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Artigos

Genetic diversity and phenotypic characterization of *Ochroma pyramidale* in plantations in Mato Grosso, Brazil

Diversidade genética e caracterização fenotípica de *Ochroma pyramidale* em plantios no Mato Grosso, Brasil

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ABSTRACT

This study evaluated balsa wood (*Ochroma pyramidale*) plantations in the search for matrices for genetic improvement. We were evaluated a total of 20 trees in plantations in Mato Grosso for genetic diversity with ISSR (*Inter Simple Sequence Repeats*) primers, as well as their diameter at breast height (DBH) and commercial height (CH). The primers amplified 111 loci (97.3% polymorphic), and the Nei genetic diversity (0.32) and Shannon index (0.48) indicate that there is genetic diversity in the plantations. The AMOVA revealed greater genetic variation within the plantations rather than among the plantations. The UPGMA group indicated the formation of nine groups, four of which had one individual each. As for phenotypic characterization, individuals 48 and 52 stand out for having higher DBH, and individuals 30 and 34 presented higher CH. Considering DBH and CH concomitantly, 12 individuals are within the standards. In the evaluated plantations, there is sufficient variability for the identification of balsa wood matrices.

Keywords: Balsa wood; Genetic variability; Molecular markers; Genetic improvement

RESUMO

Este estudo avaliou plantações de pau-de-balsa (*Ochroma pyramidale*) em busca de matrizes para o melhoramento genético. Foi avaliado um total de 20 árvores em plantações no Mato Grosso quanto à diversidade genética com iniciadores ISSR (entre sequências simples repetidas), quanto ao seu diâmetro à altura do peito (DAP) e altura comercial (HC). Os *primers* amplificaram 111 locos (97,3% polimórficos), sendo que a diversidade genética de Nei (0,32) e o índice de Shannon (0,48) indicam que há diversidade genética nas plantações. A AMOVA revelou maior variação genética dentro das plantações do que entre as plantações. O agrupamento UPGMA indicou a formação de nove grupos, sendo quatro deles com um indivíduo cada. Quanto à caracterização fenotípica, os indivíduos 48 e 52 se destacam por apresentarem DAP mais elevado, e os indivíduos 30 e 34 apresentam HC superior. Considerando DAP e HC concomitantemente, 12 indivíduos estão dentro dos padrões. Nos plantios avaliados, há variabilidade suficiente para a identificação de matrizes de pau-de-balsa.

Palavras-chave: *Pau-de-balsa,* Variabilidade genética, Marcadores moleculares, Melhoramento genético

1 INTRODUCTION

Ochroma pyramidale (Cav. ex Lam. Urb.), popularly known as balsa wood, is a forest species with wide distribution in Central and South America, in countries such as Venezuela, Colombia, Ecuador, Peru, Bolivia, as well as in the Brazilian Amazon, in the states of Acre, Amazonas and Pará (MIDGLEY *et al*., 2010; MORANTE-ALARCÓN *et al*., 2017). The economic importance of the species is related to the traits of fast growth, light-colored wood, and its low density, which favors the manufacture of plywood used in the road transport, marine, railway, aerospace, defense, and wind energy industries (MIDGLEY *et al*., 2010; MARKET RESEARCH FUTURE, 2019).

The species has been cultivated in Ecuador and Papua New Guinea as an economic activity and, for some years, has been gaining importance in the state of Mato Grosso (REIS; PALUDZYSZYN FILHO, 2011). Commercial plantations in the state of Mato Grosso, Brazil are characterized by their high phenotypic variability, due to the use of seeds from the species of wide natural distribution area and the diversity of individuals used as matrices (WEIRICH *et al*., 2008; BEHLING *et al*., 2019), which generates heterogeneity in the standard of the trees and great discontent among the foresters due to difficulties in commercialization of the wood. According to Hamrick (2004) and Nybom (2004), in general, forest species with wide natural geographical distribution have high genetic variability that is distributed among and within populations. Therefore, knowledge of the genetic variability of populations is essential for breeding programs and conservation of the species.

The detection of genetic variability through molecular characterization of genetic markers or polymorphisms allows us to deduce the degree of diversity among and within populations (JU *et al*., 2018). Among the molecular markers available, ISSRs (*Inter Simple Sequence Repeats*) are important tools for the analysis of genetic diversity among and within populations of plant species. These markers are efficient because they have high reproducibility, are low cost and do not require prior knowledge of the genome of the species under study (COSTA *et al*., 2015).

To our knowledge, there currently no studies in the literature that report the genetic diversity and population structure of balsa wood. However, these studies are important, since, for the cultivation of a species, it is necessary to have a broad knowledge of the species' biological traits. As reported by Guimarães *et al*. (2019), and according to Costa *et al*. (2015), knowledge of the genetic diversity of native species assists in the identification of parent trees with greater genetic variability that can provide seeds for conservation and genetic improvement programs.

Despite the potential of balsa wood, there is currently no genetically improved material available for Brazilian environmental conditions. Thus, the present study aimed to estimate the genetic diversity and evaluate the commercial traits of balsa wood, from different origins, to select superior trees that may be used for the breeding of the species.

2 MATERIAL AND METHODS

The evaluated balsa wood trees (*Ochroma pyramidale* (Cav. ex Lam. Urb.) came from three plantations that have been cultivated since 2011 in Guarantã do Norte County Mato Grosso State (09º 47' 15" S, 54º 54' 36" W), Brazil. One plantation was cultivated by Embrapa Agrossilvipastoril at the Canavis nursery, and two plantations were cultivated by Compensados São Francisco Company at Sinuelo Farm and Aliança Farm. A total of 20 individual were selected from each plantation and listed sequentially, Sinuelo: 1 to 20; Cavanis: 21 to 40 and Aliança: 41 to 60.

In the Sinuelo and Cavanis plantations, the seeds came from other plantations in the north of the state of Mato Grosso, while, in the Aliança plantation, the seeds came from Ecuador. For the composition of the plantations, the seeds were acquired without selection criteria as there was no information on the number of matrices and the genetic basis.

For the analysis of genetic diversity young balsa wood leaves were obtained for DNA extraction in the Molecular Biology Laboratory of Embrapa Agrosilvipastoril, located in Sinop county, Mato Grosso, Brazil. The extraction was based on the protocol proposed by Zanetti *et al*. (2019a). DNA quantification was performed using a spectrophotometer (Nanodrop, Themo Fisher Scientific) and final concentrations were adjusted to 10 ng/µL.

PCR amplifications were performed using 15 ISSR *primers* developed by the University of British Columbia (UBC). The amplification reactions (PCR) were performed in a thermocycler (T100, BioRad), and the amplification steps were 4 min at 94 ºC (initial denaturation), 35 cycles of 30 seconds at 94ºC (denaturation); 35 seconds at the anneling temperature of each primer; 2 min at 72ºC (extension) and 7 min at 72 ºC (final extension). Each 15 µL of PCR reaction contained 1x PCR buffer (10 mM Tris-HCl, 50 mM KCL, 1.5 mM $MgCl_2$), 0.25 µM of each dNTP, 0.3 µM of each primer, 0.05 U of Taq DNA polymerase and Mili-Q® water to complete the final volume. The amplification products were separated by electrophoresis in agarose gel 1.5%, stained with GelRed (Biotium, Hayward, USA), using running buffer TAE 0.5 X, under a constant voltage of 60 V. The gels were visualized using a UV transilluminator (LTB-21x26, Loccus Biotechnology) and photo-documented.

The phenotypic characteristics sampled were commercial height (CH) and diameter at breast height (DBH). The CH is the distance between the soil surface and the base of the bifurcation or trifurcation of the main trunk of the tree and was measured in meters using a digital hypsometer (HAD IV, Vertex). The DBH is obtained by measuring the circumference at the height of the chest, 1.3 m from the ground, and dividing by π.

2.1 Statistical analysis

A binary matrix (0/1) was elaborated considering presence (1) and absence (0) of the amplified fragments. Descriptive analyses of the data, including total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphism (%P) and polymorphic information content (PIC) (ANDERSON *et al*., 1993), were carried out.

Genetic diversity was estimated using the Nei genetic diversity index (He) (NEI, 1978) and the Shannon diversity index (I) (LEWONTIN, 1972). We also estimated the number of observed alleles (Na), number of effective alleles (Ne), number of polymorphic loci (NPL), the percentage of polymorphism for each plant, as well as for the species, using the program POPGENE 1.32 (YEH *et al*., 2000).

The genetic comparison between pairs of individuals was estimated using the Jaccard coefficient. Subsequently, a cluster dendrogram based on these distances was constructed using the unweighted pair group method using arithmetic means (UPGMA) and Genes software (CRUZ, 2013). The molecular variance analysis (AMOVA) was used to infer the genetic structure of the plantations by means of total decomposition in the components among and within plantations, with the aid of the GenAIEx program (PEAKALL; SMOUSE, 2012).

Bayesian statistics were used to infer the number of groups or clusters (K) in which individuals are structured. The number of established groups was from K = 1 to K = 6 and the definition of the most likely K was performed according to the criteria proposed by Evanno *et al*. (2005), and for each K value, 20 runs were performed with 200,000 initial interactions ("burn-ins") and 500,000 Monte Carlo simulations via Markov Chains (MCMC). The analyses were performed using the Structure Harvester software, by Pritchard *et al* (2000).

3 RESULTS AND DISCUSSION

Molecular analysis showed the 15 ISSR *primers* amplified 111 fragments, of which 108 were polymorphic (97.3%). The number of polymorphic fragments ranged from 3 (DICA5'CR1) to 12 (UBC 811), with an average of 7.2 bands per primer. Except for the primers UBC 835 and UBC 866, all the others revealed 100% polymorphism (Table 1).

Table 1 – Initiators used, annealing temperature (ATºC) total number of amplified bands (TNB), number of polymorphic bands (NPB), percentage of polymorphism (%P) and polymorphic information content (PIC) for the three balsa wood plantations

Primer	AT (ºC)	TNB	NPB	%P	PIC
UBC 808 - DiAG3'C	48.8	9	9	100	0.72
UBC 809 - DiAG3'G	48.2	6	6	100	0.67
UBC 810 - DiGA3'T	42.9	8	8	100	0.71
UBC 811 - DiGA3'C	43.8	12	12	100	0.60
UBC 817 - DiCA3'A	50.3	8	8	100	0.85
UBC 825 - DiAC3'T	51.4	6	6	100	0.77
UBC 828 - DiTG3'A	51.3	9	9	100	0.87
UBC 835- DiAG3'YC	50.2	7	5	71.42	0.63
UBC 842 - DiGA3'YG	48.8	8	8	100	0.81
UBC 861 - TriACC	60.6	4	4	100	0.62
UBC 866 – TriCTC	55.7	7	6	85.71	0.84
UBC 889 - DiAC5'DBD	50.1	8	8	100	0.82
UBC 890 - DiGT5'VHV	52.2	8	8	100	0.78
DiCA5'CR	58.8	3	3	100	0.50
DiGA5'CR	50.2	8	8	100	0.35
Total		111	108	97.30	-
Average		7.4	7.2	-	0.70

Source: Authors (2021)

In where: UBC: University of British Columbia. *Y = (C or T); R = (A or G); V = (A, C or G); H = (A, C or T); D = (A, G or T) and B = (C, G or T).

The ISSR markers presented values for polymorphic information content (PIC) that ranged from 0.35 to 0.87, with a mean of 0.70. They are considered moderately informative (0.25 to 0.5) to highly informative markers of content (>0.5), according to the classification of Botstein *et al*. (1980). Thus, in the present study, it can be considered that the efficiency of the primers in indicating polymorphism between two individuals is high.

The percentage of polymorphism for each of the plantations, Sinuelo (78.38%), Cavanis (85.59%), and Aliança (81.98%) was lower than that estimated for the species (97.8%), due to a reduction in the number of polymorphic loci. This suggests the existence of alleles that are unique to the genetic material of individuals, and this may be associated with the different sources of the seeds contained in each plantation, since the Sinuelo, and Cavanis plantations use seeds from the Brazilian Amazon region, and the Aliança plantation uses seeds from Equador.

The presence of unique alleles in the plantations is considered an indication of restricted gene flow, which can lead to an increase in genetic divergence and indicates a certain differentiation among populations (plantations) (KAGEYAMA *et al*., 2003). The presence of unique alleles is an indication of genetic diversity that should be explored (ALVES *et al*., 2013), and exemplifies the high genetic diversity found in balsa wood plantations, which indicates that they are suitable for the selection of matrices for the improvement of the species.

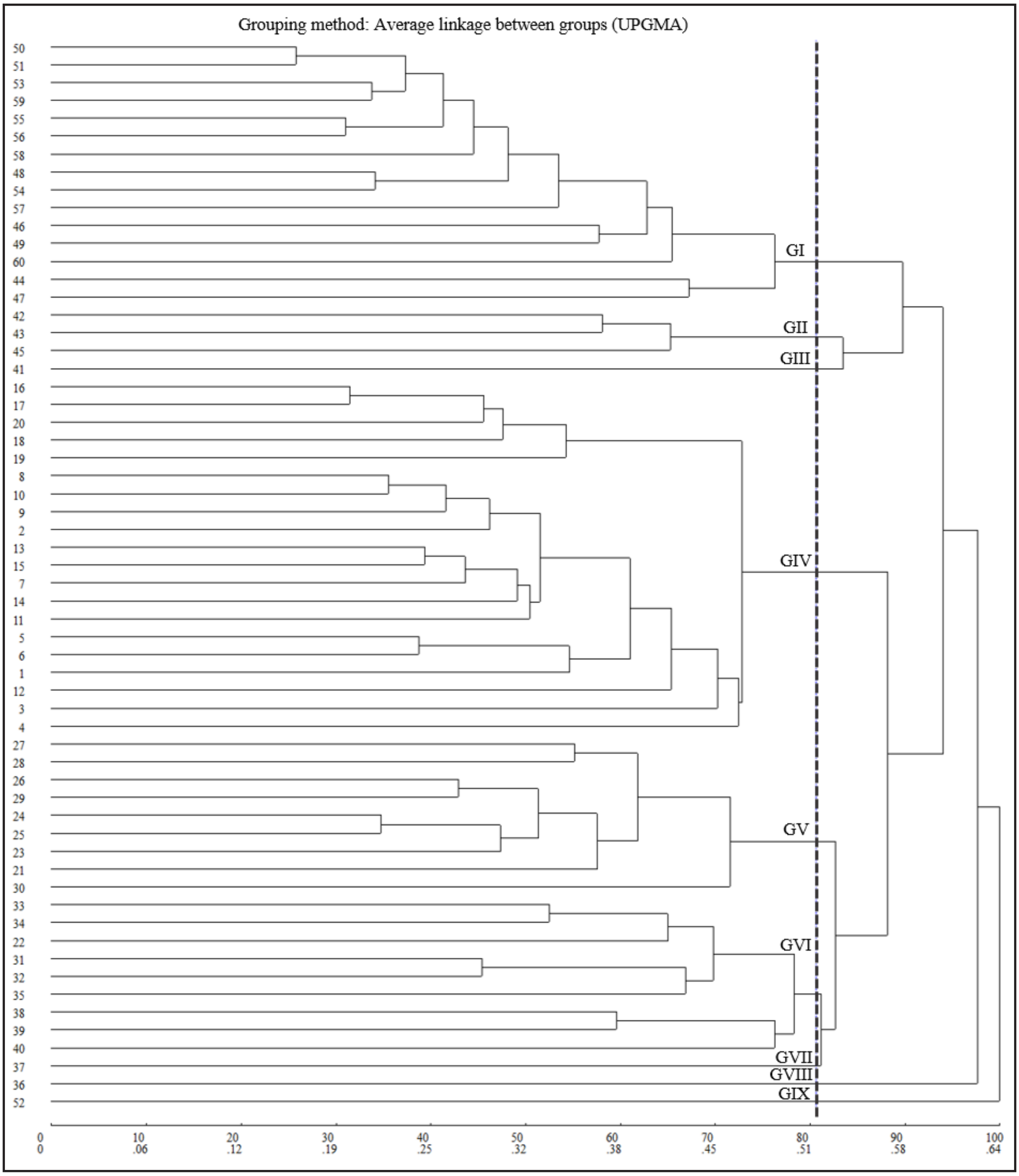
The Nei (He) genetic diversity index for balsa wood was 0.32. The number of observed alleles (Na) was 1.97, and the number of effective alleles (Ne) was 1.54. The Shannon index (I) reached the value of 0.48. The highest values for He and I were found in Cavanis (0.26 and 0.40), followed by Aliança (0.25 and 0.38) and the lowest were for Sinuelo (0.24 and 0.37).

The values found for He and I indicate that there is considerable genetic diversity in the plantations and in the balsa wood species. Similar results were observed for other tree species in commercial plantations. Giustina *et al*. (2017) found genetic diversity in candidate and neighboring trees of *Tectona grandis* (teak), with values of He from 0.15 to 0.16, and for I from 0.21 to 0.23. Silva Júnior *et al*. (2017) found high genetic diversity, He of 0.375 and I of 0.554, in forest area of *Schizolobium parahyba* var. *Amazonicum* (Brazilian firetree)

Genetic dissimilarity among individuals of balsa wood ranged from 0.17 to 0.79, which did not show identical individuals in genetic terms. The smallest genetic distance was between individuals 50 and 51, which belong to the Aliança plantation with its seeds from Ecuador, while the largest genetic distance was between individuals 40 (Cavanis) and 57 (Aliança), which have Brazilian and Ecuadorian origins, respectively. As little is known about the genetic variability of balsa wood, it is necessary to make comparisons with other similar species. Giustina *et al*. (2017) identified dissimilarity values between 0 and 0.26 in commercial teak plantations, and Silva Júnior *et al*. (2017) found dissimilarity values between 0.04 and 0.8 in Brazilian firetree forests.

From the dissimilarity matrix, the grouping obtained using the UPGMA method revealed the formation of nine groups (Figure 1). Group IV (GIV) is the most representative since it is composed of twenty of the sixty individuals evaluated, which belong to the Sinuelo plantation and has a Brazilian origin. Group I (GI) was composed of fifteen individuals from the Aliança plantation, with seeds from Ecuador. In this group, the two most similar individuals (50 and 51) were allocated. In Group II (GII) there are 3 individuals allocated (42, 43 and 45) that also belong to Aliança (Ecuadorian origin) and are probably the most dissimilar individuals within this plantation. Groups V (GV) and VI (GVI) are represented by nine individuals each; the two groups being composed of individuals from the Cavanis plantation (Brazilian origin). Groups III (GIII), VII (GVII), VIII (GVIII) and IX (GIX) had only one individual each, 41, 37, 36 and 52, respectively, being 36 and 37 of Brazilian origin, and 41 and 52 of Ecuadorian origin.

Figure 1 – Dendrogram obtained using the UPGMA method generated from the arithmetic complement of the Jaccard index as a measure of dissimilarity, in 60 balsa wood individuals, based on ISSR (Inter Simple Sequence Repeats) markers



Source: Authors (2021)

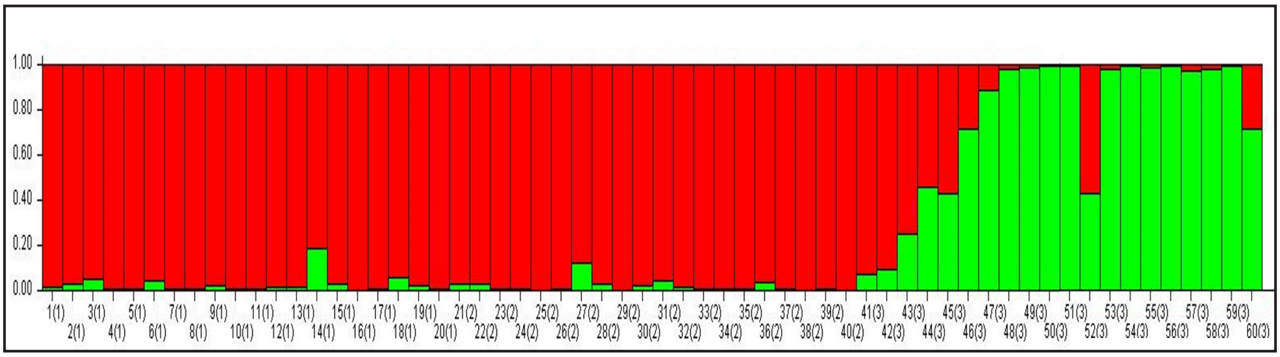
In where: UPGMA is Unweighted Pair-Group Method Average; The cutoff point of 80.6% (0.5203) was established using the Mojena procedure (1977)

There was significant genetic differentiation ($p<0.001$) among the plantations, and the largest component of variation was within plantations (71%). High genetic differentiation was found among the plantations (F_{ST} =0.29), since, according to Wright (1978), F_{ST} above 0.25 indicates very high genetic differentiation. These results suggest that there is variability to be explored among and within balsa wood plantations.

The results found are in accordance with population analyses carried out in other species, considering that the balsa wood has cross-fertilization. The studies of Sun *et al*. (2006), with RAPD and ISSR markers proved that cross-fertilized species have low levels of diversity among populations and high differentiation within populations. The low levels of genetic diversity among populations of tree species are generally due to their characteristics, i.e., life cycle, high population density, predominance of crossbreeding, and pollen and seed dispersal mechanisms (KAGEYAMA *et al*., 2003; SOUZA *et al*., 2004).

Bayesian analysis was employed to determine the genetic structure of balsa wood plantations and indicated the formation of two groups (K=2) (Figure 2). Group I (red) gathered most of the individuals, and corresponds to the Sinuelo and Cavanis plantations, which use seeds originally from the Brazilian Amazon, and Group II (green) gathered mainly the seeds from Ecuador.

Figure 2 – Genetic structure of 60 balsa wood individuals, assuming K = 2 (groups) according to the Structure program



Source: Authors (2021)

In where: *The vertical lines on the X axis represent the individuals and the colored segments on the Y axis demonstrate the association coefficient of each individual assigned to each of the inferred K.

On average, the trees presented 7.3 m of commercial height (CH), ranging from 3.6 m to 12.7 m. The average diameter at breast height (DBH) was 21.95 cm, with great variation between the minimum (16.01 cm) and the maximum (40.11 cm). As such, this confirms the high phenotypic variability among individuals (Table 2).

For selection purposes, the DBH of the species should vary between 15 and 40 cm, and the CH should be multiples of 1.8 and 2.4 m, this is due to the size of the lathe for wood processing and the dimensions of the plywood sheets and increases the efficiency in wood processing. Regarding the DBH, all trees are of the desired standard, with emphasis on individuals 48 and 52 (Ecuador). While for CH, trees that have values equal to or greater than the average added to the standard deviation, allow the factories to obtain at least five logs of 1.8 m, or three logs of 2.4 m, or two logs of 2.4 m and two of 1.8 m, as mentioned by Zanetti *et al*. (2019b). In this study, the individual 30 allocated to Group V (Cavanis plantation with seeds of Brazilian origin) has a higher CH (12.7 m). In general, this plantation has trees with higher CH (on average 8.45 m), important attribute commercial.

In relation to the two commercial attributes evaluated, twelve individuals stand out, nine from Brazil (Sinuelo plantation: 3, 17, 20, and Cavanis plantation: 23, 28, 30, 34, 35, 40) and three from Ecuador (Aliança plantation: 45, 46, 52). In other words, all these individuals are considered desirable for use as matrices, both for crossbreeding aimed at genetic improvement and to produce clones of the species.

Combining molecular results and phenotypic information, individual 52 (Ecuadorian) stands out. It is worth noting that DBH has a higher calculation of the trading volume than the height (length of the log). Therefore, tree 52 is superior among all those evaluated, with 39 cm of DBH and 10.6 m of CH. Since it remained in a single/isolated group in the analysis of genetic dissimilarity, it may present favorable alleles for the characteristics of economic importance in plantations that seek wood production, due to its growth of stem diameter and commercial height, and the collection of seeds for use in genetic improvement programs of the species is recommended.

Table 2 – Phenotypic characterization of the 60 individuals (I) of balsa wood regarding DBH (diameter at breast height) and CH (commercial height)

I (Sinuelo)	DBH	CH	I (Cavanis)	DBH	CH	I (Aliança)	DBH	CH
1	27.06	7.50	21	17.83	5.90	41	17.83	4.10
2	22.28	7.80	22	18.46	7.00	42	22.44	4.50
3	21.65	11.30	23	24.92	10.90	43	16.01	3.60
4	22.60	8.50	24	21.39	4.50	44	19.35	7.80
5	24.83	8.60	25	24.51	8.20	45	16.04	11.50
6	29.60	6.70	26	18.84	7.40	46	19.10	11.00
7	22.28	5.10	27	22.28	6.70	47	19.13	7.10
8	20.05	5.80	28	20.50	10.10	48	40.11	5.40
9	26.10	4.80	29	19.77	9.30	49	22.50	4.30
10	20.21	4.60	30	17.22	12.70	50	22.47	4.70
11	25.78	4.60	31	16.62	8.10	51	22.57	4.80
12	22.92	6.70	32	17.54	6.90	52	39.15	10.60
13	22.92	6.40	33	17.00	8.10	53	25.62	4.40
14	20.37	5.80	34	23.27	11.60	54	25.46	5.90
15	21.65	7.50	35	17.51	9.70	55	19.19	10.40
16	20.05	7.20	36	18.84	8.80	56	22.57	4.30
17	20.37	10.10	37	17.89	8.60	57	25.78	4.00
18	20.05	6.00	38	16.27	8.00	58	20.37	6.90
19	19.48	5.30	39	20.34	6.90	59	25.78	8.30
20	28.97	10.90	40	19.77	9.70	60	25.46	4.30
Average	22.96 (3.09)	7.06 (2.01)		19.54 (2.59)	8.46 (1.98)		23.35 (6.39)	6,40 (2.65)
General average DBH			21.95 (4.62)		General average CH		7.30 (2.36)	

Source: Authors (2021)

In where: *Standard deviation in parentheses.

The success of a forest improvement program, according to Silva *et al*. (2018) and Miranda *et al*. (2019), depends on genetic variability, which allows the manipulation and selection of superior genotypes, selection of origin and individual selection within base populations, thus combining desirable characteristics and maintaining genetic variability.

4 CONCLUSIONS

The balsa wood plantations evaluated in this study present genetic and phenotypic diversity and, as such, it is possible to explore such populations to obtain parent plants that possess the necessary characteristics required for genetic improvement of the species regarding Brazilian plantation conditions.

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