

Morphological characterization and genetic divergence of a cashew population in Floriano, Piauí, Brazil

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ABSTRACT. *Anacardium occidentale* (cashew) is a tropical tree species that occurs mainly in northeastern Brazil. The species has great socioeconomic importance for rural populations. We investigated the genetic diversity of a natural cashew population in a rural area of Floriano, State of Piauí, Brazil. We quantitatively evaluated 12 natural variables related to the inflorescence, the cashew nut (fruit), and the peduncle (pseudofruit) in 35 trees (numbered sequentially G1-35). The characteristics of the peduncle (pseudofruit) appeared as the variables that most contributed to genetic divergence, while characteristics related to the inflorescence contributed less to the morphological differentiation. Multivariate statistics, employing principal component analysis evidenced that the first three principal components explained 81.72% of the total variance. Cashews G-3

and G-8 were identified as the most divergent, with G-3 demonstrating the greatest peduncle lengths and weights.

Key words: *Anacardium occidentale*; Multivariate analysis; Genetic diversity

INTRODUCTION

The cashew tree (genus *Anacardium*) is native to Brazil and demonstrates wide diversity in various biomes in that country; it occurs mainly in Northeast Brazil (Bitu et al., 2015). Taxonomists recognize 11 species in the genus, of which eight are encountered in Brazil (Vieira et al., 2014).

Anacardium species have significant economic importance throughout the world, principally because of two of its natural products: the cashew nut itself (botanically, the fruit) and the pseudofruit (which is actually an expanded and fleshy peduncle) (Schweigert et al., 2016). Brazil is one of the largest producers of cashew nuts, with 109.68 thousand tons, also standing out in the production of the pseudofruit, with 1,805 thousand tons (FAO, 2016).

Biological compounds such as flavonoids, carotenoids, and vitamins (C and A) are also present in the fruit (Souza et al., 2017). In Brazil, the peduncle is consumed fresh, although approximately 10% of its production is processed into vitamin-rich juices, jellies, alcoholic beverages, and sweets. In addition, cashew extracts have been used as alternative medicines because they have anesthetic and bactericidal properties (Kubo et al., 2011; Kulis et al., 2012; Silva et al., 2013).

It is important to note the importance of plant genetic resources to cashew biodiversity studies – especially those biodiversity components that demonstrate current or potential utility for humans. Genetic resource distributions reflect combinations of both the natural and anthropic processes that transform ecosystems (Govindaraj et al., 2015).

The use of morphological descriptors stands out as one of the main instruments for the characterization and quantification of genetic diversity in cashew trees. This characterization has been carried out through a set of botanical, morphological and agronomic descriptors, which are related mainly to inflorescence, fruit and pseudofruit. In that sense, plant genetic resource conservation is a theme of enormous relevance, and those studies are largely based on analyses of genetic diversity (Thimmappaiah et al., 2009; Cota et al., 2017; Borges et al., 2018).

Biometric characterization consists in evaluating morphological traits of different parts of the plant, such as fruits and seeds (Zuffo et al., 2019). One of the tools that aids the determination of genetic divergence of a species is biometric analysis (Costa et al., 2016; Menegatti et al., 2017; Santos et al., 2018). In this context, biometrics is considered an important tool for identification as a source of information for studies of genetic diversity (Zuffo et al., 2016)

Genetic diversity in evaluations depend on the information available to researchers and can be estimated from phenotypic genetic markers. Research projects designed to quantify and evaluate the magnitude of genetic variability in cashews have been undertaken by researchers such as Santos and Santos Júnior (2015) and Pereira et al. (2016).

The purpose of this study was to characterize the genetic diversity of natural cashew populations by phenotypic markers, to generate information that could subsidize the preservation and conservation of the genetic resources of this important fruit tree.

MATERIAL AND METHODS

We evaluated 35 adult cashew trees from a natural population, located in the rural zone of Floriano, Piauí State, Brazil ($-6^{\circ}58'36.4''$ S x $-43^{\circ}05'26.7''$ W). The plants were selected according to the availability of flowers, fruits and pseudofruits. Data relative to their morphological characterizations were collected during the reproductive phases (flowering and fruiting). Field expeditions to collect inflorescences, fruits, and pseudofruits were undertaken in August and October/2017. The plants evaluated were identified as G-1 through G-35, based simply on their collection order.

After collection, the materials were transported to the Biology Laboratory of the *Campus* Amílcar Ferreira Sobral at Floriano, Piauí, for genetic characterization based on multivariate analyses that considered 12 quantitative descriptors of the inflorescences, peduncles, and cashew nuts, as described by the International Board for Plant Genetic Resources –IBPGR (now Biodiversity International; IBPGR, 1986).

The evaluated variables are described in Table 1.

Table 1. Twelve morphological descriptors selected from the list of cashew descriptors published by IBPGR (now Biodiversity International; IBPGR, 1986).

Morphological descriptors	Description
Inflorescence length	Random measurements during the most intense flowering period.
Maximum inflorescence width	The maximum distance between the inflorescence branches
Total number of inflorescence ramifications	The numbers of inflorescences in relation to the principal axis
Peduncle length	Distance from the apex to the base
Peduncle diameter	Measured at the peduncle base
peduncle base diameter	Measured at the peduncle base
Peduncle apex diameter	Measured at the peduncle apex
Peduncle weight	Mean weight of 10 randomly collected peduncles
Cashew nut length	Distance from the fixation point to the apex of the cashew nut
Cashew nut width	Maximum distance between the shoulders of the cashew nut
Cashew nut thickness	Maximum distance between the two sides of the cashew nut
Cashew nut weight	The mean weight of 10 randomly collected cashew nuts

The measures of inflorescence length and width were made using a millimeter scaled rule; the measures of peduncle and cashew nut lengths, widths, thicknesses and diameters were made using digital calipers (and expressed in millimeters); measurements of mass were made using a precision balance (and expressed in grams).

The physical measurements used 10 specimens of the inflorescences, fruits, and peduncles from each tree examined. Repeatability studies have established that 10 measurements per individual for each characteristic resulted in reliabilities $\geq 90\%$ (Costa et al., 2016).

The morphological data were submitted to multivariate analyses based on Tocher's methods of grouping optimization, the UPGMA hierarchical method, and Principal Component Analysis (PCA). The analyses were processed using the Genes version 2013.5.1 software.

RESULTS AND DISCUSSION

The phenotypic characters examined generally exhibited significant variations, principally those related to the cashew nuts and pseudofruits (peduncles). The phenotypic variance is influenced by components of uncontrolled environments, by the anthropic condition, by the soil, by the climate, by the age of the plants and by the isolation of the genetic species among the individuals (Costa et al., 2015).

The characters inflorescence lengths varied from 6 to 18 cm; the maximum inflorescence width varied from 8 to 25 cm; and the total number of inflorescence ramifications varied from 7 to 12. Peduncle weights varied from 6.62 to 27.79 g; peduncle lengths varied from 21.63 to 76.79 mm; peduncle diameters varied from 14.42 to 98.48 mm; peduncle base diameters varied from 11.4 to 46.22 mm; peduncle apex diameters varied from 11.72 to 49.13 mm; and cashew nut weights varied between 1.4 and 8.5 g.

A matrix of mean Euclidian distances was used to analyze the genetic diversity of all cashew pairs, yielding a value of 1.32. The analyses of distance estimates indicated genetic dissimilarities, which were corroborated by the maximum and minimum Euclidian distances. The minimum Euclidian distance was 0.32 between cashew trees G-24 and G-25; the maximum estimated Euclidian distance (2.84) was between cashew trees G-3 and G-8.

Divergences were observed in the 12 characters analyzed using the methodology described by Singh (1981), with the variable "peduncle length" contributing most to the observed divergence (28.85%), as can be seen in table 2. Zuffo (2018) in his study characterized pseudofruit, fruits and seeds of *Anacardium humble* and observed large biometric variations, as well as significant and positive correlation between the characteristics analyzed. Jena et al. (2016) evaluated genetic diversity among 12 promising Indian cashew cultivars using morphometrics; a high degree of variation was observed among cultivars with respect to inflorescence length, peduncle weight and nut weight.

Table 2. Relative contributions of the 12 characters measured to the genetic divergence among the cashew trees studied, based on the morphological descriptors and using the methodology described by Singh (1981).

Character	S _j	(%)
Inflorescence length	503.11	0.18
Maximum inflorescence width	703.9	0.25
Total number of inflorescence ramifications	115.46	0.04
Peduncle length	80914.37	28.85
Peduncle diameter	28278.54	10.08
Peduncle base diameter	24376.14	8.67
Peduncle apex diameter	30575.36	10.9
Peduncle weight	60124.53	21.44
Cashew nut length	18564.26	6.69
Cashew nut width	8566.06	3.05
Cashew nut thickness	10724.90	3.82
Cashew nut weight	17040.28	6.07

S_j: value of the relative contribution according to SINGH; (%): value in percentage.

The Tocher optimization algorithm separated the cashews into four groups considering the 12 characters analyzed, with an intergroup distance of 2.23. Group I comprised 80.01% of the plants, with an intragroup distance of 1.51. Groups II, III and IV comprised 5.71%, 8.57% and 5.71% of the cashews evaluated respectively (Table 3). Tocher grouping discriminated the individuals genetically, allowing the formation of groups showing internal homogeneity, but heterogeneity between groups.

Table 3. Grouping of the 35 cashew plants, obtained using the Tocher optimization method considering 12 morphological variables.

Groups	Cashew trees evaluated
I	G-24, G-25, G-5, G-31, G-32, G-33, G-34, G-15, G-17, G-7, G-28, G-18, G-35, G-26, G-12, G-13, G-22, G-6, G-27, G-1, G-20, G-4, G-23, G-16, G-19, G-21, G-10, G-29
II	G-2, G-11
III	G-8, G-9, G-14
IV	G-3, G-30

One possible explanation for that grouping could be human influences resulting from the introduction of cashew plants (as the samples were collected from rural space the observed phenotypic variations may reflect differences in the local environment/or human selection).

The results of the grouping analysis, based on the 12 morphological descriptors, were revealed through cophenetic correlation coefficients (ccc). According to Rohlf (1970), cophenetic correlation values less than 0.7 indicate the inadequacy of the grouping method. In the present study, the cophenetic correlation value of the dendrogram was 0.95 – indicating the adequacy of that method for grouping the cashews.

By establishing the division point of the dendrogram, it was possible to observe the formation of four groups. The first group was formed by cashew G-3, considered one of the most divergent - and characterized principally by demonstrating elevated peduncle lengths, peduncle diameters, peduncle base diameters, peduncle apex diameters, and peduncle weight values; the second group was formed by G-30, which, like G-3, demonstrated one of the largest values for peduncle length; the third group was composed only of G-8, which demonstrated one of the largest values for cashew nut weight; the other specimens together compose the fourth group, with members G-24 and G-25 demonstrating the smallest genetic distances (Figure 1).

The principal components analysis, based on the 12 morphological descriptors, demonstrated the necessity of only three principal components to explain 81.72% of the observed variability of the cashew plants. The analyses of the variables with the greatest scores in the constitution of Principal Component 1 (PC1), demonstrated that the characters related to the peduncle, such as its length, apex diameter, and base diameter presented positive combinations in terms of constituting that component. The determination of Principal Component 2 (PC2) revealed associations between variables related to the cashew nut, such as its length and width.

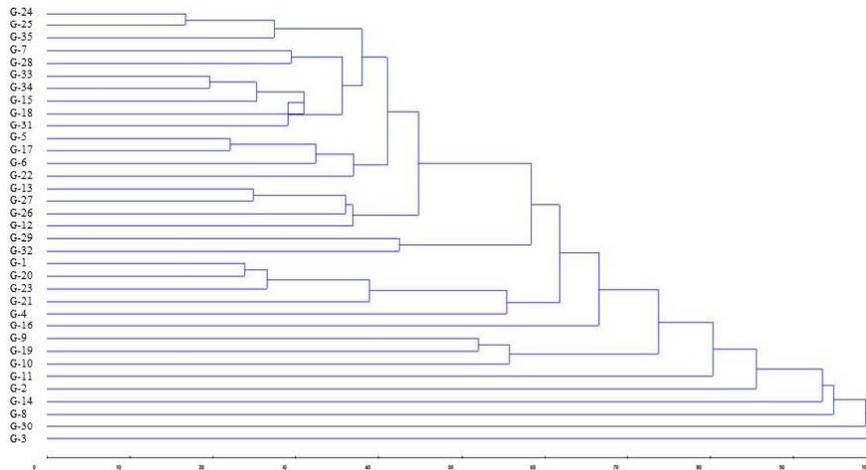


Figure 1. Dendrogram obtained from the analysis of morphological descriptors in a natural population of 35 adult cashew trees (G-1 to G-35) by means of the grouping method UPGMA. Coefficient of correlation coefficient (r) = 0.95. The plants evaluated were identified as G-1 through G-35, based on their collection order.

The variables related to peduncle lengths and base diameters aligned positively in the constitution of Principal Component 3 (PC3) (Table 4). Most of the plants demonstrated moderate to low values for characters related to the inflorescence, which supported the results obtained using the methodology proposed by Singh (1981), as the variables inflorescence length and maximum width contributed less to the genetic divergence of the plants. Archak et al. (2009) evaluated a total of 91 individuals from 4 cashew populations using morphometric data, so that analysis of diversity showed that the greatest existed in productivity indices (peduncle weight, nut weight and nut yield).

Table 4. Estimates of the eigenvalues associated with the principal components and the percentages and accumulated variances of cashew plant morphological characters.

Principal Component	AV	AV(%)	% Accumulated
PC1	293.84	50.22	59.22
PC2	109.85	21.65	71.87
PC3	44.37	9.85	81.72
PC4	20.18	7.61	89.33
PC5	13.54	4.82	94.15
PC6	5.51	2.48	96.63
PC7	4.51	2.12	98.75
PC8	4.33	0.61	99.36
PC9	0.81	0.16	99.92
PC10	0.54	0.36	99.72
PC11	0.070	0.213	99.93
PC12	0.048	0.07	100

AV: Eigenvalues (AV); AV(%): Percentage of variance explained; %: Percentage of variance cumulative ratio

The analyses of the principal components and patterns established by the Tocher and UPGMA grouping methods were in agreement in terms of cashew plant specimen

discriminations, and demonstrated significant genetic diversity between them. The results of the multivariate analyses allowed the identification of genetic variability among the morphological markers of the cashew trees evaluated here. Cashew G-3 stood out as being one of the most divergent, demonstrating the greatest values related to the peduncle; G-8 demonstrated the greatest measurements for cashew nut variables.

Biometrics analyses are important tools for examining genetic variability within populations of the same species and the relationship between these variability and environmental factors, as well as offer subsidies for variety identification in breeding programs (Goudel et al., 2013). Both the cashew nut (which constitutes the fruit) and the peduncle (which represents the pseudofruit) have significant economic value. Parameters related to fruits and seeds constitute basic information that indicates the productive and economic potential of the plant as food resource and raw material for industry (Schwartz et al. 2010).

The natural cashew population evaluated presented a great variation in the biometric value of the nut and peduncle, in which they are important characteristics for food and industry. Classical phenotype features are still extremelly useful, moreover, breeding of cashew is mostly based on traditional methods of selection of useful traits which in turn are phenotypes such as nut sinze, nut weight, sex raio, lenght of panicle and yield performace.

CONCLUSIONS

These results demonstrate the existence of genetic diversity among the morphological characters. The cashew G-3 and G-8 were found to be the most divergent, with G-3 demonstrating the greatest values for peduncle length and weight. Based on the analysis of the genetic diversity of the natural cashew population by the phenotypic descriptors, we obtained important results that could provide subsidies for the creation of commercial crop areas, since cashew production is an economical alternative for rural communities, as the fruiting period occurs mainly during the dry season, when there are few alternative agricultural production possibilities for small farmers.

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