

# Seedlings germination aspects of the *Carapa guianensis* and its ecological conservation for the Amazonian peoples

*Aspectos de la germinación de plántulas de Carapa guianensis y su conservación ecológica para los pueblos amazónicos*

*Aspectos da germinação de mudas de Carapa guianensis e da sua conservação ecológica para os povos amazônicos*

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## Research article

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## Abstract

Anthropic action with intense fruit exploitation for medicinal purposes has caused genetic erosion of 'andirobeira' (*Carapa guianensis* Aublet.), associated with unusual phasic germination, generating the need for research to obtain vigorous seedlings. Therefore, the objective of this work was to

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evaluate different substrates on seedling germination and phasic development, contributing to the conservation of this important, very threatened, species. The experimental design used was randomized blocks, with three treatments: Yellow Latosol (LA), Quartzarenic Neosol (NQ) and Haplic Gleysol (GH), and three replications of 40 plants each. The following characteristics were evaluated: seedling height (AP), root length (CR), stem diameter (DC), and leaf area (AF), along with dry weights from seeds (PSS), roots (PSR), stems (PSC) and leaves (PSF), in addition to the total dry weight (PST), relative growth rate (TCR), and net assimilation rate (TAL). The conclusions were that the days after emergence do not influence the TCR and TAL, but the substrate characteristics affect seedling development. The substrate LA, relative to NQ and GH, should be chosen to obtain seedlings with better development, resulting in superior seedlings. Indeed, the development of seedlings in NQ and GH substrates indicates that their use in the recovery of degraded areas is possible, and the andirobeira presents phasic seedling development, which must be known for effective ecological conservation. Damage to the cotyledons must be avoided to supply the period in which the seedling returns to the heterotrophic state, otherwise the subsequent development of the plant may be compromised.

**Keywords:** Amazon Forest, growth physiology, andiroba trees, Yellow Latosol

### Resumen

Acción antrópica con sobre-explotación de los frutos con fines medicinales causa erosión genética de la andirobeira (*Carapa guianensis* Aublet.), asociado a la inusual germinación fásica, genera la necesidad de investigaciones para obtener plántulas vigorosas. Por ello, el objetivo de este trabajo fue evaluar diferentes sustratos en la germinación de plántulas y en el desarrollo de fases, como contribución a la conservación de esta importante y amenazada especie. Se aplicó un diseño experimental de bloques al azar y tres tratamientos: Yellow Latosol (LA), Quartzarenic Neosol (NQ) y Haplic Gleissolo (GH), con tres repeticiones y 40 plantas cada una, evaluándose las características: altura de plántula (AP), longitud de raíz (CR), diámetro del tallo (DC), área foliar (AF), peso seco de: semillas (PSS), raíz (PSR), tallo (PSC), hojas (PSF), y total (PST), tasa de crecimiento relativo (TCR), tasa de asimilación neta (TAL). Las conclusiones fueron que los días posteriores a la emergencia no influyen en el TCR y TAL, sino las características del sustrato en el desarrollo de las plántulas; el sustrato LA, en relación con NQ y GH, debe ser elegido para obtener plántulas con mejor desarrollo, resultando en plántulas superiores; sin embargo, el desarrollo de plántulas sobre sustratos NQ y GH indica que es posible utilizarlas en la recuperación de áreas degradadas y; andirobeira presenta un desarrollo escalonado de las plántulas, que debe ser conocido para una conservación ecológica eficaz, ya que se debe evitar el daño de los cotiledones para suplir el período en que la plántula vuelve al estado heterótrofo, so pena de comprometer el desarrollo posterior de la planta.

**Palabras clave:** Selva Amazónica, fisiología del crecimiento, andiroba, semillas, Latosol Amarillo

### Resumo

Ação antrópica com intensa exploração de frutos para fins medicinais causa erosão genética da andirobeira (*Carapa guianensis* Aublet.), associada à incomum germinação fásica, gera a necessidade de pesquisas para obtenção de mudas vigorosas. Portanto, o objetivo deste trabalho foi avaliar diferentes substratos na germinação de mudas e no desenvolvimento fásico, contribuindo para a conservação desta importante espécie, muito ameaçada. O delineamento experimental empregado foi blocos casualizados, três tratamentos, sendo Latossolo Amarelo (LA), Neossolo Quartzarênico (NQ) e Gleissolo Hápico (GH) e três repetições, com 40 plantas cada, sendo avaliadas as características: altura da plântula (AP), comprimento da raiz (CR), diâmetro do colo (DC), área foliar (AF), peso seco das sementes (PSS), da raiz (PSR), do caule (PSC), das folhas (PSF), peso seco total (PST), taxa de crescimento relativo (TCR), taxa de assimilação líquida (TAL). As conclusões foram que os dias após a emergência não influenciam o TCR e TAL, mas sim as características do substrato no desenvolvimento das mudas; o substrato LA, em relação ao NQ e GH, deve ser escolhido para obtenção de mudas com melhor desenvolvimento, resultando em mudas superiores; porém o desenvolvimento de mudas nos substratos NQ e GH indica que é possível sua utilização na recuperação de áreas degradadas e; a andirobeira apresenta desenvolvimento fásico das mudas, o que deve ser conhecido para efetiva conservação ecológica, pois deve-se evitar danos nos cotilédones para suprir o período em que a muda retorna ao estado heterotrófico, sob pena de comprometer o desenvolvimento posterior da planta.

**Palavras-chave:** Floresta Amazônica, fisiologia do crescimento, andirobeira, sementes, Latossolo Amarelo.

## Introduction

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The Brazilian Amazon region continues to suffer from severe, intense, and uninterrupted degradation, with continuous and growing deforestation, according to the National Institute for Space Research, through the Satellite Amazon Forest Monitoring Project – INPE/PRODES (2021). In 2012, the smallest deforested area was registered, but as of this year, it has been on an increasing scale, reaching 10,851 km<sup>2</sup> in 2020.

This intensification of the degradation process was mainly caused by anthropic actions to convert primary forests into environments for agricultural activities, including land grabbing, and also illegal mining activities, resulting in intense degradation of soils and water resources, in addition to predatory logging, with consequent permanent genetic erosion of several species of this biome (Araújo, 2015; Palacios and Jaramillo, 2016).

In this sense, it is considered that the degraded area recovery in the Amazon requires the adoption of strategic innovations aimed at protecting its biodiversity and mitigating environmental and social problems. Thus, as a possible solution in recent years, the interest in the propagation of multiple-use native forest species for reforestation has been observed, requiring research about their multiplication. One of the species with multiple uses is the andirobeira (*Carapa guianensis* Aublet), native to Amazonian forests, which is extensively commercially exploited and, for this reason, widely demanded by the industrial sector, both for medicines and cosmetics as well as for logging (Bacca, *et al.*, 2020). However, the predatory extraction of this species has generated a decrease in its natural population (Oliveira and Macedo, 2015), with a high risk of genetic erosion (Furtado *et al.* 2021).

The andirobeira or andiroba tree has its name originating from the indigenous words “landi”, oil, and “rob”, bitter, constituting a late secondary or climax, evergreen, and canopy or sub-canopy tree species, (Kageyama *et al.*, 2004). The species is dispersed in Central America, Antilles, Tropical Africa, and throughout northern South America, including the Amazon Basin. In Brazil, andiroba is found in abundance throughout the Amazon Basin and its tributaries, mainly in regions of the lowland forests, Igapó and floodplains, along the banks of rivers and creeks, and is also present in upland forests (Ferraz, *et al.*, 2002; Boufleuer, 2004; Kenfack, 2011). It is perfectly adapted to the conditions of the Haplic Gleysol with Yellow Latosol, but it has plasticity, as it adapts to different environments (Boufleuer, 2004; Raposo, 2007).

Late and climax secondary species, emerging after the occupation by pioneers, which include the andirobeiras, exhibit less plasticity, lower relative growth rates and net assimilation, and are shade tolerant (Dos Santos *et al.*, 2019).

The success of a species in a biome is defined in the initial phases of the trees, with the seedlings' development, where the initial metabolism of the plants is heterotrophic, being supported by the nutritional reserves of the seed. With seedling development and depletion of internal reserves used for the development and growth of all plant structures, there is a gradual change to an autotrophic metabolism (Gommers and Monte, 2018), which initiates the photosynthetic phase (Rose and Poorter, 2003). Efficient reserve use implies obtaining more vigorous individuals, with greater capacities to achieve autotrophic transition (Delgado *et al.*, 2015). There is a great lack of knowledge about aspects of the heterotrophic-autotrophic transition in tropical tree species, and there are few studies on this subject for andirobeira.

The majority of research with andirobeira involves the pharmacology of the oil obtained from the seeds, ecology of adult trees, and plant and fruits phenology in adult individuals, reaching more than 99% of the scientific articles, while there are almost no articles with development of management techniques or seedling production to obtain new genetic matrices, whose purpose is to multiply the plants and thereby to avoid the genetic erosion (Oliveira and Macedo, 2015; Bacca, *et al.*, 2020; Furtado *et al.*, 2021). Lima and Pauletto (2021), show that no article from 2009 to 2019 involved aspects of germination in andirobeira. Bouffleur (2004) expresses the need for more information on the botanical and ecological aspects of andiroba species, to complement the existing studies, and without a doubt, this aspect is real because very little is effectively researched about the germination and development of seedlings.

In reforestation, it is indispensable to have studies of the morphology of seed germination, growth, and establishment of seedlings, aiming at their propagation and preservation, as these contribute to the understanding of the succession and regeneration of natural ecosystems (Melo and Varela, 2006). Given these aspects, the theme of this work has a high relevance, as it aims to evaluate the effect of different Amazonian soils on the physiology of the germination process and early growth. It also seeks to ratify and disseminate information on the phasic germination of the species, to provide information that can support the production of seedlings for use in degraded area recovery projects and reforestation, and thus make this information available for the conservation of the species.

## Data collection and analysis procedures

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The experiment was conducted in the Experimental Field of the Brazilian Agricultural Research Corporation - Embrapa Amapá, from December 2020 until April 2021, in the Fazendinha District (00° 01' 00" S and 51° 06' 35" W) in the city of Macapá, Amapá State, Brazil, according to Figure 1.



Figure 1. Map of the experiment location in Macapá municipality, Amapá State, northern Brazil. Source: Google Earth (2022)

The experimental environment was in a high-tunnel greenhouse, with a cover using a transparent polyethylene film 150 microns thick, the sides closed with a white nylon screen, and installation of a 70% shade screen, suspended at a height of 3 m inside the same (Figure 2). The average temperature and relative humidity readings inside this greenhouse were taken at 9:00 am, 3:00 pm and 9:00 pm, utilizing a thermohydrograph daily. Substrate moisture was maintained close to field capacity using a microsprinkler.





Figure 2. Greenhouse situated in the Experimental Field of the Brazilian Agricultural Research Corporation - Embrapa Amapá in the Fazendinha District, city of Macapá, Amapá State, Brazil. *Source:* Paulo André Rodrigues da Silva

The sowing to evaluate seedling development was carried out in black polyethylene plastic bags measuring 17 cm × 22 cm filled with the respective substrates, with the cultural treatments being carried out constantly and manually.

The experiment was set up in a split-plot layout within a randomized complete block design with four replications. Main plots consist of soil type (Quartzarenic Neosol, Haplic Gleysol and Yellow Latosol), and subplots consist of eight evaluations related to day intervals (7, 14, 21, 28, 35, 42, 49 and 56 days after seedling emergence), according to the Figure 3.

Substrates				Days after seedling emergence													
NQ	7	14	21	28	49	21	35	42	35	49	28	7	42	14	7	21	
	35	42	49	56	14	56	7	28	56	21	14	42	28	49	56	35	
GH	49	21	35	42	28	7	35	49	49	21	35	42	56	7	35	14	
	14	56	7	28	21	56	14	42	14	56	7	28	42	21	49	28	
LA	14	35	7	28	56	49	35	28	7	56	14	42	56	49	42	35	
	56	21	49	42	42	7	21	14	49	28	21	35	7	14	21	28	
		Replication 1				Replication 2				Replication 3				Replication 4			

Figure 3. Layout from adopted experimental design by split-plot within a randomized complete block with four replications and five andirobeira seedlings per subplot. *Source:* the authors

The analysis of variance was based on the following model, according to Cruz, Regazzi, and Carneiro (2012):  $Y_{ijk} = m + T_i + B_k + E_{ik} + A_j + (TA)_{ij} + E_{ijk}$ ; where  $Y_{ijk}$  is the observed value in the  $i$ -th treatment (substrate),  $k$ -th block and  $j$ -th subplots (days);  $m$  is the general average of substrates and days;  $T_i$  is the effect of the  $i$ -th treatment (substrate),  $k$ -th block,  $j$ -th subplots (days);  $T_i$  is the effect of the  $i$ -th treatment (substrate);  $B_j$  is the effect of the  $k$ -th replication;  $E_{ik}$  is the plot error;  $A_j$  is the effect of the  $j$ -th subplots (days after seedlings emergence);  $TA_{ik}$  is the effect of the interaction between  $i$ -th substrate and  $j$ -th day; and  $E_{ijk}$  is the subplot error.

The three main treatments consisted of different types of substrates: Quartzarenic Neosol (NQ), Haplic Gleysol (GH), and Yellow Latosol (LA), whose main characteristics are presented below, according to Santos et al. (2018).

Yellow Latosols (LA) are soils with intense weathering, popularly called old soils, defined by SiBCS (Santos et al., 2018) by the presence of a latosol diagnostic horizon and general characteristics as the presence of the clays with a predominance of iron, aluminum, silicon and titanium oxides, low activity clays (low CTC), strongly acidic and low base saturation. They usually have low fertility, except when originating from rocks richer in minerals essential to plants, high acidity, and aluminum content. This soil has good physical conditions for agricultural use, associated with good permeability because they are well-structured and very porous soil. However, due to the same physical aspects, there is low moisture retention, especially in those with a coarser texture in drier climates. The yellowish color is uniform in depth, as is the clay content. The most common texture is clayey or very clayey. Another field aspect refers to the high cohesion of the structural aggregates (cohesive soils).

Quartzarenic Neosol (NQ) soils are constituted by mineral material or little thick organic material, with an insufficient manifestation of the diagnostic attributes that characterize the different processes of soil formation, whether due to greater resistance of the parent material or other formation factors (climate, relief, or time) that can prevent or limit soil evolution. This soil has a predominance of characteristics inherited from the original material and is defined by SiBCS (Santos et al., 2018) as poorly evolved soils without the presence of a diagnostic horizon.

Neosols can have high (eutrophic) or low (dystrophic) base saturation, acidity, and high levels of aluminum and sodium. They range from shallow to deep soils and from low to high permeability. This soil class occurs in flat or gently undulating relief and has a sandy texture along the profile and a uniform yellowish color below the A horizon, which is slightly dark. Considering the occurrence relief, the erosion process is not high; however, due to the texture being essentially sandy, precautions were taken for erosion. Because of the

high depth, there is no physical limitation for root development in depth, but the presence of allic character or dystrophic character limits root development in depth, aggravated by the reduced amount of water available (essentially sandy texture). The levels of organic matter, phosphorus and micronutrients are very low. Nitrate leaching is intense due to the essentially sandy texture.

Haplic Gleysol (GH) soils are water-saturated permanently or periodically, unless artificially drained. Water remains stagnant internally, or saturation is by lateral flow into the soil. Thus, these soils are characterized by strong glazing due to the reduced humidity regime, virtually free of dissolved oxygen from water saturation throughout the year, or at least for a long period, associated with biological oxygen demand. Gleysol is defined by the SiBCS (Santos et al., 2018) as hydromorphic soil, consisting of mineral material, which has a gley horizon, which can be a subsurface horizon (C, B or E) or a superficial A horizon. The surface horizon presents colors ranging from gray to black, thickness normally between 10 and 50 cm, and low levels of organic carbon.

In this work, the methods of physicochemical analysis, as well as those of granulometric or textural analysis, including the soil sampling collection technique, following the recommendations of Smith (1976) and Graetz (1983), being adopted as the standard technique adopted and in use by the Soil Analysis Laboratory of the Brazilian Agricultural Research Corporation – EMBRAPA (1997) and the main chemical characteristics are presented in Table 1.

Table 1. Chemical characteristics of substrates used for andirobeira seedling germination.

Substrates	pH	K	Ca <sup>2+</sup> + Mg <sup>2+</sup>	H <sup>+</sup> Al <sup>3+</sup>	P
	H <sub>2</sub> O		Cmmol <sub>c</sub> dm <sup>-3</sup>		mg dm <sup>-3</sup>
LA <sup>1</sup>	4,1	0,33	9,0	5,3	6,0
LA <sup>2</sup>	5,7	1,19	ND	0,8	235,0
GH <sup>3</sup>	5,6	0,15	11,7	3,4	12,0
NQ <sup>4</sup>	5,4	0,02	0,4	0,3	3,0

1. LA: Yellow Latosol collected in nature, present in the Terra-Firme forest ecosystem, called "black earth"
  2. LA: Yellow Latosol fertilized and corrected with 2 kg of dolomitic limestone, 2 kg of triple superphosphate (SPT) and organic fertilizer
  3. GH: Haplic Gleysol, occurring in floodplains and alluvial plains by sedimentation of colloids dragged by the hydrographic network of the Amazon Basin
  4. NQ: Quartzarenic Neosol, sand fraction equal to or greater than 70% and clay fraction less than 15%
- ND: not determined



The seeds were obtained according to the recommendations of Vencovsky (1978), Figliolia (1995) and Shumacher, Hoppe, and Farias (2002), where it is recommended that to guarantee the genetic variability of the species in research, seeds from 20 random trees should be collected. Thus, the seeds were collected from four trees each at five locations, being Macapá, at the headquarters of Embrapa Amapá, in the Distrito da Fazendinha, in the municipality of Mazagão at its headquarters in Camaipí, and lastly in the municipality of Porto Grande. The seeds were obtained from ripe fruits, already released to the ground. These were selected by size from medium to large. Smaller seeds and those attacked by the lepidopteran *Hypsipyla ferrealis* Hampson were excluded from the collected lots.

The seeds were packed in plastic bags for transport and short-term storage, avoiding dehydration of the collected material, because the seeds do not tolerate desiccation as recommended by Ferraz *et al.* (2002), maintaining humidity with the slightly wet sand used for germination capacity conservation (Medeiros *et al.*, 2015), and sowing was affected one week after collection.

In the subplot, five seedlings of each treatment were evaluated every seven days, in the period between germination, which started seven days after sowing and ended the eighth week after sowing. For each plant, biometric data were collected, such as seedling height (AP, in cm), root length (CR, in cm), stem diameter (DC, in cm), leaf area (AF, in cm<sup>2</sup>), weight seed dry (PSS, in g), root dry weight (PSR, in g), stem dry weight (PSC, in g), dry weight of leaves (PSF, in g). And, from these primary data, the subsequent variables, such as total dry weight (PST, in g), relative growth rate (TCR, in g.g<sup>-1</sup>, in 7 days), net assimilation rate (TAL, in g. dm<sup>-2</sup>, in 7 days), were determined according to the indications of Lucchesi (1984) and Benincasa (1986). Measurements of seedling height, root length and stem diameter were performed using a caliper and centimeter ruler.

To determine the leaf area of the plants, four leaves were taken from each sampled plant, and four-leaf tissue discs with 0.48cm<sup>2</sup> were separated. The leaf area of the plants was calculated with the following formula:  $AF = (ND \times AD \times MSF) \times MSD^{-1}$ , where AF is the leaf area, ND is the number of sample discs, AD is the area of discs in cm<sup>2</sup>, MSF is the leaf dry matter (g), and MSD is the dry matter of discs (g).

The total dry weight of the plant, in grams, was determined using the dry weight of seeds, roots, stems and leaves calculated using the following formula:  $PST = PSR + PSC + PSF$ , where PST is the total plant dry weight, PSR is the root dry weight, PSC is the stem dry weight, and PSF is the dry weight of leaves.

According to Lucchesi (1984) and Benincasa (1986), the relative growth rate (TCR) is the most suitable measure to assess the growth of a plant, and it represents the amount (area, volume, weight) of plant material produced by a given amount of material for a predefined time interval. If TCR is calculated in terms of dry weight, the unit can be  $\text{g.g}^{-1}$  over time. The expression for the instantaneous value of the TCR is given by:  $\text{TCR} = (\text{DP} \times \text{DT}^{-1}) \times (1 \times \text{P}_0^{-1})$ , where DP is the weight variation, DT is the time interval, and  $\text{P}_0$  is the initial weight.

The net assimilation rate (TAL) represents the increase in dry matter per unit of leaf area over a predefined time interval. The result is expressed in  $\text{g.dm}^{-2}.\text{day}^{-1}$ . Its instantaneous value is given by the expression  $\text{TAL} = (\text{DP} \times \text{DT}^{-1}) \times (1 \times \text{A}^{-1})$ , where DP is the weight variation, DT is the measurement time interval, and A is the leaf area at time  $t_0$ .

The parameters obtained were subjected to statistical analysis using the statistical package “SAS System”, performing Analysis of Variance (ANOVA), by the F test for comparison of means of the Tukey test at 1%, when the results showed significant differences in the ANOVA, following the recommendations of Pimentel-Gomes (2009) and Santana and Ranal (2004).

## Discussion

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It is important to point out initially that the ability of plants to morpho-physiologically adjust (throwing or losing leaves and carrying out metabolic changes) allows for adaptive success and acclimatization in the forest site in the initial phase of growth (Larcher, 2006; Gonçalves, Santos Júnior, Nina Júnior, and Chevreuil, 2007; Wit, Galvão, and Fankhauser, 2016), this being the importance of the knowledge generated here and discussed below.

Table 2 shows significant differences for all evaluated characteristics between the substrates (T), days after seed sowing (A) and the presence of a  $T \times A$  interaction, clearly indicating that the seedling development results from different chemical and physical substrates, generating time-differentiated morphological characteristics, with a significant increase in biomass. Special attention should be paid to the  $T \times A$  interaction, which can certainly generate different combinations of responses.

*Table 2.* Synthesis of analysis of variance for ten characteristics evaluated in seedlings of *Carapa guianensis* Aublet. from the treatment (T, different substrates), repetition (R), time (A, days after seed sowing), the treatment-time interaction ( $T \times A$ ), and coefficient of variation (CV)

	General mean		Mean Square		CV
	Treatment (T)	Repetition (R)	Days (A)	TxA	
PSS	10.50 **	0,9270 <sup>ns</sup>	< 0.0001 **	0.0017 **	18.06
PSR	1.24 **	0.9270 <sup>ns</sup>	< 0.0001 **	0.0017 **	18.06
CR	10.98 **	0.6187 <sup>ns</sup>	0.0001 **	0.0001 **	7.37
DC	0.56 **	0.0127 <sup>ns</sup>	< 0.0001 **	0.0001 **	7.69
PSC	1.42 **	0.8307 <sup>ns</sup>	< 0.0001 **	0.0001 **	19.30
PSF	1.80 **	0.4077 <sup>ns</sup>	< 0.0001 **	0.0001 **	19.42
AP	24.53 **	0.0042 <sup>ns</sup>	< 0.0001 **	< 0.0001 **	9.95
AF	257.84 **	0.4077 <sup>ns</sup>	< 0.0001 **	< 0.0001 **	19.42
TCR	0.01 <sup>ns</sup>	0.9463 <sup>ns</sup>	0.0009 **	0.1288 <sup>ns</sup>	366.05
TAL <sup>a</sup>	0.00 <sup>ns</sup>	0.348 <sup>ns</sup>	0.0003 **	0.1611 <sup>ns</sup>	483.50

\* Significant at 5% probability, \*\* highly significant at 1% probability, ns not significant; CV: coefficient of variation

<sup>a</sup> the average between positive and negative values of the net assimilation rate for the evaluation period resulted in a value equal to zero; however, it is emphasized that there are positive and negative values present

PSS: seed dry mass, in grams; PSR: root dry mass in grams; CR: root length in cm; DC: stem diameter in cm; PSC: stem dry weight in grams; PSF: leaf dry weight in grams; AP: plant height in cm; AF: leaf area in cm<sup>2</sup>; TCR: relative growth rate in g.g<sup>-1</sup> over 7 days<sup>-1</sup>, and TAL: net assimilation rate in g. dm<sup>-2</sup> over 7 days.

An exception occurred to TCR and TAL with no significant differences (Table 2), which can be considered a normal behavior because physiological characteristics related to the photosynthetic process are not usual for plant selection, and therefore the heritability indices are not even tested. However, Granciere (2015) and Souza (2016) found values greater than 60% for the CO<sub>2</sub> assimilation rate, considered as high, justifying the absence of  $T \times A$  interaction due to the low response to environmental factors. This behavior indicates that the TCR and TAL are fully associated with controllable factors, certainly genetics, without any physiological plasticity in response to environmental factors, differing from the results obtained from Peixoto, Machado, Borges, Alves, and Leal (2012), which also emphasize that these characteristics provide subsidies for understanding the adaptations (ombrophylous or heliophylous species) manifested by different species under different environmental conditions.

The coefficient of variation (CV) value presented in Table 2 for the characteristics indicates a low influence of uncontrollable growth factors. The CV values are directly associated with differences in the amounts of nutrient

reserves transported from the seed to the stem and leaves, as well as the genetic variability present in this native forest species, which has not had a genetic selection, keeping some variability. TCR and TAL presented high CVs, possibly due to the dry matter accumulation in the initial seedling emergence period, followed by a fall in the process, with posterior new dry matter accumulation, generating an exponential proportion that ends up increasing the estimated CVs by a lot for the entire experimental period.

The different substrates do not influence the percentage of germination; all the seeds sown germinated (Table 3), demonstrating that the form of storage for transport from the collection site to the sowing site was adequate for the conditions of Amapá. This confirms the information of Ferraz and Varela (2003), Amoêdo (2015) and Bacca, Zuluaga, Ivez-Perez, and Palacio (2020), which also obtained high percentages of germination for andirobeira. However, it is worth remembering that Oliveira and Macedo (2015) state that the percentage of germination over time varies with soil type.

Table 3. Germination percentage (G%), seed dry weight (PSS, in grams), root dry weight (PSR, in grams), root length (CR, in cm), stem diameter (DC, in cm), stem dry weight (PSC, in grams), leaves dry weight (PSF, in grams), plant height (AP, in cm), foliage area (AF, in cm<sup>2</sup>), relative growth rate (TCR, in g.g<sup>-1</sup> over 7 days) and net assimilation rate (TAL, in g. dm<sup>-2</sup> over 7 days) of *Carapa guianensis* Aublet. seedlings, growing in Yellow Latosol (LA), Quartzarenic Neosol (NQ) and Haplic Gleysol (GH) as a function of the treatments.

Characteristic	Treatments		
	LA	NQ	GH
G%	100,00 A	100,00 A	100,00 A
PSS	10,25 B	11,50 A	9,74 B
PSR	1,85 A	0,93 B	0,93 B
CR	12,38 A	9,93 C	10,59 B
DC	0,5813 A	0,5359 B	0,5638 A
PSC	1,65 A	1,09 B	1,49 A
PSF	2,62 A	1,34 B	1,42 B
AP	28,06 A*	23,75 B	21,79 C
AF	375,33 A	192,68 B	203,06 B
TCR	0,0275 A	0,0050 A	0,0134 A
TAL	0,0026 A	0,0010 A	0,0001 A

Means followed by the same capital letters in the line do not differ statistically from each other, by the Tukey test.

\* LA fertilized

In aspects related to nutrient absorption from the substrate and leaf area, dry weight root, length root, leaf dry weight, seedling height, and foliage area

were in general higher to Yellow Oxisol (Table 3), indicating that this soil offered better conditions for the development of these parts of the seedling with the other two substrates. For seed dry weight, there is a higher value in Quartzarenic Neosol substrate, possibly due to lower demand for water and nutrition, allowing a slower physiological development, thus consuming more slowly the reserves of the seeds andirobeira.

Aspects related to the stem, such as stem diameter and stem dry weight, were higher in Yellow Latosol and Haplic Gleysol, generating seedlings with better development (Table 3). So, summarizing, in general terms, the soil with inferior characteristics was the Quartzarenic Neosol, and the Yellow Latosol was the best. These development parameters can be used to orientate the multiplication process using seeds about which type of soil is the most suitable, because the substrate characteristics, such as aeration, structure, water retention capacity and pathogen infestation, influence the germination process, which may favor or harm seed germination (Abreu *et al.*, 2017), as well as the possible development in the definitive planting site, with the degraded areas recovering objective and returning the productivity in the anthropized areas with the adoption of an Agroforestry System (Araújo, 2005).

The values observed in Table 3 for PSF and AF indicate that there is the formation of a surface available for receiving solar luminosity, therefore resulting in better photoassimilate accumulation detected by TAL (Peixoto *et al.*, 2015), which in absolute values was higher in Yellow Latosol, but statistically not differed from the other substrates, in the same way for TCR, which also did not distinguish between the different substrates; this behavior is indicative that for this species, the physiological aspects, as already mentioned, are of simple or total genetic control and inherent to andirobeira.

In the graphs, there was a division so that the scales of the measurement units would allow the visualization of the behavior according to the development time of the seedling, shown in these three graphs (Figures 1–3). Additionally, it is important to mention that the graphs were made only for the characteristics whose phasic behavior was more evident.

In the weekly results of the evaluations on days 7 until 56, in the morphological characteristics PSR, PSC and PSF, the straight line increased with the seedling development (Figure 1), a behavior that was expected; however, it is very important to emphasize the existing inflection points between 35 and 42 days, behavior not common in other species (Ferraz *et al.*, 2002). The inflection points at 28 days where the graph line stops increasing with seedling development, with resumption at 42 days, increasing again, is called phasic development, being important because the environmental requirements of emergence are different, shortly ceasing the formation of the photosynthetic apparatus to again need the internal reserves of the seed.

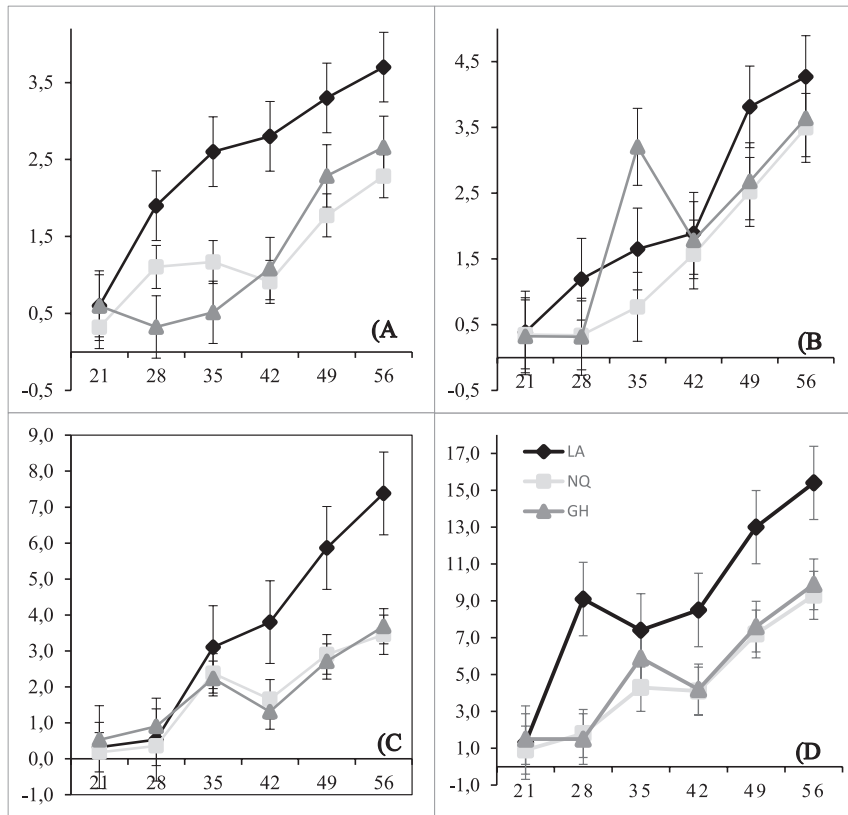


Figure 1. Development curves of andirobeira seedlings at days 7 until 56 for total dry weight of seedlings (PST, D), being the sum of dry weight from roots (PSR, A), stem (PSC, B), and leaves (PSF, C) to Yellow Latosol (LA), Quartzarenic Neosol (NQ) and Haplic Gleysol (GH); bars represent standard error.

According to Querino *et al.* (2008), the andirobeira has accelerated growth after germination, and this fact occurs because the andiroba seeds have a high level of nutrients, which favors seedling growth. This also corresponds to the expected strategy for species in early successional stages that tend to grow quickly to compete for space and light (Battilani, Santiago and Souza, 2006). Thus, when observing relative to the substrate, for LA, there is a tendency for a best seedling developmental performance for PSR, PSF and PST. For GH and NQ, the growth curves were very similar and lower than for LA (Figures 1A, 1B, 1C and 1D). Therefore, without a doubt, instead of the use of sand commonly used in the germination of Amazonian forest species was superior to the use of LA, because there is an apparent minimization of phasic germination for the andirobeira seeds.

The development and performance of germination for LA (Figures 1A, 1B, 1C and 1D) indicate that the andirobeira seed does not need stratification to



break dormancy, different from many Amazonian species (Serviço Nacional de Aprendizagem Rural, SENAR, 2018).

Regarding physiological characteristics (Figure 2), in the specific case of TCR, there is a growing addition of tissues with photosynthesis associated with the use of the seed's nutritional reserves at each evaluation. At the beginning of the seedling development process, the leaf area is left with photosynthetic capacity and growth rate both high, which makes them present high and constant TCRs at this stage of the cycle (Pedó, Martinazzo, Aumonde, and Villela, 2015), but for seedling development from andirobeira seeds, the development curve differs, presenting growth behavior in phases, with a high increase on day 28 that falls until day 42 to LA, with a subsequent increase and a new downward trend, which is an unusual oscillation among tropical forest species (Figure 2).

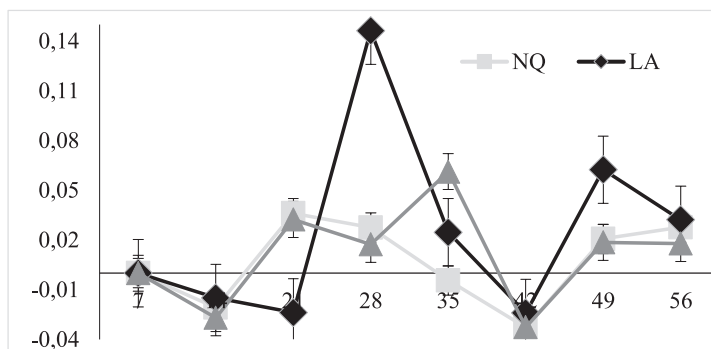


Figure 2. Development curves of andirobeira seedlings from 7 to 56 days for TCR (relative growth rate, in  $\text{g.g}^{-1}$  over 7 days) to Yellow Latosol (LA), Quartzarenic Neosol (NQ) and Haplic Gleysol (GH); bars represent standard error.

Seedlings have phasic growth, showing rapid growth right after emergence reaching up to 40 cm (*C. guianensis*) depending much more on seed size than on the species itself and then remain for weeks without showing apparent growth until again releasing a new set of leaves (Ferraz, Camargo and Sampaio, 2002; 2003). Here, the results of the phasic effect were close to the 42nd day of evaluation, where the plants were a little less than 35 cm tall, a little smaller and earlier than what was mentioned by Ferraz *et al.* (2002; 2003), possibly due to the better conditions offered by the substrates relative to sand. This emphasized the aspect mentioned by Gilbert *et al.* (2016), where growth strategies are not fixed throughout plant development.

The knowledge of phasic growth in seedling development is important, as the reserve cotyledons must not be allowed to be damaged until the plant passes through this phase period, as it will again depend on its reserves accumulated in the seed, and if removal or withdrawal is allowed, damage close to 40 days after emergence in the cotyledons compromises the formation of a vigorous seedling to be taken later to the planting site.

Therefore, in andirobeira seedlings, these rates and variations differ from several tree species studied. For example, in *Inga laurina* (Jesus *et al.*, 2016), there are no high peaks of increase in TCR, and Souza, Santos, Ferreira and Gonçalves (2017) found that *Bertholletia excelsa* was also without peaks in rates, but rather a linear and continuous increase. Regarding the type of substrate, a similarity was obtained in that, with better fertility, there was a higher TCR.

One important aspect to be highlighted is that the phasic germination behavior is more intense in the LA substrate, while in NQ and GH, the presence of this characteristic is less evident. Therefore, more fertile soils tend to increase the expression of phasic seed germination in andirobeira (Figure 2), offering initial conditions for faster seedling development, as can be seen by the graphic lines in comparison with the dry weight characteristics of Figure 1.

For TAL (Figure 3), from the 35th day from germination onwards, there were no differences in soil types with subsequent evaluations (days 42, 49, and 52), but it is noted that there were fluctuations in the values from day 14 until 35. This is not common in tropical forest species, but is characteristic of the andirobeira, according to Ferraz *et al.* (2002). Therefore, when carrying out andiroba propagation, the professional must be aware of this distinct characteristic related to the germination of the seeds and their subsequent development.

This phasic germination from andirobeira observed to TAL is coherent with the shady environments in which the andirobeira seeds generally germinate, with high heterogeneity in light availability, the andirobeira shows plasticity when starting the first leaf primordia development, associated with photosynthesis, with later stop for use of cotyledon reserves. This behavior indicates a recognition adaptation to environmental conditions, representing a mechanism of great relevance to reinforce survival and the future performance of the adult tree under different ranges of variations in light environments (Valladares and Sánchez-Gómez, 2006, Nicotra *et al.*, 2010).

Contrary to the sowing recommendation in the sand (Ferraz, 2004), and associated with the justification that the andirobeira seed has a large reserve nutrient amount (Bouffleyer, 2004), allowing the establishment of the seedling, the obtained results indicate that the best development is obtained in the LA soil. This minimizes the phasic development in the root, stem, leaves and total dry weight, despite the pronounced existence in TCR and TAL (Figure 2 and Figure 3) that was certainly counterbalanced with the reserves of the cotyledons; for this reason, the use of seeds that are perfectly formed and healthy is also indicated.

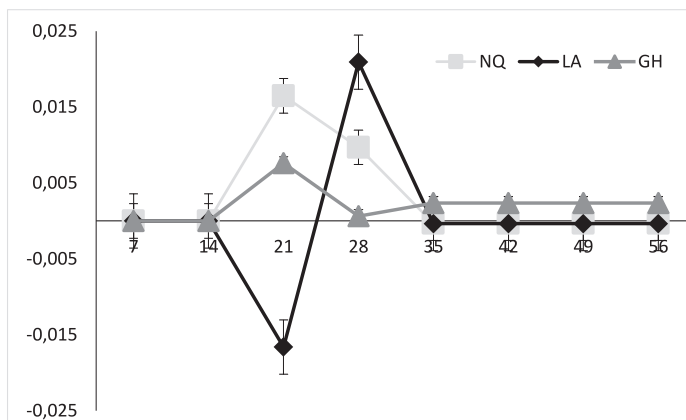


Figure 3. Development curves of andirobeira seedlings from 7 to 56 days for TAL (net assimilation rate, in g. dm<sup>-2</sup>, over 7 days) to Yellow Latosol (LA), Quartzarenic Neosol (NQ) and Haplic Gleysol (GH); bars represent standard error.

The oscillations in the TAL curve must be mentioned, emphasizing that they are less intense compared with TCR, with an increase on day 28, and it is important to cite the negative rate for LA substrate at 21 days, with subsequent intensified recovery at 28 days relative to the other substrates. This oscillation, which may seem unexplained, as already mentioned, is associated with the physiology of the species that presents a phasic development, being common in andirobeira, according to Ferraz *et al.* (2002). There is a process of formation of the first six photosynthetic individual leaves, and in the process of formation of the next leaves, the consumption becomes greater than the photosynthesis production.

For TAL (Figure 3), the results obtained were different in comparison with *Inga laurina* (Jesus *et al.*, 2016), where the seedling development did not differ between the better fertility substrate and the one with lower fertility, while here with andirobeira, there were great differences due to the substrate, similar to results to Breu Branco cited by Santos *et al.* (2012). However, it is most important to emphasize that lines in the figures present peaks for TAL and TCR, while the bibliography shows linear behavior, with no peak presence of great prominence.

According to Sánchez *et al.* (2019), the requirements for the establishment of the seedling are specific to each species and are influenced by the characteristics of the region that the species has adapted, so these characteristics are distinguished in each tropical forest species, and without a doubt, with the results obtained here, the type of substrate associated with shade conditions has a direct influence on the germination behavior of andirobeira seeds.

The results obtained here are important to meet the growing use of forest species, which need to have information on the morphology of seed germination, growth, and establishment of the seedling, aiming at its propagation and

preservation, while also contributing to the understanding of the succession and regeneration of the natural ecosystems (Melo and Varela, 2006).

This phenotypic plasticity indicates that the andirobeira, once again resorting to cotyledonary reserves, conducts a new process of leaf formation after the beginning of germination and photosynthesis. It is adapted to germinate initially in conditions of low light (ombrophilous), and therefore, care must be taken to offer the right conditions for perfect seedling development; otherwise, an adult individual with imperfections may be generated.

## Conclusions

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The days after seedling emergence did not influence TCR and TAL, but without a doubt, the physicochemical characteristics of the substrates affected the intensity of seedling development, and Yellow Latosol (LA) should be chosen to obtain seedlings with better development.

The Yellow Latosol (LA) substrate is statistically superior to the other substrates in terms of morphological characteristics, resulting in seedlings with greater dry weight and length in the metric characteristics evaluated; therefore, its use is recommended in the formation of seedlings for the recovery of degraded areas to be used to reduce the risk of genetic erosion of the species.

Even with poor soils (NA and GH), the development of seedlings was observed, which is an indication of the possibility of their use in the recovery of degraded areas.

The andirobeira presents phasic development of the seedlings, whose intensity is higher in soils with higher fertility, a germination characteristic which must be known to those who produce the seedlings to avoid damage to the cotyledons, which supply the period when the seedling returns to the heterotrophic state. Otherwise, the subsequent development of the plant may be compromised, leading to problems in production.

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