SÃO PAULO STATE UNIVERSITY "JÚLIO DE MESQUITA FILHO"
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CAMPUS DE ILHA SOLTEIRA

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STUDY OF POPULATION STRUCTURE, MOLECULAR CHARACTERIZATION OF *Aniba rosaeodora* (Ducke) ROSEWOOD IN AREAS OF POTENTIAL DISTRIBUTION OF MASISEA DISTRICT, UCAYALI, PERU

Ilha Solteira, São Paulo
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GRADUATE PROGRAM IN AGRONOMY

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Thesis presented to the Faculty of Engineering of the Ilha Solteira Campus – UNESP as part of the requirements to obtain the title of Doctor in Agronomy. Specialty: Production System.

Orientador: Prof. Dr. Alexandre Magno Sebbenn

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IMPACTO POTENCIAL DESTA PESQUISA

O pau-rosa *Aniba rosaeodora* Ducke, conhecido como “pau-rosa”, é uma espécie valiosa da Amazônia, que foi submetida a intensa exploração, que dizimou suas populações naturais, levando-a à beira da extinção e da degradação de sua diversidade genética. o presente estudo tem potencial para conservação e domesticação da espécie na Amazônia peruana que pode ser replicado no restante da Amazônia. As informações geradas podem traçar um plano de melhoramento genético da espécie, utilizando germoplasma de árvores de populações menos aparentadas, com base em padrões de dispersão de sementes e observando a composição de sua estrutura populacional. Da mesma forma, a pesquisa pode implementar uma base de sementes com aumento da diversidade genética para o manejo sustentável da floresta e o desenvolvimento de plantações comerciais em solos degradados da Amazônia, para a geração de renda econômica para os agricultores amazônicos, o que seria conter a expansão das fronteiras agrícolas na Amazônia.

POTENTIAL IMPACT OF THIS RESEARCH

The rosewood *Aniba rosaeodora* Ducke, known as "rosewood", is a valuable species of the Amazon, which was subjected to intense exploitation, which decimated its natural populations, bringing it to the brink of extinction and the degradation of its genetic diversity, so the present study has the potential for conservation and domestication of the species in the Peruvian Amazon, which can be replicated in the rest of the Amazon. The information generated can design a genetic improvement plan for the species, using germplasm from trees from less related populations, based on seed dispersal patterns and observing the composition of its population structure. Likewise, the research can implement a seed base with an increase in genetic diversity for sustainable forest management and the development of commercial plantations on degraded soils in the Amazon, to generate economic income for Amazonian farmers, which would contain the expansion of agricultural frontiers in the Amazon.

DEDICATION

To my wife Mayra, for her love, patience and understanding.

To my children Luciana, Jorge and Mayra; lights of my life and hope for tomorrow.

To my dear parents, Sadith and Jorge, for the love and effort to make me a better man, life will not be enough to thank.

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ABSTRACT

Aniba rosaeodora Ducke, known as "rosewood", is one of the most valuable tree species in the Amazon, famous for its linalool-rich essential oil, used in the cosmetics industry. However, their overexploitation has led to the decrease of their natural populations, affecting their production and genetic diversity, putting the species at risk of extinction. The species is found mainly in forests in Brazil, Colombia, Ecuador, Peru and other countries in the Amazon region, growing in habitats of plateaus and streams, and can reach up to 30 m in height. Seed dispersal depends on animals such as parrots and toucans, but their natural regeneration is threatened by predation and other factors. This study seeks to better understand the patterns of dispersion, density and population dynamics of *A. rosaeodora* in the Peruvian Amazon, in order to propose strategies for its conservation and forest management. Population dynamics play a key role in the conservation and domestication of species. From genetic assays of forest species in the Peruvian Amazon, methodologies were developed to evaluate the phenotypic variation of their traits of commercial interest. These methodologies were applied in conservation and domestication studies, through stability, adaptability and productivity tests of progenies of *Guazuma crinita* Mart, in addition to the qualitative and quantitative control of phenotypes of *Calycophyllum spruceanum* Benth. These studies, carried out in the Aguaytía river basin in the Peruvian Amazon, can be adapted for methodological application in *Aniba rosaeodora* Ducke in the region, with the aim of promoting its conservation and sustainable management.

Keywords: amazon forest, rosewood, population density, population structure, seedling dispersal, biomass, allometric model, progeny test, white bolaina, gxe interaction, capirona.

RESUMO

Aniba rosaeodora Ducke, conhecido como "pau-rosa", é uma das espécies arbóreas mais valiosas da Amazônia, famosa pelo seu óleo essencial rico em linalol, utilizado na indústria cosmética. No entanto, sua sobre-exploração tem levado à diminuição de suas populações naturais, afetando sua produção e diversidade genética, colocando a espécie em risco de extinção. A espécie é encontrada principalmente em florestas do Brasil, Colômbia, Equador, Peru e outros países da região amazônica, crescendo em habitats de platôs e riachos, podendo atingir até 30 m de altura. A dispersão das sementes depende de animais como papagaios e tucanos, mas sua regeneração natural está ameaçada pela predação e outros fatores. Este estudo busca entender melhor os padrões de dispersão, densidade e dinâmica da população de *A. rosaeodora* na Amazônia peruana, a fim de propor estratégias para sua conservação e manejo florestal. A dinâmica populacional desempenha um papel fundamental na conservação e domesticação das espécies. A partir de ensaios genéticos de espécies florestais na Amazônia peruana, foram desenvolvidas metodologias para avaliar a variação fenotípica de seus caracteres de interesse comercial. Essas metodologias foram aplicadas em estudos de conservação e domesticação, por meio de testes de estabilidade, adaptabilidade e produtividade de progênies de *Guazuma crinita* Mart, além do controle qualitativo e quantitativo de fenótipos de *Calycophyllum spruceanum* Benth. Esses estudos, realizados na bacia do rio Aguaytía, na Amazônia peruana, podem ser adaptados para aplicação metodológica em *Aniba rosaeodora* Ducke na região, com o objetivo de promover sua conservação e manejo sustentável.

Palavras-chave: floresta amazônica, pau-rosa, densidade populacional, estrutura populacional, dispersão de plântulas, biomassa, modelo alometrico, teste de progênie, bolaina branca, interação g x e, capirona.

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SUMMARY

1 INTRODUCTION

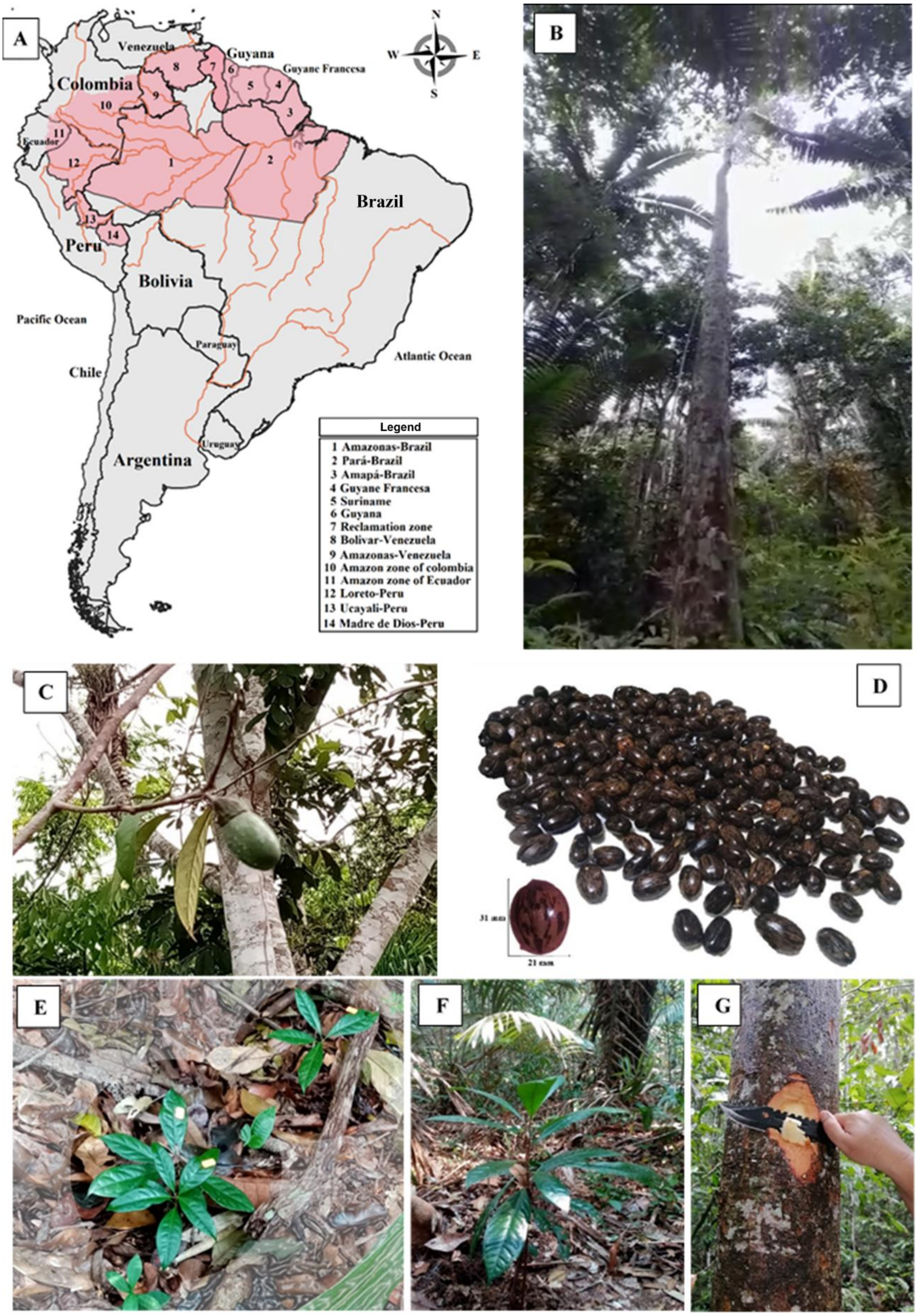
Aniba rosaeodora Ducke, or rosewood (Figure 1), is one of the most valuable native tree species in the Amazon biome. Exploited to extract its essential oil containing linalool, a secondary metabolite valued in the cosmetics industry as an excellent perfume fixative (Amusant *et al.*, 2015, 2016; Giongo *et al.*, 2017). Its overexploitation has led to the decline of its natural populations, its production and the genetic diversity of its populations, putting it at risk of extinction in the most exploited areas and leading to its inclusion in the IUCN list of threatened species (May; Barata, 2004; Barstow, 2021).

Aniba rosaeodora is a species of the Lauraceae family, with hermaphrodite flowers pollinated by insects, its seeds are dispersed by barochory and zoochory mainly by birds, particularly parrots and toucans (Spironello *et al.*, 2004). Seed production begins when individuals reach about 10 cm in diameter at breast height (DBH) (Spironello *et al.*, 2003). In adulthood, individuals can reach up to 30 m in height and 2 m in diameter at breast height - DBH (Kubitzki; Renner, 1982). In Ucayali, Peru, trees have been reported in natural populations with approximately 25 cm in DBH and a maximum total height of 26 m (Gutiérrez *et al.*, 2023; Revilla-Chávez *et al.*, 2024). The trees have a straight, cylindrical trunk, yellowish or reddish-brown bark that easily separates into large plates, with a narrow, oval crown. It occupies the intermediate or upper stratum of the forest canopy and is found in Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname and Venezuela (Kubitzki; Renner, 1982). It can be found in both dry and humid forests, with a preferred habitat on plateaus and streams (Kubitzki; Renner, 1982). Population density is generally low, ranging from 2 to 27 trees.ha^{-1} with DBH less than 10 cm in the rainfed forests of northern Manaus, Brazil (Loureiro *et al.*, 1979; Mitja; Lescure, 1996) and 0.12 and 0.16 trees.ha^{-1} for trees with DBH greater than 20 cm near Manaus (Alencar; Fernandes, 1978). The spatial distribution of adult trees is grouped into groups (five to eight trees), with tree spacing ranging from 50 to 100 m and between groups of 300 to 400 m (Alencar; Fernandes, 1978).

Seed dispersal is a fundamental element of natural regeneration that ensures the sustainability of forest resources over time when it occurs in a random and complex manner (Rodríguez; Mandujano, 2007; Calama *et al.*, 2017). This phenomenon determines the potential area of recruitment of individuals and establishes the basis for subsequent processes, which are influenced by the genetics of the tree, age, position in the forest canopy, among other factors (Campoe *et al.*, 2013). During dispersal, predation is one of the main causes of mortality in the tree's reproductive cycle, reaching up to 60% of which 50% are by insects (Spironello *et al.*, 2003; Norden, 2014; Muñoz, 2018). This can modify the dynamics of the structural composition of forests, in the distribution and abundance of seedlings, juveniles and adults, which can also be affected depending on the level of intervention of the forests (Rasal *et al.*, 2012).

In this context, the present study seeks to fill the gaps in our understanding of the species in terms of conservation and forest management, identifying the patterns of seedling dispersal, density and dynamics of the population structure of *A. rosaeodora* in a natural forest and compare the results with other studies developed to argue the potential for the conservation of the species in the Peruvian Amazon.

Figure 1 - General appearance of the species *Aniba rosaeodora*: A. Distribution map of the species in South America, B. adult tree, C. fruit with seeds, D. seeds, E. seedlings (brinzals), F. sapling (latizals), and G. stem and bark.



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2 SEEDLING DISPERSAL AND POPULATION STRUCTURE DYNAMICS OF *Aniba rosaeodora* (Ducke) IN UCAYALI, PERU[1]

ABSTRACT

Aniba rosaeodora is a valuable species from the Amazon biome that is harvested to extract linalool, a secondary metabolite used as a fixative in cosmetic perfumes, which has led it to overexploitation and to the brink of extinction, contributing to the decline and genetic erosion of its natural populations. However, forest conservation and management efforts are hampered by insufficient knowledge about the distribution of its seedlings and population structure. Therefore, the present study seeks to understand these tree dispersal patterns in a tropical forest on medium terrace soils in the Masisea district, Coronel Portillo province, Ucayali, Peru. Our results show that seeds and seedlings reach an average distance of 18.3 ± 9.3 m, up to 44 m from the seed tree. Seed dispersal and seedling emergence occur mainly by barochory and zoochory by dispersing birds, creating a population structure of 53.4%, 12.2%, 27%, and 7.4% of seedlings, saplings, stems and adult trees, respectively. With 4.3 to 7 individuals.ha^{-1}, with a low average of adult trees (0.5 individuals.ha^{-1}) in selected plots. Trees with diameters > 20 cm present groups of 2 to 5 individuals, spaced on average between 97 to 110 m. The analysis, adjusted to Meyer's exponential model (R^2= 0.98) and to Liocourt's coefficient (q= 1.5-3) indicate an unsustainable population recovery in the study area, with a high probability of coancestry indices. Being an area with potential for conservation of genetic resources for the species.

Keywords: Forest, Rosewood, Population density, Population structure, Seedling dispersal.

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[1] REVILLA-CHÁVEZ, J.M.; SAENZ-RAMIREZ, L. H; GONZALES-ALVARADO, A.C.; ALVAREZ-GARCÍA, A.J.; MORI-VÁSQUEZ, J.A.; SEBBENNA, A.M. Seedling dispersal and population structure dynamics of *Aniba rosaeodora* Ducke in Ucayali, Peru, **Acta Amazônica**, Manaus, **(Submitted: 27-Jul-2024)**

2.1 INTRODUCTION

One of the most valuable native tree species in the Amazon biome is *Aniba rosaeodora* Ducke (Lauraceae family), or rosewood. The species has been overexploited for decades to extract linalool, a secondary metabolite valued in the cosmetic industry as an excellent perfume fixer (Amusant *et al.*, 2015, 2016; Giongo *et al.*, 2017). Although linalool is present in all parts of the tree, its yield for oil extraction is 1.4 and 2.4% from wood and leaf biomass, respectively, and oil is primarily extracted from the wood (Araújo *et al.*, 1971; Teles; Mouchrek, 2022).

Overexploitation has led to the decline of both populations and production, with the consequent genetic erosion of *A. rosaeodora* natural populations, putting the species at risk of extinction in heavily exploited areas (May; Barata, 2004) and leading to its inclusion on the IUCN list of threatened species (Barstow, 2021).

Aniba rosaeodora is an insect-pollinated hermaphrodite, and its seeds are dispersed by birds, particularly parrots and toucans (Spironello *et al.*, 2004). Reproduction begins when individuals reach about 10 cm in diameter at breast height (DBH) (Spironello *et al.*, 2003).

In adulthood, individuals can reach up to 30 m in height and 2 m in diameter at breast height (DBH) (Kubitzki; Renner, 1982). In Ucayali, Peru, trees have been reported in natural populations with approximately 25 cm in DBH and a maximum total height of 26 m (Gutierrez *et al.*, 2023; Revilla-Chávez *et al.*, 2024).

Trees have a straight and cylindrical trunk, yellowish or reddish-brown bark that easily separates into large plates, and a narrow, oval crown. It occupies the intermediate or upper stratum of the forest canopy and occurs in Brazil, Colombia, Ecuador, French Guiana, Guiana, Peru, Suriname, and Venezuela. It can be found in both dry and humid land forests, with a preferred habitat on plateaus and streams (Kubitzki; Renner, 1982).

Population density is generally low, ranging for trees with DBH > 10 cm in dry land forests of north of Manaus, Brazil, from two (Loureiro *et al.*, 1979) to 27 trees.ha^{-1} (Mitja; Lescure, 1996). Near to Manaus, the density for DBH > 20 cm has been identified as ranging from 0.12 to 0.16 trees.ha^{-1} (Alencar; Fernandes, 1978). In the Tapajós National Forest, in the state of Para, Brazil, population density for trees with

DBH up to 15 cm was reported as 0.77 trees.ha^{-1} (Carvalho, 1983). The spatial distribution of adult trees is clustered in groups (five to eight trees), with spacing among trees ranging from 50-100 m and 300-400 m between groups (Alencar; Fernandes, 1978).

The natural regeneration of *A. rosaeodora* is irregular and infrequent. The recruitment of seedlings generally occurs near to seed trees through barochory. Previous studies have shown the occurrence of juvenile individuals in the area surrounding about 70% of seed trees, with approximately 50% of these juveniles distributed within a radius of four to 10 m, and no individual beyond 23 m (Araujo, 1967; Mitja; Lescure, 1993; Mitja; Lescure, 1996; Santana, 2000).

Seed dispersal also occurs by zoochory, mainly by mammals and birds (Araujo, 1967), an important aspect of seed dispersal as it influences the genetic and population structure of tree species (Dick *et al.*, 2008; Ellstrand, 2014). However, these processes are affected by forest overexploitation and the spatial fragmentation of remaining stands, which can lead to reductions in gene flow and seed dispersal between populations (Braga; Collevatti, 2011; Tambarussi *et al.*, 2015), giving rise to generations of seedlings that are increasingly related to the nearest seed tree (Manoel *et al.*, 2017). Seed dispersal is a fundamental element of natural regeneration that ensures the sustainability of forest resources over time when it occurs in a random and complex manner (Rodríguez; Mandujano, 2007; Calama *et al.*, 2017).

This phenomenon determines the potential area of recruitment of individuals and establishes the foundation for subsequent processes, which are influenced by the tree’s genetics, age, position in the forest canopy, among other factors (Campoe *et al.*, 2013). Therefore, the species may have a reduced dispersal capacity due to environmental factors, resulting in limited numbers of seeds reaching the soil.

During dispersal, predation is one of the main causes of mortality in the tree's reproductive cycle (Norden, 2014; Muñoz, 2018). The species can lose up to 60% of seed viability; on the tree, 50% of mortality can be caused by insect infestation (Spironello *et al.*, 2003) and viability can be affected by severe inbreeding processes (Sebbenn *et al.*, 2011; Sant’anna *et al.*, 2013). This can modify the dynamics of the structural composition of forests developed through natural processes, in which the distribution and abundance of seedlings, juveniles, and adults resembles an inverted J, and the sigmoid growth form, which indicates forest stands with adequate regeneration, can be affected depending on the level of intervention (Rasal *et al.*, 2012). A forest with these characteristics is classified as highly productive in ecological and economic terms (Padilla *et al.*, 2017).

Therefore, the present study seeks to understand the tree dispersal patterns and the status of the population structure of *Aniba rosaeodora* (Ducke) in a tropical forest on medium terrace soils in the Masisea district, Coronel Portillo province, Ucayali, Peru.

2.2 MATERIAL AND METHODS

2.2.1 Study site

The study was carried out in the LUSH SAC Company Forest Concession, located in the Masisea district, Province of Coronel Portillo, Ucayali, Peru (coordinates between 9º12'7.57" S 74º3'33.01" W, 9º13'18.88" S 74º2'21.05" W, 9º12'8.37" S 74º2'23.05" W, 9º13'18.25" S 74º3'31.02" W). This location is a hot spot of *A. rosaeodora* occurrence in the department of Ucayali. The concession occurs in a low terrace forest (Btb) and is a transitional zone between tropical humid forest and tropical premontane very humid forest (bh-T/bmh-PT). In this area, the mean annual temperature is 24.87 °C and the total mean annual rainfall is 1603.8 $mm.ha^{-1}.year^{-1}$ (GRU, 2017).

2.2.2 Seedling dispersal

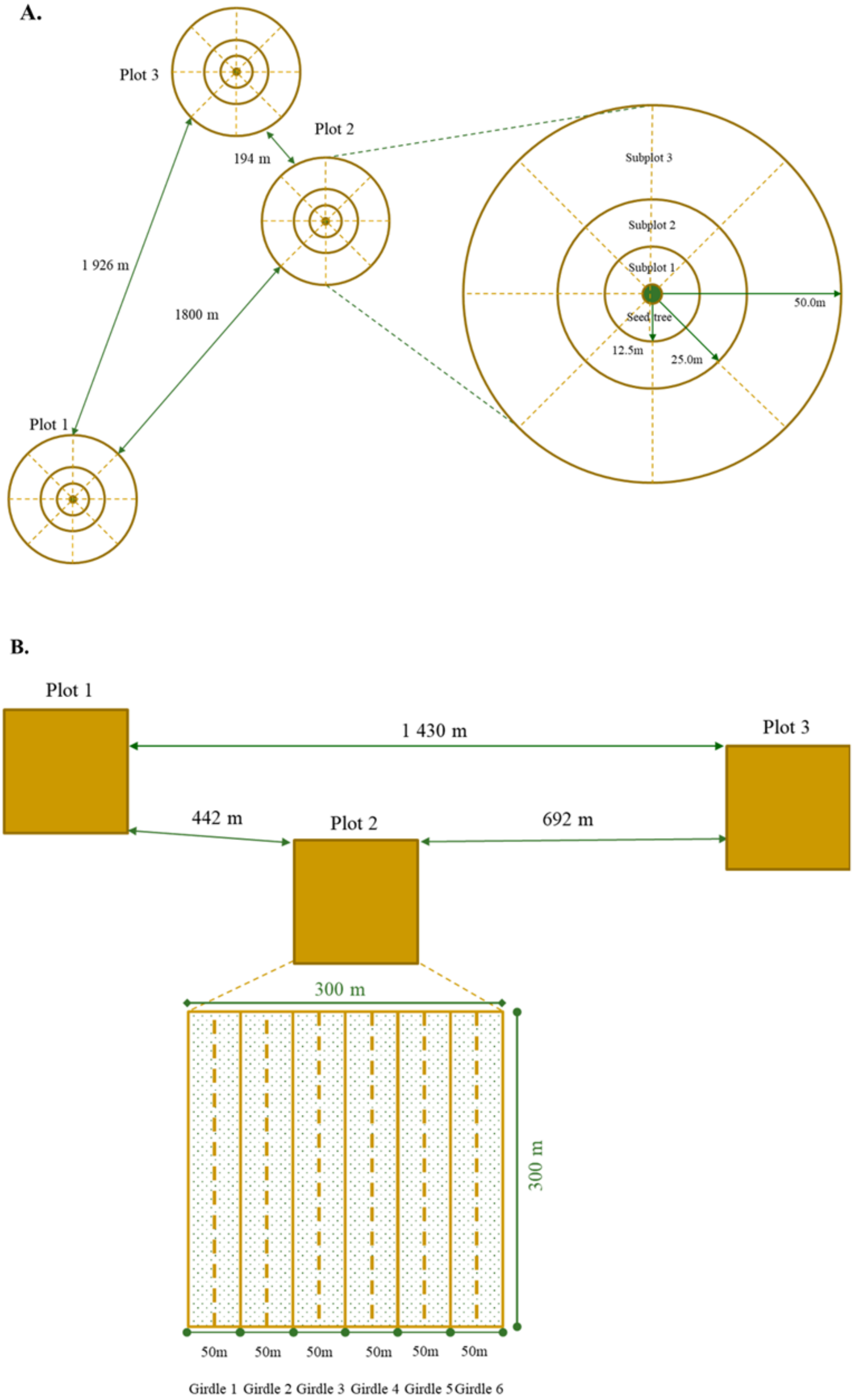
Three circular sample plots (area of 0.785 ha, for a total of 2.4 ha) were established, each with three nested concentric subplots with a radius of 12.5, 25 and 50 m, respectively (Figure 2A). The plots were installed with a seed tree as their central point. Within the plots and subplots, all *A. rosaeodora* individuals were classified by diameter category: seedling, sapling, stems, and adult. The evaluation consisted of counting the number of individuals, measuring height and diameter, and recording their geographic coordinates in WGS84 system. Diameter at breast height (DBH) was measured with a measuring tape at a height of 1.3 m above ground level or over 30 cm where the root fins end. Total tree height (H) was measured with a clinometer, from the base of the tree to the apex of its crown.

Calculated the relative frequency of seedlings (n, %), arithmetic mean of seedling density (mean), standard deviation (SD), coefficient of variation (CV%), and arithmetic mean distance to seed tree (mean), distance range (range), coefficient of variation (CV%) per plot, subplot, and for all plots using dynamic tables for frequency analysis in the Microsoft Office Excel 2019 package and R-Study (R Core Team, 2020). Seedling dispersal distance (D) was calculated as the Euclidean distance between two points ($D = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2}$, where x_i and x_j are the spatial x coordinates of seed tree i and seedling j, and y_i and y_j are the spatial y coordinates of seed tree i and seedling j, respectively). The model of seedling dispersal distance in relation to the seed tree was conducted by applying regression models, selecting the model with the highest coefficient of determination (R^2).

2.2.3 Population structure

The sample plots were established based on the *A. rosaeodora* population census conducted in the forest concession for three annual cutting plots (PCA) over an area of 823 ha. Three plots of 300 x 300 m (total area of nine ha) were established in areas with the known occurrence of *A. rosaeodora* trees, adapting the methodology of the Flora and Vegetation Inventory of the Peruvian State (MINAM, 2015). The total sampling area for three plots was 27 ha. In order to include plots with the potential for genetic conservation, with at the distance between plot 1 and 2 was 442 m, plot 2 and 3 was 692 m, and plot 3 and 1 was 1430 m (Figure 2).

Figure 2 - Sample plot distribution scheme: Figure A-plots to evaluate seedling dispersal: three plots of 100 m in diameter, each with an area of 0.785 ha (total for the three plots of 2.4 ha), and three concentric nested subplots each; Figure B-plots to study the population structure of individual categories (three plots of 300 x 300 m with an area of nine ha each, for a total of 27 ha.



In each plot, the evaluated individuals were georeferenced using UTM coordinates. In addition, the DBH (cm) and individual height (m) for four growth categories were taken: seedlings – naturally regenerating plants with a height of 30 to 50 cm; saplings – plants with diameter > than five and < 10 cm at 30 cm from the ground (D30) and one to < three m tall; stems – plants with 10 > DBH< 25 cm, and height greater than 50 cm; and adults – trees with DBH> 25 cm.

Based on the results of the natural regeneration dispersal study, a dispersal map and horizontal population structure of the species was developed. To do so, an area of seedling dispersal around mature trees (trees that produce seeds) was established, considering that the main dispersal processes of *A. rosaeodora* are barochory and zoochory.

With the abundance and DBH data, the population density (plants.ha^{-1}) of saplings, stems, and adults was determined, and frequency histograms with five diameter classes (10, 20, 30, 40, 50 cm) were developed to model the normal distribution pattern of the population. This was done by fitting Meyer's (1952) exponential equation: $y = \propto_0 e^{\propto_1 x}$, where *y* is the estimator of the total number of trees in the diameter class *x*, expressed by their class mark in centimeters; ∝i, are the model parameters, calculated for the complete sample and for the species.

The recovery status of the population was determined based on the assumption that the abundance across all classes of the population resembles an inverted J, with adjustment for the exponential model of Meyer´s (1952) and the Liocourt coefficient (q_i) (Meyer, 1952) as an indicator of the stability of the diameter classes in the population. For individuals with DBH≥ 5 cm:

$$q = \frac{N_{d_{max}}}{N_{d_{min}}},$$

where *q*= Liocourt coefficient; $N_{d_{max}}$= number of individuals in the largest diameter class; $N_{d_{min}}$= number of individuals in the lowest diameter class. Data were processed in Microsoft Office Excel 2019 using pivot tables and R-Study (R Core Team, 2020). The census dispersal and population structure maps were made using the open-source software QGIS Desktop 3.34.0 (QGIS Project, 2024).

2.3 RESULTS

2.3.1 Seedling dispersal

For the seedling dispersal analysis, plot 1 showed progeny distribution of 24.7, 48, and 27.3% in subplots 1, 2, and 3, respectively; in plot 2, 41.4, 24.1, and 34.5% of progeny were distributed in subplots 1, 2, and 3, respectively; and in plot 3, 34.6, 61.5, and 3.9% were distributed in subplots 1, 2, and 3, respectively. For the total of the three plots, 28.3, 46.3, and 25.4% were distributed in subplots 1, 2, and 3, respectively (Table 1). The mean dispersal distance measured between progenies and seed trees was 18.3 m, with a standard deviation (SD) of 9.2 and coefficient of variation (CV%) of 50%. In the evaluated plots, the maximum dispersal distance observed was 44 m. Seedling density (mean) for all plots was 0.12, 0.06, and 0.01 individuals.m^{-2}, SD was 0.04, 0.02, and 0.03, and the CV% was 73, 34, and 49% for subplots 1, 2, and 3, respectively (Table 1, Figure 3A, 3B, and 3C).

Seedling dispersal was defined by a polynomial model $y= 0.0008x^3-0.0653x^2+1.4139x-0.8204$ ($R^2= 0.46$) (Figure 4A). The plot that showed a greater frequency of total seedlings in the three plots was subplot 2 with 46.3%, with a mean distance to the seed tree (mean) of 18.7 m, SD of 18, range of 13.1-25, and CV% of 76% (Table 1).

Table 1 - Frequency, seedling density (seedlings.m^{-2}), and mean distance between seedlings to seed tree (m) for plots, subplots, and total for *Aniba rosaeodora* in a forest concession in the Masisea district, Ucayali, Peru.

		Frequency		Density (seedlings.m^{-2})			Distance to seed tree (m)			
Plot	Subplot	n	A%	Mean	SD	CV%	Mean	SD	Range	CV%
1		150	100	0.02	-	-	17.9	8.3	2.8-42.2	46
	1	37	24.7	0.08	-	-	7.4	3.0	2.8-12.4	17
	2	72	48.0	0.05	-	-	18.9	3.4	13.5-25	19
	3	41	27.3	0.01	-	-	25.6	7.9	26-42.2	44
2		29	100	0.00	-	-	20.2	13.1	3.2-44	65
	1	12	41.4	0.02	-	-	7.8	3.8	3-12.5	19
	2	7	24.1	0.00	-	-	18.7	3.5	14.4-24	17
	3	10	34.5	0.002	-	-	36.2	5.7	26.2-44	28
3		26	100	0.00	-	-	14.0	6.7	1-27.6	48
	1	9	34.6	0.02	-	-	6.3	4.3	1-12	31
	2	16	61.5	0.01	-	-	17.4	2.6	13.1-24	19
	3	1	3.9	0.0002	-	-	27.6	0	27.6	0
Total		205	100	0.03	-	-	18.3	9.2	1-44	50
	1	58	28.3	0.12	0.04	73	7.3	19	1-12.5	47
	2	95	46.3	0.06	0.02	34	18.7	18	13.1-25	76
	3	52	25.4	0.01	0.03	49	30	29	26.2-44	39

Where: n= sample size; A%= abundance of individuals per plot (%); SD= standard deviation; CV%= coefficient of variation.

2.3.2 Population structure

A total of 189 individuals were determined, distributed in the four categories found: 53.4% seedlings (ranging among plots from 26.3-67.1%); 12.2% saplings (6.7-26.3%); 27% stems (17.1-34.2%); and 7.4% adults (5.3-13.2%), maintaining the same abundance conditions in order of importance in the three evaluated sample plots. Thus, it was determined that the mean population density by diameter class was 3.7, 0.9, 1.9, and 0.5 individuals.ha^{-1} for seedlings, saplings, stems, and adult trees, respectively. Found a mean density of seven individuals.ha^{-1} (including seedlings). The proportion of the distribution structure by class of plant are maintained in the evaluated plots with 1.1 to 5.7 seedlings, 0.6 to 1.1 saplings, 1.4 to 1.9 stems, and 0.4 to 0.6 adults, with a mean of 3.7, 0.9, 1.9, and 0.5 individuals.ha^{-1} respectively, and a SD of 2.4, 0.3, 0.8, and 0.1 (Tables 2, 3, Figure 3D, 3E and 3F).

Table 2 - Population structure of *Aniba rosaeodora* in a forest concession in the Masisea district, Ucayali, Peru.

	Abundance		DBH (cm)			H (m)			Individuals.ha^{-1}	
	n	A%	Mean	Range	CV%	Mean	Range	CV%	Mean	CV%
1E	38	100	13.7	2.5-38	80.4	13.6	0.11-31	70.5	4.2*	24
Seedling	10	26.3	2.8	2.5-3.4	0.1	2.4	0.11-6.2	6.3	1.1	25
Sapling	10	26.3	8.4	5.8-9.9	2.2	12.3	7-17.6	8.3	1.1	25
Stem	13	34.2	15.9	10.3-24	21	19.5	13-31	30.6	1.4	22
Adult	5	13.2	29.6	25.1-38	24.4	21.9	12.2-27.2	10.7	0.6	29
2E	76	100	11.6	1-53.9	139.5	7.5	0.1-31	98.9	8.4*	23
Seedling	51	67.1	2.2	1-4.1	1.1	1.3	0.1-10	6.5	5.7	11
Sapling	8	10.5	7	5.5-9.9	2.5	12.8	6.5-16	10.3	0.9	30
Stem	13	17.1	17	12-22.8	15.8	21.8	16-31	21.1	1.4	28
Adult	4	5.3	39	29-55.9	109.7	29	25.3-31	7.3	0.4	32
3E	75	100	12.9	0.3-36.6	73.3	10.3	0.1-29.8	103.4	8.3*	23
Seedling	40	53.3	2.9	0.3-4.4	1.9	2	0.4-11	8.3	4.4	16
Sapling	5	6.7	7.7	5.3-9.2	2.4	12	6.34-18	19.1	0.6	31
Stem	25	33.3	15.6	14.4-23.5	18.7	20.5	9-29.8	30.9	2.8	22
Adult	5	6.7	29	25.4-36.6	20.3	24.7	17.5-28.4	19.7	0.6	31
General	189	100	12.7	0.3-53.9	96.4	9.8	0.1-31	99	7	16
Seedling	101	53.4	2.5	0.3-4.4	1.3	1.7	0.1-13	7.3	3.7	63
Sapling	23	12.2	7.7	5.3-9.9	2.5	12.4	4.2-18	10.3	0.9	33
Stem	51	27	16	10.3-24	18.1	20.6	7.4-31	27.9	1.9	41
Adult	14	7.4	32.1	25.1-55.9	59.9	24.9	13.8-31	19.6	0.5	12

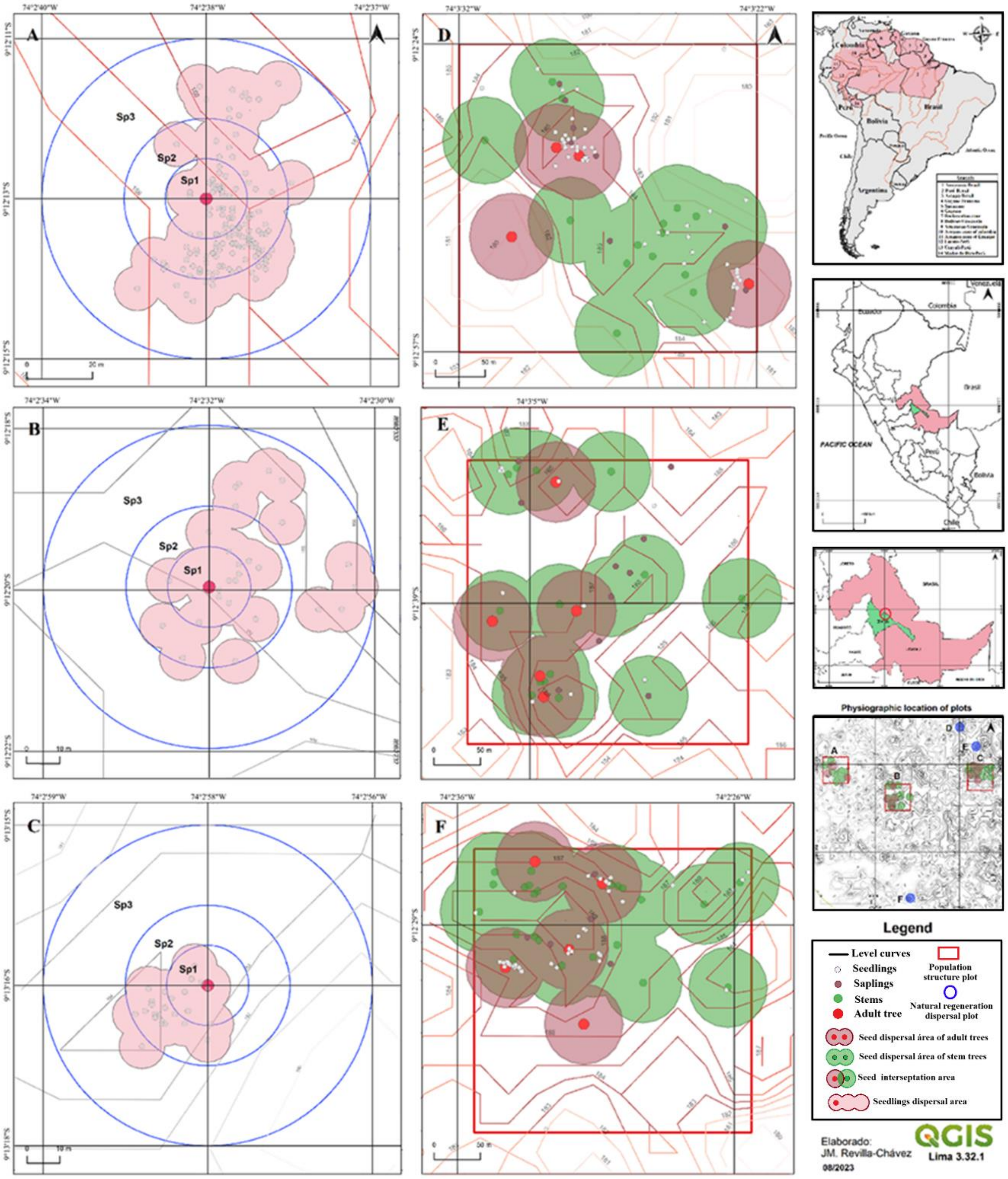
Where: n= sample size; A%= abundance of seedling, sapling, stem, and adult individuals (%); DBH= diameter at breast height; H= individual height; Mean= mean of the variable by plant class; CV%= coefficient of variation; *density includes seedlings.

Table 3 - Population structure of *Aniba rosaeodora* in a forest concession in the Masisea district, Ucayali, Peru.

Plot	Seedlings n (%)	Saplings n (%)	Stems n (%)	Adults n (%)	Total n (%)
1	10 (26.3)	10 (26.3)	13 (34.2)	5 (13.2)	38 (100)
2	51 (67.1)	8 (10.5)	13 (17.1)	4 (5.3)	76 (100)
3	40 (53.3)	5 (6.7)	25 (33.3)	5 (6.7)	75 (100)
Total	101 (53.4)	23 (12.2)	51 (27.0)	14 (7.4)	189 (100)

n= sample size.

Figure 3 - *Aniba rosaeodora* seedling dispersal and population structure by diameter class. Seedling dispersal in plots A, B and C; distribution of the population structure in plots D, E and F.



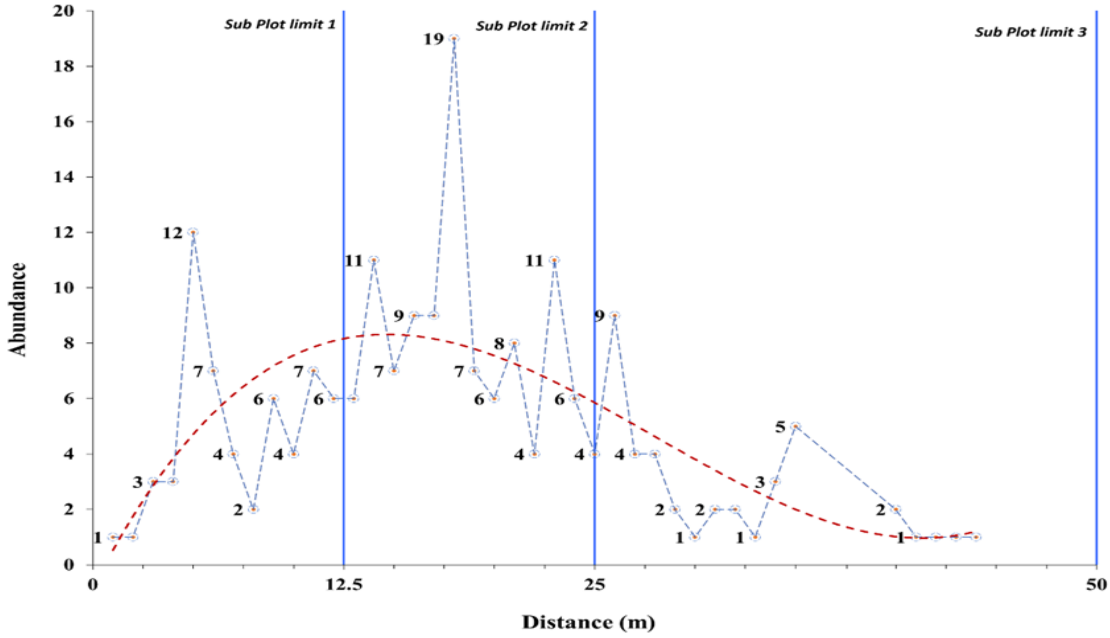
Similarly, the histograms of total frequency (PT) by diameter class to determine the restoration status of the *A. rosaeodora* population with adjustment for Meyer's (1952) exponential equation showed $y= 118.91e^{-0.852Di}$ and $R^2= 0.9837$ (Figure 4 B), with Liocourt coefficients (*q*) ranging from 1.5 to 3.0 (Table 4).

Table 4 - Regression models applied to determine the status of the total sampled *Aniba rosaeodora* in a forest concession in the Masisea district, Ucayali, Peru (Di= diametric class; P1, P2, P3, PT= plots; n=individuals by diametric class; q1, q2, q3, qt= Liocourt coefficient by plot).

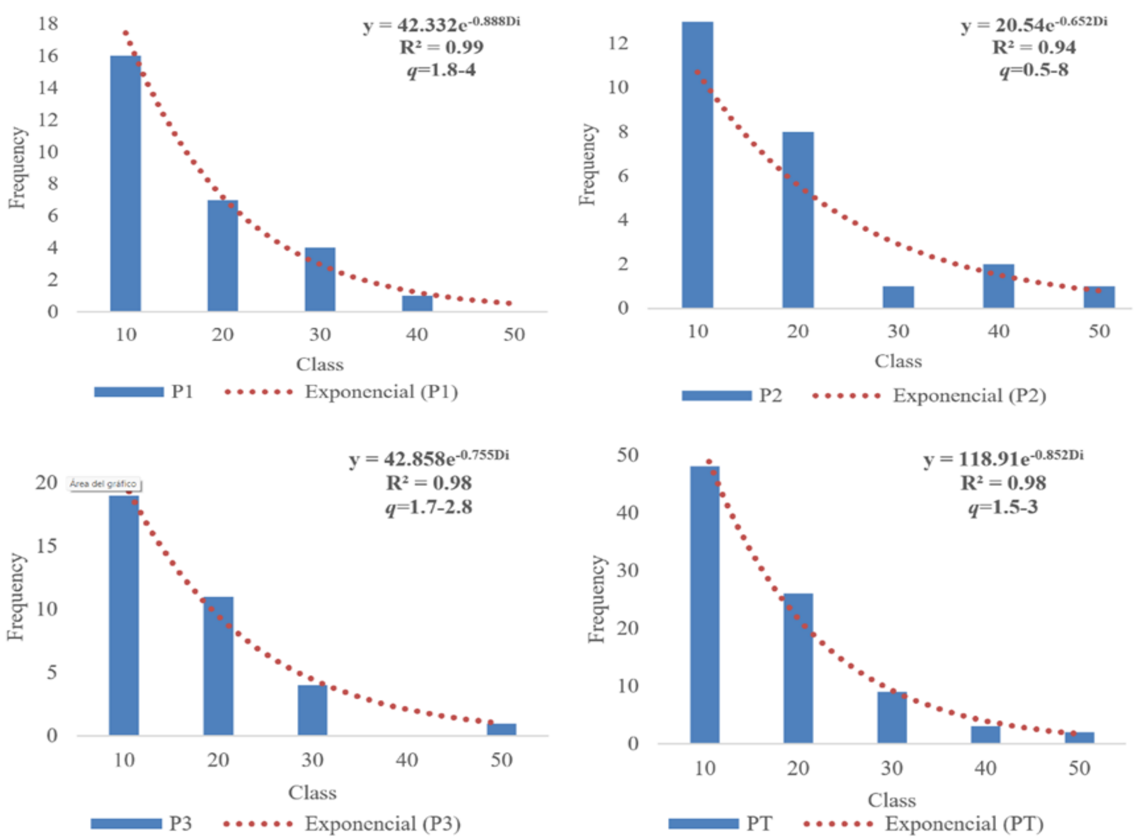
Class (Di)	P1		P2		P3		PT	
	n (%)	*q1*	n (%)	*q2*	n (%)	*q3*	n (%)	*qt*
10	16 (57.1)	2.3	13 (52)	1.6	19 (54.3)	1.7	48 (54.5)	1.8
20	7 (25)	1.8	8 (32)	8.0	11 (31.4)	2.8	26 (29.5	2.9
30	4 (14.3)	4.0	1 (4)	0.5	4 (11.4)		9 (10.2)	3.0
40	1 (3.6)		2 (8)	2.0			3 (3.4)	1.5
50			1 (4)		1 (2.9)		2 (2.3)	
Total	28 (100)		25 (100)		35		88 (100)	
Equation	$y= 42.332e^{-0.888Di}$		$y= 20.54e^{-0.652Di}$		$y= 42.858e^{-0.755Di}$		$y= 158.2e^{-0.962Di}$	
R^2	0.99		0.94		0.98		0.98	

Figure 4 - A. Abundance of individuals by mean dispersal distance (m) from the seed tree; Figure 4B. determination of population recovery potential by the inverted J method (MEYER, 1952) and Liocourt (q) through the abundance of individuals per diameter class (Di).

A.



B.



2.4 DISCUSSION

2.4.1 Seedling dispersal

The results showed that 75% of the seedlings were located near the maternal tree. A similar pattern was previously identified by Santana (2000), who reported that 79.3% of seedlings were located between four to 10 m from the seed tree. Seedling dispersal (28.1%) occurred at short distances (7.3 m) within the area of subplot 1, similar to that reported by Santana (2000) with 18.3% of seedlings located at distances of four to five m from the seed tree.

This is consistent with the projection diameter of the tree crown, which on average reached 6.1 m, thus supporting the barochory dispersal hypothesis. Similarly, seedlings (46.3%) reached an average distance of 18.7 m within the area of subplot 2, which could be attributed to the dispersal of fruits by birds (Psittacidae and Ramphastidae families) and the few mammals that feed on the pulp, which is consistent with zoochory seed dispersion (Araujo, 1967; Santana, 2000) (Figure 4A).

Previously, Spironello *et al.* (2003) found that 75% of toucans ingest the fruits and disperse them at distances greater than 50 m from the seed tree (via regurgitation, 15-30 minutes after ingesting the fruit), while 30% of parrots dispersed 40% of seeds after a few minutes. Although the seedlings in our study reached distances of up to 44 m from the seed tree, likely a result of animal dispersal, the frequencies are low.

Similar to what was referred to by Santana (2000), who found that seedlings did not occur beyond 23 m, reaching a seedling density of 0.7 and 0.19 individuals.m^{-2} at an average distance of 12 and 23 m from the seed tree, respectively. However, this pattern of short-distance seed dispersal requires further analysis, preferably based on maternity and paternity tests using genetic markers such as microsatellite loci.

Considering that the isolation of overexploited forests favors endogamous reproduction and that offspring rates increase for seedlings closer to the seed tree (Pereira *et al.*, 2020), the seedlings located in subplots 1, 2, and 3 likely have greater levels of relatedness with the nearest seed tree (Sebbenn *et al.*, 2012; Manoel *et al.*, 2017, 2021). Thus, it is necessary to carry out molecular studies to confirm the pattern for this species.

2.4.2 Population structure

The total mean population density was high when regenerants (seedlings, saplings, and stems) were included in the analysis (7 individuals.ha^{-1}). Meanwhile, adult trees showed very low population density (< 1 individual.ha^{-1}) (Table 2). These results differ from that obtained by Gutierrez *et al.* (2023) in the same area, who found a density of 0.547 individuals.ha^{-1}.

The difference between the densities is that the present study was established in plots of 300 x 300 m in areas with known occurrence of *A. rosaeodora* individuals, while Gutierrez *et al.* (2023) developed his study using strip plots of 1000 x 10 m (1 ha) spaced 350 m apart. Similarly, a study by MINAM (2017) evaluated 10 x 100 m plots in the same area and estimated an occurrence of 0.2 trees ha^{-1} (DBH ≤ 10 cm), with an population density of 2.09 individuals.ha^{-1}. Considering only plots with the presence of individuals of the species, the differences found are likely the result of the evaluation criteria used in each study.

Furthermore, the regeneration potential of the species is very heterogeneous, since Mitja and Lescure (1993) argued in a similar study that 65% of the plants identified had less than 10 young individuals in their immediate vicinity, while only 5% had abundant regeneration, leading to a high population density of 27 individuals.ha^{-1}.

Table 5 - Comparison of population densities (individuals.ha^{-1}) among several studies and species of the Lauraceae family.

Species	Individuals.ha^{-1}					Authors
	Seedling (Brinzal)	Sapling (Latizal)	Stem (Fustal)	Adult	General	
A. roseadora	3.7	0.9	1.9	0.5	7*	Present study
A. rosaeodora	-	-	0.14	-	-	Alencar; Fernandes, 1978
A. rosaeodora	-	-	-	-	2.0	Loureiro *et al.*, 1979
A. rosaeodora	6.29	3.83	0.52	-	-	Carvalho, 1983
A. rosaeodora	-	66.3	0.14	0.13	27**	Mitja; Lescure, 1996
A. rosaeodora	-	-	-	-	0.05	SPIRONELLO *et al.*, 2003
A. rosaeodora	238	0.9	1.5	0.63	2.09*	MINAM, 2017
A. rosaeodora	0.163	0.07	0.07	0.233	0.547	Gutierrez *et al.*, 2023
Aniba muca	2083	-	-	-	-	Aguirre *et al.*, 2018
Aniba guianensis	888	-	-	-	-	Aguirre *et al.*, 2018

Where: * average population density, including all diametric categories in plots with only the presence of plants; **mean population density including all plant categories; DBH= diameter at breast height; seedlings= brinzal: height ranging from 30 to 50 cm; sapling= latizal: 5 > D30 ≤ 10 cm, diameter at 30 cm from the ground (D30) and height 1≤ height < 3 m; stems= fustals: 10 > DBH ≤ 25 cm, height > 50 cm; adult: trees with DBH> 25 cm.

The histograms of total frequency (PT) by diameter class with adjustment for Meyer's exponential equation and the Liocourt coefficients (*q*) from 1.5 to 3.0 (Table 4), showed a persistent disturbance in the studied population. According to the Liocourt coefficient, the quotient between the number of individuals of consecutive diameter classes should be an almost constant ratio, which is consistent despite the varying value of that ratio for different classes.

Thus, the general form of the distribution is that of a decreasing exponential curve in the shape of an inverted J, whose pairs describe a straight line when plotted. This pattern was not observed in the present study. In regular natural forests, the species responds to multiple Eco-physiological characteristics and growth and production rates (Souza; Jesus, 1994), where young plants with smaller diameter predominate compared to a low number of individuals with larger diameter; hence the decreasing frequency distribution in the shape of an inverted J. Similarly, it can be verified that the extreme diameter classes where *q* differs appreciably from the rest occur because a large number of specimens of said class were recently extracted for various reasons (Imaña-Encinas *et al.*, 2021).

From the observed results we can assume that the impact of *A. rosaeodora* overexploitation persists in the study area, since Louman *et al.* (2001) argue that the diameter distribution of individuals in young native forests or in recovery processes presents an inverted J with stable *q* coefficients, which was not observed in the present study area (Figure 4B).

Although this behavior is typical of Lauraceae, with high levels of seed and seedling production and high density-dependent mortality, very few individuals reach the adult stage (Cuevas *et al.*, 2008). Likewise, it was found that on average there is a significant abundance of individuals located in the outer range of the maximum seed dispersal of the seed tree, which differs from that proposed by Santana (2000). This apparent difference may be due to the presence of early maturing stems that have begun reproductive dissemination, as observed in the field.

Thus, a maximum seed dispersal zone per adult tree with a radius of 44 m was identified, within which seedlings and stems were located (Figure 4A). For this reason, it is very likely that the observed population structure is the result of the presence of seed trees that are remnants of different harvesting intensities (Table 5; Alencar; Fernandes, 1978; Loureiro *et al.*, 1979; Spironello *et al.*, 2003).

However, this pattern of seedling dispersal is potentially dangerous for the sustainability of the species, considering that Guizado *et al.* (2020) found the species in this study area to be isolated compared to other *A. rosaeodora* populations in the Peruvian Amazon. Overexploited species may present high rates of co-ancestry within isolated populations, as discussed by Sebbenn *et al.* (2012), who found high rates of relatedness in seedlings close to *Swietenia macrophylla* seed trees in an over-exploited tropical forest in Bolivia.

Therefore, compared to the abundance of other species of the same family, such as *Aniba muca* and *Aniba guianesis*, *A. rosaeodora* seedlings present differently in the study area with abundant seedlings but few individuals reaching adulthood (Table 5; Aguirre *et al.*, 2018). This form of dispersal can be affected by overexploitation, which has an impact on dispersal patterns, pollination, and can modify the structure of tree populations in tropical forests.

This, in turn, can alter the dispersal potential of pollinators, change the density of flowering trees, and create habitat disturbances. *A. rosaeodora* can reach an abundance of 66.31 individuals.ha^{-1}, with 91% of individuals with a smaller diameter, while trees with DBH> 20 cm rarely reaches 0.14 individuals.ha^{-1} (Carvalho, 1983; Mitja; Lescure, 1993; Gutierrez *et al.*, 2023; Revilla-Chávez *et al.*, 2024).

Despite the presence of spatially disperse individuals, the distribution of adult trees is found clustered in groups of two to five trees spaced at distance of 97 to 110 m between trees with DBH> 20 cm (Figure 3D, 3E and 3F). This range is at the upper limit of that reported by Alencar and Fernandez (1978) who stated that trees of this species are found between 50 and 100 m apart. This distance could indicate a decrease in genetic diversity and a loss of alleles as the best phenotypes may have been removed for linalool extraction, resulting in reduced genetic drift due to changes in gene flow as a result of tree selection (Correa *et al.*, 2013). Such changes can increase the vulnerability of the species by reducing the genetic foundation for reproduction among fewer individuals or among progenies, thus increasing the co-ancestry in the population.

This results show an erratic distribution in the population recovery process compared to similar natural population models that resemble an inverted J (Rasal *et al.*, 2012). Adjusting to a potential recovery model, the studied population presents a structure similar to a flattened inverted J (Figure 4B), indicating a weak population that may struggle to recover due to the overexploitation of trees (Homma, 2003).

2.5 CONCLUSIONS

The study of *A. rosaeodora* seedling dispersal and population structure in Ucayali, Peru, indicates that the seedling dispersal pattern is irregular and the population structure does not adhere to species recovery models in tropical forests.

The population density of the study area is heterogeneous, but it is higher than the average obtained in other studies.

The study population demonstrates characteristics of an isolated population typical of an overexploited species that is at constant risk due to endogamous processes.

Because of its isolation, the population in the study area has potential for the conservation and management of the genetic resources of the species.

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3 ALLOMETRIC MODELS OF BIOMASS AND ESSENTIAL OILS OF *Aniba rosaeodora* (Ducke) IN A TROPICAL MIDDLE TERRACE SOIL FOREST OF MASISEA, UCAYALI, PERU[2]

ABSTRACT

In this study we developed an allometric model to predict the total biomass of the tropical tree of economic value *Aniba rosaeodora* to manage the sustainable use of natural populations through the use of branches and leaves, in the same way that allows us to evaluate the potential for its domestication and develop plantations. For this, 15 trees were sampled with an average diameter at breast height (DBH) of 9 cm (σ= 7.5 cm, CV%= 44%), height (Th) of 17.6 m (σ= 5.5 m, CV%= 31%), an average total biomass of 277.7 kg (σ= 203.9 kg, CV= 73%), resulting in an average of 4.5 l of essential oil (σ= 3.3 l, CV%= 75%), and with an oil yield of 1.51% (σ= 0.34%, CV%= 22.3%). Thus, 29 allometric models were selected to estimate biomass of the stem (Cb), biomass of green branches (Pb), biomass of secondary branches (Sb), leaf biomass (Lb), total tree biomass (Tb) and essential oil, from variables of easy measurement (diameter) and strong correlation (Spearman's Rho 0.63-0.99; $P < 0.05$). From the tests, the models with the best correlation coefficients, R^2 and R^2aj were selected to estimate the biomass and essential oil content of each tree. Thus, the equations whose predictor variable is the D50 was the best fit, where $Tb = (-1.73388+0.835102D50)^2$, $\rho = 0.96$; $R^2aj = 0.962$ and $Oil = \exp(-6.4554+2.54862\ln(D50))$, $\rho = 0.82$; $R^2aj = 0.930$, but by an indirect method using total tree biomass (Tb) a better fit can be obtained for essential oil, $Oil = 1/(-0.0793641+82.2948/Tb)$, $\rho = 0.84$; $R^2aj = 0.998$.

Key words: biomass, rosewood, carbon, allometric model, amazon.

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[2] REVILLA-CHÁVEZ, J.M.; SÁENZ-RAMÍREZ, L.H.; GONZALES-ALVARADO, A.C.; GARCÍA-SORIA, D.G.; SEBBENN, A.M. Allometric models of biomass and essential oils of *Aniba rosaeodora* Ducke in a tropical middle terrace soil forest of Masisea, Ucayali, Peru, **Trees, Forests and People**, Netherlands, v.16, p.100594, 2024. https://doi.org/10.1016/j.tfp.2024.100594 **(Published).**

3.1 INTRODUCTION

Aniba rosaeodora Ducke (rosewood) belongs to the Lauraceae family and is a large tree, reaching 30 m in height and up to 2 m in diameter at breast height (DBH) (Kubitzki; Renner, 1982). The species has commercial value due to its essential oil obtained from the distillation of wood, branches and leaves (May; Barata, 2004; Amusant *et al.*, 2016; Giongo *et al.*, 2017). Its oil is mainly composed of a secondary metabolite known as linalool, which is a fixing substance, with a characteristic aroma used in the manufacture of perfumes, fragrances and soaps, in large foreign multinationals (May; Barata, 2004; Teles; Mouchrek, 2022), which is why the species suffered intense predation between 1950 and 1965 for the extraction of its valuable oil in Peru (May; Barata, 2004). This situation caused its natural populations to be on the verge of extinction in Peru. In Peru the species is categorized as a vulnerable species due to DS. Nº043-2006-AG, while the governments of the Amazon basin, to protect it, included it in the 15th COP15 meeting, DOHA 2010, to *A. rosaeodora* in Appendix II, since then many studies have been developed for the conservation of the species (MINAM, 2015a).

Aniba rosaeodora is distributed in all regions of the Amazon, including Brasil, Colombia, Ecuador, French Guyana, Guyana, Peru, Suriname, and Venezuela (May; Barata, 2004). However, its wide distribution contrasts with its low densities and low frequency in natural forests, while in the Peru, its natural distribution range includes the scope of three regions of the country, these are from north to south: Loreto, Amazonas and Ucayali, *A. rosaeodora* can also be introduced, cultivated and/or wild in community areas from Loreto (Mitja; Lescure, 1996; May; Barata, 2004; Zama *et al.*, 2012; Aguirre *et al.*, 2018). Its oil is a long-used ingredient in fragrances and food products, it is colorless to pale yellow and presents a balanced combination of floral, sweet, xylem and citrus aromas, It can also yield from 0.75–3.4% of the distilled biomass (Fidelis *et al.*, 2012), which is mainly constituted by Linalol from 32-97%, which can be transformed into valuable derivatives of value for the flavor and cosmetic industries; of various medicinal uses and most of the main food categories, such as soft drinks, alcoholic beverages and confectionery products, have used rosewood essential oil and linalool as the main component (May; Barata, 2004).

In Brazil, this species is under total protection (MMA, 2014) and current legislation allows the exploitation of the species only in established plantations, while in Peru its exploitation is limited by the Non-Detrimental Extraction Opinion of "pinkwood", “from a natural environment” (Zama *et al.*, 2012; MINAM, 2015a).

Therefore, the quantification of biomass is necessary to evaluate biological, economic and nutrient productivity (Higuchi *et al.*, 1998; Yuen *et al.*, 2016), where to calculate it, the use of allometric methods has the advantage of provide greater precision, lower costs, speed and rigor in measurements to estimate biomass (Revilla-Chávez *et al.*, 2021). However, the most precise method is the destructive one in which the entire tree is weighed; this requires significant time, costs and sometimes illegal actions (Chaves *et al.*, 2014; Yuen *et al.*, 2016).

Therefore, the management of aerial biomass is used to extract oil from different parts of the plant without the need to cut down the tree, a method approved in countries like Brazil, but in Peru authorizations are not granted for the use of aerial biomass, because the method involves cutting down the tree (Amusant *et al.*, 2015; MINAM, 2015b, 2017).

Allometric modeling of trees is very useful since the methods that allow estimating the biomass of the species of interest are often high cost and take more time than necessary (Yuen *et al.*, 2016), and knowing the biomass often determines the elimination of the individual before knowing his usefulness. Furthermore, having an allometric model allows estimating the biomass from other easily measured variables, which will allow obtaining the biomass of the components of interest, such as branches and leaves (Brown, 1997) that the case is applied in. similar way for *A. rosaeodora* in biomass management; which will add value to the "sustainable oil" production chain, which can improve the viability of commercial forest and plantation management.

In this sense, allometry for biomass estimation refers to the modeling by mathematical equations of the relationships of the biomass of the complete tree, its components, to one or more biophysical elements (Kira; Shidei, 1967; Basuki *et al.*, 2009); but little has been studied in rosewood stands. Knowing these relationships means that the biomass of the stem, branches and leaves can be predicted through easily measured variables (Brown; Lugo, 1984; Brown *et al.*, 1989), which can be Applied focus on the management of aerial biomass and avoid felling the tree to obtain its benefits.

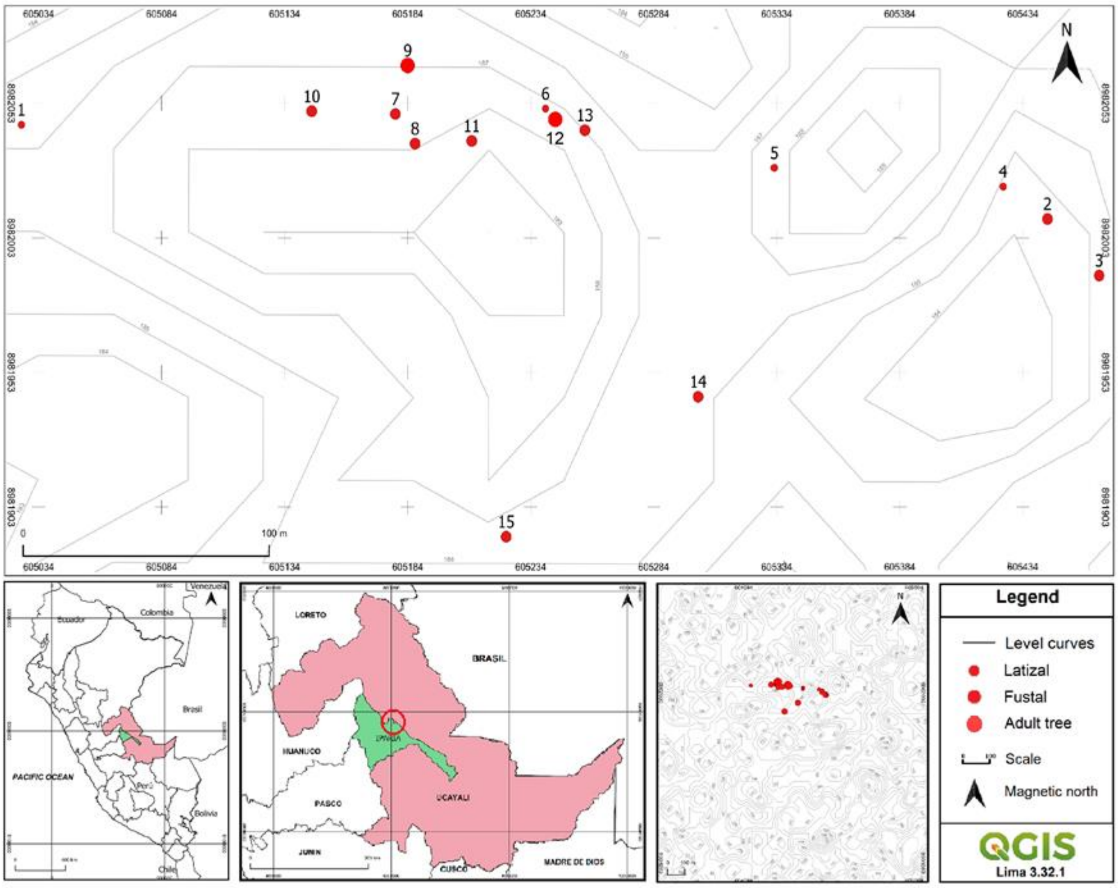
The models used to estimate tree biomass in the Amazon have produced satisfactory results (Higuchi *et al.*, 1998; Araujo *et al.*, 1999), while in Peru, there are no models that allow estimating the aboveground biomass to estimate the volume, fresh mass and dry mass that can be applicable to the management of forests and plantations. Due to that, this study aims to develop an allometric model of biomass of *A. rosaeodora* to manage the sustainable use of natural populations through the use of branches and leaves.

3.2 MATERIAL AND METHODS

3.2.1 Study site

The study was conducted in the timber forest concession area of the company LUSH PERU SAC., an area of 5,751 ha in the Masisea district, Coronel Portillo province, Ucayali, Peru (Figure *5*).

Figure 5 - *Aniba rosaeodora* trees sampled for the development of allometric biomass models.



The area is a transitional zone between humid Tropical Forest (Bht) and a very humid Tropical Forest (Bmht), with middle terrace soil (forests located on more stable soils above 10 m above water level), an elevation between 183 and 186 amsl, slope of 0 to 4%, and clayey soils. Average temperatures in the region are 24.9 °C, and average annual precipitation is 2008 mm.year^{-1} (MINAM, 2015c; GRU, 2017). These forests are located in the alluvial plain of the lowland forest, occupying the non-floodable terraces, formed by alluvial sediments coming from the materials carried by the rivers and streams that flow, deposited in the Quaternary. The instability of the river courses is giving rise to portions of low and/or middle lands where a pioneer flora is installed that colonizes the recently formed soils in a sequential and parallel manner. Its floristic composition with older species, such as *Triplaris* sp. (tangarana), *Calycophyllum* sp. (capirona), *Erythrina* sp. (amasisa), *Ficus anthelmíntica* (oje), *Inga* sp. (shimbillo), *Euterpe precatoria* (huasaí), *Trema micrantha* (atadió), *Erythrina ulei*, *Piper achromatolepis*, *Senegalia riparia*, *Calathea* sp., *Cissus erosa* (ampato huasca), *Erythrina amazonica*, *Ficus insipida* (oje), *Senna bacillaris* (mataro), *Senna ruiziana* (small mataro), *Attalea insignis* (conta), *Garcinia macrophylla* (charichuelo), *Calyptranthes densiflora* (yayo), etc. (Kalliola *et al.*, 1993), *Apuleia leicarpa, Licania caudata, Cariniana domesticata, Copaifera reticulata, Protium* sp., *Virola* sp., *Anaxagorea* sp., *Jacaranda copaia, Ormosia* sp. *Ceiba pentandra, Esterculia* sp., *Couma* sp., *Macoubea guianensis*, and *Choricia integrifolia*. The most important commercial forest species are *Apuleia leicarpa, Licania caudata, Cariniana domesticata, Copaifera reticulata, Protium* sp., *Virola* sp., *Anaxagorea* sp., *Jacaranda copaia, Ormosia* sp. *Ceiba pentandra, Esterculia* sp., *Couma* sp., *Macoubea guianensis*, and *Choricia integrifolia*. Key faunal species include *Ateles paniscus, Lagothrix lagotricha, Pecari tajacu, Tayassu pecari, Dasyprocta variegate, Dasypus novernicintus, Agouti paca, Aloutta serticulus, Saimiri sciureus, Tapirus terrestris*. (Mammals), *Boa constrictor, Geochelone denticulata, Anolis punetatus, Microlophus tigris, Melanusuchus niger* (Reptiles), *Leptadactylus pentadactylus, Procoelaebufo marinus* (Amphibians) and *Ramphastos cuvieri, Amazona mercenaria, Penelope jacquacu, Campephilus guayaquilensis, Galucidium brasi,* and *Crypturellus undulatus* (birds) (Soto, 2012).

3.2.2 Development of equations for biomass estimation

To develop the equation, the destructive method was used, separating the components of 15 trees with diameters between 6.1–32.1 cm in diameter at the height of the base (DBA, cm), which were felled 50 cm from the ground. The variables evaluated were the diameter at 50 cm from the base (D50, cm), diameter at 100 cm from the base (D100, cm), diameter at breast height (DBH, cm), stem biomass (Cb, kg), biomass of primary branches (Pb, kg), biomass of secondary branches (Sb, kg), and biomass of leaves (Lb, kg). For all components, the moisture content (CH%) was determined for deduction from the dry weight of each component (kg). Aliquot samples were taken from them, taking Graus Celsiustheir wet weights of the samples, and then taking them to the oven at ± 105 °C until constant weight, and determining their moisture content ($\mathrm{CH}(\%)$) by,

$$CH(\%) = \frac{Ph-Ps}{Ph} \times 100 \quad (1)$$

where: Ph= wet weight of the sample in grams, Ps= dry weight of the sample in grams.

The dry weights were obtained by deducing the moisture content (%) of each sample, leading by inference to the components of the tree. The dry weight of the tree was obtained from the sum of the dry weights of its components, using the methodology applied by Revilla-Chávez *et al.* (2021). The total biomass of the stem (Cb), was obtained by adding the dry weights of each of the sections of the stem (Cbs):

$$Cb = \sum_1^n Cbs \quad (2)$$

where: n=sample size and Cbs= biomass of stem sections (Kg).

The determination of biomass of green branches, Pb and Lb were deduced from the CH(%) of the samples to obtain their dry weights (Kg). The total tree biomass (Tb) of the tree was determined by the sum of all its dry components (kg):

$$Tb = Cb + Pb + Sb + Lb \quad (3)$$

The specific allometric equation was determined by the relationship of total aerial biomass of the slope variables as components of the tree, stem biomass Cb, Pb, Sb, Lb, and Tb and the independent variables DBA, D50, D100, DBH, Th, Ch_1, and Ch_2.

3.2.3 Development of equations for estimating essential oil contents

For the development of the allometric models to determine the content of essential oils, the biomass of each tree was taken, it was crushed for 3 hours and 45 min per tree, then it was subjected to steam distillation for 55 hours per tree, the process used 15 l of water for the distillation. The condensation water was cooled for 180 min, and after the process was completed, the over-swimming (essential oil) was decanted and the volume was measured in liters, then correlated with easy variables (DBA, D50, D100, and DBH) to estimate the yield of essential oils in liters.

3.2.4 Data processing

For the procedure, the variables were subjected to the Shapiro Wilk normality test to determine if they met the parameters of normality, and then the data were subjected to Spearman's rank correlation (ρ) test ($P< 0.05$) using the statistical program R with the RCmdr and RStudio packages, to identify the variables most correlated with the biomass and essential oil variables. The determination and development of the best biomass and essential oil prediction models for each of the components was done using the Stat graphics Centurion 19 program, in which simple regression models were applied to predict Tb, Cb, Pb, Lb and oil, while multiple regression model tests were applied to predict Sb, for which the Sperman correlation coefficient (ρ) and adjusted determination coefficient (R_{aj}^2) between the variables were used as selection criteria.

3.3 RESULTS

3.3.1 Biomass models

Fifteen trees were sampled with an average diameter at breast height (DBH) of 9 cm (σ= 7.5 cm, CV%= 44%), height (Th) of 17.6 m (σ= 5.5 m, CV%= 31%), an average total biomass of 277.7 kg (σ= 203.9 kg, CV%= 73%), resulting in an average of 4.5 l of essential oil (σ= 3.3 l, CV%= 75%), and with an oil yield of 1.51% (σ= 0.34%, CV%= 22.3%). The results obtained for the development of biomass prediction models of the different components of *A. rosaeodora* trees in a forestry concession in the district of Masisea, province of Coronel Portillo, department of Ucayali were carried out on a sample of trees whose statistics descriptives (

Table 6).

Table 6 - *Aniba rosaeodora* sample statistics in permanent production forests in the district of Masisea, province of Coronel Portillo, Ucayali.

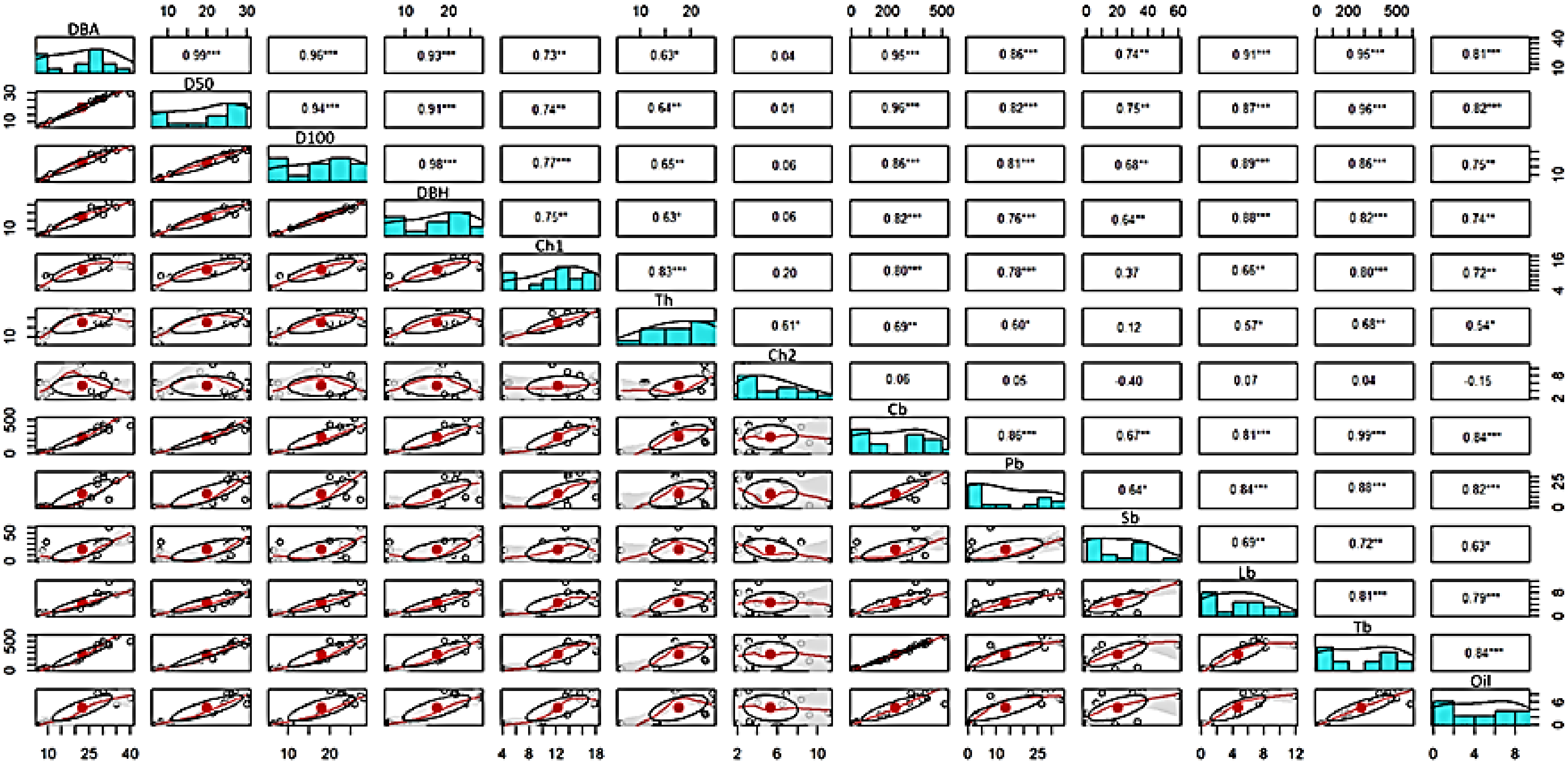
Parameter	DBA (cm)	D50 (cm)	D100 (cm)	DBH (cm)	Ch_1 (m)	Th (m)	Ch_2 (m)	Cb (kg)	Pb (kg)	Sb (kg)	Lb (kg)	Tb (kg)	Oil* (l)	Yield (%)
Number of observations	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Minimum	7	7	6.2	6.1	4.4	6.3	2	10.5	0.6	0.7	0.2	12.7	0.2	1.0
Maximum	40	30	28	27.1	18	24.1	11.1	514.8	33.3	59.4	11.8	584.5	9.1	2.1
Median	25.6	24.3	20	19	13.7	17.5	4.1	318.4	7.8	33.4	4.6	339	5.6	1.4
Mean	22.4	19.9	18.1	17.2	12.3	17.6	5.3	239.4	13.4	27.6	4.7	277.7	4.5	1.5
σ	10.5	8.8	7.8	7.5	4.5	5.5	2.6	178.3	12.9	16.3	3.4	203.9	3.3	0.3
CV%	47	44	43	44	37	31	49	74	96	59	72	73	75	22.3
Geometric mean	19.4	17.5	15.9	15.1	11.2	16.5	4.7	120.5	5.9	18.5	3	153.6	2.3	1.5
Harmonic mean	16.2	14.9	13.6	12.8	9.8	15.3	4.1	39.8	2.4	5.8	1.4	58.9	0.7	1.4

*Oil density: $0.88 g.cm^{-3}$; Where: DBA= diameter at base height (cm); D50, D100, and DBH are diameter at 50, 100, and 130 cm from the ground, respectively; Ch_1= height at first branch (m); Th=total height (m); Ch_2= canopy height (m); Cb= shaft biomass (kg); Pb= main branch biomass (kg); Sb= secondary branch biomass (kg); Lb= leaf biomass (kg); Tb=total tree biomass (kg); Oil= Essential oil (l); CV%= Coefficient of variation; σ= Standard deviation.

The results of the Shapiro Wilk normality tests and Spearman rank correlations (ρ) of the independent variables (DBA, D50, D100, DBH, CH1, and Th) and dependent variables (Cb, Pb, Sb, Lb, Tb) obtained from The sampled trees show that the variables as a whole do not meet the normality parameters and in the same way it is observed that the variables related to tree diameter (DBA, D50, D100, and DBH) have a strong correlation with the biomass components except for Sb, while the variables related to height have a lower Spearman correlation (ρ) with the biomass variables (

Figure *6*).

Figure 6 - Spearman correlation (ρ) matrix between independent and dependent variables of the biomass of *Aniba rosaeodora* in permanent production forests in the district of Masisea, province of Coronel Portillo, Ucayali. Where: DBA= diameter at base height (cm); D50, D100, and DBH are diameter at 50, 100, and 130 cm from the ground, respectively; Ch_1= height at first branch (m); Th= total height (m); Ch_2= canopy height (m); Cb= shaft biomass (kg); Pb= main branch biomass (kg); Sb= secondary branch biomass (kg); Lb= leaf biomass (kg); Tb= total tree biomass (kg); Oil= Essential oil (kg).



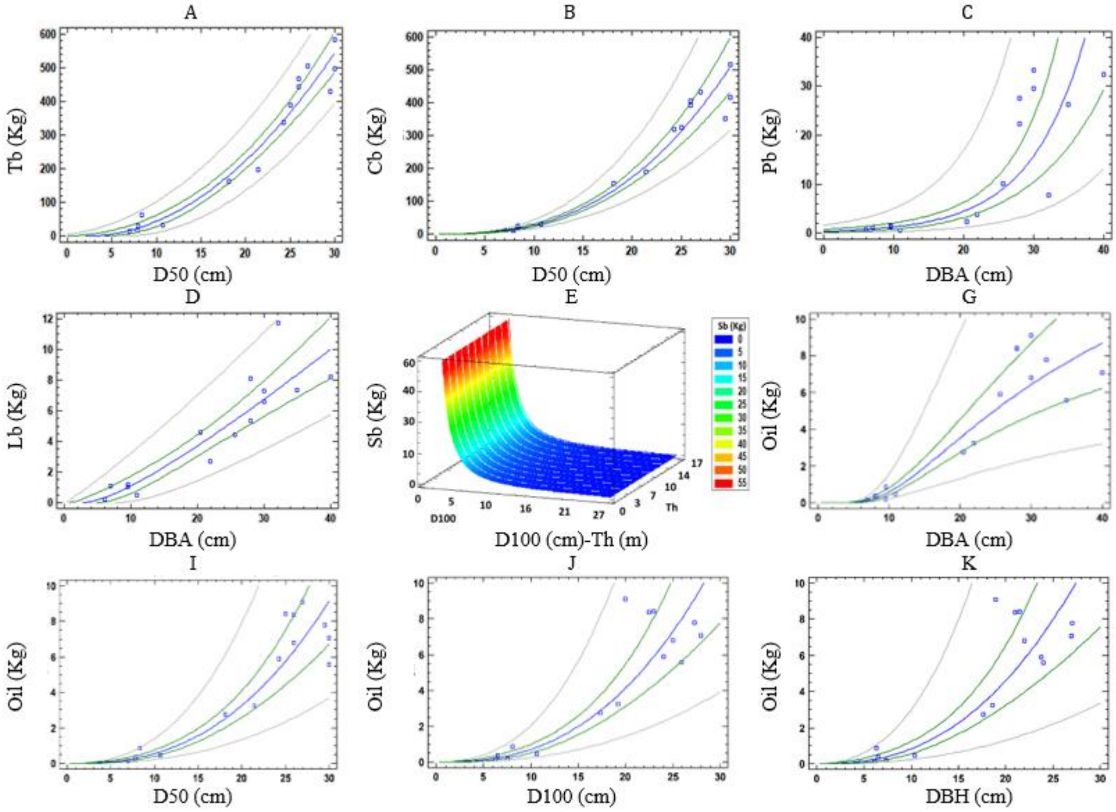
From the results of evaluations, the best models for predicting the biomass of the different components of the tree were determined (Table 7, Figure 7), having as independent variables those related to the diameter (DBA, D50, D100, and DBH) as they are the variables with greater correlation (ρ), and coefficient of determination (R_{aj}^2) with the biomass components (Cb, Pb, Lb, and Tb), which are better predicted by using simple regression models.

Table 7 - Best models, equations, Spearman correlation (ρ), and coefficient of determination (R_{aj}^2) of *Aniba rosaeodora* in permanent production forests in the district of Masisea, province of Coronel Portillo, Ucayali. A. Biomass models, B. Essential oil models.

Trait X	Trait Y	Best model	Equation	ρ	R_{aj}^2
A. Biomass models					
DBA	Cb	$Y = aX^b$	$Cb = \exp(-2.24037 + 2.3877\ln(DBA))$	0.99	0.96
DBA	Lb	$Y = (a + b\sqrt{X})^2$	$Lb = (-1.07058 + 0.669469\sqrt{DBA})^2$	0.9	0.87
DBA	Pb	$Y = \exp(a + bX)$	$Pb = \exp(-1.03636 + 0.126469DBA)$	0.92	0.84
DBA	Sb	$Y = a + bX^2$	$Sb = 4.13736 + 0.026701DBA^2$	0.74	0.39
DBA	Tb	$Y = (a + b\sqrt{X})^2$	$Tb = (-11.5289 + 5.80758\sqrt{DBA})^2$	0.95	0.94
D50	Cb	$Y = aX^b$	$Cb = \exp(-2.284207 + 2.66784\ln(D50))$	0.96	0.98
D50	Lb	$Y = (a + bX^2)^2$	$Lb = (0.751787 + 0.00258658D50^2)^2$	0.87	0.87
D50	Pb	$Y = \exp(a + bX)$	$Pb = \exp(-1.28422 + 0.153754D50)$	0.82	0.83
D50	Sb	$Y = a + bX^2$	$Sb = 2.59415 + 0.0372313D50^2$	0.75	0.39
D50	Tb	$Y = (a + bX)^2$	$Tb = (-1.73388 + 0.835102D50)^2$	0.96	0.96
D100	Cb	$Y = aX^b$	$Cb = \exp(-2.63739 + 2.68339\ln(D100))$	0.86	0.97
D100	Lb	$Y = (a + bX)^2$	$Lb = (0.0680281 + 0.105529D100)^2$	0.89	0.86
D100	Pb	$Y = \exp(a + bX)$	$Pb = \exp(-1.2178 + 0.165714D100)$	0.81	0.77
D100	Sb	$Y = a + bX^2$	$Sb = 3.536 + 0.0429738D100^2$	0.68	0.32
D100	Tb	$Y = aX^b$	$Tb = \exp(-1.52493 + 2.236931\ln(D100)$	0.86	0.91
DBH	Cb	$Y = aX^b$	$Cb = \exp(-2.28767 + 2.60697\ln(DBH)$	0.82	0.94
DBH	Lb	$Y = \sqrt{(a + bX^2}$	$Lb = \sqrt{178.551 + 2.08047DBH^2}$	0.88	0.21
DBH	Pb	$Y = \exp(a + b\sqrt{X})$	$Pb = \exp(-3.25183 + 1.2495\sqrt{DBH})$	0.76	0.7
DBH	Sb	$Y = \sqrt{(a + bX^2}$	$Sb = \sqrt{-70.1922 + 2.3357DBH^2}$	0.64	0.31
DBH	Tb	$Y = (a + bX)^2$	$Tb = (0.0995933 + 0.10909DBH)^2$	0.82	0.85
D100, Th	Sb	$Y = a + b(\frac{1}{X^2}) + c\left(\frac{X}{Y}\right) - d(Y$	$Sb = 0.06 + 4.6(\frac{1}{D100^2}) + 0.42(\frac{Th}{D100}) - 0.02(Th)$	0.97	0.97
B. Oil models					
DBA	Oil	$Y = \exp(a + b/X)$	$Oil = \exp(-3.06376 + 36.1098/DBA)$	0.81	0.92
D50	Oil	$Y = aX^b$	$Oil = \exp(-6.4554 + 2.54862\ln(D50))$	0.82	0.93
D100	Oil	$Y = aX^b$	$Oil = \exp(-6.2182 + 2.54862\ln(D100))$	0.75	0.90
DBH	Oil	$Y = aX^b$	$Oil = \exp(-5.83459 + 2.45693\ln(DBH))$	0.74	0.86
Pb	Oil	$Y = (a + b\ln(X))^2$	$Oil = (-0.76955 + 0.62158\ln(Pb))^2$	0.82	0.87
Lb	Oil	$Y = (a + b\sqrt{X})^2$	$Oil = (-0.122826 + 1.01098\sqrt{Lb})^2$	0.79	0.83
Sb	Oil	$Y = b + bX - 0.0012X^2$	$Oil = 2.07 + 0.16bSb - 0.001169(Sb)^2$	0.63	0.11
Tb	Oil	$Y = 1/(a + b/X)$	$Oil = 1/(-0.0793641 + 82.2948/Tb)$	0.84	0.99

DBA=diameter at base height (cm); D50, D100, and DBH are diameter at 50, 100, and 130 cm from the ground, respectively; Th= total height (m); Cb= shaft biomass (kg); Lb= leaf biomass (kg); Pb= main branch biomass (kg); Sb= secondary branch biomass (kg); Tb= total tree biomass (kg); Oil= Essential oil (l).

Figure 7 - Allometric biomass models with highly correlated variables for *Aniba rosaeodora*. Tb= total biomass (kg), Cb= stem biomass (kg), Pb= biomass of main branches (kg), Lb= biomass of leaves (kg), Sb= biomass of secondary branches (kg), DBA= diameter at base (cm), D50= diameter at 50 cm from ground (cm), D100= diameter at 100 cm from ground (cm), DBH= diameter at 130 cm from ground (cm); A. Tb per D50, B. Cb per D50, C. Pb per DBA, D. Lb per DBA, E. Sb per D100 and Th, G. Oil per DBA, I. Oil per D50, J. Oil per D100, K. Oil per DBH.



3.3.2 Essential oil models

Similarly, the sample data (

Table 6), determined eight best prediction models for essential oil, of which four are based on easily measured and directly measured variables (DBA, D50, D100, and DBH) and four with indirectly measured variables (Pb, Sb, Lb, Tb) (Table 7, Figure 7), of which the best equations with best fits were:

$\mathrm{Oil} = \exp(-6.4554 + 2.54862\ln(\mathrm{D50}))$, with ρ= 0.82 and R_{aj}^2= 0.93,

and $\mathrm{Oil} = 1/(-0.0793641 + 82.2948/\mathrm{Tb})$, with ρ= 0.84 and R_{aj}^2= 0.998.

3.4 DISCUSSION

3.4.1 Biomass models

Taking into account the best models resulting from our study, it can be seen that the aboveground biomass in Tomé Açu forests south of Belém de Pará in Brazil was determined using the model $\mathrm{Y} = \mathrm{aX^b}$, which resulted in the equation $\mathrm{FW} = 0.465\mathrm{DBH}^{2.202}/(1 - \mathrm{CH}(\%))$ (Araujo *et al.*, 1999), which is similar to the model used by our study to determine the DBA and Cb and D100 with Tb (Table 7), and showed high values for R^2_{aj} (0.964 and 0.965%, respectively). In the same way, this model differs from the model used by MINAM (2015b) to determine the total weight of the tree, $\mathrm{Tb} = 0.0009\mathrm{D}^{1.585}\mathrm{Th}^{2.651}$.

Likewise, our results are related to those obtained by Krainovic *et al.* (2017), who in the models applied to determine the aerial biomass per tree and the crown biomass, found in plantations a R^2_{aj} ranging from 0.63-0.8 and ranging from 0.407-0.41 with simple regression models with DBH and combined using DBH and Th, respectively. We can be observed that a weak correlation is maintained to predict the tree crown biomass component, which includes main, secondary branches and leaves, which in our study have a weak correlation, especially to predict the biomass of secondary branches (Sb: ρ ranging from 0.63-0.75, R^2_{aj} ranging from 0.102-0.386).

A similar R^2_{aj} coefficient observed in our study (0.98) was reported in the determination of the total aerial biomass of the species *G. crinita* from diameters (DBA) in plantations in Ucayali, Peru, with DHB in the range between 10-17.9 cm and using the exponential model of Tb= aDBA^b, where a R^2 of 0.97 was found (Revilla-Chávez *et al.*, 2021). Likewise, in a similar study to determine the commercial volume of *G. crinita* wood with the best model with a variable ln(V)= a+bln(DBH) had a R^2= 0.93, while the model of combined variable using the DBH and Th as V= a+bln(DBH^2)+cln(DBHTh)–dln(DBH^{2}Th) there was a better fit with R^2= 0.98 (Revilla *et al.*, 2021). Thus, the use of combined variables results in better adjustments for low correlation variables as demonstrated in our study, where the Sb variable had low correlation with its independent variables (diameters). However, this correlation improved from R^2_{aj} (0.311-0.386) with the application of a combined variable model with three variables, such as D100 and Th (Figure 7E), which shows the best R^2_{aj} in the intervention of more than one variable to predict Tb, which was confirmed by Krainovic *et al.* (2017), who obtained better fits with the application of combined variable models for the determination of total aerial biomass of *A. rosaeodora* in plantations.

3.4.2 Essential oil models

The allometric equations for oil estimation had a high correlation (ρ) with diameter traits (ρ: 0.74-0.82) and R^2_{aj} (0.86-0.93), showing that higher diameters result in high oil yield. The allometric equations for oil estimation had a similar correlation between oil and biomass (ρ: 0.63-0.84) and R^2_{aj} (0.109-0.998) to those obtained between diameter and biomass traits (ρ: 0.64-0.99; R^2_{aj}: 0.21-0.982). Thus, the equations can be used from diameters (DBA, D50, D100, and DBH) variables of easy measurement or from biomass variables (Pb, Sb, Lb, and Tb), which are obtained after adding the dry weights of the components, which requires procedures prior to the application of the equation, without implying a precise correlation, since the determination coefficient for diameters has R^2_{aj} ranging from 0.86-0.93, versus R^2_{aj} ranging from 0.109-0.998, using tree biomass and tree components as predictors. Thus, it is advisable to use any of the tree diameters because they better predict the essential oil content of *A. rosewood* (Table 7, Figure 7).

Similarly, the yields of essential oil of *A. rosaeodora* with 1.5%, σ= 0.34 l and CV%= 22.3%, correlate with those reported by Fidelis *et al.* (2012, 2013), which give a yield of 0.75-3.4% of essential oil from distilled biomass in the same species. However, is slightly higher than that reported by Guenther (1950), who states that the oil yield is between 0.7 and 1.2%, while the oil yield is between 0.7 and 1.2%. 4% of essential oil from distilled biomass in the same species, but it is slightly higher than that referred by Guenther (1950) who states that the oil yield is between 0.7 and 1.2%, while Teles and Mouchrek (2022) determined the yield of essential oil at 1.9% and Chacón-Pagan *et al.* (2006), determined a yield of 0.44-0.47%.

3.5 CONCLUSIONS

The variables that best predict the aboveground biomass of *A. rosaeodora* trees in a forest concession in the district of Masisea, province of Coronel Portillo in the department of Ucayali is the diameter. The highest correlation was between diameter at 50 cm from the ground (D50), chaft biomass (Cb) and are total tree biomass (Tb). Combined variable models improved biomass prediction fit when increased from one to two variables in the present study. The most strongly related variables for oil prediction are total tree biomass (Tb) and D50.

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4 GENETIC CONTROL OF QUANTITATIVE AND QUALITATIVE TRAITS OF *Calycophyllum spruceanum* (Benth.) IN THE PERUVIAN AMAZON[3]

ABSTRACT

Families of 38-month-old *Calycophyllum spruceanum* planted at three locations in the Peruvian Amazon were analyzed with a view to future breeding. Significant differences were detected among families, sites and combined sites for the traits trunk diameter (D), height (H), number of nodes (NN), number of branches (NB) and survival rate (SUR), except for NN at site 2 and SUR at site three and combined sites. The correlation of the genotype-environment interaction was simple for D, H, NN, and SUR (0.77-0.89). Heritability was generally highest for D, H, NN, and NB (0.12-0.7), and the genotypic coefficient of variation ranged from 8.6 to 27.4%. Genetic correlations between these traits within and among sites ranged from 0.35 to 1.0. The selection of 75 trees from different families, within blocks and locations, suggested the possibility of genetic gains between 6.1 and 29.8% for D, H, NN, and NB, and that the remaining effective population size can be exploited in future selection cycles.

Keywords: Genetic correlations, genetic variation, heritability, progeny test, tree breeding

[3] REVILLA-CHÁVEZ, J.M.; SEBBENN, A. Genetic control of quantitative and qualitative traits of *Calycophyllum spruceanum* in the Peruvian Amazon. **Crop Breeding and Applied Biotechnology**, Viçosa - Minas Gerais, v.24, n.1, p.e47262419, 2024. https://doi.org/10.1590/1984-70332024v24n1a09

4.1 INTRODUCTION

Calycophyllum spruceamun (Benth.) (Rubiaceae) is a heliophytic pioneer tree found in alluvial forests in Brazil, Bolivia, Colombia, Ecuador and Peru, up to 1000 m above sea level (Reynel *et al.*, 2003; Sears *et al.*, 2014; Ushiñahua, 2016; Saldaña *et al.*, 2021). The tree has multiple uses; its timber is exported to various parts of the world in view of the beautiful creamy brown color and high density of the wood, which makes it durable and suitable for construction (Taylor, 2005). The very straight, cylindrical bole of adult trees can reach diameter at breast heigh (DBH) ranging from 50-120 cm and tree height ranging from 20-35 m, with a crown in the upper third. A study on this species with seeds from different origins in the Peruvian Amazon reported growth to a height ranging from 1.4-1.6 m within six months and ranging from 3.5-4.7 m within one year (Sotelo-Montes *et al.*, 2003). In Peru, the response potential of the species in plantations for industrial purposes is considered highly efficient and promising (Orrego; Bustamente *et al.*, 2017). At the national and international level, the demand for *C. spruceanum* is on the rise, due to the wood properties and low production costs. In recent years, the species has been counted among the first 10 commercial species for saw wood production, with very diverse applications, e.g., as structural timber for construction, furniture and sporting goods (Orrego; Bustamente, 2017; Saldaña *et al.*, 2021).

Due to the economic and social relevance of *C. spruceanum* wood production by small farmers, the genetic variability of wild populations of the species has been the focus of some research based on genetic markers (Russel *et al.*, 2003; Dávila-Lara *et al.*, 2017; Saldaña *et al.*, 2021) and quantitative traits (Sotelo-Montes *et al.*, 2003, 2006, 2007a, 2007b, 2007c, 2008; Boivin-Chabot *et al.*, 2004; Weber; Sotelo-Montes, 2005; Dawson *et al.*, 2009; Weber *et al.*, 2009; Thauchen *et al.*, 2011). However, information about the genetic variation and control of growth and quality traits of the species is currently limited, since these studies addressed only a minor part of the species distribution.

Due to the increase in deforestation, forest fires and intense selective cutting in the environments of *C. spruceanum*, as well as the increased demand for its seeds for reforestation (Carmo *et al.*, 2017), strategies of conservation and breeding of the species are urgently needed. Provenance and progeny tests, underlying the determination of genetic variation within and among populations, genetic inheritance (heritability) and phenotypic and genetic correlations between quantitative and qualitative traits have been used for both ex-situ conservation and breeding of populations (Sebbenn *et al.*, 2005). These data are fundamental to evaluate whether a germplasm collection strategy efficiently represents and conserves the genetic variation of populations of species ex situ and for successful selection in breeding programs (Sebbenn *et al.*, 2009a). Provenance and progeny tests can therefore be used for both purposes simultaneously, *ex situ* conservation and production of somewhat improved seeds, for both commercial and environmental reforestation (Sebbenn *et al.*, 2005, 2009a). To this end, the best trees with desirable traits are selected (Sebbenn *et al.*, 2009a; Canuto *et al.*, 2016). Likewise, testing families from different origins allows a better understanding of the genetic variability in populations of a species and detects the most adaptable genotypes to the environmental conditions of a reforestation site (Menegatti *et al.*, 2016; Revilla-Chávez *et al.*, 2022).

Therefore, this study analyzed the genetic variation and control of quantitative and qualitative traits of 38-month-old *C. spruceanum* trees in progeny trials in the Peruvian Amazon, to obtain information for the implementation of second-generation seed orchards with high seed quality for forest restoration.

4.2 MATERIAL AND METHODS

4.2.1 Study sites and progeny tests

The progeny tests were established at three locations in the Aguaytía river basin, on the eastern slope of the Cordillera, in the department of Ucayali, Peru (between 9º 24' 25.87" S/ 75º 54' 38.91" W and 7º 57' 36.47" S/74º 5' 33.64" W, in an area 100 km E-W and 60 km N-S). The vegetation of the area is mainly tropical premontane forest, with a mean annual temperature of 26 °C (Holdridge, 1978). Rainfall increases with increasing elevation: at sites 1, 2 and 3, at average elevations of 150, 200, and 260 m asl, respectively, mean annual precipitation is 1656.3, 1926.5, and 2501 mm.ha^{-1}.year^{-1}, respectively (Hijmans *et al.*, 2005). However, water stress of 1, 4 and 5 months was observed at sites 3, 2 and 1, respectively (Ugarte-Guerra; Domínguez-Torrejón, 2010). Data of the progeny tests and characteristics of the three experimental sites are listed in Table 8.

Table 8 - Location, progeny test and edaphoclimatic characteristics of evaluation plots of *Calycophyllum spruceanum* at three sites in the Aguaytia river basin, Ucayali, Perú.

	Site 1	Site 2	Site 3
Number of families	200	200	200
Number of blocks	5	5	5
Number of plants per plot	2	2	2
Elevation (m)€	150	200	260
Rainfall (mm.ha^{-1}.year^{-1})€	1656	1926	2501
Number of months under water stress£	5	4	1
Terrace€	low	medium	high
Soil€	very sandy	sandy loam	clayey
Phosphorus availability (P)	low	low	high
Levels of K, Ca and MG€	medium	medium	high

€Hijmans *et al.* (2005); £Ugarte-Guerra and Domínguez-Torrejón (2010); K= Potassium; Ca= Calcium; Mg= Magnesiun.

The progeny tests were arranged in a randomized complete block design, with five blocks, 200 open-pollinated families and two plants per plots. The families were derived from seeds collected from 200 seed trees (June 1998), at 13 sites in the Aguaytia river basin: 39 families from the district of Nueva Requena; five families from the Neshuya river, at km 49.5 of the countryside highway Carretera Federico Basadre (CFB); five families from the Tahuayo river basin, at km 72 CFB; 10 families from the Curimaná river basin; six famillies from the Aguaytía river basin; seven families from the Yurac-Aguaytía river basin; 21 from Puerto Inca; six families from

Von Humboldt; 43 families from Macuya; 50 families from San Alejandro; seven families from along the CFB up to km 72; six families from along the Curimaná road; and three families from the Aguaytía river basin along the CFB to the district of Tournavista. Each block comprised a total of 400 plants, 200 plots and two plants per plot, at a spacing of 2.5 x 2.5 m, on an area of 2,500 m^2 per block. Two plant rows around the blocks were considered border rows that were not evaluated and they were separated by a 5m wide fire strip. At the time of planting, 1600 kg of earthworm humus ha^{-1} and 320 kg of rock phosphate ha^{-1} (1 kg and 200 g per plant, respectively) were applied to the experimental area. In the second and third years, fertilization was repeated, with 112 kg.ha^{-1} urea, 316 kg.ha^{-1} rock phosphate and 160 kg ha^{-1} potassium chloride (i.e., 70, 185, and 100 g of each product per plant, respectively). During the first 12 months, plants stunted by weeds, insects and rodents were eliminated.

4.2.2 Estimates of genetic parameters

The data were arranged in a randomized block design and processed as described below. The REML/BLUP procedure (restricted maximum likelihood/best unbiased linear prediction) by the linear mixed model was used in individual analyses to estimate the variance components and genetic parameters of each trait evaluated at each site, using the SELEGEN software, based on the statistical Model 1 (Resende, 2016), $y = Xr + Za + Wp + e$, where y, r, a, p and e are the data, effects of repetition (fixed) added to the overall mean, individual additive genetic effects (random), effects of portion and the error or residual vector (random), respectively. Capital letters represent the incidence matrices for the said effects. For combined site analysis, Model 4 (Resende, 2016) was used: $y = Xr + Za + Wp + Ti + e$, where y is the data vector; r the vector of repetition effects (fixed) added to the overall mean; a the vector of individual additive genetic effects (random); p the vector of plot effects (random); i the vector of the effects of the genotype-environment (GE) interaction (random); and e the error or residual vector (random). Capital letters represent the incidence matrices for the said effects. From analysis of variance, the following components of interest were estimated: σ_A^2 = additive genetic variance; σ_f^2= genetic variance among families; σ_e^2= variance due to family × replication interaction effects; $\sigma_{e(s)}^2$= variance due to the interaction between the families within and replications within environments; σ^2_{gxe}= variance of the GE interaction; and σ^2_w= phenotypic variance within family. The genetic correlations between sites ($\mathrm{r_{ge}}$), mean heritability among families (h^2_m) and within family (h^2_w) for each site were estimated by:

$$h^2_m = \frac{\sigma^2_f}{\sigma^2_f + \frac{\sigma^2_e}{J} + \frac{\sigma^2_w}{\bar{n}J}} \text{ and} \quad (1)$$

$$h^2_w = \frac{(1-0.25)\sigma^2_A}{\sigma^2_w}, \quad (2)$$

and for combined analysis of sites (s) by:

$$h^2_{m(s)} = \frac{\sigma^2_f}{\sigma^2_f + \frac{\sigma^2_{gxe}}{S} + \frac{\sigma^2_{e(s)}}{SJ} + \frac{\sigma^2_w}{\bar{n}SJ}} \text{, and} \quad (3)$$

$$h^2_{w(s)} = \frac{[(1-0.25)/\bar{n}]\sigma^2_A}{\sigma^2_{e(s)} + \frac{\sigma^2_w}{\bar{n}}}, \quad (4)$$

where S, J and $\bar{n}$ are the number of sites, replication and the harmonic mean of the number of plants per plot. We also calculated the coefficients of environmental variance ($CV_e\% = 100(\sqrt{\sigma^2_e}/x)$), genetic variance among families ($CV_g\% = 100(\sqrt{\sigma^2_f}/x)$), and additive genetic variation among families ($CV_{gi}\% = 100(\sqrt{\sigma^2_A}/x)$, where x is the mean of the trait under study. The relative correlation coefficient was estimated by $CV_r = CV_g\%/CV_e\%$. The genetic (r_g) and phenotypic (r_f) correlations between pairs of traits were computed as proposed by Resende (2002), taking only traits with genetic variation among families into consideration. The genetic gain by selection among and within families (G_{aw}) was estimated for each site for selection intensities of 37.5% of the best families (70:200) and 25% of the best trees within families (5:10), based on the equation, $G_{aw}(\%) = 100(d_a h^2_m + d_w h^2_w)/x)$, where d_a and d_w are the selection differentials among and within families, respectively. d_a was estimated by d_a=$x_{sa} - x$, where x_{sa} is the mean of the selected families and x the population mean for the traits under study. d_w was estimated by d_w=$x_{sw} - x$, where x_{sw} is the mean of selected trees within family for the traits under study.
The effective population size (N_e) after selection was estimated as $N_e = 0.5/\Theta$, where Θ is the group coancestry (Lindgren *et al.*, 1996), estimated by $\Theta =$

$[mn0.5(1 + F_p) + m\theta_{xy}n(n - 1)]/(nm)^2$ (Sebbenn *et al.*, 2009b), where *m* and *n* are the number of selected families and plants within family, respectively; F_p is the inbreeding coefficient in the parental population; and θ_{xy} the coancestry between plants within family, assumed as half-sibs (θ_{xy}= 0.125).

4.2.3 Variables evaluated

The estimation of genetic parameters was performed on the evaluation of stem diameter (D, cm), tree height (H, m), stem shape (SSh,

Table 9), number of nodes (NN, quantity), number of branches (NB, quantity) and survival (SUR, %) at 38 months of age of the variables of *C. spruceanum* progeny.

Table 9 - Score used to measure stem shape (SSh) in 38-month-old *Calycophyllum spruceanum* trees.

Class	Terminal but presence	Terminal but dominance	Branch orientation	Branch position	Stem	Score
Stem shape	yes	yes	vertical	different	straight	100
	yes	yes	horizontal	different	straight	98
	yes	yes	vertical	different	crooked	96
	yes	yes	horizontal	different	crooked	94
Branched stem	yes	yes	vertical	different	-	92
	yes	yes	horizontal	different	-	90
	yes	yes	vertical	single plane	-	88
	yes	yes	horizontal	single plane	-	86
	no	yes	vertical	different	-	84
	no	yes	horizontal	different	-	82
	no	yes	vertical	single plane	-	80
	no	yes	horizontal	single plane	-	78
	yes	no	vertical	different	-	76
	yes	no	horizontal	different	-	74
	yes	no	vertical	single plane	-	72
	yes	no	horizontal	single plane	-	70
	no	no	vertical	different	-	68
	no	no	horizontal	different	-	66
	no	no	horizontal	single plane	-	64
	no	no	vertical	single plane	-	62
Inclined stem shape	yes	yes	vertical	different	-	60
	yes	yes	horizontal	different	-	58
	yes	yes	vertical	single plane	-	56
	yes	yes	horizontal	single plane	-	54
	no	yes	vertical	different	-	52
	no	yes	horizontal	different	-	50
	no	no	vertical	single plane	-	48
	no	no	horizontal	single plane	-	46
	yes	no	vertical	different	-	44
	yes	no	horizontal	different	-	42
	yes	no	vertical	single plane	-	40
	yes	no	horizontal	single plane	-	38

4.3 RESULTS

Significant differences among families at sites and combined sites were detected for D, H, NN, NB and SUR, with exception for NN at site 2, and SUR at site 3 and in the combined analysis. These results indicated genetic variability among families for D, H, NN, NB and SUR and the possibility of breeding by selection of the best families (Table 10). The correlation of the GE interaction ($\mathrm{r_{ge}}$) was positive and high for D, H, NN, and SUR ($\mathrm{r_{ge}}$ = 0.768-0.891), indicating that the GE interaction is simple. Little family rank changes for the GE interaction between sites were observed, indicating that selection for these traits can be done efficiently at a single site. In contrast, the $\mathrm{r_{ge}}$ for NB (0.554) and SF (0.154) indicated a complex type GE interaction, where family rank changes between sites are involved, and that selection for these traits should be performed specifically for each site (Table 10).

The traits D, H, NN, and NB generally have higher mean heritability among families (h_m^2), heritability within family (h_w^2), selective accuracy (r_a), coefficient of genotypic additive genetic variation (CV_{gi}), and coefficient of relative variation (CV_r) than stem shape (SSh) and SUR, suggesting that high genetic gains by selection can be expected for D, H, NN, and NB. The coefficient h_m^2 was higher than h_w^2 for all traits (except for SUR) in the site and combined site analysis (h_m^2= 0.024-0.696, h_w^2= 0.003-0.52), indicating that higher genetic gains can be expected from selection among than within families. For SUR, the range of h_m^2 was 0.033 to 0.196 among sites (combined= 0.292). The selective accuracy (r_a) was 0.375 to 834 for D, H, NN and NB; 0.443 for SUR at site 1; and 0.54 for the combined sites, which indicated that the phenotype mean is a good predictor of the additive genetic value of families (Table 10). Our results showed that the genetic parameters were generally lower for D, H, and NN and higher for SUR at site 1 than at the other two locations. In other words, the highest genetic gains from selection can be expected at sites 2 and 3 for D, H, and NN, and at site 1 for SUR (Table 10).

Table 10 - Mean and estimates of genetic parameters for trait at site and combined sites in tests with 38-month-old *Calycophyllum spruceanum* progenies.

Trait	Mean	h_m^2	h_w^2	r_a	CV_{gi}%	CV_r	GS(%)	r_{ge}
Diameter (D, cm)								
Site 1	4.7*	0.269	0.220	0.519	11.9	0.27	18.4	
Site 2	8.6*	0.464	0.416	0.681	18.3	0.42	27.7	
Site 3	11.2*	0.512	0.383	0.715	14.5	0.46	19.5	
Combined	8.16*	0.654	0.329	0.808	15.8	0.40	26.5	0.84
Height (H, m)								
Site 1	3.2*	0.203	0.201	0.450	13.7	0.22	13.2	
Site 2	6.41*	0.555	0.520	0.745	13.5	0.44	23.9	
Site 3	8.52*	0.549	0.502	0.741	14.5	0.49	14.5	
Combined	6.4*	0.696	0.429	0.834	16.1	0.50	16.6	0.891
Stem shape (SSh)								
Site 1	94.8	0.027	0.008	0.163	1.1	0.07	0.1	
Site 2	95.3	0.030	0.012	0.172	0.9	0.08	0.1	
Site 3	95.6	0.035	0.012	0.186	0.9	0.08	0.1	
Combined	95.2	0.024	0.003	0.154	0.5	0.03	0.1	0.154
N. of nodes (NN)								
Site 1	26.9*	0.141	0.121	0.375	8.6	0.18	7.0	
Site 2	37.0	0.276	0.238	0.526	9.6	0.28	8.8	
Site 3	42.6*	0.300	0.233	0.548	8.8	0.29	6.1	
Combined	35.5*	0.473	0.263	0.687	12.0	0.35	25.5	0.85
N. of branches (NB)								
Site 1	7.9*	0.379	0.376	0.616	27.4	0.35	29.8	
Site 2	13.4*	0.321	0.288	0.567	17.9	0.32	11.7	
Site 3	17.6*	0.392	0.372	0.626	11.0	0.31	8.7	
Combined	12.9*	0.404	0.197	0.636	13.7	0.29	16.6	0.554
Survival (SUR, %)								
Site 1	86.6*	0.196	-	0.443	11.9	0.22	1.7	
Site 2	93.5*	0.039	-	0.197	3.2	0.09	0.2	
Site 3	96.9	0.023	-	0.151	1.8	0.07	0.1	
Combined	92.3	0.292	-	0.540	6.6	0.14	2.4	0.768

*P< 0.05, with 0.5 degrees of freedom for likelihood ratio test (LRT), χ^2 deviance chi-square; r_{ge} is the genetic environmental correlation; h_m^2 and h_w^2 are the mean heritabilities among and within family, respectively; r_a is the selective accuracy; CV_{gi}% and CV_r are the coefficients of additive genetic and relative variation, respectively.

Genetic (r_g) and phenotypic correlations (r_p) varied between pairwise traits at the sites (Table 11). Genetic correlations (r_g) were high and positive (≥ 0.6) between pairwise traits D, H, NN, and NB at each and all sites (r_g= 0.77-1.0), except between NN and NB at site 3 (r_g= 0.56). The phenotypic correlation (r_p) was generally high between pairwise traits at each and all sites (r_p= 0.68-0.89), except between D and NN at site 3 (0.59), D and NB at site 2 and the combined sites (0.48-0.57) and between H and NB at site 3 and combined sites (0.42-0.56), NN and NB at sites 2 and 3, and combined sites (0.36-0.55) (Table 11).

Table 11 - Genotypic (r_g, above diagonal) and phenotypic (r_p, below diagonal) correlations for diameter (D), height (H), number of nodes (NN) and number of branches (NB) of each site and combined sites in tests with 38-month-old *Calycophyllum spruceanum* progenies.

	D	H	NN	NB
Site 1				
D		0.81	0.77	0.88
H	0.89		0.79	0.96
NN	0.68	0.80		0.97
NB	0.71	0.67	0.6	
Site 2				
D		0.88	0.78	0.74
H	0.87		0.87	0.89
NN	0.67	0.74		0.85
NB	0.57	0.65	0.55	
Site 3				
D		0.85	0.82	0.71
H	0.78		0.97	0.68
NN	0.59	0.68		0.56
NB	0.65	0.42	0.49	
Combinad				
D		0.89	0.91	0.95
H	0.83		0.97	0.96
NN	0.65	0.64		1.0
NB	0.48	0.56	0.36	

The progeny test was designed to serve two purposes: the ex-situ conservation of *C. spruceamun* combined with the production of seeds with some level of breeding. Thus, since the installed blocks were isolated from each other within the sites, we chose the strategy of selecting 75 of the 200 families (among) and one plant within each family (selection within) per block at each site (Table 12).

Table 12 - Number of selected trees and families, group coancestry, and effective population size (N_e) of each site and combined sites in tests with 38-month-old *Calycophyllum spruceanum* progenies.

	Site 1	Site 2	Site 3	Combinad
Selected trees per block (1 tree/family)	75	75	75	75
Total number of selected trees per site	375	375	375	1125
Total number of selected families per site	172	173	175	199
Group coancestry per block: Θ_b	0.0067	0.0067	0.0067	0.0067
Group coancestry per site: Θ_s	0.0019	0.0019	0.0018	0.0011
Effective population size per block: $N_{e(b)}$	75	75	75	75
Effective population size per site: $N_{e(s)}$	265	264	270	472

4.4 DISCUSSION

The mean values of all traits were lowest at site 1 and highest at site 3 (Table 10). After 38 months, the mean survival rate (SUR) among sites (92.3%) indicated a high adaptation of the families to the three studied sites. The mean growth among sites for D (8.16 cm) and H (6.4 m) was greater than the mean reported for the species at other locations in Peru after 39 months (D= 5.86 cm, H= 6.21 m; Sotelo-Montes *et al.*, 2006). In contrast, the NN (35.9) and NB (12.9) were lower than the mean reported at other locations in Peru after 18 months (NN= 41.21, NB= 23.49; Sotelo-Montes *et al.*, 2003). These results showed that the families studied here have the potential to produce good quality wood (low number of knots in the wood), since the low values of NN and NB indicate wood with less defects (knots). However, our results may have been affected by the mortality rate at site 1, resulting from inter-tree competition, which may have affected growth traits such as D and H, decreasing the estimates of genetic parameters such as h_m^2, h_w^2, and r_a, because D is competition-sensitive (Leonardecz-Neto *et al.*, 2003; Pavan *et al.*, 2011). This could explain the inverse patterns of the estimated heritability and tree mortality rate. Under this condition, an efficient genetic selection at site 1 would be difficult, because D would be affected by a major environmental effect (competition), which would also affect the selection of genotypes for the best progeny for tree growth (Pavan *et al.*, 2011). This aspect also has a negative effect on the selection of genotypes for growth control with regard to tree quality. For this purpose, the number of nodes (NN) is evaluated, which is related to the branching habit. The nodes are related with the wood quality for causing defects in the trunk, since most fractures in wood structures occur due to the presence of knots (Jansons *et al.*, 2009; Corvalan-Vera, 2020; Resim-Esteves *et al.*, 2022).

After selection, NN can be mitigated by silvicultural practices such as selective thinning and pruning. However, breeding allows a more effective long-term solution, since if sufficient genetic variation for NN is available (Vargas-Hernández *et al.*, 2003), its expression can be controlled. Therefore, breeding programs include the control of traits associated with wood quality (Tong *et al.*, 2013), as well as other traits related to branching, sinuosity and stem branching (Sotelo-Montes *et al.*, 2007a; Santos *et al.*, 2010; Tung *et al.*, 2010).

For D and H, the h_m^2 was higher than reported in a test with 36-month-old *G. crinita* progenies, assessed at the same sites (Revilla-Chávez *et al.*, 2022), where h_m^2 values were low for D (0.033-0.39, site mean = 0.369) and H (0.052-0.36, site mean = 0.34). This comparison indicated a higher potential for breeding by selection of the tested *C. spruceanum* than the *G. crinita* progenies.

Our findings corroborated results of Sotelo-Montes *et al.* (2006) for the study species, who also reported high r_g between D and H (0.89-0.91), and data of Sotelo-Montes *et al.* (2003) and Tauchen *et al.* (2011), who found high r_p between D and H (0.85) and moderate r_p between NN and NB (0.45). The high r_g between D and H indicated that the two traits can be considered as one in the selection process, since the selection for trees with higher D will also induce an increase in H, and since r_g between both traits is largely influenced by the same pleiotropic genes (Vencovsky; Barriga, 1992). This result is very favorable for indirect selection, since positive associations between traits indicate that selection on one trait can lead to indirect gains in another (Sotelo-Montes *et al.*, 2007a; Revilla-Chávez *et al.*, 2022; Schoffen *et al.* 2023).

Therefore, each block was transformed into a seed orchard, resulting in a total of 172-175 families per site and 199 families across the three sites for selection. This established an effective population size of 75 within the blocks, varying from 264 to 270 between sites, and totaling an effective population size of 472 for the three sites. This selection strategy achieved the genetic gains expected by selection among and within families, which varied between traits and sites. As expected, the possibility of genetic gains for the traits with highest heritability (D, H, NN and NB) was between 6.1 and 29.8%.

4.5 CONCLUSIONS

Genetic variation among families was detected at each site and combined sites for the traits trunk diameter (D) and height (H) and number of branches (NB), as well as for number of nodes (NN) at sites 1 and 3 and combined sites, and for survival rate (SUR) at sites 1 and 2, which can be exploited by selection in breeding programs with 38-month-old *C. spruceanum* progenies. The genotype - environment interaction for the traits D, H, NN, and SUR in 38-month-old trees is simple, so selection may be carried out at only one site. The genetic control of the traits D, H, NN and NB, measured by heritability coefficients among and within families, varies from moderate to high, indicating the possibility of genetic gains by selection among and within families. The genetic and phenotypic correlations between D, H, NN, and NB for each site and combined sites are positive and high, and selection for any of these traits will result in indirect genetic gains for the others. The strategy of applying selection among and within families, within blocks and sites, serves two purposes: improvement by breeding and *ex situ* conservation, while the effective size of the remaining population can be exploited in other selection cycles.

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5 METHODOLOGY FOR HE PHENOTYPIC EVALUATION IN *Guazuma crinita* (Mart.) (MALVACEAE) TREES IN UCAYALI, PERU[4]

ABSTRACT

The objective of this study was to present a methodological tool for the phenotypic evaluation in progeny tests of *Guazuma crinita* in producer plots of the Aguaytía river basin, Ucayali, Peru, which allows field technicians to standardize the morphological evaluation criteria of trees in forest plantations. Therefore, the phenotypic traits were evaluated for plant height (m), diameter at the height of the base (cm), number of branches, number of rings, stem form, branch orientation, presence and quantity of leaves. The heritability and genetic and phenotypic correlations between traits were also estimated. Therefore, 32 morphological categories were plotted based on the significant correlations ($p \leq 0.05$) shown between the place of planting, the stem form, the orientation of the branches and the presence of leaves. For the same reason, the progeny showed low morphological patterns, being a low factor of phenotypic variability. It is concluded that the correlations between the biometric and morphological traits evaluated, allowed to validate the phenotypic evaluation procedures of *G. crinita* progeny tests at 36 months of age.

Keywords: Amazon; Aguaytia Basin; Bolaina blanca; Progeny test

[4] REVILLA-CHÁVEZ, J.M., LÓPEZ-GALÁN, E.E.; GONZALES-ALVARADO, A. C.; SÁENZ-RAMÍREZ, L.H.; MORI-VÁSQUEZ, J.A.; ROJAS-MEGO, K.C.; ABANTO-RODRÍGUEZ, C.; SEBBENN, A.M. Methodology for the phenotypic evaluation in *Guazuma crinita* trees in Ucayali, Peru. **Ciência Florestal**, Santa Maria RS, v.34, n.1, p.e71675, 2024. https://doi.org/10.5902/1980509871675 **(Published)**

5.1 INTRODUCTION

Guazuma crinita Mart. (Malvaceae) is a fast-growing tree, promising for use in agroforestry plantations and improving the quality of life of farmers in the Peruvian Amazon. The species has been subjected to intense logging, which is causing the decline of its will populations, as well as leading to its genetic erosion (Revilla-Chávez *et al.*, 2021). This limits the possibilities of using the species in the recovery of degraded areas and hinders commercial reforestation programs, which require seeds with genetic quality originating from genetic improvement programs (Sebbenn *et al.*, 2007; Kubota *et al.*, 2015; Aguiar *et al.*, 2019; Gerber *et al.*, 2021; Silva *et al.*, 2023). Much of what we know about the species in Peru arose as a result of the ICRAF Agroforestry Tree Domestication Program in the 1990s, whose philosophy is to promote the conservation of genetic resources through their use by farmers (Sotelo; Weber, 1997; Putzel *et al.*, 2013; Sears *et al.*, 2018; Tuisima *et al.*, 2016, 2020).

The economic criterion associated with productivity is the most important variable in the choice of tree species to be used by farmers in reforestation. For this, the best trees from wild populations are selected based on the phenotypic expression of traits of economic interest for collecting seeds and using them in their plantings. However, the phenotypic selection of traits in trees from wild populations, due to the interaction of genotypes with the environment, can lead to errors in choosing mother trees that produce seeds with better genetic quality (Aguirre; Fassbender, 2013). To avoid errors in the selection of mother tees, provenance and progeny tests are used, where information is obtained on the heritability of the traits, the genetic and phenotype correlations between the traits and the interaction between the genotype and the environment (Weber *et al.*, 1997; Oliva; Rimachi, 2017). Therefore, a forest domestication program can provide genetically improved germplasm for the establishment of forest plantations, being necessary to establish seed orchards with individuals with traits that the market wants.

The selection of high-yielding trees is the foundation of forest genetic improvement, and success depends on the quality and rigor that individuals are characterized (Vallejos *et al.*, 2010; Gutiérrez-Vásquez *et al.*, 2016). Most of the trials consider the types of stems, growth, stability, adaptability, resistance to pests and diseases, as they are directly related to the attributes of the wood (Zobel; Talbert, 1994; Espitia *et al.*, 2016). Proof of this is that the reports of genetic gain when using superior material is greater than 15% for the traits growth in height and growth in diameter at breast height (DBH), as well as greater than 35% in volume per unit area (Cornelius, 1994; Espitia *et al.*, 2016). Selection works by considering highly heritable traits (Zamudio; Guerra, 2002) such as stem straightness, branching, plant health, tree height, diameter et breast heigh (DBH) and volume (Cornelius, 1994). So, these traits are suggested for selection due to having a higher heritability (Murillo *et al.*, 2003; Murillo; Badilla, 2004).

Qualitative traits often have greater heritability ($h^2 > 0.5$), indicating that they are regulated by a few loci, and are less subject to environmental effects, so that a tree with a qualitative trait superior, it will be superior to a great extent in other environments (Murillo *et al.*, 2003). Thus, the bifurcation of a tree is used as a suppression factor for a tree aspiring to a plus tree, since it is considered to have high heritability (Zobel; Talbert, 1994). Therefore, the stem form is expressed as a result of the interaction between the genotype and the environment (Chambel *et al.*, 2005).

Identifying desirable individuals that have more than one trait of interest is a fundamental objective in forest improvement. But this task is not easy, because the traits are associated with each other, where the association between some traits may have a direct relationship and others may have an inverse relationship (Vencovsky; Barriga, 1992). The quantification of the association between traits can be obtained from analysis of phenotypic, genotypic and environmental correlations (Vencovsky; Barriga, 1992). In selection, correlations are useful for the indirect selection of a trait using its relationship with another, based on criteria of greater ease of measurement and identification and greater heritability, which allows inferring that the traits are genetically association and indicates the possibility of developing synchronous selection for several traits simultaneously (Vencovsky; Barriga, 1992; Correa *et al.*, 2013). For this purpose, the final product of the tree should be observed based on its intrinsic traits such as growth dynamics and tree morphology. So, the evaluation should collect comprehensive information with which the traits occur in a stand based on to the expected products. For example, in that sense for sawn wood and posts, straight and cylindrical trunks, low conicity factor, without low forks, with fine branches and horizontal position are required. On the other hand, for the production of firewood, the shape of the stem and branches is not very important, as the biomass yield is of greater interest. But, in both cases, fast growth, health is promising in seed production (Vallejos *et al.*, 2010).

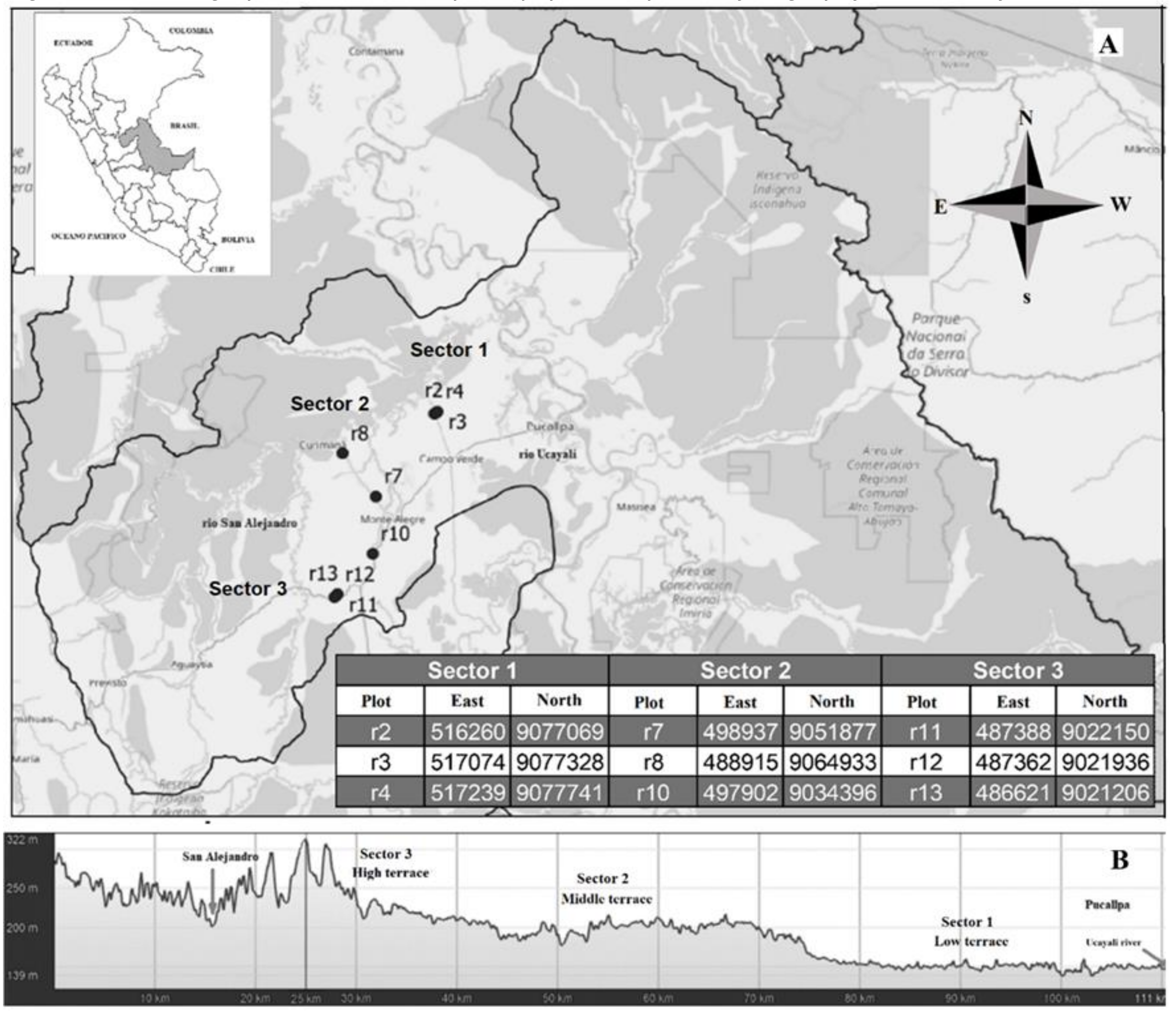
Therefore, in order to find practical methods of phenotypic selection, it is necessary to validate the use of morphological descriptors and determine the phenotypic variability mainly from the easily observed traits of the tree (Castañeda-Garzon *et al.*, 2021). From the qualitative assessment of the trees, it is possible to identify trees with exceptional, average and undesirable traits (Ortiz *et al.*, 2017; Ramírez-Garcia *et al.*, 2022). Due to that, this study aimed to develop a tool for the evaluation of qualitative traits of commercial interest, such as stem form, branches and health, as they are variables of high heritability in *G. crinita* progeny test established in the Aguaytia river basin, Ucayali, Peru.

5.2 MATERIAL AND METHODS

5.2.1 Site and establishment of the progeny test

The study was carried out in *G. crinita* progeny test of 36 months of age, established in the Aguaytia river basin developed by the World Agroforestry Center (ICRAF), in the department of Ucayali (Figure 8A). The region physiography has terraces low, medium and high (Figure 8B). With varied drainage conditions; riverbank complexes, predominantly acidic high terrace soils with low fertility; the floodable alluvial zone has soils of greater fertility; the low terraces with sandy and very clayey soils on the upper terraces of the basin; rainfall ranges from 1,400 mm in the lower part to 2,500 mm closer to the eastern Andes Mountain range with the presence of 2 life zones, Tropical Humid Forest (bh-T), Premontane Tropical Humid Forest (bh-PT) (GRU, 2017).

Figure 8 - A. Geographical location of plots (repetitions); B. Physiography of the study area.



Sector 1			Sector 2			Sector 3		
Plot	East	North	Plot	East	North	Plot	East	North
r2	516260	9077069	r7	498937	9051877	r11	487388	9022150
r3	517074	9077328	r8	488915	9064933	r12	487362	9021936
r4	517239	9077741	r10	497902	9034396	r13	486621	9021206

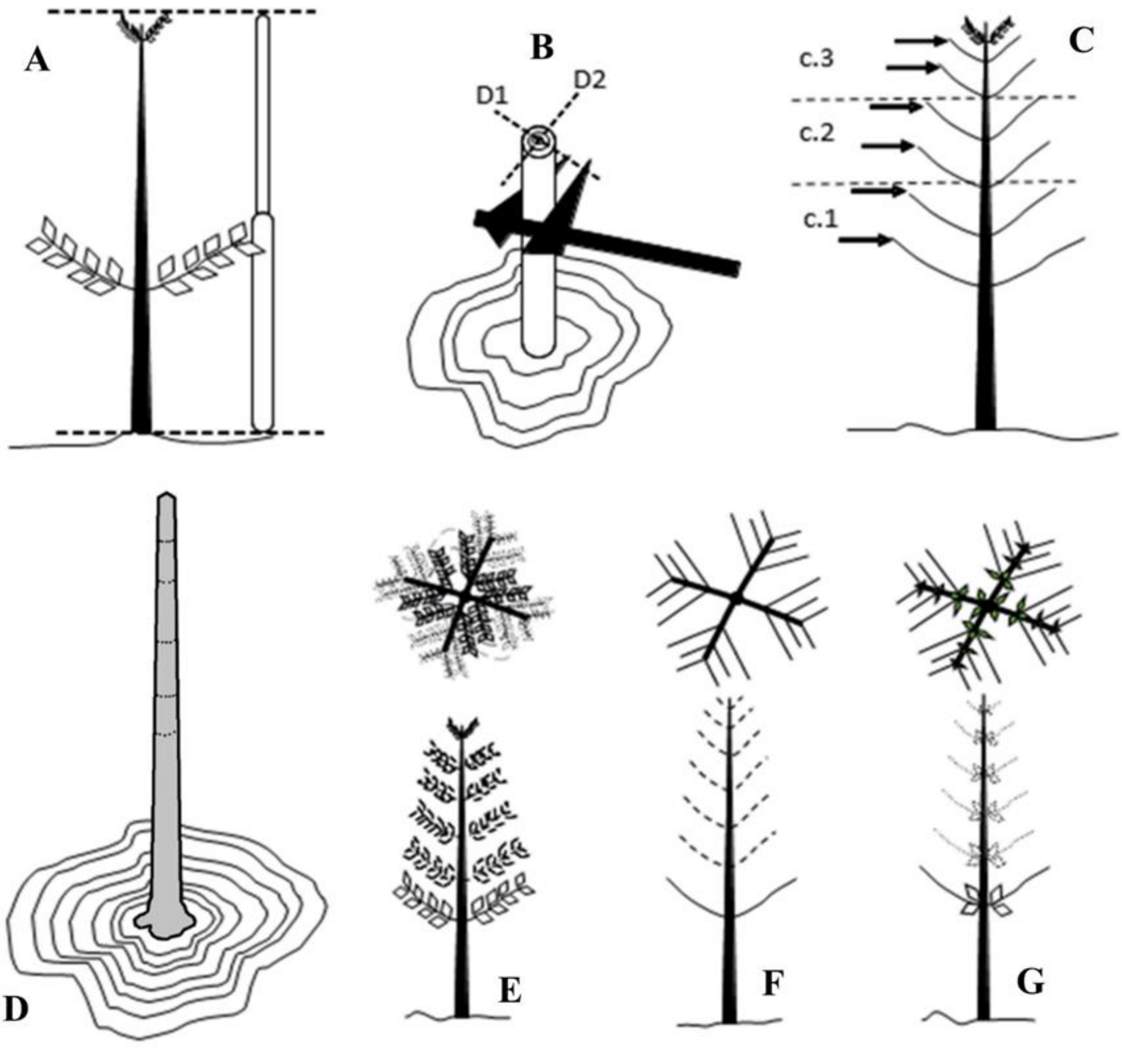
Source: Authors (2023)

The trial was established with 209 open-pollinated progenies, collected in 14 sites in the Aguaytia and Pachitea River Basins: New Requena - River (seventeen trees), Neshuya Stream - Federico Basadre Road (CFB) Km 49.5 (thirteen trees), Tahuayo Stream - CFB Km 72 (eleven trees), Curimana - River (twenty trees), Aguaytia River (nineteen trees), Yurac Stream - Aguaytia (three trees), Inca Port (eighteen trees), Von Humboldt (seventeen trees), Macuya (forty-nine trees), Santo Alexandre (seventeen trees), CFB to Km 72 (seven trees), Road to Nueva Requena (four trees), Road to Curimaná (seven trees), and Road to Tournavista (seven trees) (Revilla-Chavez *et al.*, 2022). The progeny test was planted in three diferente sites (sectors 1, 2 and 3) (Figure 8B), each trial established in a randomized complete block design with three blocks (repetitions) distributed in three environments, 200 progenies per block and two individuals per plot (400 plants per evaluable block). Each block has 0.25 ha, with a density of 1,600 trees.ha^{-1}, spacing between trees of 2.5 x 2.5 m, and with two lines of non-evaluable plants at the edge of the plots (Revilla-Chavez *et al.*, 2021).

5.2.2 Evaluation of traits

The phenotypic evaluation of the trees was performed for biometric and morphological traits. Seven procedures were used to evaluate the biometric traits total height of the tree, diameter, number of branches, number of rings, location and arrangement of branches with or without leaves (Figure 9). The tree height (cm) was measured with a telescopic ruler from the base to the upper end of the apex (Figure 9A). The evaluation of stem form was performed using the table of traits from the World Agroforestry Center's domestication of agroforestry tree Project (Table 13). The height of the tree (H, cm), the diameter at the height of the base (DBA, cm) and the diameter at breast height (DBH, cm) were measured; when it is the case (DBA< 2 cm) two diameters are measured perpendicular to each other (Figure 9B) with the help of a caliper, while with DBA or DBH> 2 cm, the diameter tape was used. The branches were evaluated by counting them separately at three levels of the tree crown, lower, middle and upper (Figure 9C, c1, c2, and c3), and at the same time it was observed whether they had leaves, axillary leaves or no leaves (Figure 9E, F and G). As morphological traits, stem form and stem health were evaluated using a graphic template of 32 most frequent stem morphotypes (Figure 10, Table 13) correlated with their biometric traits, because a qualitative trait varies from the observer's point of view, so the graphic descriptors are intended to reduce the evaluation error. For the evaluation of the symptoms, the description described in Table 13 was be used.

Figure 9 - Graphic diagrams of trait evaluation: A. measurement of the total tree height (Height); B. diameter measurement: diameter 1 (D1) and diameter 2 (D2); C. branch count: c.1. upper branches; c.2. middle branches; c.3. lower branches; D. branches with leaves; F. leafless branches; G. branches with axillary leaves.



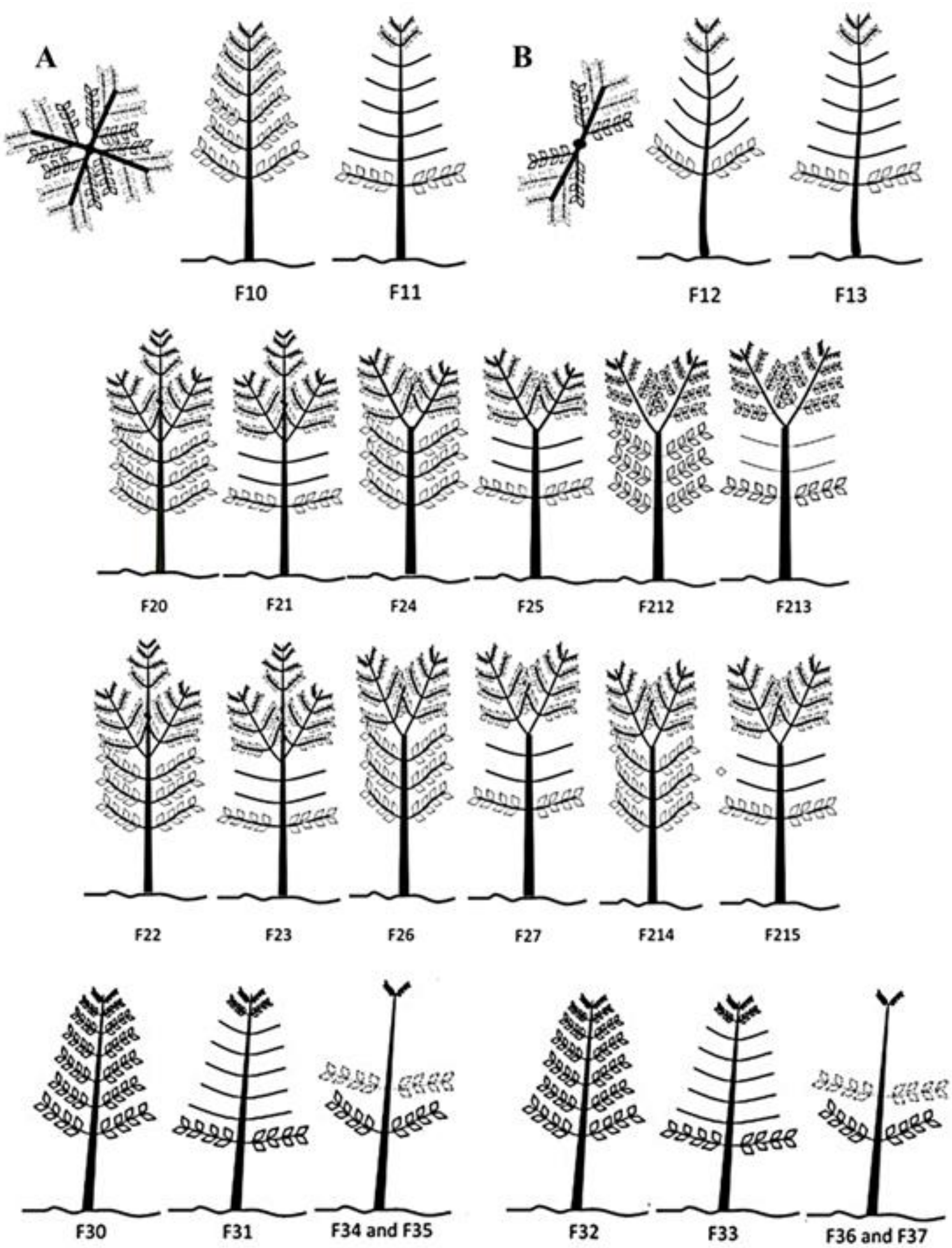
Source: Authors (2023)

Table 13 - Stem form type, forked stem, inclined stem form, and plant symptoms of the trees.

Class	Terminal bud		Branches		Stem	Code
	Presence	Dominance	Orientation	Positions		
Stem form	Sim	Sim	vertically	diferente	straight	F10
	Sim	Sim	horizontally	diferente	straight	F11
	Sim	Sim	vertically	diferente	crooked	F12
	Sim	Sim	horizontally	diferente	crooked	F13
Forked stem	Sim	Sim	vertically	diferente	-	F20
	Sim	Sim	horizontally	diferente	-	F21
	Sim	Sim	vertically	plano único	-	F22
	Sim	Sim	horizontally	plano único	-	F23
	no	Sim	vertically	diferente	-	F24
	no	Sim	horizontally	diferente	-	F25
	no	Sim	vertically	plano único	-	F26
	no	Sim	horizontally	plano único	-	F27
	Sim	no	vertically	diferente	-	F28
	Sim	no	horizontally	diferente	-	F29
	Sim	no	vertically	plano único	-	F210
	Sim	no	horizontally	plano único	-	F211
	no	no	vertically	diferente	-	F212
	no	no	horizontally	diferente	-	F213
	no	no	horizontally	plano único	-	F214
	no	no	vertically	plano único	-	F215
Inclined stem form	Sim	Sim	veritcally	diferente	-	F30
	Sim	Sim	horizontally	diferente	-	F31
	Sim	Sim	veritcally	plano único	-	F32
	Sim	Sim	horizontally	plano único	-	F33
	no	Sim	veritcally	diferente	-	F34
	no	Sim	horizontally	diferente	-	F35
	no	no	veritcally	plano único	-	F36
	no	no	horizontally	plano único	-	F37
	Sim	no	veritcally	diferente	-	F38
	Sim	no	horizontally	diferente	-	F39
	Sim	no	veritcally	plano único	-	F310
	Sim	no	horizontally	plano único	-	F311
	Fungal attack	Insect attack	Nutritional deficiencies	Animal damage		Code
Plant symptoms	no	no	no	no		0
	Sim	no	no	no		1
	no	Sim	no	no		2
	no	no	Sim	no		3
	no	no	no	Sim		4
	Sim	Sim	no	no		5
	no	Sim	Sim	no		6
	no	no	Sim	Sim		7
	Sim	Sim	Sim	no		8
	no	Sim	Sim	Sim		9
	Sim	Sim	no	Sim		10
	Sim	no	Sim	Sim		11
	Sim	Sim	Sim	Sim		12

Source: Adapted by authors from proposed to evaluate of traits from the World Agroforestry Center's domestication of agroforestry tree Project.

Figure 10 - A. Branches in diferente positions; B. Branches in a plano único; Normal stem form with branches in diferente positions F10 to F13; Forked stem: F20, F21, F24, F25, F212, F213 to F215 and shape of inclined stem with branches in diferente positions F30, F31, F34 and F35. Inclined stem form with branches in a plano único F32, F33, F36, and F37.



Source: Authors (2023)

5.2.3 Graphic of stem classification

For the evaluation of the types of stem of the tree, an alphanumeric coding system was established, which has as first digit "F" corresponds to the abbreviation of stem, plus the shape code that corresponds to "1" for the normal stem, “2” forked stem and “3” for a sloping stem and a correlative code of the existing variants regarding the form of insertion of the branches in the stem ranging from 1 to 15, resulting in the following code, for example F212 breaks down to: F212= F+2+12, where, F is the shaft, 2 is the forked stem and, 12 is no terminal bud, no dominance, branches in vertical orientation, branches in diferente positions. The method shows how the counting of the rings formed by the presence of dry knots caused by the fall of tree branches was carried out (Figure 9D). The graphs of tree types with normal and bifurcated stems (Figure 10, F10-F13, F20-F213), with variants in the form of arrangement of branches, which appear as branches in diferente positions, when they project into more than two directions opposite to each other and in more than one plane, being observed from above as a cross or more edges (Figure 10A); while the variant with branches arranged in a plano único, is observed in a single line of branches opposite each other, seen from above is a line (Figure 10B), this type of stem is presented similarly to how the rachis in a branch. The inclined tree type plots (Figure 10, F30-F37) maintain the variants of branch arrangement similar to the previous models.

5.2.4 Statistical analysis

To determine whether the data were normally distributed, the Kolmogorov-Smirnova (degree of freedom > 50) and Shapiro-Wilk (degree of freedom < 50) tests were applied using the SPSS statistical software (02-03-2022). To determine the correlations between quantitative and qualitative traits, Spearman's correlation coefficient analysis was applied between pairwise traits at level of sector, plot, progeny, diameter, height, stem form, branches and health, using RStudio software 2022.12.0 Build 353.

Site-level and joint-site analyzes of variance were performed based on the incomplete randomized block design, using the SAS program (SAS, 1999) and the GLM procedure to determine significant differences between treatments. The variance components were estimate using the REML (Restricted Maximun Likelihood) method, in combination with the VARCOMP command of the SAS statistical program due to the experimental imbalance in terms of the unequal number of surviving trees per plot.

For site-level analysis, the following mixed model was used: $Y_{ijk} = \mu + b_i + t_j + e_{ij} + d_{ijk}$, where Y_{ijk} is the phenotypic value of the k-th individual of the j-th progeny of the i-th replication; μ is the fixed term of the total mean; b_i is the fixed effect of the i-th replication; t_j is the random effect of the j-th progeny; e_{ij} is the effect of the random interaction between the j-th progeny and the i-th replication; d_{ijk} is the random effect of the k-th tree within the j-th progeny of the i-th replication. Where $i = 1 \ldots b$ (b is the number of replication); $j = 1 \ldots t$ (t is the number of progeny); $k = 1 \ldots n$ (n is the number of plants within the progeny). For joint analysis, the following mixed model was used: $Y_{ijkl} = \mu + l_i + b_{j(i)} + t_k + lt_{ik} + e_{ij(k)} + d_{ijkl}$, where Y_{ijkl} is the phenotypic value of the l-th individual of the k-th progeny of the j-th replication at the i-th site; l_i is the fixed effect of the i-th site; $b_{j(i)}$ is the fixed effect of the j-th replication within the i-th site; t_k is the random effect of the k-th progeny; lt_{ik} is the effect of the random interaction between the k-th progeny with the i-th site; $e_{ij(k)}$ is the effect of the random interaction between the k-th progeny in the j-th replication within the i-th site; d_{ijkl} is the random effect of the l-th tree within the k-th progeny of the j-th replication at the i-th site. Where $l = 1 \ldots s$ (s is the number of sites evaluated).

The estimated components of variance were: σ_f^2= genetic variance between progenies; σ_{fxs}^2= variance of genotype x site interaction; σ_e^2= environmental variance; σ_w^2= environmental variance. From the components of variance were estimated the additive genetic variance ($\sigma_a^2 = 4\sigma_f^2$) and mean heritability among progeny (h_m^2, $h_{m(s)}^2$) for each site,

$$h_m^2 = \frac{\sigma_f^2}{\sigma_f^2+\frac{\sigma_e^2}{J}+\frac{\sigma_w^2}{JK}}, \tag{1}$$

and joint sites,

$$h_{m(s)}^2 = \frac{\sigma_f^2}{\sigma_f^2+\frac{\sigma_{fxe}^2}{L}+\frac{\sigma_e^2}{LJ}+\frac{\sigma_w^2}{LJK}}. \tag{2}$$

Coefficients of genetic and phenotypic correlations were estimated between DBH and plant height *versus* stem form, branches, and health for each site and joint-site, based on Namkoong (1979).

5.3 RESULTS

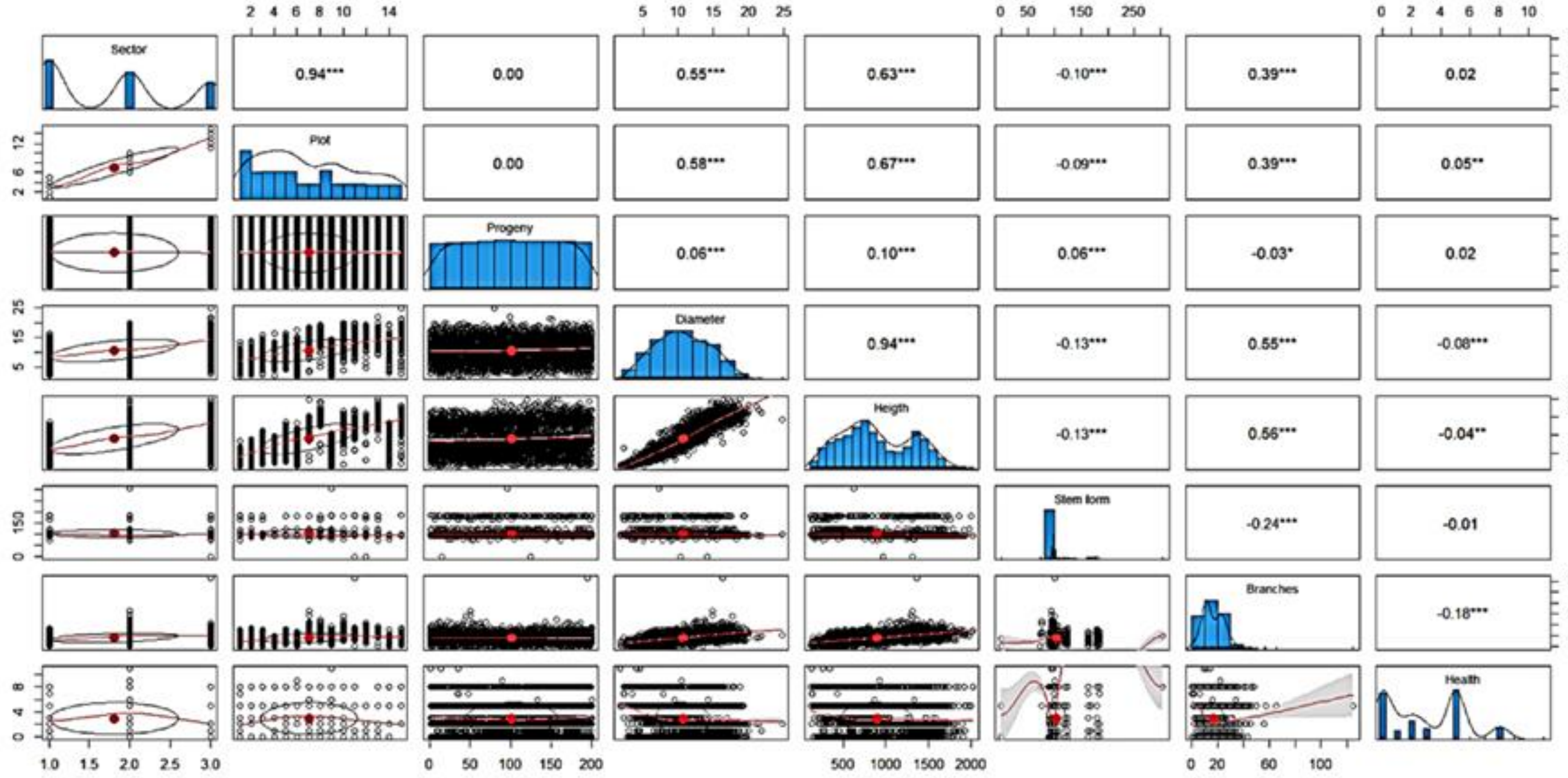
According to normality tests for growth and stem form traits, the data did not show a normal distribution at the sector level (P< 0.005, Table 14). Therefore, applying Spearman's correlation test, it was found that there was significant correlation between traits at sector, plot level with diameter, plant height, stem form, branches and health, while progeny had correlation with diameter, plant height, stem form and branches; on the contrary there was no correlation between sectors and stem form with health (Figure 11), having sector and plot a low correlation and inversely with stem form (ρ: -0.10 and -0.09, respectively). In contrast, quantitative traits such as plant diameter and height may respond better to genotype-environment interactions, as shown by the effect of sector and plot with diameter (ρ: 0.55 and 0.58, respectively) and height (ρ: 0.63 and 0.67, respectively).

Table 14 - Test of normal distribution for stem form.

	Kolmogorov-Smirnova			Shapiro-Wilk		
Sector	Test	df	P-value	Test	df	P-value
1	0.392[a]	1060	<0.001	0.345[a]	1060	<0.001
2	0.427[a]	599	<0.001	0.191[a]	599	<0.001
3	0.368[a]	596	<0.001	0.378[a]	596	<0.001

Source: Authors (2023); df is the degree of freedom; a Lilliefors significance corrections.

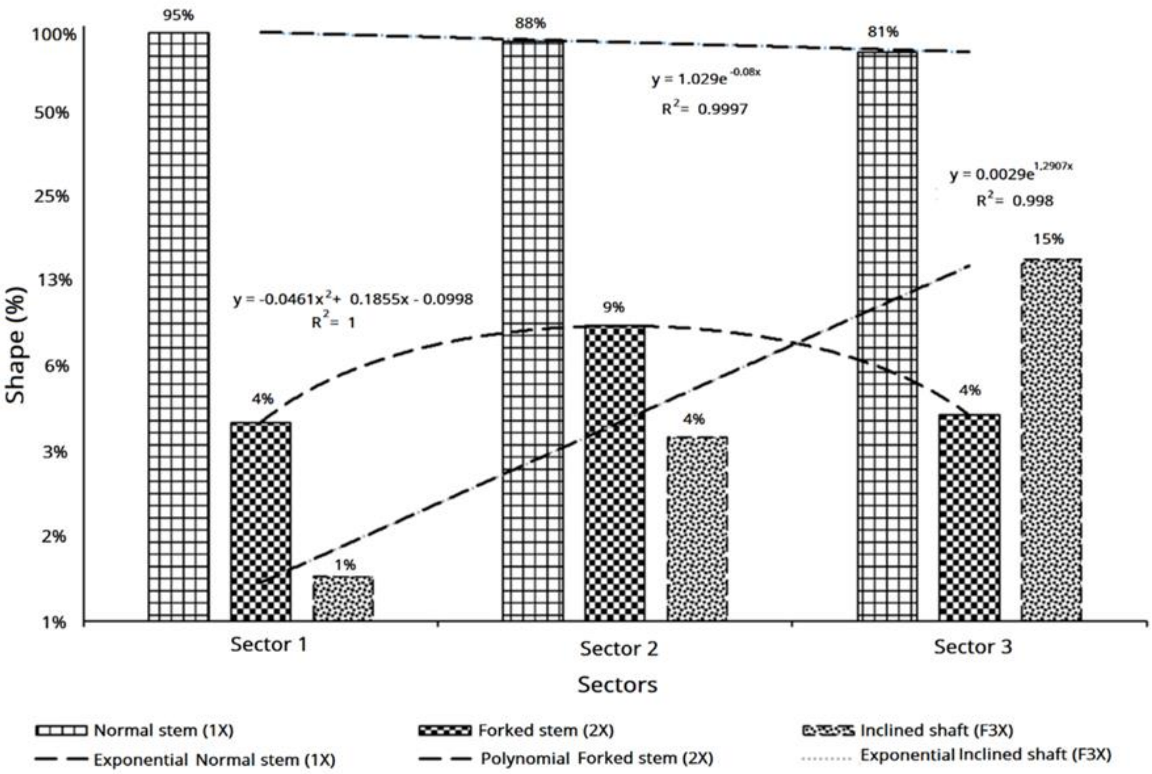
Figure 11 - Probability values (P-value) and Spearman's correlation coefficient (ρ) of for the main quantitative and qualitative traits of *Guazuma crinita* progeny test at 36 months of age in the Aguayita river basin, Ucayali, Peru.



Source: Authors (2023).
**P< 0.05; ***P< 0.01.

Taking into account that the sectors and plot were correlated with the main selection traits such as diameter, height, stem form and branches (Figure 12), we can see that in all sectors there is a higher prevalence of trees with normal stems, which is expressed by the exponential regression model Y= $1.029e^{-0.08x}$ (R^2= 0.9997) and is inversely proportional to the rise of the sector in the basin (Figure 12), while trees with inclined stems have exponential behavior directly proportional to the rise of the sector in the basin expressed with the exponential regression model Y= $0.0029e^{1.2907x}$ (R^2= 0.998), which could be attributed to the increase in the slope of the soil; while the trees with bifurcated stems have a downward concave polynomial behavior as they ascend in the basin expressed by equation Y= $-0.0461x^2+0.1855x-0.0998$ (R^2= 1).

Figure 12 - Occurrence of normal (F1X), bifurcated (F2X) and inclined (F3X) bole types in *Guazuma crinita* progeny test at 36 months of age by site (sectors) in the Aguaytia river basin, Ucayali, Peru.



Source: Authors (2023)

Significant differences were detected between progenies for DBH, height, branches and health at site 1, and for all traits at sites 2 and 3 (Table 15). Significant differences between sites were also detected for all traits, although no significant genotype-environment interactions were detected for traits. These results indicate that, although the traits have diferente performance between sites, in terms of growth, stem form and tree health, due to the absence of genotype-environment interaction, there is the possibility of genetic improvement through the simultaneous selection of the same progenies for all sites. In addition, the trees in the three study sites have generally straight boles, lack of bifurcation, and low rates of attack by fungi, insects, nutritional deficiency, and damage caused by animals.

Table 15 - Estimates of mean among family heritability (h_f^2) for trees at 36 months of age for DBH, height (H), stem form (SF), branches (BR), and health (HE), per site and joint sites.

	DBH (cm)	H (m)	SF	BR	HE
Mean					
Site 1	8.6**	6.2*	97.2	12.4**	2.76*
Site 2	11.8**	10.7**	93.6*	19.8*	2.97*
Site 3	14.0**	13.0**	95.0**	19.1*	3.16**
Joint	11.5**	9.97**	95.1	17.9	4.03
P-value-Site	<0.001	<0.001	<0.001	<0.001	<0.001
P-value-GxE	0.943	0.328	0.381	0.685	0.872
h_f^2					
Site 1	0.281	0.094	0.407	0.065	0.118
Site 2	0.273	0.352	0.409	0.094	0.127
Site 3	0.198	0.304	0.695	0.189	0.087
Joint	0.293	0.356	0.524	0.158	0.123

Source: Authors (2023)
*P-value< 0.05; **P-value< 0.001; P-value-Site and P-value-GxE for F test among sites and genotype by environmental interaction (GxE).

Mean heritability among progenies (h_f^2) was variable across locations and traits, with values ranging from low ($h_f^2 \leq 0.2$) to moderate ($0.2 > h_f^2 \leq 0.7$), where stem form presented the highest values and branches and health generally showed the lowest values. In addition, joint analyzes generally show the highest heritability values, which, associated with the fact that the genotype by environmental interaction did not alter the classification of progenies between environments, the same progenies can be selected for the production of improved seeds to meet the demand for seeds for commercial reforestation in the three locations. Stem form, tree height and DBH are the most suitable traits for selection, as they have the highest heritability values.

Genetic (r_g) and phenotypic (r_p) correlations were estimated between trait pairs at each site and joint site (Table 16). Genetic and phenotypic correlations show a direct association between DBH, height, stem form and branches and an inverse relationship between DBH and height with health. Results show generally higher (or similar) genetic correlations than phenotypic correlations between traits. Genetic and phenotypic correlations were high (> 0.7) between DBH and height, and moderate (0.2> r ≤ 0.7) between DBH and tree height with stem form and branch. Genetic and phenotypic correlations between DBH and health ranged from low (-0.04) to moderate (-0.35) and from low (-0.06) to high (-0.91) between tree height and health. These results indicate a very positive picture for selection of multiple traits in the progeny test and that it is possible to construct a selection index for simultaneous selection of progenies. For example, the results indicate that the selection of progenies with high DBH will select taller trees with straighter stamens. In addition, the inverse relationship observed between DBH health and tree height indicates that larger trees have a low incidence of fungal attack, insects, nutritional deficiency and damage caused by animals and low branches. Thus, the selection of progenies with high DBH will result in indirect selection for progenies with high sanity.

Table 16 - Genetic (r_g) and phenotypic (r_p) correlations between DBH, height (H), stem form (SF), branches (BR), and health (HE), per site and joint sites.

	Site 1		Site 2		Site 3		Joint	
	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p
DBH vs H	0.93**	0.92**	0.89**	0.83**	0.87**	0.88**	0.85**	0.93**
DBH vs SF	0.72**	0.64**	0.41*	0.45*	0.45*	0.41*	0.64**	0.63**
DBH vs BR	0.6**	0.55**	0.47*	0.3*	1.0**	0.36*	0.64**	0.33*
DBH vs HE	-0.13	-0.08	-0.36*	-0.07	-0.04	-0.04	-0.29*	-0.2*
H vs SF	1.0**	0.54**	0.51**	0.23*	0.73**	0.42*	0.55**	0.5**
H vs BR	1.0**	0.5**	0.55**	0.56**	0.55**	0.34*	0.86**	0.31*
H vs HE	-0.65**	-0.12	-0.91**	-0.06	-0.35*	-0.08	-0.16*	-0.15*

Source: Authors (2023)
**P< 0.01; *P< 0.05.

5.4 DISCUSSION

The development of methodologies for the phenotypic evaluation of *G. crinita* trees at 36 months of age is based on the principle that the traits plant height, diameter, stem form and health have a greater heritability, so these are the traits most suggested for the selection of trees in domestication programs (Vallejos *et al.*, 2010; Gutierrez-Vasquez *et al.*, 2016). From an adequate evaluation of these traits, the phenotypic plasticity of the species can be determined, being this fundamental in adaptation, survival, development, reproduction and evolution in ecosystems in permanent changes (Parejo-Farnés *et al.*, 2019).

Spearman's correlation tests found significant correlation between sector traits, at the plot level with diameter, plant height, stem shape, branches, and health, while progeny correlated with diameter, plant height, stem shape, and branches; On the contrary, the absence of correlation between the sectors and the shape of the stem with health, with the sector and the plot having a low and inverse correlation with the shape of the stem (ρ: -0.10 and -0.09, respectively), which corroborates what was referred to by Murillo *et al.* (2003), who mentioned that qualitative traits such as stem shape respond to a small number of alleles, which makes the shape a more stable feature, despite the diversity of environments. Conversely, quantitative traits such as plant diameter and height may respond better to genotype-environment interactions, as evidenced by the effect of sector and plot with diameter.

According to the evaluations resulting from this methodology, it is observed that the traits under analysis have a strong correlation with each other, due to the fact that there is an intrinsic natural relationship between them, product of the expression of their genotypes in their interaction with the environment (Parejo-Farnés *et al.*, 2019), as applied in the present study.

The methodological development for the phenotypic evaluation in 36-month-old *G. crinita* progeny test, is especially important, because by developing practical evaluation methods it can support the early selection of better phenotypes and help in genetic gain in a shorter time and in turn improve the profitability of the plantations what is preferable during the juvenile stage (De Grado *et al.*, 1999; Gorbitz *et al.*, 2020). Therefore, evaluating this trait establishes a form of classification that allows the trees to be assessed quickly and consistently (Gutiérrez-Caro *et al.*, 2018) and as corroborated in the present study. We concluded that the morphological traits measured allowed to graph the procedures for the phenotypic evaluation of *G. crinita* progeny test, maintaining the correlations that exist between them.

5.5 CONCLUSIONS

Considering the strong phenotypic correlation between biometric and morphological traits in forest species, the graphical evaluation methodology used in the evaluation of *G. crinita* progeny test at 36 months of age in the Aguaytia river basin, Ucayali, Peru, maintained correlations between the traits evaluated, which allows the use of this methodology in experiments of other tropical tree species. There are genetic differences among progenies for both growth and morphological traits, which can be exploited by selecting the best progenies and establishing an orchard to meet the demand for improved seeds for commercial plantings in the three study sites. The growth rate differed between the three study sites, but due to the simple genotype-environment interaction, it is possible to establish only one seed orchard to meet the demand for improved seeds in the three sites. Based on the highest heritability values, the most suitable traits for selection are DBH, height and stem shape. The genetic correlations observed between growth and morphological traits indicate that selection in any one of them can promote direct or indirect genetic gains in the others.

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6 PRODUCTIVITY, ADAPTABILITY, AND STABILITY IN *Guazuma crinita* (Mart.) PROGENY TESTS ACROSS THREE ENVIRONMENTS IN THE AGUAYTIA RIVER BASIN, UCAYALI, PERÚ[5]

ABSTRACT

Guazuma crinita is a fast-growing tree with potential for use in agroforestry systems, due to its rapid wood production, which can contribute significantly to the livelihoods of small-scale farmers in the Peruvian Amazon. However, indiscriminate logging due to high demand is leading to the disappearance of natural forests. As such, the International Council for Research in Agroforestry (ICRAF) began a domestication program to reduce pressure on natural populations of the species. The objective of the present study was to use analyses of genetic parameters, adaptability (PRVG), productivity (MHPRVG), and stability (MHVG), to select *G. crinita* genotypes from a three-year-old progeny test established in the Aguaytía River Basin, in Ucayali, Peru. The test was established in three diferente sites, with three blocks, 200 progeny per block, and two individuals per plot. The measured traits were diameter at breast height (DBH), total height (H), and total aerial biomass (B). Significant differences in traits between progenies were detected, but with no genotype x environment interaction (GxE). However, the genotypic correlation among sites was important (> 0.702), suggesting that genetic improvement is possible by selecting the same progeny across sites. The mean heritability among progenies was moderate for all traits (0.34-0.369) and selective precision through combined site analysis was relatively high (0.583-0.608). Based on selection for DBH through combined analysis, MHVG, PRVG, and MHPRVG, 50 superior progenies (25.9%) were identified for all environments. These should be prioritized in breeding programs as they can offer stable genetic variability for future selection cycles.

Keywords: Biomass, G x E interaction, native tree species, plantations, White Bolaina, tree improvement.

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6.1 INTRODUCTION

Guazuma crinita Mart. (Malvaceae), commonly known as White Bolaina, is a fast-growing timber forest species that develops in alluvial forests of the Peruvian Amazon (Coral *et al.*, 2020). The range of the species is distributed from Central America to the south of Brazil, Bolivia, and Peru, and individuals can reach more than 30 m in height and up to 0.5 m in diameter (Reynel *et al.*, 2003). The species shows good potential for agroforestry plantations and wood production, as it can be harvested at a very young age and can contribute significantly to the livelihoods of small-scale farmers in the Peruvian Amazon (Rochon *et al.*, 2007; Putzel *et al.*, 2013). It is a versatile species with excellent adaptability to a wide variety of locations (Maruyama *et al.*, 1997; Flores, 2000). Due to the high workability of its wood, *G. crinita* is widely used in general carpentry, as well as for the manufacture of brooms, toothpicks, medical pallets, and toys. Above all, the wood is used for the manufacture of paneling, which is preferred by consumers with limited economic resources due to the low price. As such, it has cornered a large part of the regional and national market (Aróstegui, 1975; Encarnación, 1983).

In Peru, the species is found at a high density in the regions of Loreto, Amazonas, Ucayali, Huánuco, San Martín, Madre de Dios, Junín, and Cerro de Pasco (Reynel *et al.*, 2003). Because of this abundance, the production of Bolaina increased significantly from 2004 to 2019, growing from 23,110.02 to 70,054.15 m^3, as a result of high market demand (SERFOR, 2019). However, to date, the demand for *G. crinita* wood continues to grow, causing concern that such demand might lead to over-exploitation of natural stands through selective extraction and the eventual decimation of the species. Such processes can result in a loss of natural regeneration due to the elimination of seed-trees, leading to genetic erosion and the fragmentation of natural stands in the short and medium term, and reducing the yield of harvesting cycles (Casas *et al.*, 2016). In this context, it is necessary to select productive genotypes that can be successfully used in conservation and improvement programs for reforestation. This requires an understanding of the interaction between genotypes and environments (GxE) in order to minimize or leverage the effects and increase efficiency in the selection of genotypes (Pupin *et al.*, 2018; Murillo *et al.*, 2019).

Selection is generally based on growth traits, which present complex patterns of heritability, making it difficult to predict responses of genotypes to diferente environments (Namkoong *et al.*, 1966). Information on the nature and magnitude of this interaction is essential, particularly for selection based on traits with low levels of heritability (Yan; Kang, 2002). Barros *et al.* (2008) defined the GxE interaction as the inconsistent performance of genotypes in diferente environments. While Namkoong *et al.* (1966) point out that GxE interaction can generate erroneous and inflated estimates of genetic variability. Which directly affects the selection and reduce genetic gain, making it difficult to select genotypes with broad adaptability. One of the alternatives to minimize the effects of GxE interaction is the choice of genotypes with simultaneously high productivity, stability, and adaptability (Torres-Dini *et al.*, 2016; Chaves *et al.*, 2021), which can be done using predictive methods, such as the Harmonic Mean of the Relative Performance of Genotypic Values (MHPRVG). This method allows for the use of unbalanced data and heterogeneity of variances, providing stability values and genotypic adaptability, as well as generating results on the scale or magnitude of the evaluated trait. As such, the method enables direct interpretation of results as genetic values and genetic gain estimation based on its productivity, stability, and adaptability (Resende 2007; Torres-Dini *et al.*, 2016; Pupin *et al.*, 2018).

Considering that the environment can result in phenotypic variation of up to 80%, this effect together with genotypic variation are the most relevant for selection if the effects of GxE interaction are considered (Yan; Kang, 2002). The GxE interaction reduces the correlation between phenotype and genotype (Rao *et al.*, 2011), interferes with genetic gains, and creates greater uncertainty in selection (Vencovsky *et al.*, 2012). Therefore, to study GxE interaction, it is necessary to establish multi-environment experimental trials (Mustapha; Bakari, 2014). The evaluation of GxE interaction can lead to the adoption of two selection strategies: i) stratification of heterogeneous areas into smaller and homogeneous subregions for selection; ii) selection of stable genotypes based on its performance in a variety of environments (Funga *et al.*, 2017; Souza *et al.*, 2020). In this context, Weber and Sotelo (2008) assessed the genetic differences in *G. crinita* biomass yield between provenances and suggested the best provenance to be used in future plantations.

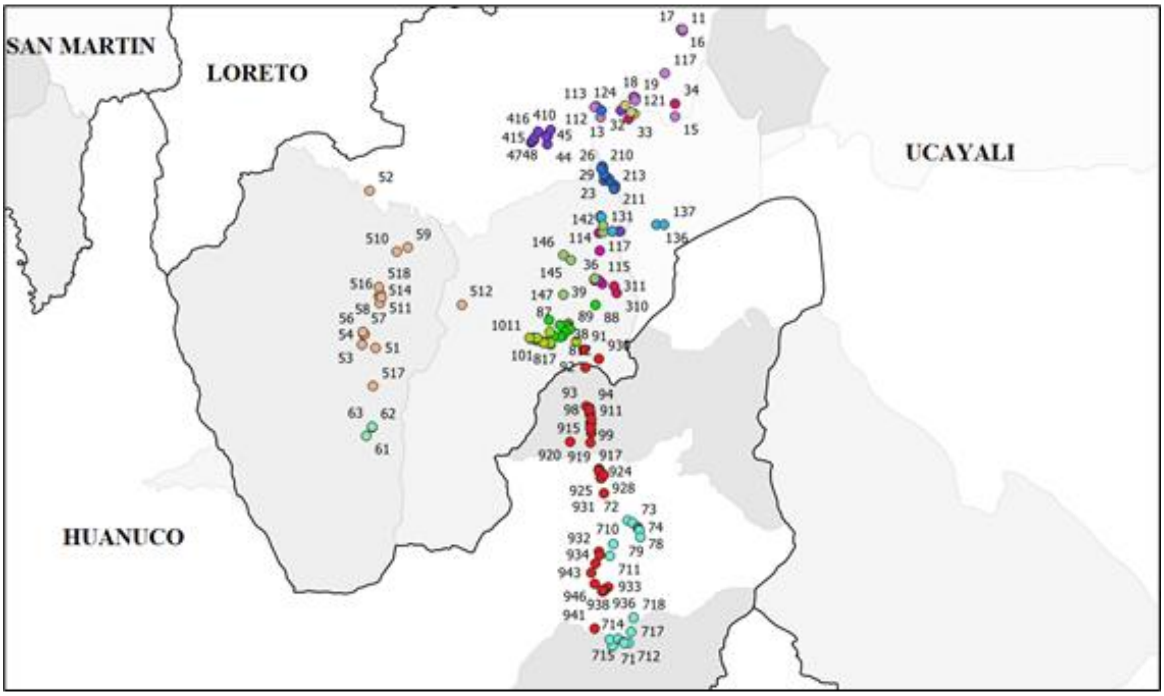
The present study aimed to estimate genetic parameters and select *G. crinita* genotypes from a series of three-year-old progeny tests established in three diferente environments in the Aguaytía river basin, Ucayali, Peru, based on adaptability (PRVG, relative performance of genetic values), productivity (MHPRVG), and stability analysis (MHVG, harmonic mean of breeding values).

6.2 MATERIAL AND METHODS

6.2.1 Sampling and establishment of progeny test

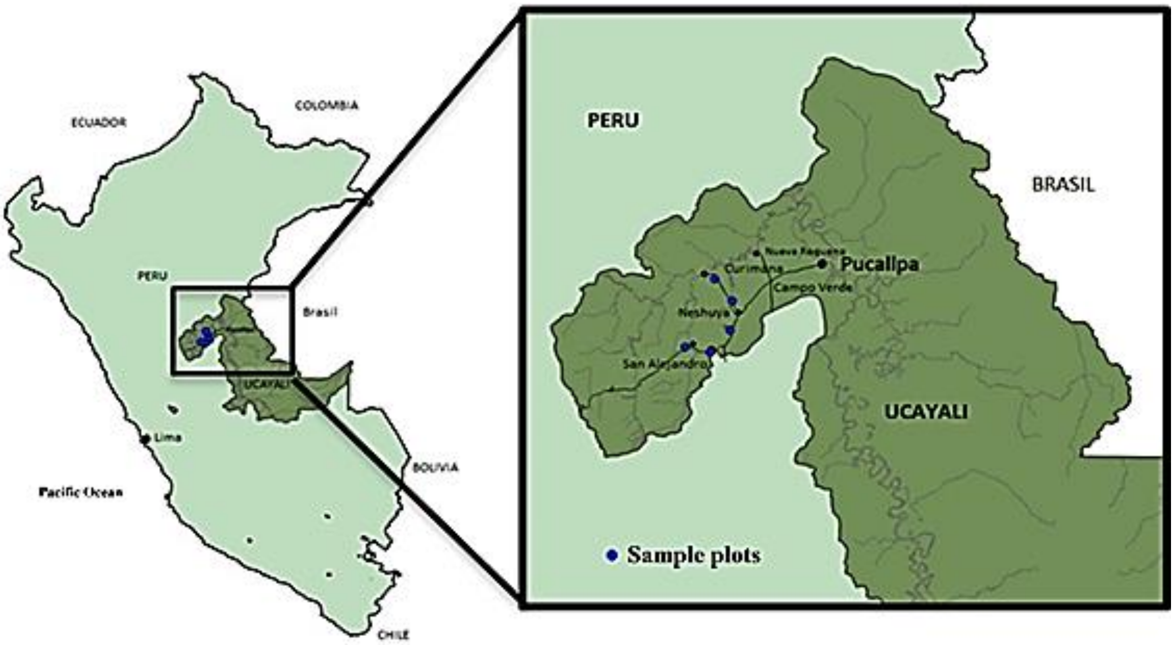
Open-pollinated seeds from 209 mother trees were collected from 14 sites in the Aguayita and Pachitea River Basins: New Requena - River (seventeen trees); Neshuya Stream - Carretera Federico Basadre (CFB) Km 49.5 (thirteen trees); Tahuayo Stream - CFB Km 72 (eleven trees); Curimana - River (twenty trees); Aguaytia River (nineteen trees); Yurac Stream - Aguaytia (three trees); Inca Port (eighteen trees); Von Humboldt (seventeen trees); Macuya (forty-nine trees); Santo Alexandre (seventeen trees); CFB to Km 72 (seven trees); Road to Nueva Requena (four trees); Road to Curimaná: (seven trees); and Road to Tournavista (seven trees) (Figure 13).

Figure 13 - Collection of seeds from 209 trees for progeny testing *G. crinita*: 1= New Requena - River (seventeen trees); 2= Neshuya Stream (thirteen trees); 3= Tahuayo Stream (eleven trees); 4= Curimana-River (twenty trees); 5= Aguaytia River (nineteen trees); 6= Yurac Stream (three trees); 7= Inca Port (eighteen trees); 8= Von Humboldt (seventeen trees); 9= Macuya (forty-nine trees); 10= Santo Alexandre (seventeen trees); 11= CFB up to Km 72 (seven trees); 12= Road to Nueva Requena (four trees); 13= Road to Curimaná (seven trees); 14= Road to Tournavista (seven trees).



This study was carried out on the west side of the Aguayita River Basin, in lowland forest at elevations between 180 m and 300 m, with terrain ranging from undulating at higher elevations to flat at lower elevation (Ugarte-Guerra; Domínguez, 2010). For the study, we evaluated progeny in plots established by the International Council for Research in Agroforestry (ICRAF), distributed in terraced soils, at three sites in the basin: lower basin between the areas of Pucallpa, Campo Verde, and Nueva Requena (site 1); middle from Neshuya to Curimaná (site 2); and upper basin from Von Humboldt to San Alejandro (site 3) (Figure 14).

Figure 14 - Location of the study area.



Sites have predominantly acidic soils and low natural fertility, with rainfall ranging from 1500 mm in the lower basin to 2500 mm in the upper basin. The average annual temperature is 26 °C (

Table 17).

Table 17 - Environmental variables of the Aguaytia River Basin.

Parameter	Site 1	Site 2	Site 3
Soil*			
Type of soil	Sandy Franc	Clay Franc	Clayey
Clay (%)	21.1	35.7	52.4
Loam (%)	33.8	38.5	27.5
Sand (%)	45.1	25.8	20.1
pH	4.63	5.34	6.52
Phosphor: P (ppm)	3.79	7.76	3.46
Acidity (pH)	2	2.08	0.2
Potassium: K (ppm)	0.23	0.22	0.2
AC	1.43	10.2	32.3
Magnesium: Mg	0.84	2.02	3.43
Cation exchange capacity: CICE ($meq.100g^{-1}$)	4.5	14.52	36.14
Sat. To (%)	39.7	18.5	0.6
Organic matter content: C.O. (%)	0.8	1.2	1.6
Physiography	Low terrace	Medium terrace	High terrace
Elevation (masl)	160	172	232
Climate**			
Precipitation ($mm.ha^{-1}.year^{-1}$)	1504	1997	2545
Mean annual temperature (°C)	25.4	25.4	25.8

Source: *ICRAF (2003); **USAID-PERU (2012).

Each of the three trials was established with a randomized complete block design, with five repetitions (blocks) per site, for a total of 15 repetitions with 200 progenies. For the present study, we used only nine repetitions (Figure 14). Each progeny test in replicate was randomly in a two seedlings (half-sibs) plot, for a total of 400 plants, spaced at 2.5 x 2.5 m, over an area of 2500 m^2. All replicates had perimeter of two tree rows which were excluded from the assessment. At the time of planting, 1600 kg ha^{-1} of earthworm humus and 320 $kg.ha^{-1}$ of phosphate rock were applied (1 kg of earthworm humus and 200 g of phosphate rock per plant applied at a 2.5 x 2.5 m distance). During the second and third year, the experiment was fertilized with 112 kg of $urea.ha^{-1}$, 316 $kg.ha^{-1}$ of phosphoric rock, and 160 $kg.ha^{-1}$ of potassium chloride, corresponding respectively to 70, 185, and 100 g of each product per plant (Ugarte-Guerra; Domínguez, 2010). In the third year, 50% thinning was conducted, resulting in approximately 200 individuals within each repetition; the remaining planting density was maintained until year six (Revilla-Chávez *et al.*, 2021a). At 36 months-old were evaluated: i) total height (H, m); ii) diameter at breast height (DBH, cm); iii) total aerial biomass (B, kg); and iv) survival (SUR, %). Total aerial biomass was determined through applying the allometric model $B= 0.04253*DBH^{2.5027}$ (Revilla-Chavez *et al.*, 2021b) for trees up to 21.8 cm (DBH).

6.2.2 Estimate of variance components and genetic parameters

REML/BLUP procedure using mixed linear models was used, in order to estimate the genetic parameters for each trait across the three sites. SELEGEN-REML/BLUP software (Resende, 2016) was employed with statistical model 93, $y = X_r + Z_a + W_p + e$, where: *y*, *r*, *a, p,* and *e* are the vectors data, replication, genetic, plots, and error, respectively. The incidence matrices for the above effects are represent by the uppercase letters *X*, *Z,* and *W* (Resende, 2007). The following variance components were estimated: genetic variance among progeny (σ_p^2), additive genetic variance ($\sigma_a^2 = 4\sigma_p^2$), environmental variance among plots (σ_c^2), residual variance (environmental + non additive) (σ_e^2), variance of genotype x environmental interaction (σ_{ge}^2), and individual phenotypic variance, $\sigma_f^2 = \sigma_a^2 + \sigma_c^2 + \sigma_e^2$. From the variance components, the following parameters were estimated:

Narrow-sense individual heritability, $h_i^2 = \sigma_a^2/\sigma_f^2$;

Mean heritability among progeny,

$$h_m^2 = 0.25\sigma_a^2 \big/ 0.25\sigma_a^2 + \frac{\sigma_c^2}{r} + \frac{0.75\sigma_a^2+\sigma_e^2}{nr}; \quad (1)$$

Coefficient of individual genotypic variation,

$$CV_{gi}\% = 100\left(\sqrt{\sigma_a^2}/m\right); \quad (2)$$

Coefficient of experimental variation,

$$CV_e\% = 100\left(\sqrt{[0.75\sigma_a^2 + (\sigma_e^2/n)] + \sigma_c^2}/m\right), \quad (3)$$

where n is the number of plants per plot;

Selective accuracy among progeny, assuming full survival, $r_a = \sqrt{h_m^2}$;

Genotypic correlation between progeny in various environments,

$$r_g = \sigma_a^2/(\sigma_a^2 + 4\sigma_{ge}^2); \quad (4)$$

Coefficient of determination of the plot effect, $C_p^2 = \sigma_c^2/\sigma_f^2$;

Coefficient of determination of the genotype x environment interaction (GxE) effect,

$$C_{ge}^2 = \sigma_{ge}^2/\sigma_f^2. \quad (5)$$

Analyses of adaptability (PRVG), productivity (MHPRVG), and stability (MHVG) were conducted using all three progeny tests (sites). For this, the MHPRVG method predicted by BLUP (Resende, 2007) was used, based on SELEGEN-REML/BLUP software, according to mathematical model 51: $y = X_r + Z_a + W_p + T_i + e$, where *y*, *r*, *a, p, i,* and *e* are the vectors data, replication, genetic, plots, GxE interaction, and error term. The incidence matrices for the above effects are represented by uppercase letters *X*, *Z, W,* and *T* (Resende, 2007).

6.3 RESULTS

6.3.1 Genetic parameters

At three years of age, *G. crinita* progeny in site 1 presented lower mean DBH, H, and B than in site 2 and site 3 (Table 18). The chi-square test (χ^2) on deviance analysis was statistically significant (P< 0.05) among progenies for all traits in site 1 and 2, indicating the possibility for genetic improvement by selection among progenies. For the site combined analysis, mean DBH was 12.6 cm, H was 11.2 m, and B was 27.55 kg. The χ^2 was statistically significant (P< 0.05) for all traits among progenies and plots, indicating the possibility of genetic improvement through selection among progenies (Table 19).

The coefficient of determination of the effects of plots (C_p^2) was low for all traits in sites 2 and 3 (≤ 0.106), for DBH and B in site 1 (≤ 0.239), and DBH in the combined analysis (0.025), but intermediate in the combined analysis for H (0.528) and B (0.63). Thus, no strong environmental heterogeneity was found within pairwise blocks for the studied traits. For the site combined analysis, the coefficient of determination of GxE interaction (C_{ge}^2) was low for all traits (< 0.03) and the genotypic correlation by environment (r_{ge}) was high for all traits (0.702-0.743), indicating that the interaction is simple. The individual heritability (h_i^2) was moderate for all traits in sites 1 and 3 (ranging from 0.226-0.27 and 0.374-0.494, respectively), as was heritability at the mean progeny level (h_m^2: site 1, 0.218-0.263; site 3, 0.36-0.435). In site 2, h_i^2 and h_m^2 were low for all traits (< 0.06). Consequently, the selective accuracy (r_a) was higher for all traits in sites 1 and 3 (0.466-0.66) than site 2 (0.181-0.228), indicating that there is greater possibility for obtaining genetic gains with the selection of progenies within sites 1 and 3, with highest mean for all traits. For the site combined analysis for all traits, h_i^2 was lower (0.041-0.049) than h_m^2 (0.34-0.369), and r_a was relatively high (0.583-0.608), indicating the possibility for improvement through selection among progenies across all sites.

Table 18 - Estimation of genetic parameters for the traits diameter at breast height (DBH), height (H), and total aerial biomass (B), per site and for all sites, of *Guazuma crinita* progeny tests.

Parameter	Site 1			Site 2			Site 3			All sites		
	DBH (cm)	H (m)	B (kg)	DBH (cm)	H (m)	B (kg)	DBH (cm)	H (m)	B (kg)	DBH (cm)	H (m)	B (kg)
Coefficient of determination of the plot effects: C_p^2	0.239	0.522	0.189	0.106	0.104	0.104	0.088	0.089	0.085	0.246	0.528	0.63
Coefficient of determination of GxE interaction effects: C_i^2	-	-	-	-	-	-	-	-	-	0.014	0.015	0.021
Individual heritability: h_i^2	0.226	0.27	0.267	0.025	0.04	0.04	0.42	0.374	0.494	0.041	0.045	0.049
Mean heritability at progeny level: h_m^2	0.222	0.218	0.263	0.033	0.052	0.051	0.39	0.36	0.435	0.369	0.34	0.343
Seletivas accuracy: r_a	0.472	0.466	0.513	0.181	0.228	0.227	0.625	0.6	0.66	0.608	0.583	0.586
Genotypic correlation among site: r_{ge}	-	-	-	-	-	-	-	-	-	0.743	0.742	0.702
Coefficient of individual genetic variation: $CV_{gi}\%$	12	14.8	28.7	2.6	2.6	7.2	10	8.2	25.3	3.7	3.4	8.7
Coefficient of experimental variation: $CV_e\%$	19.6	24.3	41.5	12.2	9.5	26.9	10.8	9.4	24.9	14	13.7	34.9
Overall mean: $\overline{m}$	8.9	6.3	11.6	14.3	13.5	34.7	14.6	13.9	36.5	12.6	11.2	27.5
LRT: Likelihood ratio test, χ2 deviance chi-square	3.7*	3.6*	5.3*	0.02	0.07	0.06	6.5*	5.4*	8.8*	10.3*	10.4*	11.1*

*P< 0.05, with 0.5 degree of freedom.

Table 19 - Deviance analysis (ANADEV).

Source of variation	DBH (cm)		Height (m)		Total aerial biomass (kg)	
	Deviance	LRT (χ^2)	Deviance	LRT (χ^2)	Deviance	LRT (χ^2)
Progeny	5956.46	10.31*	25409.19	10.37*	12676.85	11.11*
Plot	5974.87	28.72*	25557.11	158.29*	12767.08	101.34*
Progeny x Plot	5946.59	0.44	25399.28	0.46	12666.48	0.74
Complete Model	5946.15	-	25398.82	-	12665.74	-

*P< 0.05, with 0.5 degree of freedom; LRT: Likelihood ratio test, χ2 deviance chi-square.

The coefficient of genotypic variation (CV_{gi}%) was variable among traits and sites. The CV_{gi}% as a mean across all sites was higher for B (7.2-28.7%, mean of 8.7%) than DBH (2.6-12%, mean of 3.7%) and H (2.6-14.8%, mean of 3.5%). However, for all traits, CV_{gi}%was lower in site 2 (2.6-7.2%) than in site 1 (12-28.7%) and site 3 (8.2-25.3%), indicating high variation among progenies for all traits in site 1 and 2. The coefficient of experimental variation (CV_e%) was higher for all traits in site 1 (19.6-41.5%) than sites 2 and 3, where the values were similar for all traits (9.4-26.9%). The B trait showed the highest CV_e% in all sites and as a mean across sites (24.9-41.5%).

6.3.2 Adaptability, productivity, and stability

From the evaluations carried out based on the sources of variation (progeny, plot, and progeny x plot interaction), we found that there are significant differences in the evaluated DBH, H, and B traits at 5% probability in the progenies and plots. But no differences were found for the GxE interaction (

Table 20). In the simultaneous selection by DBH through the combined analysis (MHVG, PRVG, and MHPRVG), individual analysis for MHVG, PRVG, and MHPRVG, 50 progenies (25.9%) stood out (

Table 20). The ranking of these progenies considering all four methods was relatively consistent. Based on a combined analysis, it is possible to select genetic materials across all sites with a consistent adaptability, productivity, and stability.

Table 20 - Best progeny of *Guazuma crinita* with selection intensity of 25.9%, based on DBH, in the combined analysis of genotypic stability (MHVG), genotypic adaptability (PRVG), and productivity (MHPRVG).

Ranking	Combinad	MHVG	PRVG	MHPRVG	Ranking	Combinad	MHVG	PRVG	MHPRVG
1	76	76	76	76	26	417	945	945	945
2	134	134	134	134	27	136	136	136	136
3	416	416	416	416	28	137	46	46	46
4	77	77	77	77	29	916	137	137	137
5	29	61	29	29	30	46	1016	417	417
6	61	29	61	61	31	1016	918	1016	1016
7	515	515	515	515	32	918	417	918	918
8	58	38	58	58	33	28	28	28	28
9	38	58	38	38	34	42	31	31	31
10	71	110	71	71	35	31	42	42	42
11	911	74	110	110	36	942	12	942	942
12	110	113	911	911	37	107	942	12	12
13	113	71	113	113	38	932	512	932	932
14	1014	911	74	74	39	12	932	107	107
15	74	35	1014	1014	40	420	810	420	420
16	1011	1014	35	35	41	131	420	512	512
17	35	943	943	943	42	936	131	131	131
18	943	1011	1011	1011	43	512	107	810	810
19	935	45	45	45	44	11	813	813	813
20	144	513	513	513	45	813	11	936	936
21	45	55	935	935	46	810	936	11	11
22	513	935	55	55	47	32	53	811	811
23	55	946	144	144	48	108	811	53	53
24	946	144	946	946	49	39	32	39	39
25	945	916	916	916	50	811	39	32	32

6.4 DISCUSSION

6.4.1 Genetic parameters

At three years of age, *G. crinita* progenies showed variation among traits and sites. These results indicate the possibility for improvement by selecting the best progenies within sites and across all sites. The low values for h_i^2, h_m^2, r_a, and $CV_{gi}\%$ and the absence of significant differences among progenies for all traits in site 2, indicates that the possibilities of obtaining genetic gains in this site, with selection within and among progenies is limited. In contrast, for site 1 and 3, there are significant differences among progenies. The h_i^2 in sites 1 and 3 showed a moderate genetic control for all *G. crinita* traits, with results falling within the range of those reported for other tropical tree species at an early age (Aguiar *et al.*, 2019; Murillo *et al.*, 2019; Machado *et al.*, 2021; Resende *et al.*, 2021). The h_m^2 was also moderate for traits (0.34-0.369), and r_a in the combined analysis was relatively high (0.466-0.513). Despite of the low number or blocks and plants per progeny in the test, enough and large genetic variation was presented among progenies to enable performing selection and to expect genetic progress. These results indicate that genetic gains can be obtained by selecting progenies with the highest mean for the studied traits in sites 1 and 3. Therefore, to implement a second-generation progeny test, or for the genetic conservation of the species, the selection of progenies will be effective.

Despite of the low number or blocks and plants per progeny in the test, enough and large genetic variation was presented among progenies to enable performing selection and to expect genetic progress.

The $CV_{gi}\%$ for B was at least twice that for DBH and H in all sites, indicating large variation among progenies for B. Theoretically, B is indicated as an effective trait for selection. However, because DBH and B are directly related (Lima *et al.*, 2016; Araujo *et al.*, 2021; Revilla-Chávez *et al.*, 2021a), with a higher DBH resulting in greater B, and DBH is an easy to measure, we chose to use DBH as the variable for selection. Furthermore, the coefficient of determination of the effects of plots (C_p^2) was lower for DBH (0.246) than for H and B (> 0.5). Thus, in this experimental trial, no environmental heterogeneity was found within the pairwise blocks for DBH.

In the combined site analysis, h_i^2 was low for all traits (< 0.05), suggesting an unfavorable situation for improvement by mass selection in the experiment. Although h_i^2 values for all traits in *G. crinita* were low, they were within the range of values reported for other young tropical trees, such as *Calycophyllum spruceanum* (Sotelo *et al.*, 2006), *Dipteryx alata* (Pagliarini *et al.*, 2016), *Myracrodruon urundeuva* (Canuto *et al.*, 2017), and *Theobroma grandiflorum* (Chaves *et al.*, 2021). In contrast, h_m^2 was moderate for traits (0.34-0.369), and r_a in the combined analysis was relatively high (0.583-0.608). These results indicate that genetic gains can be obtained with the selection of progenies with the highest mean for all traits. Thus, selection precision was high compared to the $CV_{gi}\%$, h_m^2, and r_a, respectively. This again suggests that selection to implement a second-generation progeny test, or for genetic conservation of the species, would be effective. The observed absence of significant difference for the coefficient of determination of GxE interaction (C_{ge}^2) and the high genotypic correlation by environment (r_g) for all traits (0.702-0.743) indicates that the interaction is simple. Thus, in a breeding program, this type of interaction would be easier to be managed than that of a complex interaction (Vencovsky *et al.*, 2012; Pupin *et al.*, 2018; Souza *et al.*, 2020).

6.4.2 Analysis of adaptability, productivity, and stability

Significant differences were observed among progenies for DBH, H, and B, but no differences were found for GxE interaction across sites. These results are probably related to several factors, including the fact that the species occurs across a wide natural range. Thus, the species-environment interaction is probably minimal within a restricted area. Results suggest that such an environmental interaction is only found if there is a significant variation in climate and edaphic conditions. On the other hand, species that have a limited distribution and small population size usually present low levels of genetic diversity (White *et al.*, 2007). Coral *et al.*, (2016) reported differences with limited clustering of 44 superior *G. crinita* individuals based on DBH and H from the same experimental unity and that utilized in the present study.

6.4.3 Progeny selection

In the simultaneous selection by DBH considering the combined analysis, MHVG, PRVG, and MHPRVG, 50 progenies stood out. The consistent ranking of these progenies across the four methods applied is related to the positive correlation between genotypic behavior and environments. Therefore, the progeny selected based on the combined analysis are the most productive, the most stable, and the most adaptable. Both for a genetic improvement program and for the genetic conservation of the species, the most productive progenies are not significantly influenced by the environment and, consequently, do not present GxE interaction. These progenies should be prioritized as they present genetic traits that will help maintaining the species in the face of adverse conditions and contribute to *ex situ* gene conservation of this tree species.

Based on combined adaptability, productivity, and stability for DBH trait, it is possible to select genetic materials across all environments in these progeny tests. Fifty progenies with the highest DBH were selected (25.9% of the total progeny in the combined analysis of all environments), providing a good representation of all genetic material across all environments. These progenies showed adequate adaptability, productivity, and stability across all sites.

These results were similar to those found for stability, which is the variation in genotypic performance in diferente environments (Resende, 2007; Moraes *et al.*, 2012; Pupin *et al.*, 2018). This selection is consistent with that reported by Dawson *et al.* (2012), who argue that the genetic variation of a trait in a given area is high and the environmental variation is low, then selecting genetically superior individuals is possible Similar results were reported for *C. spruceanum* which were sampled and analyzed in the same river basin (Sotelo *et al.*, 2006).

6.5 CONCLUSIONS

We found genetic variation among *G. crinita* progenies for all traits, but no GxE interaction for the studied traits, indicating that the same group of progenies can be selected for all three sites. Compared to H and B, DBH was the most effective variable to select the best progenies in the assessment of genotypic adaptability, productivity, and stability of 200 *G. crinita* progenies at three years of age. Based on the combined analysis of genotypic adaptability, productivity, and stability, with a selection intensity of 25.9%, and considering the highest DBH values, 50 progenies are indicated as ideal for the development of a genetic improvement program.

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APPENDIX

7.1 General appearance of the species *A. rosaeodora*: A. Adult tree trees with DBH>25 cm, B. Stems=fustals: 10 > DBH < 25 cm, height > 50 cm, C. Sapling (latizals) 5 > D30 < 10 cm, diameter at 30 cm from the ground (D30) and height 1 ≤ height < 3 m, D. Seedlings (brinzals) height ranging from 30 to 50 cm, E. Shoots.

7.2 Collection of tissue samples for DNA diversity studies and vegetative propagation of *A. rosaeodora*: A. leaf tissues taken from saplings, B. bark tissue extraction procedure, C. bark tissues taken from an adult tree, D. seedlings collected for clonal propagation garden, E. clonal garden.

7.3 Collection of biomass samples for the development of allometric models of biomass and essential oil of *A. rosaeodora*: A. measurement of diameter of sample trees, B. green leaves, C. stump of felled tree, D. secondary branches, E. trunk of felled tree, F. separation of trees by leaves, branches and trunk, G. weighing of samples to determine moisture content, H. transport of trunks for oil extraction.