AGRO-MORPHOLOGICAL CHARACTERIZATION AND GENETIC DIVERSITY OF *Paullinia cupana* var. *sorbilis*

Pablo Roberto da Silva Ozorio[1], André Luiz Atroch[2], Firmino José do Nascimento Filho[2]

[1]Federal University of Amazonas. E-mail: pabloozorio@hotmail.com
[2]Embrapa Amazônia Ocidental. E-mail: andre.atroch@embrapa.br, firmino.filho@embrapa.br

ABSTRACT

This study aimed to describe and quantify the genetic diversity between 18 guarana cultivars (*Paullinia cupana* var. *sorbilis*) from the guarana breeding program based on agro-morphological traits, to identify cultivars for use in crosses in guarana breeding program. Dissimilarity between cultivars was calculated using Gower's algorithm and a dendrogram was obtained using the unweighted pair group method with arithmetic mean (UPGMA) algorithm. High genetic variability and diversity was identified between guarana cultivars via 20 morpho-agronomic traits. Two groups and six subgroups of genetic diversity were identified. Crosses between clones of different subgroups may produce high variability and diversity in segregating populations, increasing the capacity to select for superior genotypes in these populations.

Keywords: *Paullinia cupana* var. *sorbilis,* genetic variability, hybridization, plant breeding

CARACTERIZAÇÃO MORFOAGRONÔMICA E DIVERSIDADE GENÉTICA ENTRE CULTIVARES DE GUARANAZEIRO

RESUMO

Este trabalho objetivou caracterizar e estimar a diversidade genética entre dezoito cultivares de guaraná (*Paullinia cupana* var. *sorbilis*) com base em descritores morfoagronômicos, para identificar cultivares para uso em cruzamentos no programa de melhoramento genético. A dissimilaridade entre as cultivares foi calculada utilizando o coeficiente de Gower e um dendrograma foi obtido utilizando o método UPGMA (unweighted pair group method with arithmetic mean). Existiu grande variabilidade e diversidade genéticas entre as cultivares de guaraná avaliadas, que puderam ser acessadas usando 20 descritores morfoagronômicos. Foram

identificados oito grupos e seis subgrupos de diversidade genética entre as cultivares. Os cruzamentos entre cultivares de diferentes subgrupos possivelmente produzem alta variabilidade e diversidade nas populações segregantes, aumentando a probabilidade de seleção de genótipos superiores nessas populações.

Palavras-chave: *Paullinia cupana* var. *sorbilis*, variabilidade genética, cruzamento genético, melhoramento vegetal

INTRODUCTION

Guarana, *Paullinia cupana* Kunth var. *sorbilis* (Mart.) Ducke (Sapindaceae), is a native Brazilian species of considerable economic and social importance. It is a perennial shrub native to Amazonian rainforests, where it has been long used for its caffeine-rich seeds (SMITH & ATROCH, 2007). Guarana seeds contain more caffeine than any other plant in the world, with levels ranging from 2 to 7.5%, which is about four times as much caffeine as occurs in coffee (BECK, 2005). Guarana seeds also contain traces of theobromine and theophylline, these being other alkaloids in the xanthine group to which caffeine belongs (PIZZA et al., 1999).

Brazil is the only commercial producer of guarana in the world, supplying both domestic and international markets. It is an important crop in the Brazilian Amazon, mainly in the states of Amazonas, Acre, Pará, Rondônia, and Mato Grosso. In recent decades, guarana production has expanded beyond Amazonia, and today the northeastern state of Bahia produces almost half of the national guarana crop (ATROCH & NASCIMENTO FILHO, 2018). In 2017, the total area destined to harvest with guarana in Brazil was around 10,719 ha with a harvested area of about 13,664 ha, a production of 2,663 t of dry seeds and a productivity of 250 $kg.ha^{-1}$ (IBGE, 2019). The largest producers are the states of Bahia (58% of national production), Amazonas (32%), Mato Grosso (7%), Rondônia (2.5%), and Pará (0.5%), with a total value of production in 2013 of US$ 7.6 million (IBGE, 2019).

Phenotypic selection of superior parents began in 1976 at the Maues Experimental Station, and initially 36 genitors were identified in a population originating from 3074 plants grown by local farmers. The plants had an age range of 9-20 years. In 1984, a national network for evaluating open-pollinated progeny and clones was set up at Embrapa centers in northern Brazil and at CEPLAC/CEPEC in Bahia state. These experiments continued until 1994.

In 1996, Embrapa Western Amazon set up a state network for evaluating 32 promising clones with the intention of assessing their behavior under varied environmental conditions in Amazonas State. Between 1999 and 2000, Embrapa Western Amazon distributed the first 12 clonal guarana cultivars for planting in Amazonas State. Embrapa has already recommended a total of 18 guarana cultivars for planting in the state of Amazonas, with productive potential up to 10 times higher than the average state productivity, and they are currently being distributed for planting (ATROCH & NASCIMENTO FILHO, 2018).

However, clonal selection narrows the genetic base. Accordingly, to increase variability, it is necessary to use controlled crosses or the use of recurrent selection. For the choice of parents, it is necessary to know which genotypes will be used in the crosses and in the progeny formation. Therefore, such studies are of great importance for the continuity of the guarana breeding program.

Guarana genetic resources are preserved in an active germplasm bank at Embrapa Western Amazon, in Manaus, since the development of new cultivars can take 20 to 30 years. It is, therefore, very important, when choosing appropriate selection methods, to know the variability and form of inheritance of traits that are of interest to the breeder (NASCIMENTO FILHO & ATROCH, 2002). So, it is especially important in plants with longer maturation cycles.

Yield is the most important selection criterion in the guarana breeding program. The minimum period for its evaluation is four to five years (NASCIMENTO FILHO et al., 2009; ATROCH, 2004). Other variables that are also important in deciding the superiority of given genotypes are main branch length, number of branches and number of leaves, all indicative of a plant's ability to establish itself and survive after planting in the field (NASCIMENTO FILHO & ATROCH, 2002). Adaptability and stability under various environmental conditions, cropping systems, locations, and year-to-year variations are also measured (NASCIMENTO FILHO & ATROCH, 2002).

The objective of the current study was to evaluate the genetic variability of clonal guarana cultivars held in the Embrapa germplasm bank and available for planting in the state of Amazonas, and to identify divergent genotypes that, when hybridized, may increase genetic variability in segregating populations.

MATERIAL AND METHODS

The experiment was carried out in a completely randomized block design with 18 cultivars, four replications, three plants per plot, with rows spaced 5.0 m and plants spaced 5.0 m, in a clay oxisol, with fertilization and cultivation at Embrapa Western Amazon, at Manaus Experimental Station State of Amazon, latitude 3º8'5"S, longitude 60º1'W GRT, located in the humid tropical forest biome with a rainy tropical climate.

Agro-morphological data were recorded from two randomly selected plants from each treatment in each replication. Mode data were used for statistical analysis for the following traits: **a)** plant architecture: erect, semi erect, decumbent; **b)** plant branches length: short, medium, long; **c)** leaf shape: oval, elliptical, oblong; **d)** young leaf color: light green, dark green, purplish green, brown, purple; **e)** leaf anthocyanin pigmentation: absent, present; **f)** green leaf color: light, medium, dark, yellowish; **g)** intensity of leaf anthocyanin pigmentation: low, medium, high; **h)** bullate on the upper side of the leaf blade surface: low, medium, strong; **i)** brightness of leaf blade upper surface: low, medium, high; **j)** leaf rudiments on rachis: absent, present; **l)** leaf rudiments on rachis, shape: winged, margined, smooth; **m)** fruit density on raceme: low, medium, high; **n)** raceme length: short, medium, long; **o)** fruit shape: elliptic, obovate, spherical; **p)** fruit color: yellow, orange, reddish yellow, yellowish red, orange red, red, dark red; **q)** fruit pericarp surface (FRPS): smooth, rough; **r)** fruit size: small, medium, large; **s)** fruit pericarp brightness intensity: low, medium, high; **t)** fruit ripening season: early, normal, late; **u)** Yield: data harvest (1985 to 1994 period).

The yield (kg.plant^{-1}.year^{-1}) was recorded across a ten year period (1985 to 1994), and an analysis over variance (ANOVA) was performed considering the annual mean for each year.

Level of genetic diversity was calculated using the general coefficient of Gower (GOWER, 1971). The Gower method allows the simultaneous analysis of standardized quantitative and qualitative data in the determination of genetic distance. The Gower algorithm is expressed as follows:

$$Sij = \frac{\sum_{k=1}^{p} Wijk.Sijk}{\sum_{k=1}^{p} Wijk}$$

where: k is the k_{th} variable (k = 1, 2, …; p = total number of characteristics evaluated); i e j, two individuals; $Wijk$ is a weight given the comparison ijk, assigning value 1 for valid

comparisons and value 0 for invalid comparisons (when the value of the variable is absent in one or both individuals)

The dendrogram was based on genetic distance matrix of Gower using the unweighted pair group method with arithmetic mean (UPGMA) method as a grouping criterion. The cut-off point was based on Mojena (1977). Analyzes were performed using Genes software. The cophenetic correlation coefficient of Sokal & Rohlf (1962) was calculated using Genes software.

This study was carried out under a license to access the genetic patrimony of Brazil for bioprospecting purposes from CGEN and granted to the Guarana Genetic Breeding Project under number Special Authorization No. 001/2009 Process No: 02000.002921/2009-89 CGEN Deliberation No: 237 of 11/12/2008, and CGEN registration number A1BCD7A.

RESULTS AND DISCUSSION

There was variation in the morphological data for all characters analyzed from the guarana cultivars. A percentage was obtained for each qualitative trait (Table 1).

Traits related to branch length showed a higher percentage of plants with medium branch lengths. Additionally, most plants showed a semi-erect architecture (Table 1). Plant architecture and branch length are important traits in determining proper planting spacing. Plants erect and short branches allow a higher planting density.

For leaves, the highest proportion of plants had oval leaves, green purplish in color, with a low intensity of anthocyanin pigmentation in young leaves. In mature leaves, the predominant color was dark green, low surface bullate, with medium brightness on the upper surface. For most plants, leaf rudiments were absent from the rachis, but when present, they were winged in shape (Table 1). Qualitative descriptors are often used to characterize germplasm because of their high heritability, easy measurement and low genotype-environment interaction.

Thus, the estimation of genetic diversity is facilitated by the use of many qualitative descriptors.

Fruit density was high in most of the plants studied. Fruits were most commonly spherical in shape, medium sized, with a smooth pericarp surface, occurring on long racemes, and with a medium intensity yellowish-red color.

For ripening, it showed that 50% of the plants had early ripening fruit, and 50% had normal ripening (Table 1). The precocity is an important trait, as it represents the possibility of a larger harvesting schedule when several clones are planted. Allowing to optimize the farm labor.

Table 1. Phenotypic proportions for morpho-agronomic traits between 18 guarana cultivars from the germplasm bank at Embrapa Amazônia Ocidental.

Morpho-agronomic traits	Phenotypic percentage
Branches length	61.1% medium; 38.9% long
Plant architecture	61.1% semi erect; 27.8% decumbent; 11.1% erect
Leaf shape	55.6% oval; 33.3% elliptical, 11.1% oblong
Young leaf color	44.5% purple green; 33.3% light green; 11.1% brown; 11.1% dark green
Young leaf anthocyanin pigmentation	94.4% presence; 5.6% absent
Green leaf color	44.5% medium; 33.3% dark; 16.6% yellowish; 5.6% light
Intensity of young leaf anthocyanin pigmentation	38.9% low; 27.8% medium; 33.3% high
Upper side of the leaf blade surface bullate	33.3% low; 38.9% medium; 27.8% strong
Brightness of the upper side of the leaf blade	38.9% medium; 33.3% high; 27.8% low
Leaf rudiments on rachis	66.7% absent; 33.3% present
Leaf rudiments shape on rachis	66.7% winged, 33.3% margined
Fruit density	66.7% high; 33.3% medium
Raceme length	83.3% long; 16.7% medium
Fruit shape	66.7% spherical; 27.8% obovate; 5.5% elliptical
Fruit color	27.8% yellowish red; 22.2% red; 16.7% orange red; 11.1% yellow; 11.1% orange; 11.1% reddish yellow
Fruit pericarp surface	83.3% smooth; 16.7% rough
Fruit size	77.8% medium, 16.7% big; 5.5% small
Fruit brightness intensity	50% medium; 27.8% high; 22.2% low
Fruit ripening season	50% precocious; 50% medium

Analysis of variance found no variability between the guarana clonal cultivars for the variable 'yield' (Table 2).

Table 2. Analysis of variance for yield between 18 guarana clonal cultivars from the germplasm bank at Embrapa Amazônia Ocidental.

Source of variation	DF	MS
Cultivars	17	23833780^{ns}
Error	141	
Mean	6946	
CV (%)	69.06	

ns: not significant

Based on analysis of the 20 agro-morphological traits, it was possible to identify genetically dissimilar cultivars (Table 3). According to the genetic distance measurement of Gower (1971), the pairs of the most similar genotypes were BRS CG505 and BRS CG612 (0.823), BRS Amazonas and BRS CG608 (0.820), BRS Mundurucânia and BRS CG189 (0.807), BRS Cereçaporanga and BRS Andirá (0.805) (Table 3). In addition, pairs with least similar genotypes were BRS CG189 and BRS CG850 (0.585), BRS CG372 and BRS CG850 (0.573) (Table 3).

Figure 1 shows a dendrogram of 18 guarana cultivars based on Gower genetic distances and UPGMA clustering. It was observed two distinct groups and six subgroups. Subgroup 1 is formed by BRS CG648 and BRS CG189; subgroup 2 by BRS CG505, BRS CG610, BRS CG882 and BRS CG612; subgroup 3 by BRS Amazonas, BRS Marabitana and BRS Saterê; subgroup 4 includes BRS Luzéia and BRS Maués; subgroup 5 BRS CG850, BRS Cereçaporanga and BRS CG608; while subgroup 6 is formed by BRS CG372, BRS CG611, BRS Mundurucânia and BRS Andirá.

The value of the Sokal & Rohlf (1962) cophenetic correlation coefficient was 0.86, and a Mantel test approximated to a normal curve (z stat = 31.28**) (Figure 1).

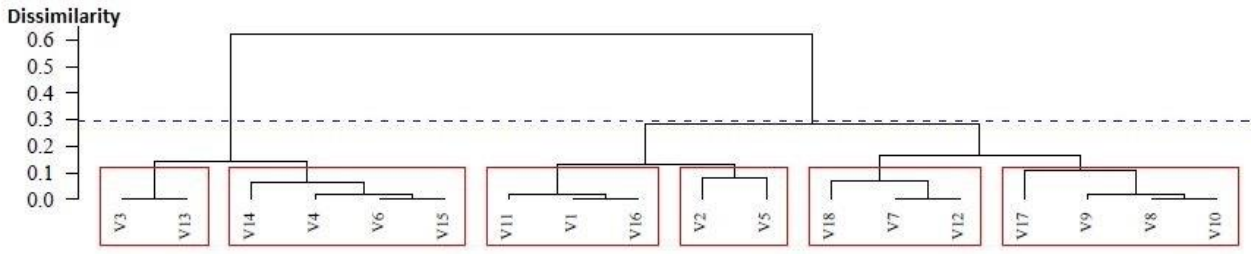
Yield is the most important variable the reproductive partners for a breeding population of guarana. The low variability in yield observed here between guarana clonal cultivars occurred because the genotypes were selected and recommended for planting in the state of Amazonas, and have similar yields (Table 2).

Table 3. Gower genetic distance based on 20 morph-agronomic traits of 18 guarana clonal cultivars from the germplasm bank at Embrapa Amazônia Ocidental.

Cultivars	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18
v1	0,17	0,83	0,69	0,08	0,71	0,34	0,17	0,19	0,17	0,02	0,34	0,83	0,64	0,71	0,00	0,06	0,27
v2		1,00	0,87	0,08	0,88	0,52	0,34	0,36	0,34	0,19	0,52	1,0	0,81	0,88	0,17	0,24	0,45
v3			0,14	0,91	0.12	0.48	0.65	0.64	0.65	0.81	0.48	0.00	0.19	0.12	0.83	0.76	0.55
v4				0,77	0,02	0,34	0,52	0,50	0,52	0,67	0,34	0,14	0,05	0,02	0,69	0,62	0,41
v5					0,79	0,43	0,26	0,27	0,26	0,10	0,43	0,91	0,72	0,79	0,08	0,15	0,36
v6						0,36	0,53	0,52	0,53	0,69	0,36	0,12	0,07	0,00	0,71	0,64	0,43
v7							0,17	0,16	0,17	0,33	0,00	0,48	0,29	0,36	0,34	0,27	0,07
v8								0,02	0,00	0,16	0,17	0,65	0,46	0,53	0,17	0,10	0,10
v9									0,02	0,17	0,15	0,64	0,45	0,52	0,19	0,12	0,08
v10										0,16	0,17	0,65	0,46	0,53	0,17	0,10	0,10
v11											0,33	0,81	0,62	0,69	0,02	0,52	0,26
v12												0,48	0,29	0,36	0,34	0,27	0,07
v13													0,19	0,12	0,83	0,76	0,55
v14														0,07	0,64	0,57	0,36
v15															0,71	0,64	0,43
v16																0,07	0,27
v17																	0,21

V1- BRS Marabitana; V2- BRS Luzéia; V3- BRS CG648; V4- BRS CG610; V5- BRS MAUÉS; V6- BRS CG882; V7- BRS CEREÇAPORANGA; V8- BRS MUNDURUCÂNIA; V9- BRS CG611; V10- BRS ANDIRÁ; V11- BRS AMAZONAS; V12- BRS CG608; V13- BRS CG189; V14- BRS CG505; V15- BRS CG612; V16- BRS SATERÊ; V17- BRS CG372; V18- BRS CG850.

The guarana cultivars dendrogram derived using the UPGMA hierarchical method (Figure 1) shows two groups and six subgroups formed from 18 genotypes, indicating that overall genetic diversity is high.



V1- BRS Marabitana; V2- BRS Luzéia; V3- BRS CG648; V4- BRS CG610; V5- BRS MAUÉS; V6- BRS CG882; V7- BRS CEREÇAPORANGA; V8- BRS MUNDURUCÂNIA; V9- BRS CG611; V10- BRS ANDIRÁ; V11- BRS AMAZONAS; V12- BRS CG608; V13- BRS CG189; V14- BRS CG505; V15- BRS CG612; V16- BRS SATERÊ; V17- BRS CG372; V18- BRS CG850.

Figure 1. Dendrogram of Gower genetic distance between 18 guarana cultivars from the germplasm bank at Embrapa Amazônia Ocidental based on UPGMA clustering.

The value of the Sokal & Rohlf (1962) cophenetic correlation coefficient was 0.86, considered high, with good concordance between the dissimilarity matrix and dendrogram. A Mantel test (z stat = 31.28**) found good approximation to a normal curve (Figure 1). The cophenetic correlation indicates that the genetic distance matrix was well represented graphically by the dendrogram.

Because guarana has now been shown to be polyploid (FREITAS et al., 2007), some of this variability may be attributable to epistasis among the multiple genes coding for different morphological traits (STEBBINS, 1985). Polyploids are subject to phenotypic, physiological and chemical changes (LEVIN, 1983), which could also explain the high level of morphometric variability observed in guarana. Genetic changes are based on alterations in the arrangement of DNA sequences, resulting in permanent changes in the molecule or gene loss. Possible alterations in the sequence or in the chromosomes could derive from unequal crossing-over, recombination of chromosomes homeologists, aneuploids, gene conversion, insertions, deletions, or point mutations. Epigenetic changes, such DNA methylation, histone modification, RNA interference, and dosage compensation can alter the gene expression pattern, without changing the DNA sequence (WOLFFE & MATZKE, 1999).

The studied guarana clones showed great phenotypic variability for all the traits analyzed to date, but there is low genetic variability. As noted above, this paradoxical situation is likely due to its recent domestication as a polyploid. However, populations show sufficient genetic variability for a number of traits so that selection is possible of superior individuals that have large numbers of desirable characteristics that can be used either directly by producers or in breeding programs. This affirmation is based on 30 years of study, during which guarana has been characterized and evaluated for both morphometric variability and molecular genetic variability (NASCIMENTO FILHO et al., 2001; ANGELO et al., 2014).

Valois et al. (1979) observed that the guarana reproduction method and the percentage of female to male flowers in an inflorescence could be responsible for the low correlation between inflorescence size, number of buds, number of fruits, and number of seeds per fruit. They also showed that these factors are genetically variable, for example: inflorescence size (CV=30.6%), number of buds (CV=24.9%) and number of seeds (CV=27.9%) and, consequently, can be significantly increased by selection.

Nascimento Filho et al. (1992) studied 26 characters related to the aerial part and root system of guarana plants, and found high variability for all traits between the clones studied. They obtained genotypic determination coefficients of over 70% for the most of the variables studied, showing that simple breeding methods could be applied to give good selective gains.

Evaluating guarana genetic diversity has two advantages: the identification of genitors with maximum genetic diversity for future breeding programs, and the identification of genitors with maximum genetic similarity for use in vegetative propagation.

With the aim of identifying productive, divergent, guarana clones that could be used in a crossing program to obtain hybrids with high heterosis, as well as materials for vegetative propagation, Nascimento Filho et al. (2001) evaluated 148 guarana clones for main branch length, number of branches and leaves, and dry seed production in kilograms per plant. Phenotypic variability analysis was significant for all traits evaluated. To analyze genetic divergence among groups of clones, they used average Euclidean distance, Tocher's optimization method and the nearest neighbor method. Genetic distance estimates allowed seven separate groups to be distinguished, with the majority of clones (85%) in one group, which indicates that genetic divergence among the clones currently used in the guarana genetic improvement program at Embrapa Western Amazon is not extensive

(NASCIMENTO FILHO et al., 2001). This set of results maybe indicates the influence of recent domestication via polyploidy on guarana phenotypic and genetic variability.

Two groups and six subgroups of genetic diversity were identified in the current study. Crosses between clones of different subgroups may produce high variability and diversity in the segregating populations, increasing the probability for selection of superior genotypes in these populations. From such crosses the evaluation and selection of half-sib guarana progenies could be performed in a continuous recurrent selection program.

The importance of characterization is evident in the conservation and use of plant genetic resources, particularly in germplasm banks and breeding programs, since agro-morphological characterization is the first step to understanding the variability of any plant species in established collections (BURLE & OLIVEIRA, 2010).

In the morphological and agronomic characterization of cassava (*Manihot esculenta* Crantz), Albuquerque et al. (2009) found great variability among clones. Rocha et al. (2010), using the Gower algorithm to study genetic divergence in tomato (*Solanum lycopersicum* L.), showed that the germplasm bank studied accessions possessed ~~a~~ high genetic divergence.

In a study using agro-morphological to assay the nature and extent of genetic variability and diversity of Indian turmeric (*Curcuma longa*) descriptors, Bahadur et al. (2016) used the Mahalanobis D^2 statistic for each analyzed character and Tocher´s method to group the accessions into clusters, and concluded that these analytical techniques can be helpful when selecting diverse parents and for widening indigenous turmeric gene-pools for future breeding programs.

CONCLUSION

Guarana cultivars show high genetic variability and genetic diversity when assessed using 20 agro-morphological traits. The study identified two groups and six subgroups of genetic diversity. Hybridization between clones of different subgroups is expected to produce greater variability and diversity in segregating populations, enhancing the probability of recovering superior genotypes in the resulting progeny.

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