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Research Article

On-farm conservation of cassava in Cuiabá, Mato Grosso state, Brazil: ethnobotanical aspects and genetic diversity¹

Melca Juliana Peixoto Rondon², Auana Vicente Tiago³, Eulalia Soler Sobreira Hoogerheide³

ABSTRACT

Family farmers are important in the on-farm conservation of cassava, since they maintain distinct ethnovarieties in their plantations. This research aimed to analyze ethnobotanical aspects and the genetic diversity of cassava ethnovarieties maintained by farmers in the Baixada Cuiabana, Mato Grosso state, Brazil, in the Rio dos Couros community. A semi-structured questionnaire was applied for the ethnobotanical characterization of the cassava ethnovarieties. Subsequently, young leaves of the cassava plants were collected for DNA extraction. The ethnobotanical survey identified 29 cassava ethnovarieties, with the most cultivated ones being Pão, Amarelona and Cacau. The farmers characterize the cassavas with the following characteristics: palatability, pulp color, origin and similarities with animals and vegetables. Genetic relationships were observed among the ethnovarieties and five groups were identified, with formation of subgroups. In these groupings, it is possible to highlight the isolation of the Matrinxã, Paraguinha and Broto Roxo ethnovarieties as the most genetically divergent ones.

KEYWORDS: *Manihot esculenta*, genetic erosion, plant genetic resources.

RESUMO

Conservação *on farm* de mandioca em Cuiabá, Mato Grosso: aspectos etnobotânicos e diversidade genética

Agricultores familiares são importantes na conservação *on farm* de mandioca, visto que mantêm em suas plantações distintas etnoveriedades. Objetivou-se analisar aspectos etnobotânicos e a diversidade genética de etnoveriedades de mandioca mantidas por agricultores da Baixada Cuiabana, Mato Grosso, na comunidade Rio dos Couros. Um questionário semiestruturado foi aplicado para a caracterização etnobotânica das etnoveriedades de mandioca. Posteriormente, folhas jovens das mandiocas foram coletadas para extração de DNA. O levantamento etnobotânico identificou 29 etnoveriedades, sendo as mais cultivadas a Pão, Amarelona e Cacau. Os agricultores caracterizam as mandiocas com os seguintes caracteres: palatabilidade, cor da polpa, origem e semelhanças com animais e vegetais. Observaram-se relações genéticas entre as etnoveriedades e cinco grupos foram identificados, com formação de subgrupos. Nesses agrupamentos, foi possível destacar o isolamento das etnoveriedades Matrinxã, Paraguinha e Broto Roxo como as mais divergentes geneticamente.

PALAVRAS-CHAVE: *Manihot esculenta*, erosão genética, recursos genéticos vegetais.

INTRODUCTION

Family farming is intrinsically linked to the food and nutrition security of the Brazilian population. It does not only boost local economies, but also contributes to sustainable rural development by establishing an intimate relationship and lasting family bonds with its housing and production environment (Bittencourt 2020).

In Brazil, cassava is commonly cultivated by family farmers, and is one of the most traditional agricultural crops, with effective cultivation in all

regions of the country (Aplevicz & Demiate 2007). As farmers maintain different varieties in their plantations, they play an important role in the on-farm conservation of cassava, which, in addition to promoting its conservation, also expands the genetic variability of the species, and the exchange of propagation material (setts) is one of the main factors that contribute to the increase in diversity (Figueredo et al. 2019, Tiago et al. 2019).

Brazil is considered the origin and a center of diversity of cassava, and has a wide genetic variability conserved *in situ*. Its cultivation by small

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rural or indigenous producers maintains the genetic variability of the species conserved on farm (Tiago et al. 2016, Salomão 2010). In the region known as Baixada Cuiabana, in the Mato Grosso state, north of the Mato Grosso Pantanal, the traditional communities show a great dedication to the production of cassava, with extensive knowledge about the management of cultivars, and the processing of all its by-products has been traditionally carried out for a long time by families, thus ensuring their main source of income (Amorozo 2002, Marchetti 2012).

The great genetic diversity presented by the cassava crops is due to natural selection during the evolution of the species, domestication, ease of cross-pollination, high heterozygosity and abrupt dehiscence of the fruits, which continuously originate a multitude of new genotypes (Fukuda & Silva 2002). The vegetative propagation via human actions and activities ensures the perpetuation of the species that are maintained and propagated among farmers in the community itself, or even by various regions. Therefore, the characterization and evaluation of cassava germplasm is essential for its efficient use in breeding work and conservation studies. In addition, ethnobotanical and population genetics studies enrich the understanding about the maintenance of the agricultural diversity of this crop, and may even indicate priority areas for conservation (Oler & Amorozo 2017).

As such, this study aimed to analyze the ethnobotanical aspects and estimate the genetic diversity of cassava ethnovarieties conserved on farm by farmers in the Mato Grosso state, Brazil.

MATERIAL AND METHODS

The study was carried out at the Rio dos Couros community, in the Baixada Cuiabana region, municipality of Cuiabá, which is located in the MRH-335 microregion (15°30'S and 15°40'S; and 55°35'W and 55°50'W), in the central-south mesoregion of the Mato Grosso state, Brazil (RadamBrasil 1982), from July 2014 to July 2015. The Baixada Cuiabana region covers 14 municipalities (Acorizal, Barão de Melgaço, Campo Verde, Chapada dos Guimarães, Cuiabá, Jangada, Nobres, Nossa Senhora do Livramento, Nova Brasilândia, Poconé, Rosário Oeste, Santo Antônio do Leverger, Várzea Grande and Planalto da Serra) and has several traditional communities (Souza & Amaral 2015).

The community includes about 40 farmers who develop various agricultural activities in their properties, such as cassava cultivation, cassava flour manufacturing and management activities, as well as activities linked to the community's small-producers association. Among the total number of people in the community, only ten can be classed as farmers who cultivate cassava in their properties.

A semi-structured questionnaire was applied to the farmers, which contained questions related to the characteristics of each ethnovariety maintained by them, such as criteria for ethnovarieties identification, root morphological aspects, types of use, cultivation time and ethnovarieties origin. The research was registered at the SisGen platform under the number A3DF14E.

For the molecular study, young leaves of each cassava ethnovariety maintained by the ten farmers were collected. The leaf samples were inserted into 2.0 mL polypropylene tubes with loading buffer (1 mL of saturated NaCL-CTAB solution, 70 g of NaCL and 3 g of CTAB dissolved in 200 mL of distilled water). Then, the material was identified and sent to the laboratory, where it was stored in a freezer at -4 °C, until DNA extraction.

The DNA extraction was performed following the Doyle & Doyle (1990) protocol, based on the CTAB method (cetyltrimethyl ammonium bromide), with modifications: STE buffer for macerating the leaves instead of liquid nitrogen, increased concentration of polyvinylpyrrolidone (PVP) from 1 to 2 % and β -mercaptoethanol from 0.2 to 2 % in the extraction buffer, in addition to reducing the incubation time from 60 to 30 min, at 65 °C.

To verify the quality of the extracted DNA, the samples were placed on 1 % agarose gel, stained with GelRed (Biotium, Hayward, USA), quantified based on absorbance at 260 nm and purity analysis based on their absorbance ratio at 260 and 280 nm, using a spectrophotometer (Nanodrop-2000, ThermoScientific). After quantification, the DNA concentrations were standardized to 100 ng μ L⁻¹. For the amplification of the material, 15 microsatellite loci (SSR) were selected (Chavarriaga-Aguirre et al. 1998, Mba et al. 2001) and labeled with fluorochrome 6-FAM (blue) and HEX (green) (Table 1).

The amplification reactions were conducted using a final volume of 10 μ L, containing 1 μ L of buffer [0.05 % (w/v) bromophenol blue, 40 % (w/v) sucrose, 0.1 M EDTA pH 8.0 and 0.5 % (w/v) sodium

Table 1. Microsatellite loci used in the molecular characterization of cassava ethnovarieties from the Rio dos Couros community, Cuiabá, Mato Grosso state, Brazil.

Locus	Fluorochrome	Motif	Classification	(bp)
SSRY-21**	FAM	(GA) ₂₆	Simple perfect	172-212
SSRY-28**	HEX	(CT) ₂₆ (AT) ₃ AC(AT) ₂	Compound imperfect	160-214
SSRY-27**	FAM	(CA) ₁₄	Simple perfect	245-297
SSRY-35**	HEX	(GT) ₃ GC(GT) ₁₁ (GA) ₁₉	Compound imperfect	174-310
SSRY-8**	FAM	(CA) ₁₄ CT(CA) ₂	Simple imperfect	268-320
GAGG-5*	HEX	NP	-	108-150
GA-12*	FAM	NP	-	119-180
GA-21*	HEX	NP	-	104-146
GA-131*	FAM	NP	-	75-141
SSRY-43**	HEX	(CT) ₂₅	Simple perfect	229-275
SSRY-47*	FAM	(CA) ₁₇	Simple perfect	216-280
SSRY-126*	HEX	(GT) ₂ T(GT) ₅ (GC) ₄	Compound imperfect	225-297
GA-136*	FAM	NP	-	145-185
GA-140*	HEX	NP	-	154-192
SSRY-40*	HEX	(GA) ₁₆	Simple perfect	211-269

* Chavarriaga-Aguirre et al. (1998); ** Mba et al. (2001). NP: motif has not been published; (bp): amplitude of amplification - base pairs.

sulfate (SDS)]; 0.8 µL of dNTPs (2.5 mM); 0.13 µL of broth and 0.25 µL for each primer [forward and reverse (20 mm), respectively]; 0.2 µL of Taq DNA polymerase (5 u); 0.25 µL of HEX and 0.25 µL FAM (2 mm) markers; and 100 ng of DNA, with the volume being completed with ultrapure Milli-Q water.

The reactions were carried out in a thermocycler (T100, Bio-RAD) under the following conditions: initial denaturation at 94 °C for 5 min; 30 cycles followed by denaturation at 94 °C for 30 sec; annealing temperature of 45 °C for 45 sec and 72 °C for 45 sec, and eight cycles at 94 °C for 30 sec, 53 °C for 45 sec, 72 °C for 45 sec, and a final extension of 72 °C for 10 min. The amplifications were submitted to electrophoresis in 1.5 % agarose gel, with 0.5 x TAE buffer, and constant voltage of 80 V for 40 min, and then visualized on an ultraviolet light transilluminator (L-PIX Image, Loccus Biotecnologia) to check the quality of the reactions.

The genotyping of the samples was performed via capillary electrophoresis, in an automatic DNA analyzer (ABI 3130xl GeneticAnalyzer, Applied Biosystems, Foster City, California, USA). The recognition of the size of the amplified fragments was performed with a DNA of known size (Rox 500, Applied Biosystems) using the GeneMarker® software v.2.6.3 (Softgenetics).

The ethnobotanical data were interpreted descriptively. For the classification of the nomination of cassava ethnovarieties, the criteria described by

Marchetti (2012) were used. The genetic diversity of cassava ethnovarieties was estimated via allele frequencies, number of alleles per locus (A), expected (*He*) and observed (*Ho*) heterozygosity, allele fixation index (*f*) and percentage of polymorphic loci (%P), using the Genetic Data Analysis (GDA) software (Lewis & Zaykin 2001).

The PowerMarker v. 3.25 software (Liu & Muse 2005) was used to construct the dendrogram with the unweighted pair-group method using arithmetic averages (UPGMA), based on the Nei (1973) distance, and to analyze the polymorphic information content (PIC).

RESULTS AND DISCUSSION

In total, 29 ethnovarieties were cited as being cultivated by the ten farmers, with an average of three ethnovarieties/farmer, a result obtained descriptively, according to the frequency of citations by the producers. A similar study was conducted by Oler (2012), in two communities in the municipality of Porto Estrela, Mato Grosso state, who found a total of 57 ethnovarieties for 30 farmers, with an average of 1.9, i.e., lower than what was found in the present research.

The ethnovarieties cited as being maintained by the farmers were: Pão, Vassourinha, Abóbora, Brancona, Urubu, Amarelona, Canela de Ema, Osso, Matrinxã, Orelha de Onça, Manteiga, Cacau, Liberatona, Menina Branca, Latadeira, Aipim,

Branquinha, Juriti, Liberatinha, Broto Roxo, Galhadeira, Pele Roxa, Estrondadeira, Paraguinha, Roxona, Piauí, Guarapé, Amarela and Liberata. The cassava ethnovarieties of the Rio dos Couros community are named based on palatability, pulp color, place of origin and similarities with animals and vegetables (Table 2).

The most cited ethnovarieties (27.59 %) were Pão (six times), Amarelona (four times), Cacau (three times) and Brancona, Osso, Manteiga, Juriti and Galhadeira (twice for each). According to the farmers, Pão is the most cultivated ethnovariety in the community, because it presents good yields and adapts well to the predominantly stony soil. This type of soil hinders the performance of cassava, i.e., good root yield, due to the limitation that it imposes on root development (Bengough et al. 2011), leaving the roots smaller and firmer. In regions where the climate is predominantly dry and hot, soils tend to be shallow and stony, due to the reduced amount of rainfall. An important feature of cassava is the ability to adapt to different types of soils. Thus, it is preferable to cultivate it in looser and deeper soils, because, in addition to allowing the thickening of the roots, these soils facilitate harvesting (Souza & Fialho 2003). Of the 29 ethnovarieties mentioned, 21 are maintained by a single farmer (72.41 %), revealing a high risk of genetic erosion.

Of the 29 ethnovarieties cited, 22 have been already ethnobotanically described. This difference occurred because, as there was an interval between the collection of leaf tissue and the application of the questionnaire, due to the need to wait for authorization from the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) for access to traditional knowledge, some farmers lost certain

ethnovarieties, and others were no longer found in the community during this interim.

Most of the ethnovarieties presented white pulp, except for Matrinxã, which has a different aspect in the pulp coloration and is similar to the color of a fish (Table 3). The yellow coloration is related to carotenoid contents, especially β -carotene, which is a precursor of vitamin A and represents a nutritional benefit (Fagundes et al. 2021). The genetic breeding programs of cassava for cooking are focused on the selection of varieties with this characteristic, and aim at the nutritional improvement of the reserve roots (Silva et al. 2014). Thus, these ethnovarieties are sources of genes of interest in breeding programs that target this trait.

The ethnovarieties Estrondadeira, Amarelona, Osso and Brancona are intended only for flour production, and Paraguinha, according to the farmers, has a higher yield than the others, in addition to multiple uses. The same is true for Broto Roxo, which can be used for the production of starch, cassava flour and cooking. In this sense, the ethnovarieties that have two uses, i.e., used for cooking and flour production, are Cacau, Manteiga, Galhadeira, Liberata, Pele Roxa, Abóbora, Branquinha, Liberatona and Abóbora. The ethnovarieties Pão and Cacau had a greater preference among the producers, as they present a good yield and palatability, indicating that sensory characteristics are fundamental for a certain ethnovariety to be adopted or not (Souza et al. 2016).

Regarding the ethnovarieties origin, it was identified that the majority (32 %) was acquired from neighbors, predecessors (inherited) (18 %) or donated by friends (22 %). The remaining forms of acquisition (23 %) are donations from relatives, spontaneous origin or from other communities, municipalities or states. There were also farmers

Table 2. Classification of cassava ethnovarieties from the Rio dos Couros community, Cuiabá, Mato Grosso state, Brazil, according to Marchetti (2012).

Identification criteria	Ethnovarieties
Coloring (stem, petiole and leaves)	Brancona, Amarelona, Menina Branca, Broto Roxo, Pele Roxa, Roxona, Branquinha, Amarela
Morphological features (architecture, stem branching and root growth)	Vassourinha, Liberatona, Latadeira, Liberatinha, Galhadeira, Estrondadeira, Liberata
Similarity to living things (animals)	Urubu, Canela de Ema, Osso, Matrinxã, Orelha de Onça, Juriti
Similarity to living things (vegetables)	Abóbora, Aipim, Cacau
Origin	Piauí, Guarapé, Paraguinha
Palatability (taste/texture)	Pão, Manteiga

Source: Souza et al. (2016).

Table 3. Characterization of 22 cassava ethnovarieties found in the Rio dos Couros community, Cuiabá, Mato Grosso state, Brazil, their root-pulp color and different uses.

Acronym	Ethnovarieties	Pulp	Uses
RC1	Pão	White	CC
RC2	Vassourinha*	-	CC
RC3	Abóbora*	-	CC/FP
RC5	Urubu*	-	CC
RC6	Amarelona	White	FP
RC7	Canela de Ema	White	CC
RC9	Osso	White	FP
RC10	Matrinxã	White with red stripes	CC
RC11	Orelha de Onça	White	CC
RC12	Manteiga	Yellow	CC/FP
RC13	Cacau	White	CC/FP
RC17	Liberatona	Yellow	CC/FP
RC23	Branquinha	White	CC/FP
RC24	Juriti	White	CC
RC26	Liberatinha	White	CC
RC28	Broto Roxo	White	CC/FP/SP
RC30	Liberata	White	CC/FP
RC31	Pele Roxa	White	CC/FP
RC32	Estrondadeira	White	FP
RC34	Paraguainha	White	CC/FP/SP
RC38	Brancona	White	FP
RC44	Galhadeira	White	CC/FP

* The cassava ethnovarieties were lost by the farmers, but they provided information about the type of use. CC: cassava for cooking; FP: flour production; SP: starch production. Source: Souza et al. (2016).

who did not know the origin of the material (5 %). Nine of these ethnovarieties were introduced to the Rio dos Couros community over a ten-year period (40.91 %). The ethnovarieties known as Amarelona, Cacau, Manteiga and Paraguainha have been in the possession of the same owner for more than 20 years. The remaining ethnovarieties (13-59.09 %) have been owned by them for at least five years.

The estimated genetic diversity for the 29 ethnovarieties is shown in Table 4. The number of mean alleles per locus was 5.6. The expected (H_e) and observed (H_o) heterozygosity stood out with values between 0.67 and 0.72, respectively, and the observed heterozygosity was higher than expected in nine of the 15 analyzed loci (Table 4). Based on the H_e , H_o and f , it can be inferred that there is no inbreeding among the evaluated individuals.

Thus, it is possible to observe that the studied cassava ethnovarieties presented genetic diversity among themselves. This is possibly related to the selection made by farmers, which ensures a high vigor and heterozygosity among the ethnovarieties

Table 4. Estimation of parameters of genetic diversity for the 15 microsatellite loci in 29 cassava ethnovarieties cultivated in the Rio dos Couros community, Cuiabá, Mato Grosso state, Brazil.

Loci	A	H_e	H_o	f	PIC
SSRY21	8	0.77	0.78	0.00	0.73
SSRY28	8	0.82	0.82	0.01	0.79
SSRY27	7	0.65	0.64	0.05	0.62
SSRY35	5	0.74	0.48	0.37	0.69
SSRY8	6	0.67	0.76	-0.12	0.62
GAGG5	2	0.44	0.66	-0.47	0.34
GA12	4	0.66	0.76	-0.13	0.59
GA21	4	0.52	0.52	0.03	0.42
GA131	8	0.82	1.00	-0.20	0.80
SSRY43	6	0.76	0.90	-0.15	0.73
SSRY47	7	0.80	0.94	-0.14	0.77
SSRY126	2	0.13	0.00	1.00	0.12
GA136	5	0.67	0.85	-0.24	0.61
GA140	7	0.75	0.71	0.07	0.71
SSRY40	5	0.78	0.93	-0.18	0.74
Mean	5.6	0.67	0.72	-0.05	0.62

A: number of alleles per locus; H_e : expected heterozygosity; H_o : observed heterozygosity; f : allele fixation index; PIC: polymorphic information content.

that are maintained via vegetative propagation, thus explaining the low inbreeding. The average polymorphic information content (PIC) for the 15 analyzed loci was 0.62 (Table 4), with 80 % of these loci presenting values greater than 0.50, what indicates that the primers are considered highly informative for genetic diversity studies (Botstein et al. 1980).

The dendrogram in Figure 1 represents the genetic relationships of the ethnovarieties, for which five distinct groups were identified. The GI group, which presented the greatest distance in relation to the other groups, isolated the Matrinxã (RC10) ethnovariety and demonstrated that it is the most divergent one, in relation to the others. This fact can be explained by the difference in the alleles found among the ethnovarieties, as it is the only ethnovariety with a root-pulp coloring that is white with red stripes, in addition to being among the ethnovarieties cultivated for more than ten years in the community and used for cooking. Therefore, these factors may favor its isolation, in relation to the other ethnovarieties.

The GII group consisted of two ethnovarieties, known as Orelha de Onça (RC11) and Branquinha (RC23), both of which stood out for the characteristic coloration of the white pulp (Table 3). The same fact was observed in the GIV Group, but consisted

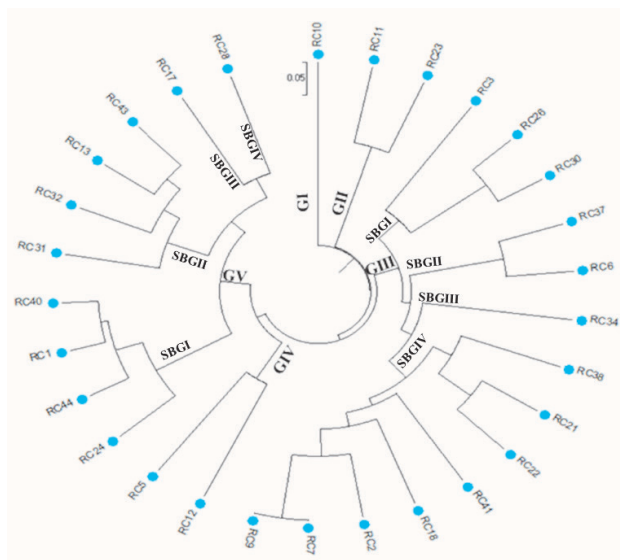


Figure 1. Dendrogram based on the Nei (1973) distance, by the unweighted pair-group method using arithmetic averages (UPGMA), for the 29 cassava ethnovarieties found in the Rio dos Couros community, Cuiabá, Mato Grosso state, Brazil. RC1: Pão; RC2: Vassourinha; RC3: Abóbora; RC5: Urubu; RC6: Amarelona; RC7: Canela de Ema; RC9: Osso; RC10: Matrinxã; RC11: Orelha de Onça; RC12: Manteiga; RC13: Cacau; RC17: Liberatona; RC18: Menina Branca; RC21: Latadeira; RC22: Aipim; RC23: Branquinha; RC24: Juriti; RC26: Liberatinha; RC28: Broto Roxo; RC30: Liberata; RC31: Pele Roxa; RC32: Estrondadeira; RC34: Paraguainha; RC37: Amarela; RC38: Brancona; RC40: Roxona; RC41: Piauí; RC43: Guarapé; RC44: Galhadeira.

of the ethnovarieties Urubu (RC5) and Manteiga (RC12). In this group, the RC12 stands out for its characteristic pulp, that has a yellow coloration, but there is no information for the RC5 (Table 3). Based on the previous grouping, for the ethnovarieties RC11 and RC23, it is possible to infer that perhaps this ethnovariety also has the same characteristics as the RC12, and may, therefore, be allocated in the same group.

The GIII and GV groups were the most numerous ones, with 14 and 10 ethnovarieties, respectively, as well as the formation of subgroups (Figure 1). Observing the subgroups of the GIII group, these can be divided into four subgroups. As these are formed by a mixture of ethnovarieties, the groupings are therefore not related to the types of use (cooking, flour production and starch), except for the SBGI subgroup, which was constituted only by the ethnovarieties, namely RC34 (Paraguainha), which is

destined for multiple uses (Table 3), and is different from the other subgroups. This is an ethnovariety that has had the same owner for more than 20 years, and, therefore, presents exclusive genetic characteristics, when compared to the others studied. The same fact was observed in the subgroups of the GV group, but with the isolation of two ethnovarieties, in this case, RC17 and RC28 (Liberatona and Broto Roxo, respectively). RC28 was also indicated by farmers as having multiple uses.

In general, it was observed that the Matrinxã, Paraguainha and Broto Roxo ethnovarieties are the most genetically divergent ones, a fact also confirmed via the ethnobotanical study.

This result demonstrates that there is an exchange of plant material (setts) among farmers, which extends beyond municipalities and even regions (Pedri et al. 2021). The number of ethnovarieties sampled in this study confirms this fact, since a significant number of ethnovarieties has been cultivated for less than 5 years, i.e., they have been recently introduced into the community. Oler & Amorozo (2017) and Figueredo et al. (2019) emphasize the importance of this flow of setts, which guarantees the expansion of the collection through new introductions and also guarantees the genetic variability of the species. This suggests that the inclusion of setts followed by clonal propagation is important for the maintenance and expansion of genetic diversity in cultivated varieties (Alves-Pereira et al. 2017).

The studied ethnovarieties have potential for future researches on genetic breeding that explore their productive potential, since they are cultivated to meet the local needs of each farmer.

CONCLUSIONS

1. The farmers of the Rio dos Couros community (Cuiabá, Mato Grosso state, Brazil) name the cassava varieties primarily for their palatability, pulp color, place of origin and similarities to animals and vegetables;
2. Pão is the ethnovariety most frequently cultivated by them, because it has a good yield and adaptability to the predominantly stony soil, and the most cited ethnovarieties were Pão, Amarelão and Cacau;
3. The cassava collection of the community has a high genetic diversity, with the Matrinxã, Paraguainha

and Broto Roxo ethnovarieties being the most divergent ones;

4. The ethnobotanical characterization and the analysis of genetic diversity complemented each other and, together, helped to explain the divergence between the ethnovarieties, mainly by highlighting the color of the root pulp and types of uses.

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