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Molecular Characterization of Fifteen Ethnovarieties of Cassava Cultivated in the Northern Region of the State of Mato Grosso, Brazil

Caracterização Molecular de Quinze Etnovariedades de Mandioca Cultivadas na Região Norte do Estado de Mato Grosso, Brasil

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Abstract

Cassava has roots that are rich in carbohydrates. It is cultivated in many regions of Brazil and is used as a food for both humans and animals. Recent studies have indicated that local cassava varieties grown in the plantations of family farmers constitute a form of genetic resource that must be conserved. In this context, the objective of this study was to evaluate, via ISSR molecular markers, the genetic diversity of 15 cassava ethnovarieties cultivated by farmers in seven municipalities of the state of Mato Grosso. The extraction of plant DNA was performed based on the CTAB method. For the PCR amplification, 15 ISSR primers were used. The amplified fragments were analyzed and encoded as binary characters that were used to calculate the % of polymorphism and the PIC. Dissimilarity between ethnovarieties was evaluated and grouping was performed using the UPGMA method, the Tocher optimization method and Bayesian analysis. The 15 primers amplified a total of 139 fragments, 101 of which were polymorphic (72.94%). The PIC had a mean of 0.47. Genetic dissimilarity values ranged from 0.20 to 0.50. The UPGMA clustering method permitted the formation of nine groups and the Tocher optimization method six groups. Bayesian analysis divided the 15 cassava ethnovarieties into two genetic groups. The results indicate that there is genetic diversity among the cassava ethnovarieties cultivated in the plantations of the family farmers. There was no separation of ethnovarieties by location of collection, which may be associated with the exchange of plant material among farmers in the sampled municipalities.

Keywords: *Family Farmers. Genetic Diversity. Manihot esculenta. Molecular Markers. Genetic Resources.*

Resumo

A mandioca apresenta raízes rica em carboidratos, é cultivada em diversas regiões do Brasil, sendo utilizada tanto na alimentação humana quanto animal. Estudos recentes indicam que as variedades locais de mandioca cultivadas nas roças dos agricultores familiares constituem uma forma de recurso genético que deve ser conservado. Neste contexto, objetivou-se com este estudo avaliar a diversidade genética de 15 etnovariedades de mandioca cultivadas por agricultores em sete municípios do estado de Mato Grosso, por meio de marcadores moleculares ISSR. A extração do DNA vegetal foi realizada com base no método CTAB. Para amplificação via PCR foram utilizados 15 *primers* ISSR. Os fragmentos amplificados foram analisados e codificados como caracteres binários utilizados para calcular a % de polimorfismo e o PIC. Foi avaliada a dissimilaridade entre as etnovariedades e realizado o agrupamento por meio do método UPGMA, Tocher e análise Bayesiana. Os 15 *primers* amplificaram um total de 139 fragmentos, sendo 101 polimórficos (72,94%). O PIC apresentou média de 0,47. Os valores de dissimilaridade genética variaram entre 0,20 a 0,50. O método de agrupamento UPGMA permitiu a formação de nove grupos e o método de otimização de Tocher seis grupos. A análise bayesiana dividiu as 15 etnovariedades de mandioca em dois grupos genéticos. Os resultados indicam que há diversidade genética entre as etnovariedades de mandioca cultivadas nas roças dos agricultores familiares. Não houve separação das etnovariedades por localidade de coleta, o que pode estar associado com a troca de material vegetal realizada entre os agricultores nos municípios amostrados.

Palavras-chave: Agricultores Familiares. Diversidade Genética. *Manihot esculenta*. Marcadores Moleculares. Recursos Genéticos.

1 Introduction

Cassava (*Manihot esculenta Crantz*) is an important food source for many people around the world due to its high nutritional value (LEBOT, 2009). The plant has numerous utilities in the countries that produce it, which increases its worldwide socioeconomic importance. As such, it is the main source of carbohydrates for millions of people, especially in the American, African and Asian continents (GUSMÃO *et al*., 2016).

The cultivation of cassava in many regions of Brazil is due to its edaphoclimatic adaptation, high root productivity and resistance to pests and diseases (LEANDRO, 2007). In the state of Mato Grosso, cassava is cultivated mainly by family farmers, who act as the guardians of local diversity, since they maintain different ethnovarieties in their plantaions and exchange propagative material among themselves, thus ensuring the diversity and maintenance of the collection (GALERA; VALLE, 2007; MARTINS; OLIVEIRA, 2009).

In this sense, studies aimed at the molecular characterization of these varieties grown in the plantaions of family farmers are very important, as they generate useful information in the choice of strategies for *ex situ, in situ* and on farm conservation (FERREIRA, 2011). The existing genetic diversity among cassava ethnovarieties can be accessed through studies with

molecular markers, which allow one to access the variability directly in the genome and identify variations in DNA, while excluding the effects of the environment (FERREIRA; GRATTAPAGLIA, 2008).

Among the available molecular markers that stand out, the ISSRs (inter simple sequence repeats) (ZIETKIEWICZ *et al*., 1994) present a high degree of polymorphism and reproduction and do not require prior knowledge of the genome (TURCHETTO-ZOLE; ZANELLA, 2017). Different studies carried out in recent years have shown the efficiency of these markers in evidencing the genetic diversity that exists within the species *M. esculenta* (TIAGO *et al*., 2016; FIGUEREDO *et al*., 2019; AFONSO *et al*., 2019; ASHA *et al*., 2019; ASHA *et al*., 2020; CUCHI *et al*., 2022).

Thus, the objective of this study was to evaluate, via ISSR molecular markers, the genetic diversity of fifteen ethnovarieties of cassava cultivated by farmers in seven municipalities of the state of Mato Grosso.

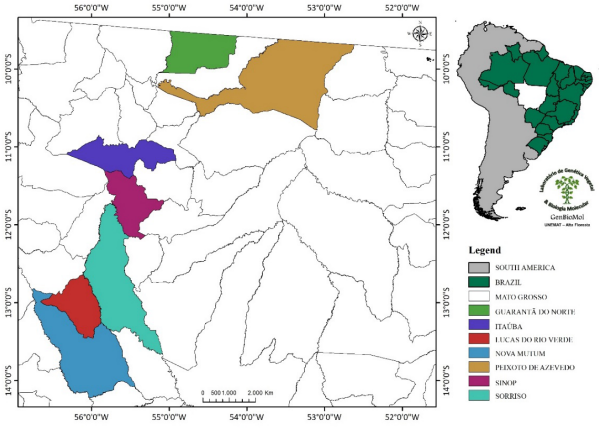
2 Material and Methods

2.1 Sampling and collection of plant material

Fifteen ethnovarieties of cassava were sampled in seven municipalities located in the north of the state of Mato Grosso (Figure 1). These ethnovarieties were selected for being cultivated for sale in local markets and municipal fairs and for consumption in public schools.

The terms "ethnovarieties" or "local varieties" are cited by Cleveland *et al*. (1994) in relation to populations that originate from local selection processes that are carried out by farmers. Therefore, in this context, "ethnovarieties" is used to refer to cassava varieties whose planting and use are carried out by small traditional producers who act as guardians of the genetic variability and the cultural knowledge of cassava.

Figure 1 - Geographical location of the in seven municipalities sampled on the BR163 highway, Mato Grosso, Brazil



Source: the authors.

Young leaves of the 15 cassava ethnovarieties were collected (Table 1) for subsequent DNA extraction. The leaf material was identified while in the field, stored in aluminum foil envelopes, packed in plastic ziplock bags and kept in a thermal box with ice. Then, the material was sent to the laboratory of Plant Genetics and Molecular Biology (GenBioMol) of the Center for Research and Technology of the southern Amazon (CEPTAM) of the State University of Mato Grosso Carlos Alberto Reyes Maldonado, University campus of Alta Floresta, MT, and stored in a freezer at a temperature of -20 °C.

Table 1 - Code, ethnovariety and place of collection of the 15 cassava ethnovarieties sampled in the state of Mato Grosso, Brazil

Code	Ethnovariety	Place of collection
ETHNO 01	*Cascatinha*	Nova Mutum
ETHNO 02	*Liberata*	Nova Mutum
ETHNO 03	*Branca*	Lucas do Rio Verde
ETHNO 04	*Folha roxa*	Lucas do Rio Verde
ETHNO 05	*Mandioca de fritar sem cozinhar*	Sorriso
ETHNO 06	*Capelari*	Sorriso
ETHNO 07	*Branca*	Sorriso
ETHNO 08	*Amarelinha*	Sorriso
ETHNO 09	*Amarelinha*	Sinop
ETHNO 10	*Casca roxa*	Itaúba
ETHNO 11	*Branca 1*	Itaúba
ETHNO 12	*Branca 2*	Itaúba
ETHNO 13	*Amarelinha*	Peixoto de Azevedo
ETHNO 14	*Castelinha*	Guarantã do Norte
ETHNO 15	*Casca branca*	Guarantã do Norte

Source: the authors.

2.2 Molecular characterization

2.2.1 Extraction of total DNA

DNA was extracted from approximately 100 mg of leaf tissue based on the CTAB protocol (cetyltrimethylammonium bromide) described by Doyle and Doyle (1990), with modifications as cited by Tiago *et al*. (2016) The concentration of polyvinylpyrrolidone (PVP) was increased from 1% to 2% and β-mercaptoethanol from 0.2% to 3% in the extraction buffer, in addition to reducing the incubation time from 60 min to 30 min at 65 °C.

The quality and concentration of the extracted DNA were confirmed by electrophoresis in 1% agarose gel, prepared in 1x TBE buffer (Tris-Borate-EDTA) and stained with ethidium bromide solution (10 mg mL^{-1}), which, in turn, was prepared from 1 g of ethidium bromide for each 100 mL of distilled and autoclaved water. This solution was used to stain the 1% agarose gel, adding 1 µL for each 50 ml of gel. Quantification was performed by comparison with DNA-λ (50 ng μL^{-1}). Subsequently, the extracted DNA was diluted to a concentration of 20 ng/μL^{-1} and then stored at -20 °C for subsequent amplifications.

2.2.2 Amplification via PCR

For DNA amplifications via PCR (Polymerase Chain Reaction) 15 ISSR primers (Inter Simple Sequence Repeat) of 15-18 nucleotides in length were used, which were developed

by the University of British Columbia (UBC), Vancouver, Canada (Table 2).

Table 2 - Code, sequence and annealing temperature of ISSR primers used for molecular characterization of 15 cassava ethnovarieties.

Primer code	Primer sequence (5'----3')	Tm (ºC)*
-TRI$(GTG)_5$	GTGGTGGTGGTGGTG	58.9
UBC 807 - Di$(AG)_8$3'T	AGAGAGAGAGAGAGAGT	47.0
UBC 808 - Di$(AG)_8$3'C	AGAGAGAGAGAGAGAGC	48.8
UBC 811 - Di$(GA)_8$3'C	GAGAGAGAGAGAGAGAC	52.8
UBC 815 - Di$(CT)_8$3'G	CTCTCTCTCTCTCTCTG	52.8
UBC 828 - Di$(TG)_8$3'A	TGTGTGTGTGTGTGTGA	51.3
UBC 834 - Di$(AG)_8$3'YT	AGAGAGAGAGAGAGAGYT*	49.2
UBC 835 - Di$(AG)_8$3'YC	AGAGAGAGAGAGAGAGYC*	51.0
UBC 840 - Di$(GA)_8$3'YT	GAGAGAGAGAGAGAGAYT*	47.4
UBC 844 - Di$(CT)_8$3'RC	CTCTCTCTCTCTCTCTRC	48.6
UBC 856 - Di$(AC)_8$3'YA	ACACACACACACACACYA*	51.0
UBC 857 - Di$(AC)_8$3'YG	ACACACACACACACACYG*	52.0
UBC 868 - Tri$(GAA)_6$	GAAGAAGAAGAAGAAGAA	50.0
UBC 888 - Di$(CA)_7$5'BDB	BDBCACACACACACACA	49.0
UBC 891- Di$(TG)_7$5'HVH	HVHTGTGTGTGTGTGTG	47.0

*Y = C or T; R = A or G; V = A, C or G; H = A, C or T. * Annealing temperature.
Source: the authors.

The amplification reactions were performed in a final volume of 20 µL, which contained: 4.6 µL of H_2O, 2 µL of DNA (20 ng), 2 µL of 10x buffer (1 M KCl; 1 M Tris pH 8.3; 10% Tween 20), 3.2 µL of $MgCl_2$ (25 mM), 3 µL of primer (0.2 mM), 4 µL dNTP (0.1 mM of each dNTP), 1 µL DMSO and 0.2 µL Taq polymerase (5 U/µL).

The amplification program is described by Silva *et al*. (2011), with an initial phase of denaturation at 94 ºC for 4 min, followed by 35 cycles under the following conditions: 30 seconds for denaturation at 94 ºC; 35 seconds for annealing at 47 – 58.9 ºC (varying according to the primer used) and two minutes for extension at 72 ºC. The final extension was 72 ºC for seven minutes.

The amplification products were separated via electrophoresis in 1.5% agarose gel in a horizontal vat, with 1 x TBE buffer, with a constant voltage of 80 V for approximately four hours. After the electrophoretic run, the gels were stained in a container with ethidium bromide (EtBr) solution (0.6 µg/mL^{-1}) for 20 minutes. Then, They were visualized and photodocumented in UVB transilluminator LTB-21X26 (Loccus Biotecnologia®), and the size of the amplified fragments were compared with the 100pb Kapa Universal molecular marker.

2.3 Data analysis

The amplified fragments were analyzed and encoded as binary characters, which were converted into a binomial matrix: presence (1) or absence (0) of bands. The binary matrix was used to calculate the polymorphism percentage of each primer using Equation 1:

$$\text{Equation 1: } P = \frac{npb}{tnb} \times 100$$

where P = percentage of polymorphism; npb = number of polymorphic bands and tnb = total number of bands.

To determine the discriminatory power of each primer, the polymorphism information content (PIC), proposed by Anderson *et al.* (1993), was calculated depending on the number of alleles detected and their distribution and frequency in the individuals studied. The PIC values for each marker were determined using Equation 2:

$$\text{Equation 2: } PIC = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where is the frequency of the "*j*" allele in the "i" marker (the sum extends across all alleles).

The calculation was based on the number of alleles detected per marker for a given locus and the relative frequency of each allele in the set of genotypes analyzed. The PIC value ranges from "0" (for monomorphic profiles) to "1" (for highly polymorphic profiles).

The dissimilarity between cassava ethnovarieties was obtained through the binary matrix, which was converted into a genetic dissimilarity matrix using the Jaccard index via the arithmetic complement. This coefficient consists of comparing the number of presences of common bands and the total number of bands involved, though excluding the number of joint absences (MEYER, 2002) via Equation 3:

$$\text{Equation 3: } D_{ij} = 1 - S_{ij}$$

Where:

$$S_{ij} = \frac{a}{a + b + c}$$

In this case, a = the number of times the presence of the band occurs in both individuals; b = the number of times the presence of the band occurs only in individual i, and c = the number of times the presence of the band occurs only in individual j.

From the dissimilarity matrix, obtained via the Jaccard index, grouping analyses of ethnovarieties were performed using the grouping methods UPGMA, nearest neighbor (NN) and WARD.

For each group tested, the cophenetic correlation

coefficient (CCC), stress and distortion and cutoff point were calculated according to Mojena (1977). Subsequently, the grouping method that best represented the divergence of the material under study was selected.

The output matrix generated by the Jaccard index was also used to group individuals via the Tocher optimization method. All analyses were performed using the GENES program (CRUZ, 2013).

The Structure program, version 2.3.4 (PRITCHARD *et al*., 2000), based on the Bayesian clustering model, was used to infer the number of groups (K) by using Markov Chain Monte Carlo (MCMC). To determine the best K found in the population, we used the output file of the Structure program based on STRUCTURE HARVEST (EARL *et al*., 2012) determined by ΔK. With the data partitioned into two groups (K=2), as obtained by the Structure program, it was possible to reveal the distribution of genetic diversity within the group of cassava ethnovarieties studied.

3 Results and Discussion

The 15 primers amplified a total of 139 fragments, 101 of which were polymorphic (72.94%) (Table 3). This evidences the existence of genetic variability among the ethnovarieties evaluated. The total number of fragments (TNF) per primer ranged from seven (UBC 828 and 857) to 12 (UBC 891), with an average of 9.27 per primer. The number of polymorphic fragments (NPF) ranged from four (UBC 857 and UBC 868) to 11 (TRI and UBC 888).

Table 3 - Total number of amplified fragments (TNF), number of polymorphic fragments (NPF), percentage of polymorphism (%P) and polymorphic content index (PIC) for the 15 ISSR primers in relation to the 15 cassava ethnovarieties collected in the state of Mato Grosso, Brazil

Primer	TNF	NPF	%P	PIC
TRI (GTG)	11	11	100.00	0.61
UBC 807	10	07	70.00	0.53
UBC 808	08	05	62.50	0.45
UBC 811	09	07	77.77	0.47
UBC 815	10	07	70.00	0.40
UBC 828	07	07	100.00	0.60
UBC 834	08	06	75.00	0.38
UBC 835	08	05	62.50	0.48
UBC 840	09	07	77.77	0.51
UBC 844	10	08	80.00	0.51
UBC 856	08	06	75.00	0.43
UBC 857	07	04	57.14	0.39
UBC 868	11	04	36.36	0.32
UBC 888	11	11	100.00	0.62
UBC 891	12	06	50.00	0.33
Total	139	101	-	-
Mean	9.27	6.73	72.94	0.47

Source: the authors.

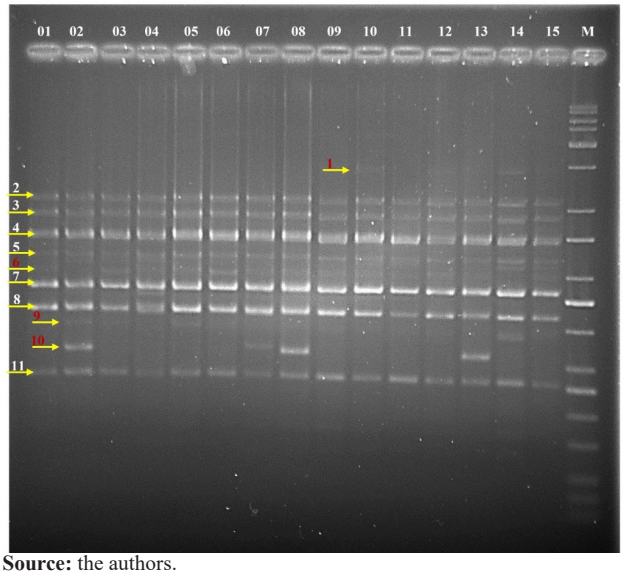
Similar results were found by Tiago *et al*. (2016), who found a total of 61.67% polymorphism when evaluating 17 ethnovarieties of cassava cultivated by farmers in the municipality of Alta Floresta, Mato Grosso. Silva *et al*. (2011) found a higher amount of polymorphism (89.7%) in their study with cassava genotypes from different countries (Brazil, Indonesia and Thailand).

Melo (2016) obtained a total mean value of 88.3% polymorphism among the genotypes evaluated, while Vidal *et al*. (2015) using 24 ISSR primers in different cassava genotypes obtained a percentage of 57.1% polymorphism. The number of polymorphisms observed is due, among other factors, to the particularity of the ethnovarieties under study, as well as the number of individuals evaluated (Silva *et al*., 2018).

The electrophoretic profile of 15 individuals of *M. esculenta* with the primer UBC 868 can be seen in Figure 2.

Figure 2 - Amplification products of genomic DNA of 15 cassava ethnovarieties with UBC 868 primer. M: marker 100 bp. Numbers in red indicate polymorphic fragments

Primer 868 Tri$(GAA)_6$



Source: the authors.

The polymorphic information content (PIC) for each marker varied from 0.32 (UBC 868) to 0.62 (UBC 888) with a mean of 0.47, and the primers UBC 828, TRI and UBC 888 presented the highest PIC values (0.60, 0.61, and 0.62 respectively) (Table 3), being, therefore, the most informative (BOTSTEIN *et al*., 1980) and highly recommended for future work with the species.

In this study, no primer presented a PIC lower than 25%, with the lowest value found being 0.32% (UBC 868), which is therefore considered by Botstein *et al*. (1980) as moderately informative, and thus reveals the discriminative potential of the 15 primers used and the efficiency of the ISSR-PCR technique in studies of quantification and organization of genetic diversity in cassava. In their work, Tiago *et al.* (2016) found PIC values that ranged from 0.04 to 0.61, with a mean of 0.39, these values being lower than found in this study (0.47).

The genetic dissimilarity values among the 15 cassava

ethnovarieties range from 0.20 to 0.49. The most genetically similar ethnovarieties were ETHNO 05 (*Mandioca-de-fritar-sem-cozinhar*) and ETHNO 06 (*Capelari*), which are shown in Figure 3, while the most dissimilar were ETHNO 05 (*Mandioca-de-fritar-sem-cozinhar*) and ETHNO 15 (*Casca branca*).

Figure 3 - Characteristics of the most and least similar roots of the cassava ethnovarieties. Most similar ethnovarieties - ETHNO 05 (A and B) and ETHNO 06 (C and D) have a rough root texture and a brown skin color; ETHNO 15 (E and F) was the most dissimilar in relation to ETHNO 05 and has smooth root texture and white skin color

Source: the authors.

Among the three methods tested, the UPGMA grouping method (Table 4) was selected since it presented a higher CCC value, lower stress and lower distortion (0.77, 10.44 and 1.09, respectively), and was the method that best graphically represented the genetic diversity that exists among the ethnovarieties, since CCC values greater than 0.7 reflect good agreement among the matrices. In this study, the CCC showed an association of 77% between the distances obtained in the dissimilarity matrix.

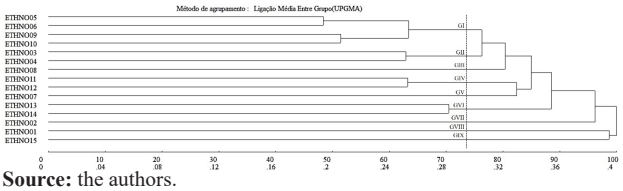
Table 4 - Cophenetic correlation coefficient (CCC), stress and distortion of Ward, UPGMA and nearest neighbor (NN) clustering methods

	WARD	UPGMA	NN
CCC	0.49**	0.77**	0.70**
Stress (%)	-	10.44	21.69
Distortion (%) -	-	1.09	34.28

** Significant at the level of 1% via the t-test.
Source:the authors.

Cruz and Carneiro (2003) state that the higher the CCC value, the lower the distortion in the grouping of individuals, which is usually obtained by the mean linkage method (UPGMA). The UPGMA grouping method with the 15 cassava ethnovarieties, using a cut-off point of 74.55%, permitted the formation of nine groups (Figure 4).

Figure 4 - Dendrogram obtained by the UPGMA method and arithmetic complement of the Jaccard index as a measure of dissimilarity in the 15 cassava ethnovarieties based on ISSR markers



Source: the authors.

Group I comprised four ethnovarieties (ETHNO 5; ETHNO 6; ETHNO 9 and ETHNO 10), which is equivalent to 26.67% of the 15 ethnovarieties evaluated. The two most similar individuals (ETHNO 05 and ETHNO 06) are found in this group. However, there is genetic variability within this group, since there was the formation of two subgroups.

Evaluating the genetic diversity of the cassava cultivars, also using ISSR markers, Ramalho *et al*. (2012) obtained the formation of six groups and observed that some of these groups were formed according to the color of the pulp of the cassava roots. Such a characteristic was also observed in this study in the Group IV, in which ETHNO 11 and ETHNO 12 have a pulp with a white coloration.

The Tocher optimization method led to the formation of six groups, and in Group I, eight ethnovarieties were allocated, which corresponds to 53.33% of the evaluated material (Table 6). The remaining groups consisted of either two or only one individual. It is noteworthy that, as with the UPGMA method, ETHNO 01 (*Cascatinha*), ETHNO 02 (*Liberata*) and ETHNO 15 (*Casca branca*) formed isolated groups (Figure 4), which reinforces the divergence of these ethnovarieties when compared to the others. This divergence may be related to the particular characteristics of the roots, such as texture, color of the skin, cortex and fresh pulp (Figure 5).

Table 6 - Grouping by the Tocher method, based on Jaccard's dissimilarity matrix from molecular analysis using the ISSR markers of the 15 cassava ethnovarieties

Group	Genotypes
I	ETHNO 05 ETHNO 06 ETHNO 09 ETHNO 10 ETHNO 03 ETHNO 04 ETHNO 11 ETHNO 08
II	ETHNO 13 ETHNO 14
III	ETHNO 07 ETHNO 12
IV	ETHNO 15
V	ETHNO 01
VI	ETHNO 02

Source: the authors.

In the Tocher grouping, it is common that the first group concentrates a greater number of genotypes. This type of analysis tries to maintain homogeneity within groups and heterogeneity between groups. This means that the greater number of individuals in a given group indicates that such individuals have greater genetic similarity with each other and that, in turn, individuals in the latter group show greater

genetic divergence when compared to the former (ELIAS *et al*., 2007).

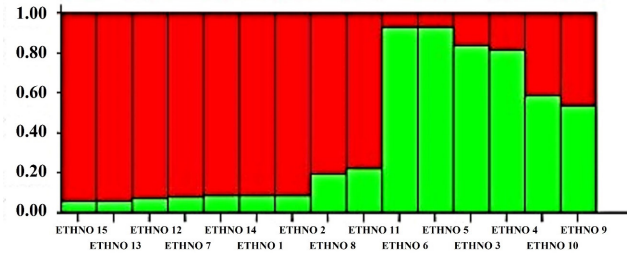
Figueredo *et al*. (2019) obtained the formation of four groups from the Tocher method, in which 76.47% of the ethnovarieties studied were allocated to Group I; while, in the present work, the first group formed by the Tocher optimization method groups 53.33% of the ethnovarieties evaluated. Using the Tocher method, Tiago *et al.* (2016) also obtained the formation of six groups, which clustered 12 genotypes and corresponds to 70.6% of the genotypes evaluated.

Figure 5 - Ethnovarities that formed isolated groups in the two methods of clustering (UPGMA and Tocher). ETHNO 01 (A; B); ETHNO 02 (C; D) AND ETHNO 15 (E; F).

Source: the authors.

The Bayesian analysis, obtained via the Structure program, divided the 15 cassava ethnovarieties into two genetic groups (k = 2) (Figure 6). The genetic group represented in green allocated the ethnovarieties present in Groups I and II of the UPGMA, and in Group I of the Tocher method, with the exception of ETHNO 11 and ETHNO 8, the other ethnovarieties constituted the red group.

Figure 6 - Grouping of the 15 ethnovarieties of cassava, in two genetic groups (red and green), obtained using the Structure program. The vertical lines along the X-axis represent the individuals and the colored segments along the Y-axis demonstrate the association coefficient of each individual assigned to each of the Ks



Source: the authors.

In a study of the genetic diversity of traditional cassava accessions in the state of Minas Gerais, Gonçalves *et al*. (2017) revealed, according to ΔK, the formation of four distinct groups, and observed a mixture of the four subpopulations formed, as was observed in our study.

The results obtained in this study are indicators of the existence of genetic diversity among cassava ethnovarieties cultivated by farmers in the north of the state of Mato Grosso. Oler (2017) explains that the interaction between farmers and the process of exchange of vegetative propagation material (setts), especially with the insertion of material from more geographically distant places, causes a greater genetic dissimilarity between ethnovarieties and this may have occurred in the municipalities of Mato Grosso sampled herein.

4 Conclusion

Genetic diversity was found among the cassava ethnovarieties evaluated and, among them, the *Cascatinha* (ETHNO 01), *Liberata* (ETHNO 02) and *Casca branca* (ETHNO 15) ethnovarieties have the greatest potential for future studies since they are the most genetically dissimilar.

The family farmers of the municipalities sampled in the state of Mato Grosso carry out the exchange of setts among themselves, which is an important practice for the maintenance and conservation of the local diversity of the species.

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