

Genetic diversity of bushy cashew (*Anacardium humile* A. St.-Hil.) based on characteristics of fruits

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Abstract - Knowledge of the genetic variability among genotypes of bushy cashew is important to establish strategies for pre-breeding and use in breeding program. The objective of this study was to evaluate the genetic diversity of bushy cashew by means of the evaluation of physical, chemical and physicochemical characteristics of cashew apples and cashew nuts. The work was carried out with cashew nuts and cashew apples collected in the harvest of 2016 of genotypes in the “*ex situ*” biological collection of *Anacardium humile* A. St.-Hil. in the area of genetic resources of the Universidade Federal de Goiás - Regional Jataí. The results were submitted to descriptive analysis and multivariate analysis, estimating the mean Euclidean distance obtained from the fruiting attributes analyzed. The measure of similarity and clustering of the provenances was done by the optimization algorithm of Tocher and dendrograma UPGMA. There was a coincidence of clusters, which confirms the distribution of genetic diversity of the genotypes in the germplasm bank of *Anacardium humile* A. St.-Hil. evaluated. The results obtained can be used for the selection of individuals of the species in future works of breeding and conservation of the specie.

Index Terms: Anacardiaceae, germoplasm bank, multivariate analysis.

Diversidade genética de Cajuzinho-do-cerrado (*Anacardium humile* A. St.-Hil.) com base nas características dos frutos

Resumo - O conhecimento da variabilidade genética entre genótipos de Cajuzinho-do-cerrado é importante para estabelecer estratégias de pré-melhoramento e uso em programa de melhoramento. Objetivou-se com este trabalho avaliar a diversidade genética de Cajuzinho-do-cerrado por meio da avaliação de características físicas, químicas e físico-química de frutos e pseudofrutos. O trabalho foi realizado com frutos e pseudofrutos coletados na safra de 2016 dos genótipos da coleção biológica “*ex situ*” de *Anacardium humile* A. St.-Hil. na área de recursos genéticos da Universidade Federal Goiás - Regional Jataí. Os resultados foram submetidos à análise descritiva e à análise multivariada estimando-se a distância euclidiana média obtida a partir dos atributos da frutificação analisados. A medida de similaridade e agrupamento das procedências foi feita pelo algoritmo de otimização de Tocher e dendrograma UPGMA. Houve coincidências entre os agrupamentos, o que confirma a distribuição da diversidade genética dos genótipos no banco de germoplasma de *Anacardium humile* A. St.-Hil. avaliado. Os resultados obtidos podem ser utilizados para a seleção de indivíduos da espécie em futuros trabalhos de melhoramento e conservação da espécie.

Termos para indexação: Anacardiaceae, banco de germoplasma, análise multivariada.

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Brazil stands out as one of the main centers of genetic diversity of wild fruit trees in the world (SANTOS & SANTOS JUNIOR, 2015), being the Brazilian Savannah a ecosystem in renewable natural resources, with fruit species with peculiar characteristics (AVIDOS & FERREIRA, 2000; MORZELLE, 2015). Considering that native species are well adapted to the local environment, low impact management for seeds collection coupled with the development and proper cultivation practices could bring economic, social and environmental benefits (SOUZA et al., 2018).

Among the innumerable native fruit trees that have high potential for commercial exploitation, it could be emphasized the bushy cashew (*Anacardium humile* A. St.-Hil.). This specie is a source of energy in food and medicinal use, but is at risk of extinction due to the impact caused by the fragmentation of their populations, either by inadequate extraction or by the expansion of agricultural frontiers (SILVA et al., 2001).

The identification and correct characterization of the genetic diversity of a species is the initial step for its subsequent use in a plant breeding program, which may result in economic gains (DANNER et al., 2011; DANTAS et al., 2012). The conservation of the diversity being is initial points in the domestication of vegetables (DANTAS et al., 2012).

Several methods can be used in the evaluation of genetic diversity, whose choice is based on the precision desired by the researcher, the way the data were obtained and the ease of analysis (RODRIGUES et al., 2010). The techniques of multivariate analysis have been routinely used because they simultaneously consider the evaluated characteristics of the genotypes, in addition to the correlation between them (CONDÉ et al., 2010). Among the multivariate statistical techniques, the principal component analyzes, canonical variables and agglomerative methods (CRUZ et al., 2012).

The volume of studies still incipient on the species opens possibilities for research in order to know its needs to plan future strategies of preservation and use of the specie. Therefore, the objective of this study was to evaluate the genetic diversity between genotypes based on the physical, chemical and physico-chemical characteristics of cashew apple and cashew nut of bushy cashew.

The study was conducted using material obtained from the Biological Collection “*ex situ*” of *Anacardium humile* A. St.-Hil, in the area of Genetic Resources of the Universidade Federal de Goiás - Regional Jataí. This experimental area has tropical climate classification of savannah (Aw), with rainy period from october to april and dry from may to september. The annual temperature ranges from 13°C to 32°C and the annual rainfall of 1700 mm.

Cashew apples and cashew nuts were harvested from 27 genotypes. From each genotype were collected from 5 to 15 units, according to the production. One of the

criteria for harvesting was the coloring of the epidermis of the cashew apple, which should be totally yellow or totally red in color, that is, completely mature cashew apple.

After harvesting were taken to the laboratory and analyzed for: cashew apple mass (AM) and cashew nut (NM) mass in digital scale, expressed in grams; cashew apple length (AL), cashew apple base diameter (ABD), cashew apple apex diameter (AAD), cashew nut length (NL), cashew nut width (NW), cashew nut thickness (NT) being determined in millimeters using a pachymeter. The color of the epidermis of the cashew apple (measured in the equatorial region of one of the faces of the cashew apple) was given by the coordinate C* (CEA) and angle hue or h° (hEA), and pulp through, also, by the coordinate C* (CPA) and h° (hPA) determined using a colorimeter Konica Minolta, model CR-10. This equipment measures reflected light, using a Cartesian coordinate system L*, a* and b*, of which units or points of approximate visual uniformity are obtained (KONICA MINOLTA, 2018).

The cashew apple were also analyzed for soluble solids content (SS) using a portable refractometer, expressed in °Brix; to the titratable acidity (TA) by titrations under stirring with 0.1 N NaOH solution using 1% phenolphthalein as indicator, expressed in % of citric acid; soluble solids ratio and acidity (*ratio*) was obtained by dividing the soluble solids values by the titratable acidity content, and the vitamin C (VIT C) determined by titration with Tillman’s reagent [2,6 dichloropheno-indofenol (sodium salt) to 0,1%] (AOAC, 2016), expressed in mg of ascorbic acid/ 100g of pulp and carotenoids (CRT) which were extracted with acetone and analyzed in a refractometer, the absorbances were determined in 470, 646,8 e 663,2 nm and the levels of carotenoids determined according to the equations of Lichtenthaler (1987) and expressed in %.

The results were submitted to descriptive analysis, obtaining mean, minimum, maximum and coefficient of variation (CV) (%); and relative importance of the characters for total variation (S.j) (%), using the criterion proposed by Singh (1981).

It was also used to the multivariate analysis by estimating the mean Euclidean distance obtained from the 27 bushy cashew genotypes, evaluated on the basis of the fruiting attributes analyzed. The measure of similarity and clustering of the accessions was made by the algorithm of optimization of Tocher and dendrogram UPGMA (Unweighted Pair Group Method using Arithmetic averages) (CRUZ et al., 2012).

The cut off criterion used to determine the number of groups in the UPGMA method was based on the relative size of the 27 levels of fusions (MOJENA, 1977). The cophenetic correlation coefficient (CCC) was calculated between the genetic dissimilarity matrix and the matrix of the cophenetic values, in order to verify the consistency of the cluster.

Data analyzes were performed with the aid of the GENES computer program (CRUZ, 2013). The dendrogram was made with the aid of software Statistic, versão 5.0.

The variables measured to determine the diversity contained in the accesses of the germplasm bank obtained coefficient of variation values ranging from 8.969 to 50.258% (Table 1), indicating that these variables present different degrees of heterogeneity. There was no discrepancy between the values of the relative contribution of the variables to genetic dissimilarity, was relatively well distributed, varying from 2.998 to 9.431%. The soluble solids content (9.431%), cashew apple length (8.508%) and the C* coordinate of the epidermis of the cashew apple (8.337%) were the variables that contributed most to total variation (or genetic dissimilarity).

According to Cruz et al. (2012), the relative importance of the characters helps in discarding variables, enabling a better choice of attributes to be considered in a genetic divergence assessment. However, in the present work it is not possible to discard variables, since both have S.j (%) with close values.

The UPGMA dendrogram (Figure 1) discriminated six groups among the 27 genotypes studied, showing access 1 allocated separately, while the other genotypes were divided into the other groups. This difference between access 1 and the others can also be observed in the Tocher cluster (Table 2), which generated two groups, where genotype 01 occupied one group while the others grouped together.

The groups of UPGMA were divided by the cut off point of 0.293, which equals 55% of the distance, forming six groups. The clustering provided by this method was adequate for the representation of the genetic divergence and group formation between the accesses used for presenting co-expressed correlation (CCC) equal to 0.81. This coefficient measures the fit between the dissimilarity matrix and the simplification matrix, due to the clustering method and can be used to increase the reliability of the conclusions regarding the interpretation of dendrograms (CRUZ et al., 2012).

For Monteiro et al. (2010), the closer to the unit is the cophenetic correlation coefficient, the better the dissimilarity matrix representation in the dendrogram form. This makes it possible to make inferences through the analyzed variables and revealing a good fit between the graphic representation of the distances and their original matrix, reinforcing the reliability of the results. CCC values above 0.80 indicate good representativity between distances (CRUZ et al., 2011).

In order to better verify the diversity by the Tocher method, access 1 was removed and a new clustering was done (Table 3), where six groups were formed. The access 1 was mainly differentiated in characters related to coloration, where it had reddish epidermis (hEA= 9.70),

while the others had a yellowish coloration (hEA : 9.70); and the larger dimensions of cashew nut (NL: 22.70 mm, NW: 22.63 mm, NT: 13.70 mm and NM: 3.57 g). They are group 1 that contains 14 genotypes (05, 15, 26, 10, 19, 21, 13, 23, 07, 11, 14, 04, 16, 09) that, did not present higher mean values in relation to the other groups in any of the characters, corresponds to a genotype of group 2 and the highest part of the genotypes that make group 3 of UPGMA, with the exception of genotypes 08 and 18, which together with genotype 03 (08, 18, 03) of group 4 of UPGMA set group 2 stood out characters *ratio* (11.33 - 13.78) and NL (13.67 - 17.60 mm); group 3 (20, 25, 17) corresponds to group 5 of UPGMA and stood out in the mean values of the variables AL (32.04 - 37.92 mm) and CRT (0.20 - 0.48%); group 4 with SS (12.40 - 13.00° Brix) and VITC (102.84 - 279.64 mg of ascorbic acid / 100g of pulp) in contrast and results from two genotypes present in group 4 of UPGMA (12, 22); group 5 presented higher in the mean values of the AT (1.41 - 1.64 %) results from two genotypes from group 2 of the UPGMA (02, 06); and group 6 corresponds to group 6 of the UPGMA (24, 27), which presented larger dimensions of cashew apple (ABD: 20.80 - 21.10 mm, AAD: 31.82 - 33.95 mm and AM: 12.16 - 18.78 g) and cashew nut (NW: 15.31 - 16.50 mm, NT: 12.79 - 12.93 mm and NM: 1.84 - 2.75 g).

Vasconcelos et al. (2007) emphasize that the Tocher method presents the peculiarity of gathering a greater number of genotypes in the first groups and generally individuals in isolation in the last groups. In this type of study, this characteristic of the method becomes interesting, since it allows identifying genetically dissimilar individuals and not only groups.

The coincidence of clusters confirms the distribution of the genetic diversity of the genotypes in the germplasm bank evaluated. However, it shows that the genotypes grouped in the same group have similar characteristics among them, being able to later select for maintenance and / or future use only some genotypes that prove to be more productive and / or based on molecular analyzes.

This study showed genetic variability within the studied population of *Anacardium humile* A. St.-Hil. The results obtained can be used for the selection of individuals of the species in future works of breeding and conservation of the species.

Table 1- Average, minimum, maximum, coefficient of variation (CV) and relative contribution (Sj) of the traits, for the dissimilarity of the genotypes of bushy cashew

Traits	Average	minimum	maximum	CV (%)	S. j (%)
AL	24.432	16.505	37.918	24.646	8.508
ABD	15.208	9.424	21.105	18.385	6.165
AAD	24.345	17.578	33.949	16.221	6.261
AM	7.345	2.179	18.779	50.258	5.468
NL	15.465	11.990	22.700	13.190	3.903
NW	14.583	11.513	22.627	14.084	3.674
NT	11.437	9.349	13.692	8.969	6.003
NM	1.419	0.626	3.575	42.141	4.421
SS	9.027	5.287	13.000	25.297	9.431
AT	1.137	0.657	1.652	22.241	6.943
<i>ratio</i>	8.173	3.877	13.78	33.340	8.146
VITC	137.971	74.086	279.643	33.214	5.347
CRT	0.235	0.103	0.479	33.298	4.667
CEA	39.962	30.333	49.233	13.165	8.337
hEA	82.060	9.700	101.067	22.267	4.303
CPA	27.693	10.367	43.000	26.463	5.426
hPA	98.668	49.633	114.033	10.896	2.998

AL: cashew apple length (mm); ABD: cashew apple base diameter (mm); AAD: cashew apple apex diameter (mm); AM: cashew apple mass (g); NL: cashew nut length (mm); NW: cashew nut width (mm); NT: cashew nut thickness (mm); NM: cashew nut mass (g); SS: content soluble solids of the cashew apple (%); TA: content titratable acidity of the cashew apple (% citric acid); *ratio*: soluble solids / titratable acidity; VITC: content vitamin C of the cashew apple (mg of ascorbic acid / 100g of pulp); CRT: content carotenoids of the cashew apple (%); CEA: C * coordinate of the cashew apple epidermis; hEA: hue angle of the epidermis of the cashew apple (h°); CPA: coordinate C * of the pulp of the cashew apple; hPA: hue angle of the pulp of the cashew apple (h°).

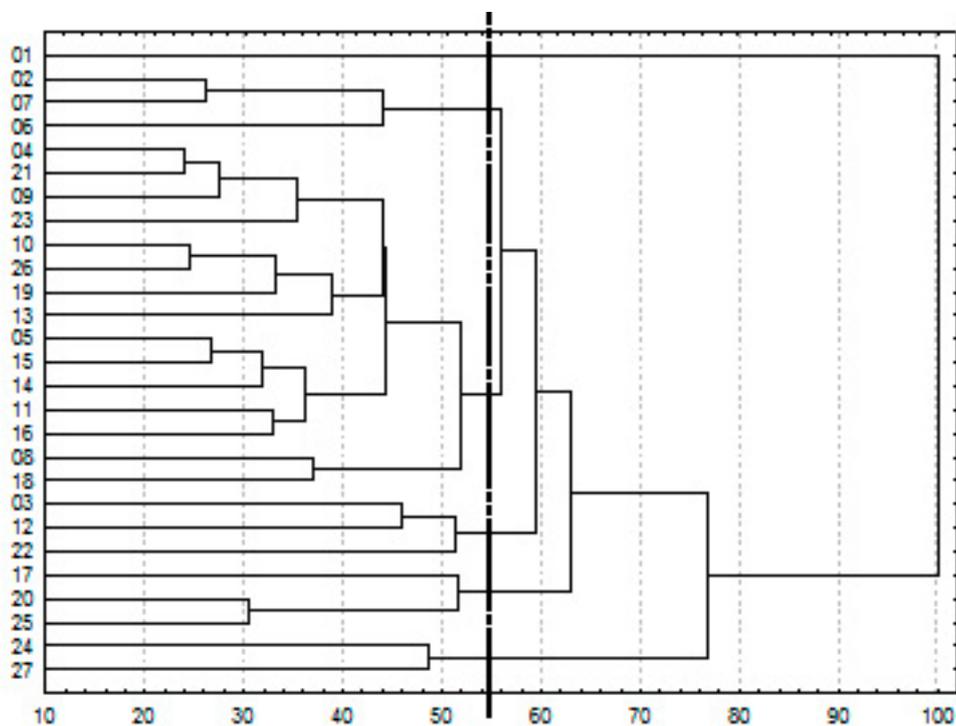


Figure 1- Dendrogram generated by the UPGMA method from dissimilarities expressed by mean Euclidean distance between 27 genotypes of bushy cashew, from 17 cashew nut and cashew apple traits. Coefficient of Cophenetic Correlation (CCC): 0.81

Table 2. Groups established by the Tocher method, based on the 17 traits for 27 genotypes of bushy cashew

Group	Genotype
	04 21 9 23 05 15 26 19 11 10 13 07
1	16 14 08 02 18 25 12 03 20 22 17
	27 06 24
2	01

Table 3. Groups established by the Tocher method, based on the 17 traits for 26 genotypes of bushy cashew

Group	Genotype
1	05 15 26 10 19 21 13 23 07 11 14 04
	16 09
2	08 18 03
3	20 25 17
4	12 22
5	02 06
6	24 27

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