm)    | 2.70    | 11.15 | 13.90   | 11.20 | 1.38               | 12.35                    |
| Width (mm)     | 2.50    | 11.73 | 19.10   | 16.60 | 1.84               | 15.66                    |
| Thickness (mm) | 6.40    | 10.61 | 15.20   | 8.800 | 1.62               | 15.31                    |
| Weight (g)     | 0.38    | 0.929 | 1.702   | 1.322 | 0.25               | 26.49                    |

Source: Authors (2023)

The results showed that *P. cupana* fruits varied widely. Length ranged from 11.00 mm to 24.40 mm, with a mean of  $16.82 \pm 3.14$  mm and a coefficient of variation of 18.68%. The width ( ) of the fruits ranged from 10.9 to 33.0 mm, with an average of  $17.77 \pm 3.63$  mm, and the thickness had an average of  $19.62 \pm 3.80$  mm, ranging from 12.2 mm to 32.80 mm (Table 1).

The weight of the fruits ranged from 0.90 g to 14.30 g, with an average of  $4.58 \pm 2.06$  g, while the number of seeds per fruit ranged from 1 to 3, with an average of  $1.92 \pm 0.77$  (Table 1).

The variables fruit weight and number of seeds per fruit showed greater variations, with respective coefficients of 45.07% and 40.08%, indicating high data dispersion. These variations may be related to the occurrence of more than one flowering period of the species and the harvesting of fruits at different stages of ripeness (Atroch & Filho, 2018). The biometric values of the fruits in this study are similar to those found by Mendonça et al. (1992), who characterized fruits with lengths of 18 to 25 mm. However, the width of the fruits observed by these authors (16 to 22 mm) was lower than that found in the present study, which ranged from 10.9 to 33 mm.

These variations may be related to a supposed genetic divergence between the matrices collected. According to Gonçalves et al. (2019), biometric responses, such as length, texture, width, fruit weight, and seed weight per fruit, may indicate genetic variability or phenotypic plasticity, providing important information for genetic improvement programs.

The high variation in fruit weight can be of great importance for identifying matrices with high productive performance and for selecting superior individuals. This genetic variability was identified by Sousa et al. (2009), who detected genetic differences in 64 guaraná clones located at Embrapa Amazônia Ocidental, based on the morpho-botanical characterization of fruits and seeds.

Regarding the number of seeds per fruit, a maximum of three and a minimum of one seed per fruit were found (Table 1). The production of fruits with more than one seed is an important characteristic for the reproduction of the species, since guaraná seeds have intrinsic difficulties in germinating, as the species belongs to the so-called recalcitrant group. Seeds classified as recalcitrant cannot withstand severe dehydration or low temperatures, losing their viability extremely quickly (Peixoto, 2017).

Among all the characteristics evaluated, fruit weight and number of seeds per fruit are of greatest importance to producers in the Southern Bahia Lowlands. These variables can represent a good financial return, since the commercial value of the species lies in the fruit pulp and seeds, which are the main inputs for the pharmaceutical and soft drink industries.

The seeds with guaranazeiro aril had an average length of  $11.73 \pm 1.8$  mm, a minimum of 2.70 mm, and a maximum of 13.90 mm, with a range of 11.20 mm (Table 1). The average width of the seeds was  $11.73 \pm 1.84$  mm, ranging from 2.50 mm to 19.10 mm, with a range of 16.60 mm (Table 1). The thickness of the seeds had a mean of  $10.61 \pm 1.62$  mm, with a minimum of 6.40 mm and a maximum of 15.20 mm, range of 8.80 mm (Table 1). The average seed weight was  $0.929 \pm 0.25$  g, ranging from 0.38 g to 1.702 g, with a range of 1.322 g (Table 1).

Seed size can influence both emergence and better vegetative development of *P. cupana* seedlings. Larger seeds usually have well-formed embryos and greater reserves (Carvalho & Nakagawa, 2000; Silva, 2019a).

Another important variable that contributes to emergence and the formation of quality seedlings is seed weight, which showed the greatest variation (26.49%) among the biometric variables evaluated. Seed weight is correlated with the amount of reserves, proteins, and biochemical activities. The increase in mitochondrial protein in seedlings from heavy seeds is indicative of a high rate of respiration and high energy production (ATP), giving heavy seeds greater development potential (Kryzianowski & França-Neto, 2001).

The selection of seeds by volumetric weight during the sexual propagation of the guaraná tree can be considered a positive strategy for the production of quality seedlings, enabling better emergence and germination rates for the species in the future.



However, it should be noted that the biometric characterization of *P. cupana* fruits and seeds is of physiological and commercial importance to producers in the southern region of Bahia.

### ***Emergence***

A significant difference was observed between the treatments evaluated (Table 2). The commercial substrate and vermiculite presented the highest emergence percentages of *P. cupana*, with 70 and 73%, respectively. These substrates probably offered adequate physical, biological, and chemical conditions for the emergence of the guaranazeiro, providing good water retention and adequate temperature.

Table 2: Average values for emergence and emergence speed of *Paullinia cupana* var. *sorbilis* seedlings subjected to different substrates.

| <b>Substrates</b> | <b>Emergence</b> | <b>ES (days<sup>-1</sup>)</b> |
|-------------------|------------------|-------------------------------|
| Commercial        | 70               | 102.26c                       |
| Sawdust           | 47c              | 121.73ab                      |
| Vermiculite       | 73a              | 117.51bc                      |
| Sand              | 60b              | 125.83a                       |

Source: Authors (2023)

Commercial substrates have physical and chemical characteristics that are favorable for the initial development of seedlings. In addition to having good water retention, they aid in the metabolic activation of seeds, subsequently favoring germination and seedling emergence (Silva et al., 2019b). Bao et al. (2014) evaluated the production of *Matayba guianensis* seedlings in different substrates and containers and observed that the percentage and speed of emergence were higher when commercial substrate (Plantmax) was used in Styrofoam containers.

Similar results were found in the emergence of other species of the Sapindaceae family. Coelho et al. (2010), when testing different substrates for the emergence of tingui (*Magonia pubescens* St. Hil.), observed that the percentage and speed of emergence were favored by the use of vermiculite substrate, while sand substrate negatively interfered with these parameters.

These results confirm that vermiculite and commercial substrates are significantly important for the germination and emergence of species of the Sapindaceae family.

Among the important characteristics of vermiculite substrate are its ease of moisture retention and high thermal expansion index. Water molecules heated rapidly at high temperatures can cause an increase in the volume of minerals, thus making expanded vermiculite chemically active, biologically inert, and low in density (Ugarte et al., 2008). Considering that guaraná seeds are recalcitrant, substrates that maintain stable moisture and provide high water content to the seeds throughout their physiological development are necessary.

The emergence speed showed similar behavior to the emergence results, with vermiculite and commercial substrates providing the shortest emergence time (Table 2). The commercial substrate promoted the emergence speed of *P. cupana* seeds with 102.26 days<sup>-1</sup>, followed by the vermiculite substrate with 117.51 days<sup>-1</sup>.

Sawdust and sand were the substrates that obtained the lowest emergence percentages and the longest emergence speeds in the production of *P. cupana* seedlings (Table 2). The sand substrate has low moisture retention due to rapid water drainage, which causes the substrate surface to dry out, impairing seed emergence (Ribeiro et al., 2022).

Sawdust can cause problems due to excessive moisture retention, and water accumulation in the substrate can cause anoxia in guaranazeiro seeds. In this case, mixing with substrates with lower water retention capacity is recommended to improve drainage and reduce water accumulation (Boodt & Verdonck, 1972).

According to Paixão et al. (2019), an agricultural substrate must retain water without reducing oxygen availability to the roots. The authors emphasize that, during seedling production, the substrate is one of the components that most interferes with growth, through factors such as structure and texture.

Figure 2 shows the cumulative emergence of *P. cupana* seedlings. The first seedlings emerged 85 days after sowing in the sand and vermiculite substrates and 92 days in the sand and sawdust substrates.

In the vermiculite and commercial substrates, 140 days after sowing, more than 50% of the seedlings had already emerged, unlike the sand and sawdust substrates, in which less than 40% of the seedlings had emerged during this period. It was also observed that the sand composite substrate showed rapid initial emergence, but this speed decreased over time.

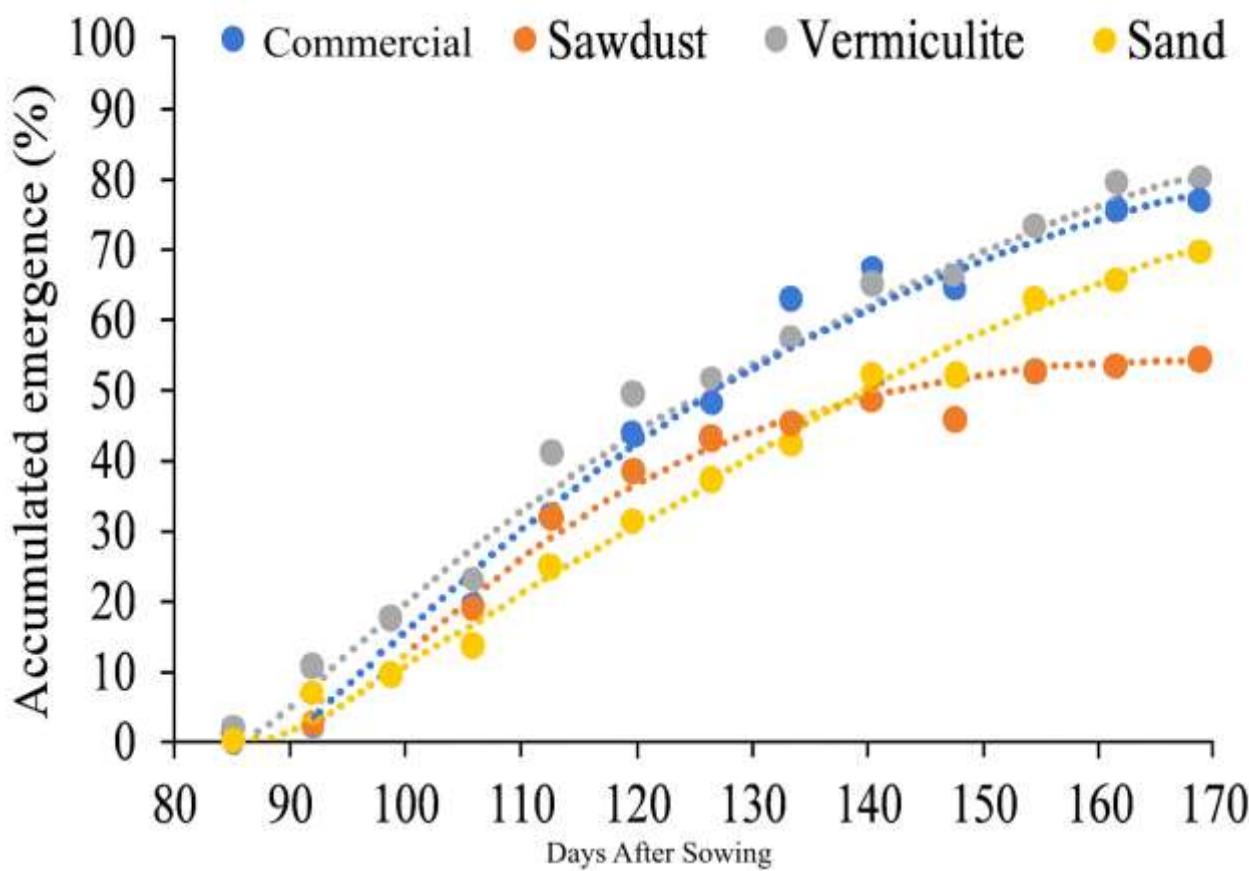


Figure 1 : Accumulated emergence of *Paullinia cupana* var. *sorbilis* seedlings subjected to different substrates. Source: (author, 2023)

The speed of seedling establishment allows for early light absorption for photosynthesis, stimulating the plants' physicochemical processes and subsequently promoting better root and vegetative development, which can favor the success of seedlings in the field (Taiz & Zeiger, 2013). In the present study, the sand and sawdust substrates delayed the cumulative emergence of *P. cupana*, impairing seedling establishment.



### Seed and fruit morphology

The fruit of *P. cupana* is globular in shape, dry and dehiscent, opening when ripe to release the seeds. The outer epicarp is orange to bright red in color and has a tip at the apex, a remnant of the style. The mesocarp is succulent and orange in color (Figure 3).



Figure 3: Morphometry of *Paullinia cupana* var. *sorbilis* fruits, epicarp and style. Source: (author, 2023)

Guaraná seeds are black or dark brown in color, with a shiny, smooth testa. The aril, which is white in color, covers the seeds, and the structure of the aril is fleshy and soft (Figure 4). The morphological characteristics of the seeds and fruits observed in this study are consistent with those described by Mendonça et al. (1992), who evaluated the morphology of guaranazeiro seeds and fruits.

The external hilum of guaranazeiro seeds is brown in color and consists of a small scar that connects the seed to the fruit, located in the central position. The micropyle is a small brown scar that corresponds to the ovule (Figure 4) and constitutes the opening through which the water absorption process occurs.

The dispersion of guaranazeiro seeds is generally anthropocoric. Similar characteristics were observed by Karl-Arens (1956), who evaluated the external and internal morphology of the seeds, highlighting a thin, hard testa and embryos without endosperm, whose two cotyledons contain reserve substances.



Figure 04: Morphometry of *Paullinia cupana* var. *sorbilis* seeds, A- Seed with aril, B-Seed without aril. A= Seed and aril, B=Hilo and Micropyle. Source: (author, 2023)

Knowledge of the morphological aspects of fruits and seeds is of great relevance for the identification and conservation of species (Silva et al., 2014). In the case of the species under study, morphology is of significant importance for genetic improvement studies and for the establishment of local germplasm banks.

The morphological characterization of fruits and seeds has positive impacts on defining the ideal harvest time for guaraná fruits. Producers in the southern lowlands of Bahia can determine the exact time for harvesting based on the color, texture, and shape of the fruits and seeds. Unlike other crops, this species does not have a homogeneous fruit harvest (Santos et al., 2022), which is one of the main problems encountered in guaraná production.

### ***Morphology of seedlings and saplings***

The germination of *Paullinia cupana* is hypogea, with the seedlings being cryptocotyledonous, whose cotyledons remain attached to the integuments. The primary root is taproot, a common characteristic in dicotyledons, and the species develops embryos with two or more cotyledons. The main root supports the lateral and secondary roots, which are called branched radicles and are cream-yellow in color (Figure 5).

The epicotyl connects the aerial part to the root part and, in the Sapindaceae family, is usually hairy. The hypocotyl is well developed, erect, green in color, and is the organ responsible for connecting the plumule and the radicle, showing potential for transplanting. The plumule, barely visible with green leaves, is the organ that gives rise to the aerial part of the seedlings.

The seedling has two pairs of developed eophylls or metophylls, composed of unifoliate leaves, usually attached to the petiole. Previous characterizations classify the eophylls of *P. cupana* as simple, alternate, and opposite, with an ovate shape, serrated edges in the upper half and smooth edges in the lower half, an obtuse base, an acute apex, and a partially winged petiole (Mendonça et al., 1992), characteristics similar to those found in the present study.

Barreto & Ferreira (2011) highlight that the external morphology of seedlings and saplings allows for immediate and unambiguous identification, providing important contributions to studies with the investigated



species, both in laboratories (micropagation or in vitro), seedling production nurseries, and in field experiments.

The morphometry of guaranazeiro seedlings can provide information to producers about when the external structures are adequate and the seedlings are in ideal morphological and physiological conditions for subsequent classification as quality seedlings. Under these circumstances, nursery growers are able to bring more vigorous and uniform seedlings to the field.

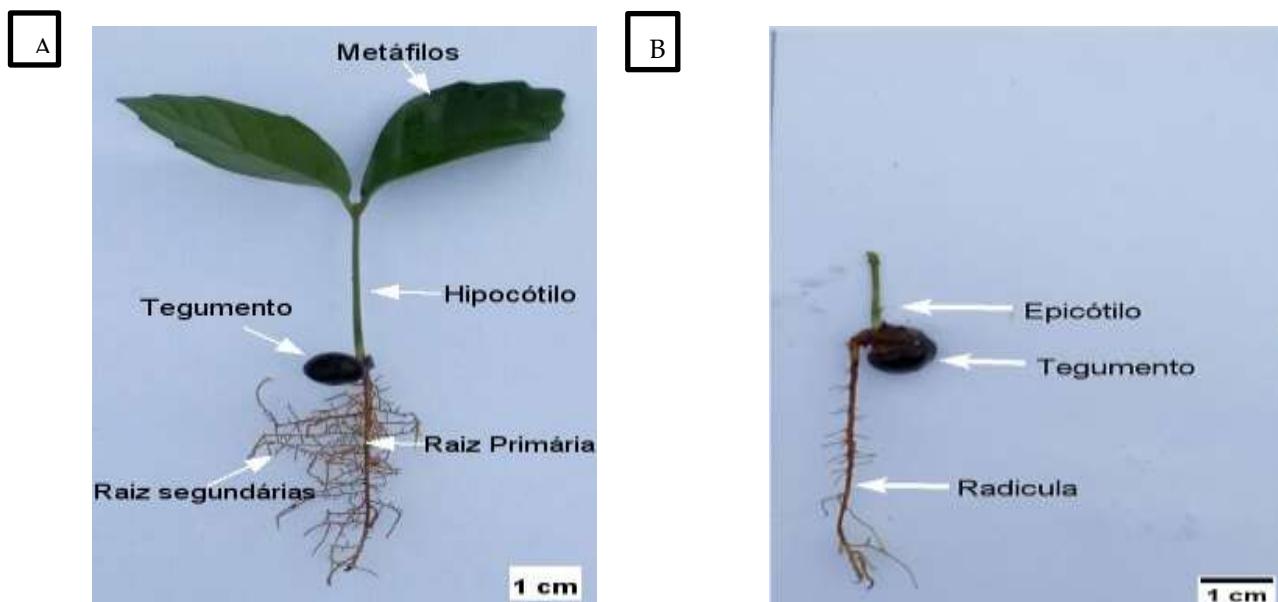


Figure 05: Morphometry of *Paullinia cupana* var. *sorbilis* seedling. A= Metaphylls, Hypocotyl, Tegument, Primary Root, Secondary Root, B= Epicotyl, Tegument, and Radicle. Source: (author, 2023)

## Conclusion

The high variations in fruit weight and seed quantity per fruit may indicate possible genetic diversity among the matrices collected. However, to confirm this variability, studies of the genetic structure of the *Paullinia cupana* population in the Southern Bahia Lowlands are necessary.

The characterization of guaranazeiro seeds is of paramount importance for classifying the physiological quality of the seeds and stands out as an alternative for selecting the aril before roasting.

Commercial substrate and vermiculite enabled the best rates of emergence speed and emergence of *Paullinia cupana* seedlings.

Morphometric aspects of the seeds and seedlings of the species constitute relevant information that can aid in studies of germination, taxonomy, and seedling production.

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