SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 18, NUM. 2 2022
doi: 10.14808/sci.plena.2022.021201

Are floral characters efficient in the phenotypic differentiation of cassava landraces?

Caracteres florais são eficientes na diferenciação fenotípica de etnovariedades de mandioca?

E. C. M. de Pedri[1*]; A. V. Tiago[1]; E. S. Cardoso[1]; E. S. S. Hoogerheide[2]; A. A. B. Rossi[1]

[1]*Faculty of Biological and Agrarian Sciences, Laboratory of Plant Genetics and Molecular Biology, State University of Mato Grosso Carlos Alberto Reyes Maldonado, 78580-000, Alta Floresta-Mato Grosso, Brazil*
[2]*Embrapa Agrossilvipastoril , 78550-970, Sinop-Mato Grosso, Brazil*

**elicmbio@gmail.com*
(Recebido em 01 de fevereiro de 2021; aceito em 18 de fevereiro de 2022)

Manihot esculenta Crantz assumes a prominent role in the world for being one of the most cultivated foods in the tropics. The aim of this study was to verify whether floral characters are efficient to detect phenotypic variability among cassava landraces cultivated in the northern region of Mato Grosso state, Brazil. For this, at six months after planting, the period of the first flowering, the phenotypic characterization of the female and male flowers of 20 cassava landraces was carried out, using the characters: color of the sepals, color of the disc, color of the ovary, color of the stigma and color of the anthers. The relative frequency of the phenotypic classes of each descriptor was analyzed. Qualitative data were subjected to genetic diversity analysis by the procedure for multicategorical data (multiple classes). The floral characters were efficient in the phenotypic differentiation of the cassava landraces. The color of the sepals, color of the disc and color of the ovary were the characters that contributed to the variation found, therefore, they are useful for preliminary evaluations of the species. The UPGMA method grouped the cassava landraces into four distinct groups, which showed the phenotypic variability and confirmed the efficiency of the floral characters in separating the landraces evaluated in different groups and subgroups. With the analyses carried out, it was possible to propose a new phenotypic class for the color of the sepals (greenish purple), going from three to four classes, which allowed a better distinction among the evaluated material.
Keywords: genetical diversity, flowers, *Manihot esculenta*.

Manihot esculenta Crantz assume papel de destaque no mundo por ser um dos alimentos básicos mais cultivados nos trópicos. O objetivo deste estudo foi verificar se caracteres florais são eficientes para detectar a variabilidade fenotípica entre etnovariedades de mandioca cultivadas na região norte do estado de Mato Grosso. Para tanto, aos seis meses após o plantio, período da primeira floração, foi realizada a caracterização fenotípica das flores femininas e masculinas de 20 etnovariedades de mandioca, utilizando os caracteres: cor das sépalas, cor do disco, cor do ovário, cor do estigma e cor das anteras. A frequência relativa das classes fenotípicas de cada descritor foi analisada. Os dados qualitativos foram submetidos à análise de diversidade genética pelo procedimento para dados multicategóricos, múltiplas classes. Os caracteres florais foram eficientes na diferenciação fenotípica das etnovariedades de mandioca. A cor das sépalas, cor do disco e cor do ovário foram os caracteres que contribuíram para a variação encontrada, sendo, portanto, úteis para as avaliações preliminares da espécie. O método UPGMA agrupou as etnovariedades de mandioca em quatro grupos distintos, evidenciando variabilidade fenotípica e confirmando a eficiência dos caracteres florais na separação das etnovariedades avaliadas em grupos e subgrupos distintos. Com as análises realizadas foi possível propor uma nova classe fenotípica para o caracter cor das sépalas (roxa-esverdeada), passando de três para quatro classes, permitindo melhor distinção entre o material avaliado.
Palavras-chave: diversidade genética, flores, *Manihot esculenta*.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz), also known as manioc, is the only species of the genus Manihot domesticated for human consumption [1]. Originating in South America [2], cassava is widely cultivated in Brazil, especially by family farmers, for its easy propagation, adaptation to edaphoclimatic conditions and productive potential. Its tuberous roots are intended for human and animal food as well as for processing agro-industries [3, 4].

The inflorescences of cassava are formed at the upper end of the stems, it is a monoic plant, that is, it presents male and female flowers arranged on the same plant, with male ones located in the upper region, in greater number, and female ones in lesser number, in lower region [5]. The species is allogamous, with preferential cross-pollination, protogenic, in other words, its female flowers open before male flowers. However, the simultaneous development and opening of flowers can occur, which also favors self-fertilization [6, 7].

The female flowers of cassava have five free sepals, a smooth and well-developed disc and on this disc there is na ovarian super ellipsoidal and trilocular with one ovule per loculus. There are three styles, short, attached to each other and at their ends is the well-developed, wide, wavy and fleshy stigma [8].

In male flowers, the sepals are joined to about half their length, involving the disc under which two series of stamens are inserted; the anthers have an elongated shape and are inclined towards the interior of the flower [8]. The main pollinators of the species are coleopterans, wasps, bees (*Apis mellifera* Linnaeus) and irapuá bees (*Trigona spinipes* Fabricius), which are attracted by the odor of flowers, pollen and nectar [9].

The maintenance of the cassava genetic diversity is mainly due to the work that farmers perform in their fields, since they cultivate different landraces, with different characteristics and names, and constantly introduce new genotypes into their crops [10]. Farmers hardly discard low-yielding landraces, even if they have to keep them in smaller quantities [11]. Furthermore, despite being propagated vegetatively, cassava maintained it sexual reproduction capacity, which allows the formation of new genotypes, generated via seeds, which are then incorporated into the cultivation system and maintained by vegetative reproduction, thus increasing the genetic basis of the species [9, 12].

The morphological characterization of the cassava landraces allows phenotypic differentiation and the detection of possible duplications [13]. When it still in the field, the characterization can be performed using morpho-agronomic descriptors and the complementary ones (flowers, fruits and seeds) [14]. The floral characters have the advantage of being evaluated at the beginning of the reproductive cycle of the plants [15], for cassava, it occurs at six months after planting.

Despite the importance of the species, there are few studies on the floral and reproductive biology of cassava, promoting an underutilization of available genetic resources [9]. Therefore, the objective of this study was to verify whether floral characters are efficient to detect the phenotypic variability existing among cassava landraces cultivated in the northern region of the Mato Grosso state.

2. MATERIALS AND METHODS

2.1 Sampling

Cuttings (propagating material) were collected from 20 landraces of cassava grown in ten municipalities located on BR 163, in the northern region of the Mato Grosso state: Nova Mutum (NMU), Lucas do Rio Verde (LCA), Sorriso (SOR), Sinop (SNP), Itaúba (ITA), Nova Santa Helena (SHE), Terra Nova do Norte (TNO), Peixoto de Azevedo (PXT), Matupá (MTA) and Guarantã do Norte (GUA) (Table 1; Figure 1).

Table 1: Cassava landraces cultivated in the northern region of the Mato Grosso state, Brazil.

Code	Landraces	Code	Landraces
NMU03	*Cascatinha*	SHE01	*Amarelinha*
NMU09	*Mandioca branca*	SHE03	*Branca quatro meses*
LCA07	*Folha roxa*	TNO02	*Vassourinha*
LCA09	*Talo roxo*	TNO03	*Cacauzinha*
SOR07	*Liberata*	PXT01	*Cacau*
SOR09	*Cacau*	PXT03	*Branca 01*
SNP03	*Mandioca pão*	MTA04	*Casca roxa 01*
SNP04	*Galhuda branca*	MTA07	*Casca roxa 02*
ITA04	*Amarela*	GUA03	*Casca branca*
ITA05	*Branca 01*	GUA04	*Casca roxa*

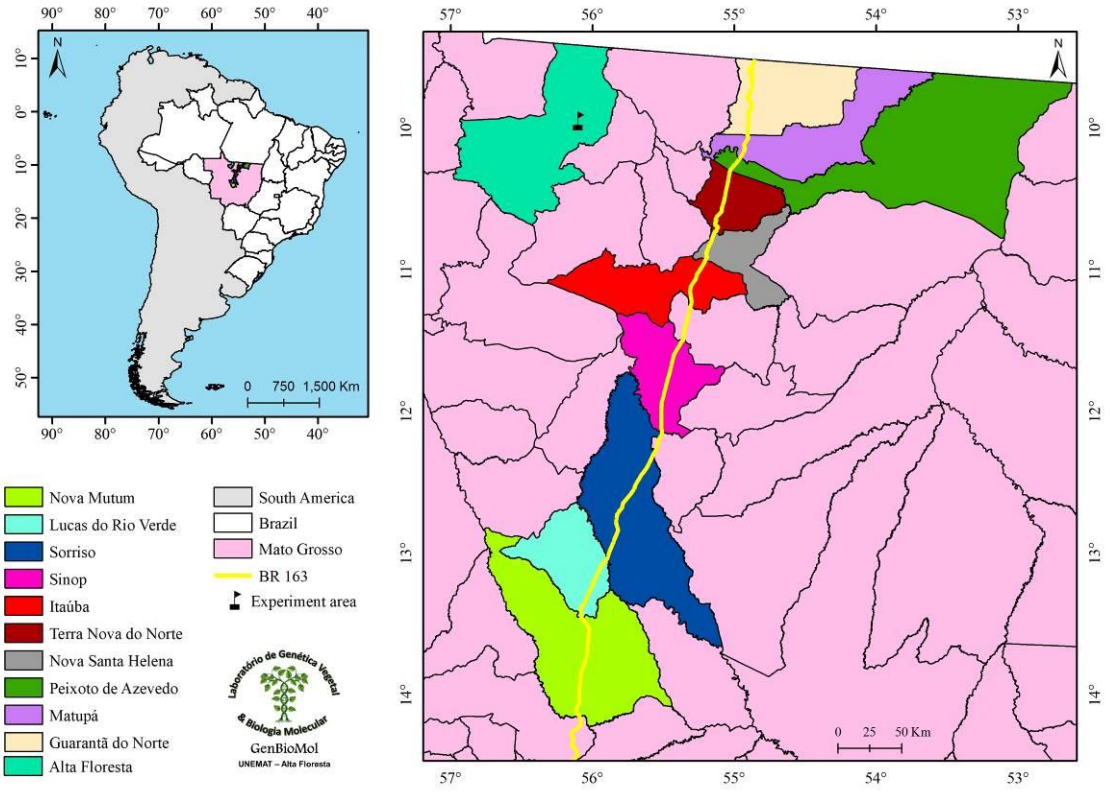

Figure 1: Geographic location of the ten municipalities for collecting cassava landraces, located on BR 163, in the northern region of the Mato Grosso state (Brazil) and the experiment area, located in the rural area of Alta Floresta, Mato Grosso, Brazil.

For multiplication and later evaluations, the propagating materials of the 20 landraces were planted in Nossa Senhora Aparecida Farm (9° 57'00.8" S; 56° 05'44.4" W), Central Community, in the municipality of Alta Floresta, Mato Grosso (Figure 1). The region's climate is of the Am type, with a dry and rainy season, an average temperature between 23 °C and 29 °C and annual precipitation ranging between 2,500 and 3,100 mm [16].

The soil in the area used for planting the landraces is classified as a dystrophic Red-yellow Latosol with a clay texture according to the triangle textural [17]. The soil preparation was carried out 15 days before planting and consisted of plowing and harrowing. Manual weeding was carried out to control invasive plants, which represents the only applied phytosanitary treatment.

The landraces were planted in simple lines, with 15 plants (cuttings) each, with spacing between plants and lines of 1.0 m and lateral borders, without repetition. The cuttings used for planting were 15-20 cm long, placed horizontally at 10 cm deep.

Six months after planting (May/2019), when the first bloom of the landraces occurred, flowers were collected and evaluated from the five central plants of each row, two females and two male flowers per plant, totaling 20 flowers per landrace.

2.2 Phenotypic characterization

For the phenotypic characterization of the cassava flowers, five qualitative morphological characters developed for the species *Manihot esculenta* (Table 2; Figure 2) were used, as described by Fukuda and Guevara (1998) [14], with adaptations related to the color of the sepals.

Table 2: Floral characters used in the phenotypic evaluation of the twenty ethnovarieties of cassava cultivated in the northern region of Mato Grosso state, Brazil.

Characters	Phenotypic classes
Sepals color	1-white or beige, 2-orange, 3-green, 4-red, 5-purple, 6-greenish-purple
Disc color	1-white or beige, 2-orange, 3-green, 4-red, 5-purple
Ovary color	1-white or beige, 2-orange, 3-green, 4-red, 5-purple
Stigma color	1-white or beige, 2-orange, 3-green, 4-red, 5-purple
Anther color	1-creme, 2-yellow, 3-others

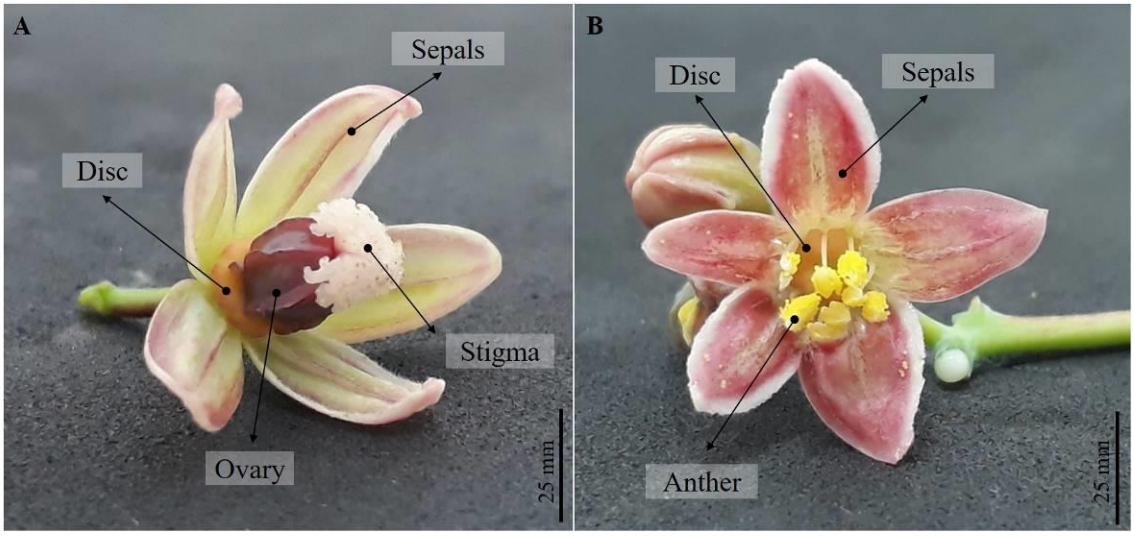

Figure 2: Detail of the floral characters evaluated for the sepals, disc, ovary and stigma of the female flower (A) and for the sepals, disc and anthers of the male flower (B) of the Talo Roxo landrace (LCA09) collected in the municipality of Lucas do Rio Verde, Mato Grosso state, Brazil.

2.3 Data analysis

The morpho-qualitative data were tabulated with the aid of the Excel 2013 software to present the relative frequency of the phenotypic classes of each character, which was obtained using formula 1:

$$\text{Relative frequency} = \frac{\text{Absolute frequency}}{\text{Total sampled}} \times 100 \quad (1)$$

Subsequently, the analysis of genetic diversity was carried out using the procedure for multiclassic data, multiple classes, from the Genes program. This methodology consists of obtaining an index, where several characters are considered simultaneously, and each descriptor can present one or more phenotypic classes [18].

To assess the consistency of the grouping, ie, to verify the ability of the dendrogram to reproduce the dissimilarity matrix, the values of the co-phenetic correlation coefficient (CCC), of the hierarchical UPGMA methods (unweighted pair group method with arithmetic mean), WARD and Nearest neighbor (Simple connection) [19] were calculated, with a significance level of 1%.

To determine the cut-off point in the dendrogram, the method of Mojena (1977) [20] was used, which is based on the relative size of the levels of fusions (distances) in the dendrogram. Statistical analyzes were performed using the Genes v. 2019.89 program [21].

The spatial location data for this study were obtained from the Brazilian Institute of Geography and Statistics (IBGE) and processed in Esri ArcGIS® software to create the thematic map.

3. RESULTS AND DISCUSSION

In the characterization, in some landraces we detected variations in the color of the sepals, completely purple flowers were observed in some landraces (Figure 3A) and in others, purple-greenish flowers (Figures 3B, C and D). Thus, considering that the morphological characters act in the phenotypic differentiation among varieties and that present a fundamental role in the dissemination of the characteristics of new genetic materials, a purple-green coloration was proposed and added to the analyzes, fitting as a new phenotypic class, for better distinction among the evaluated material.

Figure 3: Variations in the 'color of the sepals', in terms of the tone of purple. A) Landrace Folha Roxa (LCA07) with totally purple sepals; B, C and D) Landraces Liberata (SOR07), Amarela (ITA04) and Cacau (PXT01) with greenish-purple sepals.

The frequency distributions of the evaluated floral phenotypic characters are shown in Table 3. Phenotypic variability is observed among the 20 cassava landraces for three evaluated characters (Figure 4). The 'color of the stigma' and 'color of the anthers' showed no variation, all landraces presenting female flowers with a 'white or beige' color stigma and male flowers with 'yellow' anthers.

*Table 3: Floral characters and their respective phenotypic classes, number of landraces and relative frequency of the 20 cassava landraces (*Manihot esculenta *Crantz).*

Characters	Phenotypic classes	No. of landraces	Relative frequency (%)
Sepals color	6-greenish-purple	10	50%
	1-white or beige	06	30%
	3-green	02	10%
	5-purple	02	10%
Disc color	2-orange	16	80%
	4-red	03	15%
	5-purple	01	5%
Ovary color	3-green	13	65%
	5-purple	07	35%
Stigma color	1-white or beige	20	100%
Anther color	2-yellow	20	100%

For the color of the sepals, the greenish-purple (50%) and white or beige (30%) classes showed a higher frequency among the landraces. Regarding the disc color, there was dispersion among the classes, ensuring a higher frequency for those with orange (80%) and red (15%) color. The color of the ovary showed low percentage variation in the two phenotypic classes, with a green color prevailing (65%). According to Asare et al. (2011) [22], the morphological characteristics are useful for the preliminary evoluation of plant species, as it is an easy and quick approach to detect diversity.

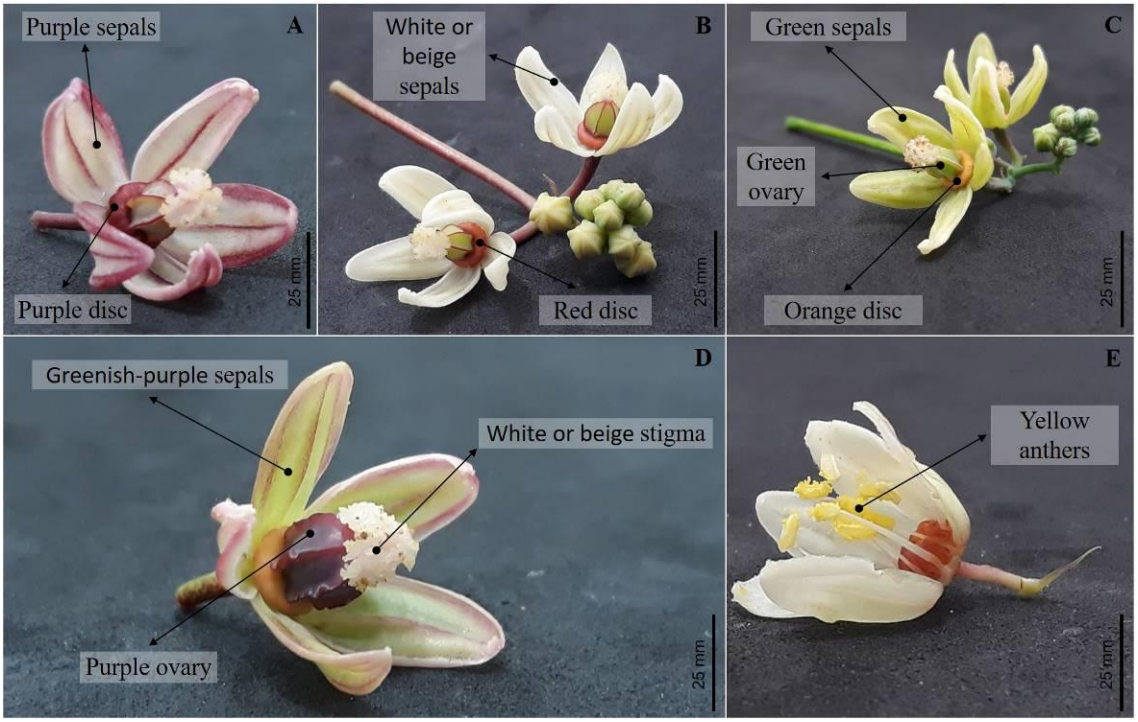

Figure 4: Phenotypic classes found among the flowers of the 20 evaluated cassava landraces. A) female flower with purple sepals and purple disc; B) white or beige sepals and red disc; C) green sepals, green ovary and orange disc; D) greenish-purple sepals, purple ovary and white or beige stigma; E) male flower with yellow anthers.

Analyzing the frequency of the characters and their phenotypic classes by sampled municipality, regardless of the evaluated landrace, it was found a greater phenotypic variability within the municipality of Lucas do Rio Verde (LCA), with eight distinct characteristics (purple and greenish-purple sepals, orange and purple disc, green and purple ovary, white or beige stigma and yellow anthers) among the eleven characteristics evaluated (Figure 5). The least variability was detected in the municipality of Matupá (MTA), with five characteristics, namely: greenish-purple sepals, orange disc, purple ovary, white or cream stigma and yellow anthers.

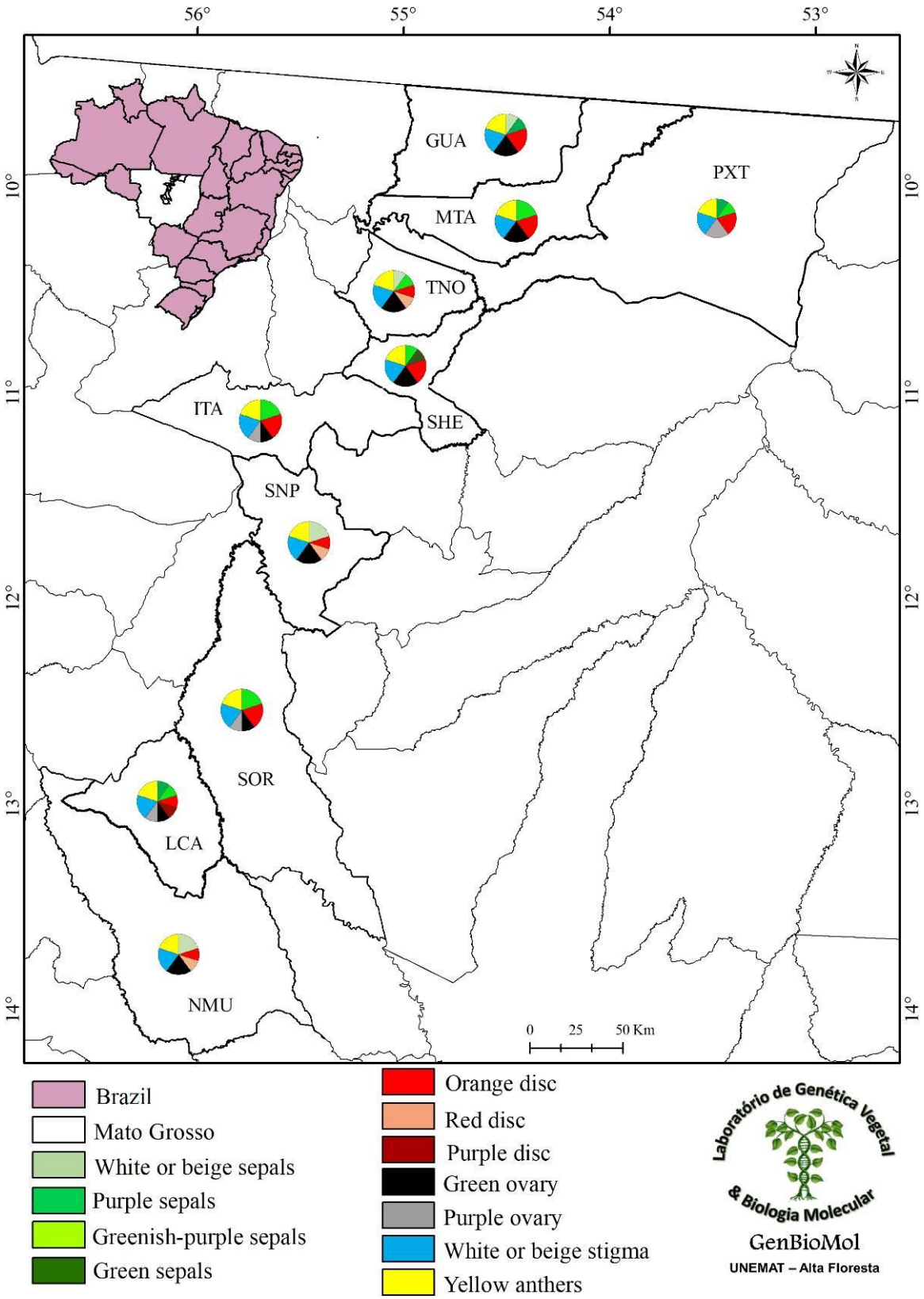

Figure 5: Frequency of floral characters and their phenotypic classes by sampled municipality, regardless of the evaluated landrace. Each circle represents a municipality: the colors within the circle comprise the characters and their classes. The municipalities are represented by code, being: NMU: Nova Mutum; LCA: Lucas do Rio Verde; SOR: Sorriso, SNP: Sinop; ITA: Itaúba; SHE: Nova Santa Helena; TNO: Terra Nova do Norte; PXT: Peixoto de Azevedo; MTA: Matupá and GUA: Guarantã do Norte.

The UPGMA method was chosen for cluster analysis of the landraces, as it presents, among the tested methods, the highest CCC value (Table 4) and according to Rohlf (1970) [23] CCC values above 0.70 reflect a good agreement between matrices. The cofenetic correlation coefficient is a measure of agreement between the original dissimilarity values and those represented in the dendrogram. The higher its value, the better is the adjustment of the dissimilarity matrix and the graphical representation of the distances [19].

Table 4: Cophenetic Correlation Coefficient (CCC) of the UPGMA (unweighted pair group method with arithmetic mean), WARD and Nearest Neighbor (simple connection) cluster methods.

Grouping methods	CCC
UPGMA	0,84**
WARD	0,67**
Nearest Neighbor	0,71**

** Significant at 1% probability, by t test.

Based on the evaluated floral phenotypic characters, the UPGMA grouping method divided the twenty cassava landraces into four distinct groups (Figure 6). Group I have nine landraces, due to its orange disc and green ovary. The three subgroups (Sub) were formed based on the color of the sepals, subgroup I consists of landraces that present white or beige sepals (SNP03, GUA04 and NMU03) (Figure 7A), subgroup II by landraces with green sepals (SHE01 and GUA03) (Figure 7B) and subgroup III by landraces with purple-green sepals (SHE03, TNO03, SOR07 and ITA05) (Figure 7C).

Group II was formed by seven landraces that presented orange disc and purple ovary. In this group, subgroup I allocated the landraces with greenish-purple sepals (Figure 7D) and subgroup II allocated the PXT03 landrace that presents purple sepals (Figure 7E). Group III comprised the SNP04, TNO02 and NMU09 landraces that showed identical phenotypic pattern (white or beige sepals, red disc and green ovary) (Figure 7F), this similarity caused duplication in the dendrogram.

The LCA07 landrace is very different from the others, as it formed an exclusive group (GIV) and remained isolated from the other landraces in the dendrogram. The purple color of its disc contributed to the isolation of this landrace (Figure 7G), as it is the only landrace among those evaluated with disc in this color.

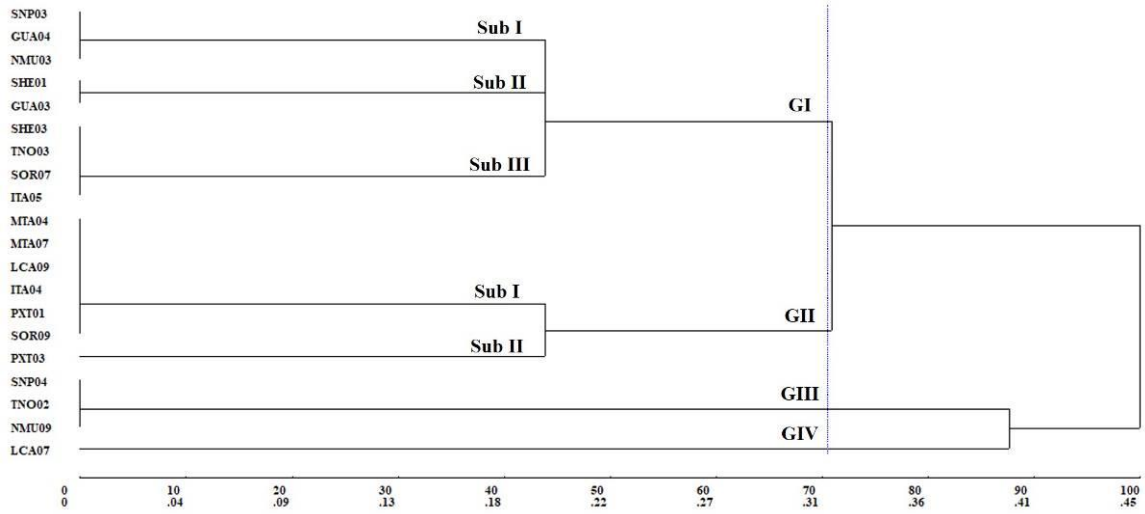

Figure 6: Analysis of genetic dissimilarity among 20 cassava landraces based on morpho-qualitative floral characters, using the UPGMA clustering method.

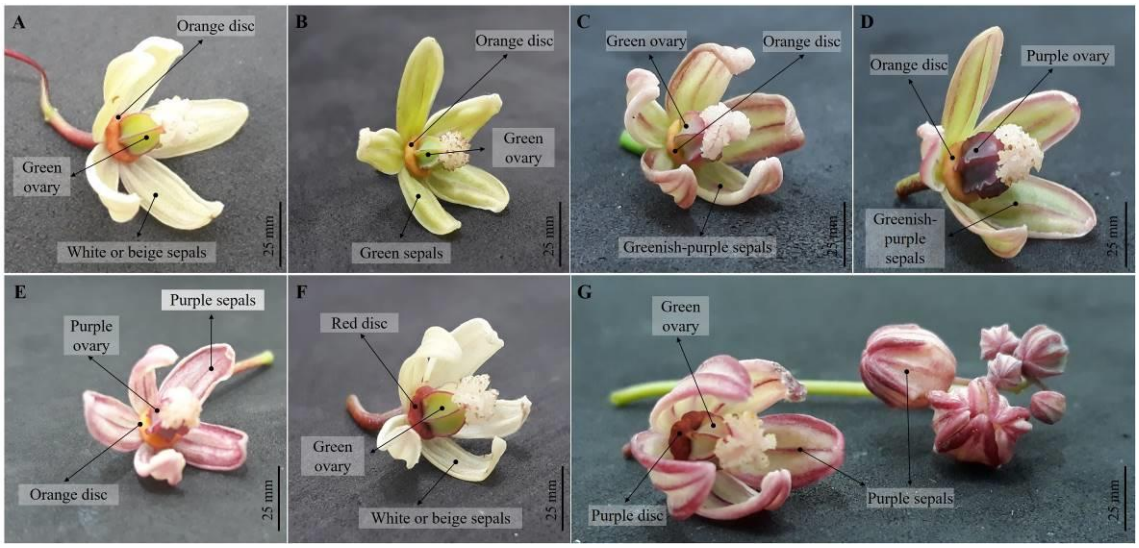

Figure 7: Phenotypic characters detected among the flowers of the twenty cassava landraces analyzed, according to groups (G) and subgroups (Sub) formed in the dendrogram. A) GI, Sub I: Cascatinha (NMU03); B) GI, Sub II: Casca branca (GUA03); C) GI, Sub III: Liberata (SOR07); D) GII, Sub I: Cacau (PXT01); E) GII, Sub II: Branca 01 (PXT03); F) GIII: Folha roxa (LCA07); G) GIV: Mandioca pão (SNP04).

The results obtained in this study demonstrate that the characterization based on multi-categorical data contributes to the phenotypic differentiation among landraces. The UPGMA clustering method was efficient in separating the landraces with the formation of distinct groups. These results were also evidenced by other researchers with cultivated species, such as Passiflora spp. [24] and *Capsicum chinense* [15]. According to Costa et al. (2009) [25], characterization through morphological descriptors is indispensable in diversity studies, since they are easy to detect and measure, have high heritability and suffer little environmental influence, as the characters analyzed in this study.

4. CONCLUSION

Floral characters were efficient in detecting phenotypic variability among cassava landraces cultivated in northern region of Mato Grosso state, Brazil. The characters 'color of the sepals', 'color of the disc' and 'color of the ovary' contributed to the variation found, and therefore, are useful for preliminary evaluations of the species.

Through the analyzes carried out, it was possible to propose a new phenotypic class for the character 'color of the sepals' (greenish-purple), going from three to four classes and allowing a better distinction among the material evaluated.

5. ACKNOWLEDGMENTS

The present research was supported by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Grant code 001.

6. BIBLIOGRAPHIC REFERENCESS

1. Mantovani ALB. Manejo da agrobiodiversidade por pequenos agricultores do agreste da Paraíba: o caso da mandioca (*Manihot esculenta* Crantz – Euphorbiaceae) [master's dissertation]. João Pessoa (PB): Universidade Federal da Paraíba; 2009. 104 p.
2. Allem AC. The origins and taxonomy of cassava. In: Hillocks RJ, Thresh JM, Bellotti AC, editors. New York (US): CAB International; 2002. p. 1-16.

3. Moura EF, Farias Neto JT, Sampaio JE, Silva DT, Ramalho GF. Identification of duplicates of cassava accessions sampled on the North Region of Brazil using microsatellite markers. Acta Amaz. 2013;43(4):461-68. doi: 10.1590/S0044-59672013000400008
4. Tumuhimbise R, Melis R, Shanahan P, Kawuki R. Genotype x environment interaction effects on early fresh storage root yield and related traits in cassava. Crop J. 2014;2(5):329-37. doi: 10.1016/j.cj.2014.04.008
5. Ospina B, Ceballos H. La yuca en el tercer milênio: sistemas modernos de producción, procesamiento, utilización y comercialización. In: Ceballos H, Cruz GA, editors. Taxonomia y morfologia de la yuca. Cali (CO): CIAT; 2002. p. 16-32.
6. Graner EA. Notas sobre florescimento e frutificação da mandioca. Bragantia. 1942 Jan;2(1):1-12.
7. Lara ACC, Bicudo SJ, Brachtvogel EL, Abreu ML, Curcelli F. Melhoramento genético da cultura da mandioca (*Manihot esculenta* Crantz). Rev Raízes Amidos Trop. 2008;4:54-64.
8. Mattos PLP, Farias ARN, Ferreira Filho JR. Mandioca: o produtor pergunta, a Embrapa responde. Brasília (DF): Embrapa Informação Tecnológica; 2006. 176 p.
9. Silva RM, Bandel G, Faraldo MIF, Martins PS. Biologia reprodutiva de etnovariedades de mandioca. Sci Agrícola. 2001 Jan/Mar;58(1):101-7. doi: 10.1590/S0103-90162001000100016
10. Sambatti JBM, Martins PS, Ando A. Distribuição da diversidade isoenzimática e morfológica da mandioca na agricultura autóctone de Ubatuba. Sci Agrícola. 2000 Jan/Mar;57(1):75-80. doi: 10.1590/S0103-90162000000100013
11. Fregene MA, Suarez M, Mkumbira J, Kulembeka H, Ndedya E, Kulaya A, et al. Simple sequence repeat marker diversity in cassava landraces: genetic diversity and differentiation in an asexually propagated crop. Theor Appl Genet. 2003;107:1083-93. doi: 10.1007/s00122-003-1348-3
12. Elias M, Penet L, Vindry P, Mckey D, Panaud O, Robert T. Unmanaged sexual reproduction and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (*Manihot esculenta* Crantz), in a traditional farming system. Mol Ecol. 2001;10:1895-907. doi: 10.1046/j.0962-1083.2001.01331.x
13. Gusmão LL, Mendes Neto JA. Caracterização morfológica e agronômica de acessos de mandioca nas condições edafoclimáticas de São Luís, MA. Rev Faculdade Zoot, Vet Agr. 2008;15(2):28-34.
14. Fukuda WMG, Guevara CL. Descritores morfológicos e agronômicos para a caracterização de mandioca (*Manihot esculenta* Crantz). Cruz das Almas (BA): CNPMF; 1998. 38 p.
15. Vasconcelos CS, Barbieri RL, Neitzke RS, Priori D, Fischer SZ, Mistura CC. Determinação da dissimilaridade genética entre acessos de *Capsicum chinense* com base em características de flores. Rev Ceres. 2012 Jul/Ago;59(4):493-8. doi: 10.1590/S0034-737X2012000400009
16. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. Koppen's climate classification map for Brazil. Meteorol Zeitschrift. 2013;22(6):711-28. doi: 10.1127/0941-2948/2013/0507
17. Empresa Brasileira de Pesquisa Agropecuária (Embrapa). Sistema Brasileiro de Classificação de Solos. 3. ed. Rio de Janeiro: Embrapa Solos; 2013. 353 p.
18. Cruz CD. 2006. Programa Genes: análise multivariada e simulação. Viçosa (MG): UFV. 175 p.
19. Cruz CD, Carneiro PCS. Modelos biométricos aplicados ao melhoramento genético. Viçosa (MG): UFV; 2003. 585 p.
20. Mojena R. Hierarchical grouping methods and stopping rules: an evaluation. The Computer Journal. 1977;20:359-63. doi: 10.1093/comjnl/20.4.359
21. Cruz CD. Genes Software – extended and integrated with the R, Matlab and Selegen. Acta Sci Agron. 2016 Oct/Dec;38(4):547-52. doi: 10.4025/actasciagron.v38i4.32629
22. Asare PA, Galyuon IKA, Sarfo JK, Tetteh JP. Morphological and molecular based diversity studies of some cassava (*Manihot esculenta* crantz) germplasm in Ghana. Afr J Biotechnol. 2011;10(63):13900-8. doi: 10.5897/AJB11.929
23. Rohlf FJ. Adaptive Hierarchical Clustering Schemes. Systemat Zool. 1970;19(1):58-82.
24. Oliveira JS, Faleiro FG, Junqueira NTV, Vieira EA, Viana ML. Caracterização fenotípica e diversidade genética de *Passiflora* spp. baseada em descritores multicategóricos. Rev Ciên Agr. 2017;60(3):223-34. doi: 10.4322/rca.2427
25. Costa FR, Santana TN, Sudré CP, Rodrigues R. Marcadores RAPD e caracteres morfoagronômicos na determinação da diversidade genética entre acessos de pimentas e pimentões. Ciên Rural. 2009;39(3):696-704. doi: 10.1590/S0103-84782008005000099