

SELECTION OF *Handroanthus impetiginosus* MOTHER TREES TO SUPPORT SEED COLLECTION AREAS

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ABSTRACT – *Handroanthus impetiginosus* is a Brazilian native tree threatened by illegal logging that needs conservation strategies. Due to the need to obtain seeds of high genetic and physiological quality, this study aimed to select mother trees, aiming at the installation of Seed Collection Areas of *H. impetiginosus*. Therefore, the genetic diversity and physiological quality of seeds from 63 individuals of a natural population of *H. impetiginosus* in Rio Grande do Norte, Brazil, were evaluated. The molecular markers revealed that the studied population has high intrapopulation genetic diversity (Nei's genetic diversity = 0.35; and Shannon index = 0.52). Subsequently, germination and seed vigor tests evaluated the physiological quality of seeds from 63 trees. Of this total, 45 individuals produced seeds of high and intermediate physiological quality. Therefore, individuals with a Nei's genetic distance < 0.72 and germination greater than 50% can be selected as mother trees, and the population has the potential to propose a Seed Collection Area.

Keywords: Genetic quality; Açú National Forest; Ipê-roxo.

SELEÇÃO DE ÁRVORES MATRIZES DE *Handroanthus impetiginosus* PARA SUBSIDIAR ÁREAS DE COLETA DE SEMENTES

RESUMO – *Handroanthus impetiginosus* é uma árvore nativa brasileira ameaçada pela exploração ilegal de madeira e necessita de estratégias para fins de conservação. Devido à necessidade de obtenção de sementes de alta qualidade genética e fisiológica, objetivou-se com este estudo selecionar árvores matrizes, visando a instalação de Áreas de Coleta de Sementes (ACS) de *H. impetiginosus*. Para tanto, avaliou-se a diversidade genética e a qualidade fisiológica das sementes de 63 indivíduos de uma população natural localizada no estado do Rio Grande do Norte, Brasil. O conjunto de marcadores moleculares revelou que a população estudada apresenta grau intermediário de diversidade genética intrapopulacional (diversidade genética de Nei = 0,35; índice de Shannon = 0,52). Posteriormente, foi avaliada a qualidade fisiológica das sementes por meio de testes de germinação e de vigor de sementes. Deste total, 45 indivíduos produziram sementes de qualidade fisiológica alta e intermediária. Assim, os indivíduos com identidade genética de Nei < 0,72 e germinação superior a 50% podem ser selecionados como árvores matrizes e a população apresenta potencial para proposição de uma ACS.

Palavras-Chave: Qualidade genética; Floresta Nacional de Açú; Ipê-roxo.



1. INTRODUCTION

The neotropical species *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (Bignoniaceae), popularly known as pink trumpet tree, has a wide geographical distribution, occurring in the Caatinga, Cerrado, Amazon Forest, Atlantic Forest and Pantanal biomes, being considered the timber tree with the second highest commercial value and the most exploited in Brazil, due to the excellent physicochemical properties of its wood (Silva-Junior et al., 2018). It is a perennial species, with anemochory dispersal, cross fertilization and self-incompatibility (Bittencourt Junior, 2017). It also has pharmacological properties (Ahmad et al., 2020; Campos and Albuquerque, 2021; Ryan et al., 2021; Silva et al., 2021) and is used in forest restoration and urban silviculture, because of the exuberance of its flowering (Chaves et al., 2018).

Due to predatory exploitation, *H. impetiginosus* is on the list of near-threatened species - NT (BGCI; IUCN, 2019), which justifies conducting studies that assist in its conservation *in situ* and *ex situ*. This situation is associated with the advance of anthropic activities, which leads to the fragmentation of natural areas, favoring the isolation of populations and the greater probability of genetic erosion (Telles et al., 2014).

Genetic variability directly affects the evolutionary dynamics of populations (Nybom, 2004; Inza et al., 2018). Thus, the analysis of genetic diversity in forest populations provides information for the conservation of species and assists in the identification of genetically different individuals, supporting studies of selection of mother trees. It is known that seed collection requires a representative sampling of the genetic variability of populations in order to conserve the evolutionary potential of the species and meet the minimum sampling criteria for genetic conservation (Fajardo et al., 2014; Inza et al., 2018; Felix et al., 2021).

Some of the factors that hinder the collection of native seeds are forest fragmentation and local extinction of populations due to anthropization. In addition, forests with higher levels of conservation are located in Conservation Units (CU), but these units have legal restrictions on the use of flora. Consequently, seed collection has often been carried out in areas with a diverse history of disturbance and without the

technical criteria for selection of mother trees (Ribeiro et al., 2016). The methods for choosing mother trees are fundamental for planting success; however, much of the literature on the subject does not disclose the criteria for choosing the mother trees, for instance they do not establish minimum distances between mother trees, minimum percentage of germination and genetic diversity (Passos and Cruz, 2015). These data demonstrate the need for more complete information and guidelines to ensure the correct choice of mother trees. In this context, such studies could contribute to the advancement of knowledge on the production of forest seeds and seedlings based on the selection of mother trees, considering the peculiarities of each species and environments of occurrence.

In fact, growing concerns about environmental issues have led to more specific and restrict legislation, in addition to the development of government programs prioritizing forest restoration, thereby increasing the demand for native forest seeds with physiological and genetic quality (Felix et al., 2021). In addition to the quantitative aspect of seedling production, nurserymen should pay attention to the quality of seeds and seedlings. Seeds used for restoration of degraded areas should have high physiological quality and genetic variability to meet the demands of reforestation programs. Among the main criteria for the proper collection of seeds is the representative sampling of the genetic variability of the population, in order to conserve the evolutionary potential of the species (Ribeiro et al., 2016; Felix et al., 2021).

Thus, to ensure high-quality seeds it is essential to select mother trees with good seed production capacity, associated with proper monitoring of the production process and seed collection (Passos and Cruz, 2015). Among the CUs that allow sustainable use and conduction of scientific research are the National Forests (FLONAs), which can serve as places of study for the selection of native mother trees (MMA, 2019). After the selection of individuals, these areas can be called Seed Collection Areas (SCA), which are indicated for obtaining seeds for forest restoration purposes, because they combine the production of high-quality seeds with the conservation of natural resources (Araújo et al., 2018).

Therefore, this study aimed to select mother trees of *Handroanthus impetiginosus*, based on the genetic

diversity of individuals and physiological quality of seeds, in order to provide support for the installation of a Seed Collection Area.

2. MATERIAL AND METHODS

Seeds and leaf tissue samples for DNA extraction were collected from 63 individuals (lots) of *Handroanthus impetiginosus*, located in the Açu National Forest (FLONA) (5°34'20"S and 36°54'33"W), a Federal Conservation Unit located in the municipality of Assu, Rio Grande do Norte, Brazil. The Açu FLONA has phytophysiognomic aspect marked by tree-shrub vegetation, remnant of the Caatinga biome (Souza et al., 2014). According to Köppen's classification, the climate is considered Bsw, that is, dry, very hot and with irregular rains concentrated generally from December to April, with an annual average of 600 mm. The average annual temperature is 28.1 °C and the average annual relative humidity is 70% (Costa et al., 2010). The relief is characterized as gently undulating and undulating, with an average altitude of 100 m (Lira et al., 2010).

Each marked tree was evaluated for height, diameter at breast height (DBH) and diameter at ground level (DGL). The tree individuals were sampled with a minimum distance equivalent to twice their height, according to criteria described by Sebbenn (2002). Individuals that visually showed high flower production, without any pests or diseases, were also considered as auxiliary criteria in the selection. Access to the genetic heritage of this study is registered in the National System of Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) and identified by the code A5DB85E. For each individual, two to three juvenile leaf samples were collected, placed in 2-mL plastic tubes containing 2X cetyltrimethylammonium bromide (CTAB), identified and subsequently stored at -20 °C until the moment of DNA extraction.

DNA was extracted by the CTAB method, as proposed by Doyle and Doyle (1987), with some modifications, using 100 mM Tris pH 8.0, 1.4 M NaCl, 20 mM EDTA pH 8.0, 2% (w.v⁻¹) CTAB, 1% (w.v⁻¹) PVP-40 and 0.2% (v.v⁻¹) β-mercaptoethanol preheated at 60 °C in water bath. After extraction, DNA was diluted with TE (10 mM Tris-HCl; EDTA mM pH 8.0) and quantified by spectrophotometry

(Epoch™ microplate spectrophotometer). The stages of polymerase chain reactions (PCR) and electrophoresis were performed according to Pimenta et al. (2022), using 8 ISSR primers (UBC - University of British Columbia).

PopGene software version 1.32 (Yeh et al., 1997) was used to evaluate the number of observed alleles (*Na*), number of effective alleles (*Ne*), Nei's (1973) genetic diversity (*h*) and Shannon index (*I*) for the population; *h* and *I* values can range from 0 to 1 (Uramoto et al., 2005). The similarity dendrogram based on Nei's (1978) genetic identity was generated by UPGMA (*Unweighted Pair Group Method with Arithmetic Mean*), using the NTSYS program v.2.11 (Rohlf, 1993). The correlation between Nei's genetic distance and geographic distance (km) was analyzed by the Mantel test using the GenAlEx program v. 6.503 (Peakall and Genalex, 2012), implemented to Excel, with 999 permutations.

To evaluate the physical and physiological quality of the seeds, the fruits were collected when they were still attached to the trees at the beginning of the natural dehiscence process, but preceding seed dispersal. The fruits were manually processed, separating healthy seeds from those considered empty, infested and/or attacked by insects and fungi. Then, the physical and physiological quality of the seeds of each lot were evaluated by the following determinations and tests: a) moisture content of the seeds: performed using the oven method at 105 ± 3 °C for 24 h (Brasil, 2009), using two subsamples of 4.5 ± 0.5 g of seeds, whose results were expressed as percentage; b) thousand-seed weight (TSW) and number of seeds per kilogram, obtained according to methodologies proposed by Brasil (2009); c) germination: the seeds were previously disinfested in 2.5% (m.v⁻¹) sodium hypochlorite (NaClO) solution for 5 min and rinsed three in distilled water (Brasil, 2013). Subsequently, they were sown for germination on paper towel moistened with distilled water, in a proportion equivalent to 2.5 times the weight of the dry paper, arranged in rolls and placed in transparent plastic bags, in a germination chamber at temperature of 25 °C (Brasil, 2013) under photoperiod of 12 h. Seeds that originated normal seedlings, with all their essential structures, on the 21st day after sowing, were considered germinated (Brasil, 2013); d) germination speed: the number of seeds germinated daily after

sowing in the germination test was counted to calculate the germination speed index (GSI), which has no unit of measurement, as it is a dimensionless quantity (Maguire, 1962); e) seedling length: determined together with the germination test, by randomly sampling 15 normal seedlings and measuring them with a graduated ruler, with results expressed in cm.sdl^{-1} , obtained by dividing the sum of the total length obtained in each replicate by the number of normal seedlings sampled in each replication (ISTA, 1981); f) seedling dry mass: normal seedlings obtained in the seedling length test had their cotyledons removed, placed in paper bags, dried at 80 °C for 24 h (Nakagawa, 1999), and then weighed on a precision analytical scale (0.001 g), with results expressed in milligrams (mg.sdl^{-1}), obtained by dividing the weight measured at the end of drying in each replicate by the number of normal seedlings sampled in each replicate; g) emergence: the seeds of each lot were sown at 1.0 cm depth in a bed with sand, in full sun, wetted with daily watering, and the total of normal seedlings emerged on the 33rd day after sowing, when the emergence was stabilized, was considered, with results expressed as percentage; h) emergence speed:

conducted together with the seedling emergence test, using the number of seedlings emerged daily and calculating the emergence speed index (ESI), which has no unit of measurement, as it is a dimensionless quantity (Maguire, 1962).

The experiment was conducted in a completely randomized design, except for the emergence test, which was performed in randomized blocks. The germination and GSI tests were performed with eight replicates of 50 seeds each, totaling 400 seeds per lot (Brasil, 2013). The other tests were performed with four replicates of 50 seeds each, except for seedling length and dry mass tests, which were performed with 8 replicates of 15 seedlings. The following tests were performed: Shapiro-Wilk for normality of residuals and homogeneity of variances at 5% probability level, parametric analysis of variance (ANOVA) by the F test, and the Scott-Knott means comparison test at 5% probability level. To facilitate understanding during the discussion of the text, the letters obtained in the Scott-Knott means comparison test were treated as groups, with letters a, b, c, d, e, f, g, h and i named as groups 1 to 17. In addition, for the morphological parameters of the trees, Pearson's simple correlation

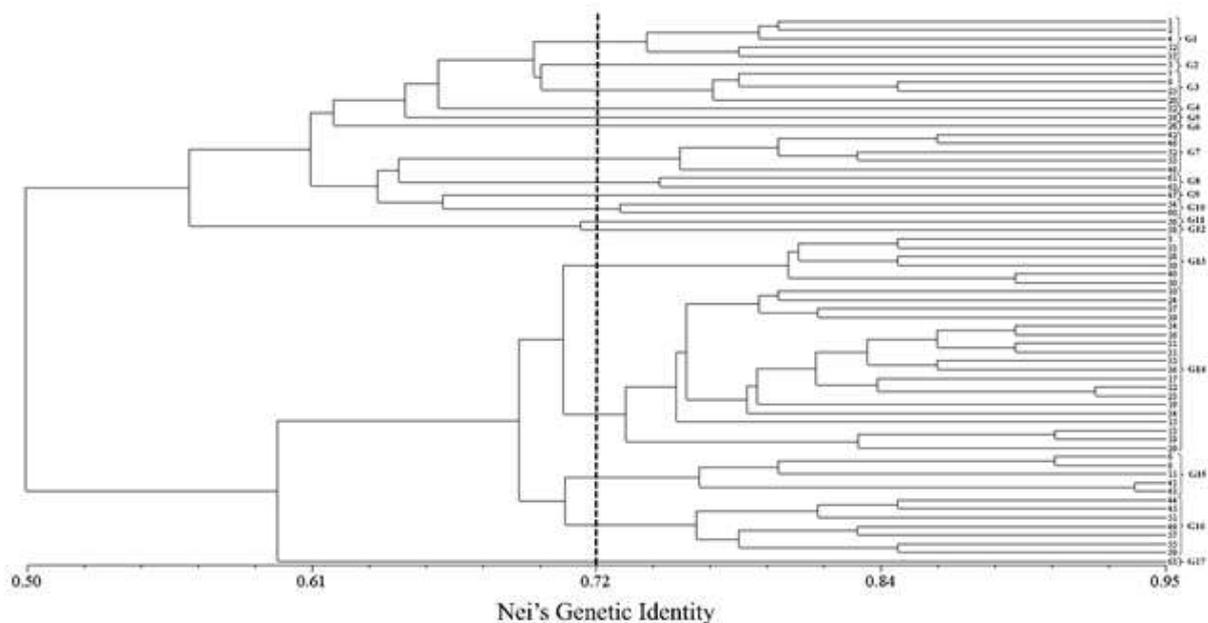


Figure 1 – Dendrogram of Nei's genetic identity of 63 individuals of *Handroanthus impetiginos* from the Açú National Forest, Assu, Rio Grande do Norte, Brazil.

Figura 1 – Dendrograma da identidade genética de Nei de 63 indivíduos de *Handroanthus impetiginos* provenientes da Floresta Nacional de Açú, Assu, Rio Grande do Norte, Brasil.

Table 1 – Physiological aspects of *Handroanthus impetiginosus* seeds from the Açú National Forest, Assu, Rio Grande do Norte, Brazil.
Tabela 1 – Aspectos fisiológicos das sementes de *Handroanthus impetiginosus* provenientes da Floresta Nacional de Açú, Assu, Estado do Rio Grande do Norte, Brasil.

Tree	G	GSI	SL	SDM	E	ESI
	%	-	(cm)	(mg)	%	-
1	87 a	4.04 e	5.1 d	7.9 e	55 b	1.51 d
2	71 c	4.01 e	6.4 d	12.2 d	44 d	1.45 d
3	61 d	2.82 g	8.5 c	13.3 c	48 c	1.57 d
4	78 b	3.18 f	5.1 d	7.2 e	54 c	1.60 d
5	81 b	4.41 d	8.3 c	12.4 d	57 b	1.86 c
6	93 a	5.52 b	7.4 c	15.6 c	55 b	1.73 c
7	66 d	3.29 f	11.8 b	20.1 b	65 a	2.03 b
8	72 c	4.24 e	7.9 c	13.5 c	59 b	1.91 c
9	90 a	4.86 c	8.4 c	13.4 c	53 c	1.80 c
10	91 a	4.52 d	7.5 c	11.7 d	40 d	1.19 e
11	91 a	4.83 c	9.0 c	13.6 c	61 a	2.04 b
12	70 c	3.87 e	8.0 c	15.0 c	45 d	1.42 d
13	76 c	2.80 g	7.4 c	10.7 d	56 b	1.81 c
14	84 b	5.02 c	14.8 a	22.6 a	64 a	2.16 a
15	54 e	2.32 h	9.8 c	16.6 c	42 d	1.48 d
16	71 c	3.81 e	10.1 c	13.9 c	53 c	1.75 c
17	81 b	4.55 d	10.4 c	17.4 c	65 a	2.09 b
18	61 d	2.19 h	10.7 c	14.4 c	59 b	2.43 a
19	46 f	1.74 i	4.6 d	15.1 c	35 e	1.23 e
20	43 f	1.68 i	3.1 e	5.3 f	36 e	1.83 c
21	26 h	0.91 j	9.6 c	14.8 c	20 f	1.81 c
22	65 d	3.64 f	10.1 c	20.7 b	63 a	2.21 a
23	43 f	1.42 i	7.6 c	16.0 c	41 d	1.94 c
24	69 c	2.41 h	9.1 c	15.1 c	64 a	2.11 b
25	30 h	1.15 j	5.9 d	10.5 d	25 f	2.00 b
26	79 b	1.55 i	8.0 c	15.8 c	50 c	1.84 c
27	72 c	4.12 e	4.5 d	8.5 d	34 e	1.27 d
28	79 b	4.55 d	11.8 b	18.8 b	59 b	1.98 b
29	82 b	6.3 a	9.5 c	14.2 c	65 a	2.15 a
30	73 c	4.65 d	5.3 d	10.2 d	44 d	1.50 d
31	45 f	1.65 i	9.2 c	21.1 b	40 d	2.13 b
32	31 h	1.03 j	9.8 c	19.6 b	29 f	1.43 d
33	57 e	2.27 h	8.5 c	10.6 d	50 c	1.91 c
34	20 i	0.77 j	13.9 a	20.9 b	18 f	2.28 a
35	29 h	1.03 j	4.6 d	7.3 e	20 f	1.88 c
36	38 g	1.23 j	8.2 c	14.3 c	38 d	1.46 d
37	55 e	2.28 h	3.8 e	7.6 e	55 b	1.77 c
38	65 d	3.16 f	8.3 c	19.0 b	57 b	2.00 b
39	47 f	1.72 i	8.5 c	15.1 c	45 d	1.79 c
40	51 e	2.27 h	9.4 c	14.1 c	50 c	2.03 b
41	70 c	3.28 f	13.8 a	26.0 a	54 c	1.72 c
42	36 g	1.39 i	9.5 c	15.2 c	30 e	1.92 c
43	70 c	3.36 f	16.8 a	25.1 a	66 a	2.21 a
44	78 b	4.10 e	8.9 c	15.4 c	51 c	1.69 c
45	63 d	2.58 g	14.1 a	21.6 b	60 a	1.97 c
46	68 c	2.41 h	7.5 c	15.8 c	53 c	1.81 c
47	73 c	3.32 f	9.7 c	15.9 c	49 c	1.77 c
48	43 f	1.69 i	10.4 c	18.5 b	40 d	2.02 b
49	74 c	2.66 g	9.8 c	14.9 c	50 c	1.66 c
50	78 b	3.46 f	5.0 d	8.7 d	44 d	1.59 d

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Table 1 ...
Tabela 1 ...

51	51 e	2.44 h	3.6 e	6.7 e	38 d	1.37 d
52	86 a	4.90 c	15.2 a	23.7 a	66 a	2.20 a
53	24 h	0.85 j	5.4 d	9.0 d	23 f	0.75 f
54	69 c	2.30 h	2.7 e	4.4 f	27 f	1.04 e
55	78 b	3.98 e	10.7 c	20.2 b	60 a	1.92 c
56	89 a	4.30 d	14.0 a	24.8 a	59 a	2.04 b
57	40 f	1.53 i	10.2 c	15.6 c	32 e	1.07 e
58	17 h	0.72 j	3.3 e	7.7 e	15 f	1.98 b
59	68 c	2.33 h	9.5 c	14.4 c	53 c	1.79 c
60	15 h	0.45 h	3.0 e	5.1 f	11 f	0.98 e
61	34 g	1.30 j	2.4 e	3.6 f	23 f	0.82 f
62	51 e	2.17 h	10.2 c	19.6 b	49 c	2.28 a
63	51 e	2.40 h	1.0 e	0.8 e	33 e	1.00 e
CV (%)	7.51	10.63	10.78	15.10	8.26	10.30

Significant at 5% probability level according to the Scott-Knott test. Statistically superior values are presented from the letter "a". CV: Coefficient of variation; G: Germination; GSI: Germination speed index; SL: Seedling length; SDM: Seedling dry mass; E: Emergence; ESI: Emergence speed index.

Significativo ao nível de 5% de probabilidade de acordo com o teste de Scott-Knott. Os valores superiores estatisticamente são apresentados a partir da letra "a".

CV: Coeficiente de variação; G: Germinação; IVG (Índice de velocidade de germinação); CP: Comprimento de plântulas; MSP: Massa seca de plântulas; E: Emergência; IVE: Índice de velocidade de emergência.

(rp) in relation to the physiological parameters of the seeds was performed, and the correlations between the variables studied was analyzed by the t-test at 5% probability level. The analyses were performed with the statistical program R (R Core Team, 2017).

3. RESULTS

In the study population of *H. impetiginosus*, the mean value of observed alleles (N_a) was 1.98 ± 0.13 and the number of effective alleles (N_e) was 1.61 ± 0.32 . The Nei's diversity index for the study population was $H_e = 0.35 (\pm 0.15)$, while the Shannon index (I) was $0.52 (\pm 0.18)$, allowing the identification of an intermediate degree of intrapopulation genetic diversity.

From the clustering of Nei's genetic identity (Figure 1), groups with genetic identity of 0.50 were formed. In the dendrogram, from a cutoff point of 0.72 of Nei's genetic identity, it was possible to identify 17 groups. The pairs of individuals with the highest genetic identity were 41 and 43 (0.93) and 22 and 23 (0.91), which were grouped into smaller clusters in the cluster analysis using the UPGMA method, which suggests that these individuals have greater genetic similarity to each other.

The largest geographical distance between the individuals was 1,609 m and the smallest, 11.65 m, with an average distance of 599 m between the trees. In the present study, the Mantel test identified no correlation between the genetic distance matrix and the geographic distance matrix ($r = 0.0001$ and $P = 0.32$).

The height of the trees ranged from 4 to 15 m, with an average of 8.1 m. In addition, the trees obtained mean DGL of 18.86 cm, with maximum and minimum values of 48.4 and 9.1 cm, respectively. The

Table 2 – Quality classification criteria for seeds from *Handroanthus impetiginosus* individuals, based on the Scott-Knott test at 5% probability level.

Tabela 2 – Critérios de classificação de qualidade de sementes dos indivíduos de *Handroanthus impetiginosus*, com base no teste de Scott-Knott ao nível de 5% de probabilidade.

Variable analyzed	Classification (vigor levels)
Germination	High: 93 – 78% (a, b)
	Intermediate: 76 – 51% (c, d, e)
	Low: 47 – 20% (f, g, h, i)
Germination Speed Index	High: 6.33 – 4.83 (a, b, c)
	Intermediate: 4.64 – 3.15 (d, e, f)
	Low: 2.82 – 0.77 (g, h, i, j)
Emergence	High: 66 – 59% (a, b)
	Intermediate: 58 – 48% (c, d)
	Low: 44 – 23% (e, f)
Emergence Speed Index	High: 2.42 – 2.14 (a, b)
	Intermediate: 2.12 – 1.65 (c, d)
	Low: 1.59 – 1.26 (e, f)
Seedling Length	High: 16.76 – 13.80 cm.sdl ⁻¹ (a, b)
	Intermediate: 11.78 – 7.35 cm.sdl ⁻¹ (c, d)
	Low: 6.42 – 2.36 cm.sdl ⁻¹ (e)
Seedling Dry Mass	High: 26.37 – 18.54 mg.sdl ⁻¹ (a, b)
	Intermediate: 17.35 – 13.34 mg.sdl ⁻¹ (c, d)
	Low: 12.43 – 3.62 mg.sdl ⁻¹ (e, f)

* Classification of the groups into vigor levels was performed according to the Scott-Knott test at 5% probability level. Groups with statistically higher means are presented from the letter "a".

*A classificação dos grupos em níveis de vigor foi realizada de acordo com o teste de Scott-Knott a nível de 5% de probabilidade. Os grupos com maiores médias estatisticamente são apresentados a partir da letra "a".

Table 3 – Selection of mother trees according to the criteria of genetic diversity and physiological quality of *Handroanthus impetiginosus*.
Tabela 3 – Seleção das árvores matrizes conforme os critérios de diversidade genética e qualidade fisiológica de *Handroanthus impetiginosus*.

Nei's dendrogram groups	Individuals of each group (Genetic diversity)	Selected individuals* (Physiological and genetic quality)
1	1; 2; 4; 12; 37	1; 2; 4; 12; 37
2	5	5
3	7; 9; 20; 25	7; 9
4	32	-
5	38	38
6	26	26
7	42; 46; 48; 52; 53	46; 52
8	61; 62	62
9	47	47
10	54; 60	54
11	56	56
12	58	-
13	3; 28; 30; 33; 40; 50	3; 28; 30; 33; 40; 50
14	10; 13; 14; 15; 16; 17; 18; 19; 21; 22; 23; 24; 27; 29; 31; 34; 35; 36; 39	10; 13; 14; 15; 16; 17; 18; 22; 24; 27; 29
15	6; 8; 11; 41; 43	6; 8; 11; 41; 43
16	44; 45; 49; 51; 55; 57; 59	44; 45; 49; 51; 55; 59
17	63	63

*Selected individuals: trees classified according to the parameters of genetic and physiological quality.

*Indivíduos selecionados: árvores classificadas segundo os parâmetros de qualidade genética e fisiológica.

moisture content of *H. impetiginosus* seeds ranged from 7.1 to 9.2% during the physiological tests of germination and vigor.

The determination of thousand-seed weight and number of seeds per kilogram showed that seeds of *H. impetiginosus* trees have different weights and volumes, with values ranging from 80.5 to 168.3 g and an average of 8,590 seeds per kilogram.

Significant effect at 5% probability level was observed for all studied variables related to the physiological aspects of the seeds, indicating that there are differences in physiological performance of germination (Table 1) and vigor for seeds from the different individuals of *H. impetiginosus* according to the statistical analyses performed.

The individuals were classified with high, intermediate and low physiological quality, according to the classification criteria, based on the statistical

analysis (Table 2). In the studied population of *H. impetiginosus*, 71.43% of the trees (individuals of groups 1, 2, 3, 4 and 5) produced seeds with germination greater than 50%, which was the minimum percentage considered for the selection of mothers trees, since it is the limit for seeds of forest species (Tables 2 and 3).

All trees of groups 1 and 2 (27% of the sampled individuals) produced seeds considered to have high germination, whose percentages were equal to or greater than 78%, and individuals belonging to groups 3, 4 and 5 (44% of the total sampled trees) produced seeds considered to have intermediate quality, with germination range of $\geq 50\% < 78\%$ (Tables 2 and 3). Exactly 29% of the trees (individuals of groups 6, 7, 8 and 9) produced seed lots with viability of less than 50%.

The trees that produced seeds with germination above 50% had an average of 8.83 cm.sdl⁻¹ (S_x : 0.50) for seedling length and 14.75 mg.sdl⁻¹ (S_x : 0.68) for

Table 4 – Pearson correlation coefficients between germination and emergence tests with the parameters height, diameter at breast height (DBH) and diameter at ground level (DGL) of *Handroanthus impetiginosus* trees.

Tabela 4 – Coeficientes de correlação de Pearson entre os testes de germinação e emergência com os parâmetros de altura, diâmetro à altura do peito (DAP) e diâmetro à altura do solo (DAS) das árvores de *Handroanthus impetiginosus*.

Physiological aspect	Height	DBH	DGL
Germination	0.5984 ^{ns}	0.7774 ^{ns}	0.4705 ^{ns}
Emergence	0.9626 ^{ns}	0.4321 ^{ns}	0.9466 ^{ns}

ns not significant at 5% probability level.

ns não significativo ao nível de 5% de probabilidade.

Table 5 – Germination for composing seed lots from selected and sampled mother trees of *Handroanthus impetiginosus*.

Tabela 5 – Germinação para a composição de lotes de sementes provenientes de matrizes selecionadas e amostradas de *Handroanthus impetiginosus*.

Seed lots	Germination (%)
Selected trees	72 a
All trees	61 b
F value	20.21**

** Significant at 1% probability level by the F test.

** Significativo ao nível de 1% de probabilidade pelo teste F.

seedling dry mass, with an average of 3.49 (Sx: 0.16) for GSI. Only the trees that had seeds with germination above 50% (Table 1) were considered for the selection of mother trees. According to the dendrogram (Figure 1), 17 groups were obtained.

Regarding the height, DBH and DGL of the trees and germination (Table 1), there was no significant correlation between the variables (Table 4). Trees that produced seeds with germination greater than 50% showed averages of 14.13 cm, 18.38 cm and 8.05 m for DBH, DGL and height, respectively. On the other hand, individuals that produced seeds with germination below 50% obtained averages of 14.43 cm, 20.37 cm and 7.58 m for DBH, DGL and height, respectively.

The selected *H. impetiginosus* trees have higher germination for the composition of seed lots aiming at an SCA, when compared with the total number of trees evaluated at the study site (Table 5).

4. DISCUSSION

According to the Nei's diversity index, which compares the evolution of the species (Nybom, 2004), genetic variability showed higher results than those estimated for species with similar life history. The expected genetic diversity for long-living perennial species is 0.25 (Nybom, 2004), and considering the characteristics of *H. impetiginosus* (perennial species, anemochory dispersal and cross fertilization), the *He* value of 0.35 was higher than expected for species with similar life parameters. The genetic diversity of *H. impetiginosus* was also higher than expected for species with cross pollination (0.27), anemochory seed dispersal (0.27) and climax successional stage (0.30) (Nybom, 2004).

The results obtained from the Nei's diversity index ($H_e = 0.35$) and the Shannon index ($I = 0.52$) allowed the identification of an intermediate degree

of intrapopulation genetic diversity when compared to the results obtained in studies with ISSR for other forest species (Rajasekharan et al., 2017; Araújo et al., 2020; Lopes et al., 2020).

The genetic variability found within the population may be associated with the conservation status and absence of anthropic activity, as it is a Conservation Unit (CU). The Açú National Forest is the first FLONA of Rio Grande do Norte and the third in the Northeast region, with 215 ha. The south and southeast boundaries have no connection with other forest remnants; however, the border areas on the east and west sides of the CU, for being well preserved, function as ecological corridors, enabling the flow of genes and the movement of the biota (ICMBio, 2019).

Genetic characterization using ISSR molecular primers is useful for the selection of mother trees (Felix et al., 2021). The results obtained provide support for the selection of individuals based on the greater genetic divergence determined by molecular markers. This is especially important for seed collection, since they are essential for the formation of seed lots with greater genetic variability. This strategy makes it possible to ensure conservation of the genetic resources of the species, favoring the evolutionary dynamics of natural populations, besides promoting programs for the recovery of native flora and contributing to avoid future problems such as physiological disorders, mortality due to low resistance to weather or attacks of pests and inbreeding.

The results indicated that the study population does not show a positive and significant relationship between the geographic distance of the individuals and the genetic distance, according to the Mantel test. In this context, the genotypes evaluated in the population have random distribution, which demonstrates that currently the individuals do not follow the pattern of isolation by distance. Thus, no aggregate spatial pattern of genotypes was observed, hence requiring joint evaluation of the genetic divergence and physiological quality of the seeds to support the selection of mother trees of the species in this population.

Physiological quality is a tool for differentiation and evaluation of lots, which is performed through viability and vigor tests. In the studied population of *H. impetiginosus*, trees that produced seeds with

germination greater than 50% were selected (Marcos Filho, 2005). This level of viability for native forest seeds was considered adequate in a study conducted by Felix et al. (2021), because the species are not domesticated and do not undergo genetic improvement, especially when it comes to allogamous plants, as is the case of the species in the present study. The results of moisture content corroborate the allowed parameters, since it should remain below 13% to avoid intensive processes of deterioration, which can result in loss of vigor (Guareschi et al., 2015). Regarding the variations obtained in thousand-seed weight, it can be suggested that they are associated with several factors, including the genetic variability existing between the trees of the same species and the environmental conditions (Teles and Barreira, 2018).

Like GSI, seedling dry mass and seedling length were efficient to distinguish seed vigor and select the best mother trees. Seedling length allowed classifying 9 individuals (groups 1 and 2) as with high quality and 46 other individuals (groups 3 and 4) as with intermediate quality. Seedling dry mass distinguished 16 individuals (groups 1 and 2) as producers of seeds with high physiological quality and 36 individuals (groups 3 and 4) as producers of seeds with intermediate quality. There was a reduction in seedling quality performance for the individuals 37, 51, 54 and 63.

The trees that showed superior parameters for seedling length, seedling dry mass and GSI were the individuals of groups 1 and 2. It is important to highlight that the non-significant correlation observed between morphological characteristics (height, DBH and DGL) and germination and emergence can be explained by the fact that the physiological quality of the seeds is related to many biotic and abiotic factors, as observed by Araújo et al. (2018) for the species *Mimosa caesalpiniiifolia* Benth. In view of the above, after the results of the viability and vigor tests and according to the criteria established for the classification of seeds into three levels of vigor, it was possible to classify the seeds produced by the trees belonging to groups 1 and 2 as with high physiological quality and the seeds collected from the individuals of groups 3 and 4 as with intermediate quality.

According to the groups formed by the dendrogram, more genetically distant individuals should be selected. In addition, the ideal is that

at least one individual from each group formed in the dendrogram is selected as a mother tree, in order to select less similar individuals in relation to genetic diversity. Thus, the selected individuals of *H. impetiginosus* can be considered as mother trees, according to the established criteria of high and intermediate genetic diversity and physiological quality of the seeds, especially trees whose seeds showed germination higher than 50%.

Therefore, the classification of viability and vigor tests, together with the results of genetic diversity of the individuals, showed that 45 trees can be selected as producers of high-quality seeds, as demonstrated in the results for physiological quality and the genetic diversity obtained. The composition of the seed lot formed by selected mother trees (physiological quality and genetic diversity) of *H. impetiginosus* is superior to that of the seed lots of all trees analyzed (selected or not selected). Therefore, the genetic diversity represented by these mother trees contributes to the formation of new forest stands with superior characteristics. Thus, the selection of mother trees of *H. impetiginosus* based on physiological quality and genetic diversity is proven to be an important step for seed production, as it increases the final quality of the seed lot.

The methods for choosing mother trees are fundamental for planting success; however, much of the literature on the subject does not disclose these criteria and the results obtained in this study can support projects aimed at the conservation and restoration of natural populations of *H. impetiginosus* with similar edaphoclimatic characteristics, supporting an adequate forest restoration with seeds adapted to the conditions. Thus, the studied population has the potential to be used as a Seed Collection Area of *H. impetiginosus*, because it has individuals with high and intermediate physiological quality, in addition to intermediate genetic diversity. Finally, it is emphasized that this study was based on a population of *H. impetiginosus* in the Caatinga biome, so studies need to be conducted in other areas of natural occurrence, since the species has a wide geographical distribution.

4. CONCLUSIONS

The selection of mother trees of *Handroanthus impetiginosus*, with a view to the installation of Seed Collection Areas, can be performed from individuals

with Nei's genetic identity < 0.72 and which produce seeds with germination greater than 50%.

AUTHOR CONTRIBUTIONS

PIMENTA JMA was responsible for conducting the experimente, data analysis and writing of manuscript. SOUZA WMAT assisted in conducting the experimente. PACHECO MV, VIEIRA FA and FAJARDO CG contributed with the supervision of methodology and correction. FERRARI CS reviewed and correct the work.

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