Conjecturas

DOI: 10.53660/CONJ-1835-2M10

Phenotypic variability in *Manihot esculenta* Crantz flowers in northern Mato Grosso, Brazil

Variabilidade fenotípica em flores de *Mahihot esculenta* Crantz no norte de Mato Grosso, Brasil

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ABSTRACT

The characterization of ethnovarieties of cassava cultivated by family farmers is essential for the selection of superior genotypes in breeding programs of the species. The aim of this study was to evaluate the genetic variability of 15 ethnovarieties of cassava cultivated in the municipality of Alta Floresta, Mato Grosso, via the morphological descriptors of the flowers. For the characterization, eight qualitative and quantitative morphological descriptors were used, as described for the species *Manihot esculenta*. The manioc flowers (female and male) showed variability in the color of the ovary, disc and sepals. Female flowers have varied sizes, from 7.12 to 9.03 mm in length and in width from 2.80 to 3.72 mm. For the male flowers, the variation in length was from 6.43 to 8.12 mm and width from 2.61 to 3.61 mm. The UPGMA grouping, using a cut-off point of 75.74%, allowed for the formation of two genetic groups, of which the first group (GI) was composed of 86.67% of the evaluated ethnovarieties and the second group (GII) was composed by 13.33%. The floral characteristics were efficient for use in phenotypic differentiation and show genetic variability among the ethnovarieties.

Keywords: Cassava; Genetic diversity; Morphological characterization.

RESUMO

A caracterização das etnovariedades da mandioca cultivada por agricultores familiares é essencial para a seleção de genótipos superiores em programas de melhoramento da espécie. O objetivo deste trabalho foi avaliar a variabilidade genética de 15 etnovariedades de mandioca cultivadas no município de Alta Floresta, Mato Grosso, por meio dos descritores morfológicos das flores. Para a caracterização foram utilizados oito descritores morfológicos qualitativos e quantitativos, conforme descrito para a espécie *Manihot esculenta*. As flores de mandioca (femininas e masculinas) apresentaram variabilidade na coloração do ovário, disco e sépalas. As flores femininas possuem tamanhos variados, de 7,12 a 9,03 mm de comprimento e de 2,80 a 3,72 mm de largura. Para as flores masculinas, a variação no comprimento foi de 6,43 a 8,12 mm e na largura de 2,61 a 3,61 mm. O agrupamento UPGMA, utilizando um ponto de corte de 75,74%, permitiu a formação de dois grupos genéticos, sendo o primeiro grupo (GI) composto por 86,67% das etnovariedades avaliadas e o segundo grupo (GII) composto por 13,33%. As características florais foram eficientes para uso na diferenciação fenotípica e mostram variabilidade genética entre as etnovariedades.

Palavras-chave: Mandioca; Diversidade genética; Caracterização morfológica.

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the main subsistence products for a large part of the world's population and is one of the most important sources of carbohydrates. As such, it occupies a prominent place among the crops exploited in tropical countries (AGUIAR, 2003; CARDOSO et al., 2014; SOARES et al., 2017). Belonging to the Euphorbiaceae family, which comprises more than 1,700 species, it is the oldest cultivated plant in Brazil, and currently the most widespread plant in the Brazilian territory (CEBALLOS, 2002; MORALES, 2015).

Cassava is a monoecious species, with male and female flowers in the same inflorescence, of the raceme type, which present short and acute basal bracts, and have variable coloration depending on the genotype (RIOS, 2006; CARVALHO; FUKUDA, 2006). Located at the end of the branch, the male flowers of the cassava are smaller and in greater numbers when compared to the female ones, which are in the basal part of the inflorescence (CEBALLOS et al., 2004). Its pollination is crossed, and the female flowers of the same inflorescence begin anthesis ten days before the male flowers; however, it is possible for simultaneous anthesis to occur in different inflorescences, but from the same plant, thus causing self-pollination (MARTIN, 1976; RIOS, 2006). Despite maintaining active sexual reproduction, over time, cassava has been propagated vegetatively by human interference (SILVA et al., 2021), and this gives it a wide genetic diversity (OLER, 2017). The exchange of setts between farmers also provides for this diversification and conservation of the species (CEBALLOS et al., 2004; MARTINS; OLIVEIRA, 2009).

The wide genetic diversity that exists may present characteristics of interest, either for use in genetic improvement programs or for the direct use by farmers, which can be evaluated through morphological characterization (ELIAS et al., 2007; PINTO, 2017). This evaluation consists in the differentiation and selection of varieties of the same species and can be made based on observations or measurements of visually differentiable morphological characteristics (CAMPOS et al., 2010; NICK et al., 2010; TEIXEIRA et al., 2014). The characterization also allows us to observe ethnovarieties with certain similar attributes and the identification of repeated material that has received different names in different places (CAMPOS et al., 2010).

Many researchers and farmers use morphological characterization as an instrument in the analysis of the potential of each of the genotypes available in their collection, as

well as to help in the choice of the best material to be used, according to the desired purpose (RAMOS, 2007). Genetic pre-breeding research in the state of Mato Grosso, such as those carried out by Campos (2010), Oler (2017), Figueiredo (2018), Pedri (2019) and Tiago (2020), has sought to identify new potential in cassava culture with the criteria desired by consumers and farmers. In addition, the presence and magnitude of diversity has been recorded.

Therefore, the objective of this study was to evaluate the genetic variability of 15 ethnovarieties of cassava cultivated in the municipality of Alta Floresta, MT, via morphological descriptors of its flowers.

MATERIAL AND METHODS

Area of study

The study was conducted using 15 ethnovarieties of cassava (Table 1), which were collected in 11 rural properties located in the settlement sector of Vila Rural I and II, in the municipality of Alta Floresta, MT, and later maintained in the working collection of Tiago (2016) at Sítio São Paulo, which is located in the Nova Esperança community, Estrada Segunda Oeste, on highway MT 208.

Table 1. Codes assigned to the 15 ethnovarieties of cassava, popular name used by farmers and place of origin.

Code	Popular name	*Place of origin	Collection place
AF01	Cacau Roxa	Alta Floresta	Rural I Linha I
AF02	Cacau Arara	Alta Floresta	Rural II Linha I
AF03	Mandioca Cenoura	Alta Floresta	Rural I Linha I
AF04	Cacau Branca	Alta Floresta	Rural I Linha I
AF05	Cacau Pinheiro	Alta Floresta	Rural I Linha I
AF06	Mandioca Pão	Alta Floresta	Rural I Linha I
AF07	Mandioca Vassourinha	Alta Floresta	Rural I Linha II
AF08	Branca Comum	Alta Floresta	Rural I Linha III
AF09	Mandioca do Ano	Alta Floresta	Rural I Linha II
AF11	Branca do Baiano	Alta Floresta	Rural II Linha I
AF12	Cacau Amarela	Alta Floresta	Rural II Linha I
AF13	Mandioca Amarela I	Carlinda	Rural II Linha I
AF14	Mandioca Amarela II	Carlinda	Rural II Linha I
AF17	Amarela III	Paraná	Rural II Linha I
AF18	Amarela da Bahia	Bahia	Rural II Linha I

*The place of origin indicates where the farmers acquired the ethnovarieties to assemble their collections.

Source: Prepared by the authors.

Morphological characterization

For the morphological characterization, the following eight morphological descriptors, of which six are qualitative (Table 2), and two are quantitative: length and width of the sepals (Figure 1), were used as described by Fukuda and Guevara (1998) for the species *Manihot esculenta.*

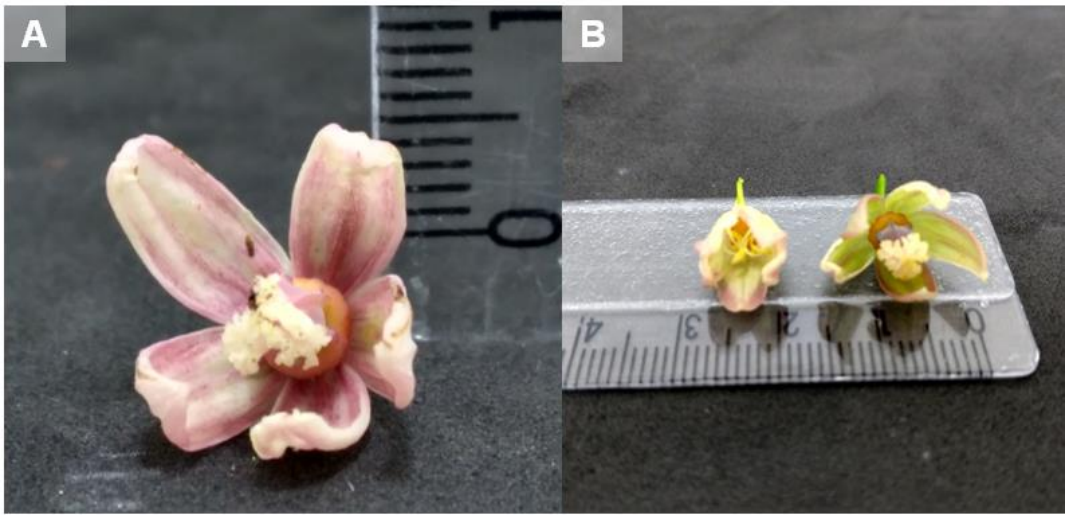
During anthesis, female and male flowers of the 15 ethnovarieties of cassava were collected at eight months post-planting. For each ethnovariety, a repetition of five plants was used, five female and five male flowers were collected, totaling ten flowers per plant and 50 per ethnovariety.

Table 2. Morphological characteristics, complementary descriptors described by Fukuda and Guevara (1998) and used in the description of the 15 ethnovarieties of cassava cultivated in the municipality of Alta Floresta, MT.

Descriptors	Phenotypic classes
Sepal color	(1) White or cream; (2) Orange; (3) Green; (4) Red; (5) Purple and (6) Green/purple
Stigma color	(1) White or cream; (2) Orange; (3) Green; (4) Red and (5) Purple
Disc color	(1) White or cream; (2) Orange; (3) Green; (4) Red; (5) Purple and (6) Yellow
Ovary color	(1) White or cream; (2) Orange; (3) Green; (4) Red and (5) Purple
Anther color	(1) Cream and (2) Yellow
Pollen	(0) Absent or (1) present

Source: Prepared by the authors.

Figure 1. Quantitative descriptors measured in female and male flowers of cassava ethnovarieties. (A) length of sepals (mm); (B) width of sepals (mm).



Source: Prepared by the authors.

Statistical analysis

Qualitative and quantitative floral characteristics

To determine the relative frequency of qualitative morphological characteristics found among the 15 ethnovarieties of cassava, Equation 1 was used:

(1

$$\text{Relative frequency} = \frac{\text{Absolute frequency}}{\text{Sample total}} \times 100$$

The data for relative frequency were graphically presented with the aid of Excel 2013 software, and the quantitative descriptors were presented as means for each ethnovariety.

Genetic variability through joint analysis

To analyze the genetic variability among cassava ethnovarieties, the qualitative variables were tabulated in the form of scores according to the phenotypic class of the evaluated characteristic, as described in Table 1. The quantitative data for the length and width of the flowers were presented as means. Subsequently, the qualitative and quantitative data were analyzed jointly, according to the methodology proposed by Gower (1971). The dissimilarity matrix generated by the sum of qualitative and quantitative matrices was used to group the cassava ethnovarieties by the method UPGMA (Unweighted Pair Group Method using Arithmetic Averages), nearest neighbor (SL) and Ward, and the grouping method best defined the representation of ethnovarieties determined by the cophenetic correlation coefficient (CCC), stress and distortion. The formation of the genetic groups, consisting of the 15 ethnovarieties of cassava, was determined by means of the Mojena criterion (1977). All the analyses were performed with the aid of the GENES v. 2018.23 program (2013).

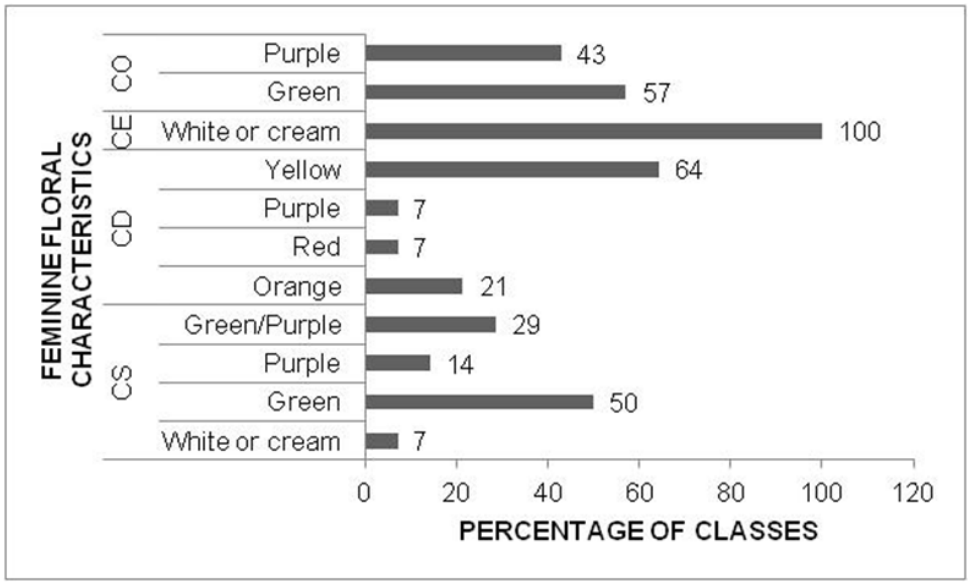
RESULTS AND DISCUSSION

Qualitative and quantitative floral characteristics

As shown in Figure 2, the majority of female flowers stood out for having a green ovary (57%), a yellow disc (64%) and green sepals (50%). For the coloring of the stigma, white or cream was the only color that predominated among all ethnovarieties (100%). Results similar to those found in this study were found by Pedri (2020), who evaluated the floral characteristics of 12 ethnovarieties of cassava. Their results show that there is

a predominance of green coloring for the ovary and the stigma has white or cream color. This classification found in both works may be an indication that the ethnovarieties cultivated by farmers in the state of Mato Grosso have, as a general characteristic, predominantly green ovaries and stigmas of white/cream colors.

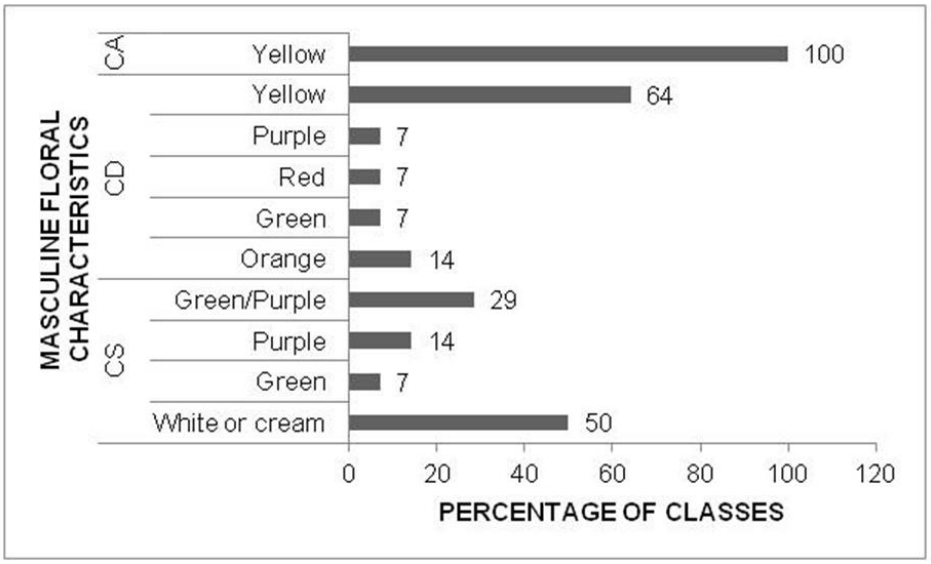
Figure 2: Percentage of qualitative characteristics present in female flowers among the 15 cassava ethnovarieties cultivated in the municipality of Alta Floresta, MT. Color of the ovary (CO); color of the stigma (CE); color of the disc (CD) and color of the sepals (CS).



Source: Prepared by the authors.

Regarding the male floral characteristics, in relation to the presence of pollen, all the flowers presented this characteristic. The colors of the anthers were presented in a single class, that is, 100% were yellow, with a predominance of the color of the disc also in yellow (64%). Unlike the female flowers, most male flowers stand out for the color of the sepals, which were white or cream (50%), and the remaining flowers of the ethnovarieties are divided between purple/green, purple and green (Figure 3).

Figure 3: Percentage of qualitative classes for male floral characteristics among the 15 ethnovarieties of cassava cultivated in the municipality of Alta Floresta, MT. Color of the anthers (CA); color of the disc (CD) and color of the sepals (CS).



Source: Prepared by the authors.

The coloration of the sepals and the disc coloration of the female and male flowers were the characteristics that presented the most polymorphism in this study. Pedri (2020), when studying the phenotypic characterization using flower and fruit descriptors, also found that the color of the sepals was the descriptor that stood out with the highest polymorphism among the ethnovarieties studied. The phenotypic variability of female and male flowers among cassava ethnovarieties evaluated in this study can be observed in Figure 4.

Figure 4. General characteristics presented by female (A, B, C and D) and male (E, F and G) flowers of cassava ethnovarieties. (A) sepals of purple coloration – white or cream stigma – purple ovary – red disc; (B) green sepals – white or cream stigma – green ovary – yellow disc; (c) white or cream sepals – white or cream stigma – green ovary – yellow disc; (D) sepals green/purple - white or cream stigma – purple ovary – yellow disc; (E) green/purple sepals – yellow anthers – purple disc; (F) purple sepals – yellow anthers – purple disc; (g) white or cream sepals – yellow anthers – yellow disc.

Source: Prepared by the authors.

Previous studies have shown that farmers use the combination of easily perceptible and distinguishable characteristics, such as leaf pigmentation, and stem and flower coloration, to recognize an ethnovariety (PINTO, 2010). Therefore, floral characteristics can be considered an important resource in the characterization of cassava ethnovarieties, and can be included with other descriptors in variability studies for the selection of cassava accessions. Female flowers have varied sizes, which range from 7.12 mm to 9.03 mm in length and from 2.80 mm to 3.72 mm in width. For male flowers, the variation in length was from 6.43 mm to 8.12 mm and in width from 2.61 mm to 3.61 mm (Table 3).

Table 3. Length and width of female and male cassava flowers as described by Fukuda and Guevara (1998).

Ethnovarieties	Female		Male	
	L (mm)	W (mm)	L (mm)	W (mm)
Cacau Roxa	8,25	3,09	7,36	2,84
Cacau Arara	7,56	2,80	6,75	2,77
Mandioca Cenoura	8,92	3,49	8,12	3,38
Cacau Branca	8,49	2,96	7,52	3,18
Cacau Pinheiro	9,03	3,36	7,55	3,07
Mandioca Pão	8,41	3,47	7,52	3,61
Mandioca Vassourinha	7,12	2,89	6,43	2,72
Branca Comum	7,88	3,08	7,15	3,04
Mandioca do Ano	8,76	3,35	7,05	3,09
Branca do Baiano	9,03	3,60	7,63	3,44
Cacau Amarela	7,92	2,83	7,20	2,98
Mandioca Amarela I	8,02	2,91	7,42	2,86
Mandioca Amarela II	8,22	2,97	6,75	2,81
Amarela III	8,42	3,35	6,56	2,61
Amarela da Bahia	8,82	3,72	6,85	3,22

L = length; W = width

Source: Prepared by the authors.

Genetic variability through joint analysis

The values expressed by the different grouping methods in relation to the cophenetic correlation coefficient for the eight morphological descriptors, obtained through joint analysis, are shown in Table 3. The UPGMA clustering method compared to the nearest neighbor (SL) and Ward presented the highest CCC (0.78%) and, consequently, the lowest values of distortion and stress. Therefore, the UPGMA method is recommended for offering a better consistency in the clustering of the data of the ethnovarieties evaluated using joint analysis (Table 3). According to Rohlf (1970) and Cruz and Carneiro (2003), the CCC values, when above 70%, indicate that the matrices present good agreement and, therefore, there is a reduction in the values of distortion and stress of the grouping. This fact was also observed in this study.

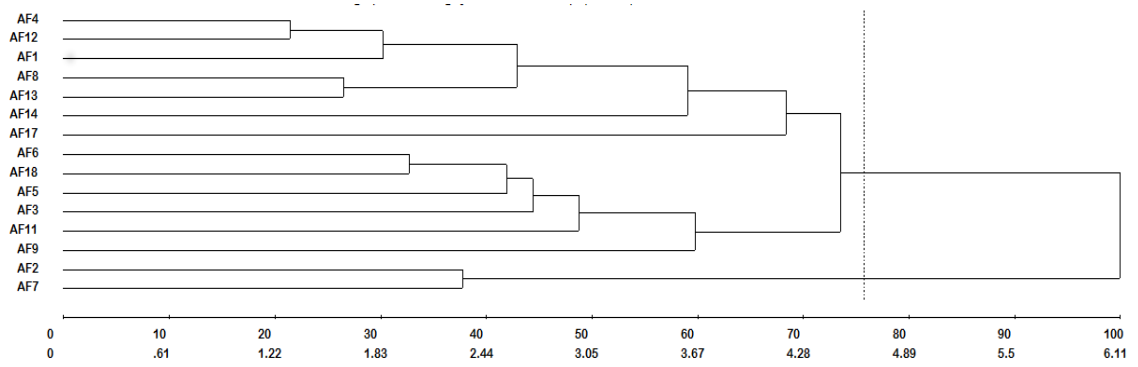
Table 3. Cophenetic correlation coefficient (CCC), stress and distortion generated during the grouping of the 15 cassava ethnovarieties using the methods UPGMA, nearest neighbor (SL) and Ward.

Grouping method	CCC (%)	Distortion (%)	Stress (%)
UPGMA	0,78	4,43	21,06
SL	0,69	59,98	41,68
WARD	0,63	-	-

Source: Prepared by the authors.

The UPGMA grouping with the 15 ethnovarieties of cassava, using a cut-off point of 75.74%, permitted the formation of two genetic groups (Figure 5), of which, the first group (GI) was composed of the majority of ethnovarieties evaluated (87%). The second group (GII) was formed by only two ethnovarieties, popularly known as *Cacau Arara* (AF02) and *Mandioca Vassourinha* (AF07).

Figure 5: Dendrogram of genetic dissimilarity among the 15 ethnovarieties of cassava obtained by the UPGMA method using the joint analysis of the morphological descriptors for the flowers.



Source: Prepared by the authors.

The UPGMA separates an original group of observations into several subgroups in order to obtain homogeneity within and heterogeneity between the subgroups, in addition to being able to visualize accessions within the groups with greater affinity (BERTAN, et al., 2006; VENDRAMINI, et al., 2011). Thus, it can be observed that in the group GI, the 13 ethnovarieties were allocated for having similar floral characteristics, such as the white or yellow color of the stigma, the yellow anthers, and the presence of pollen (37.5% of the features) It is noted that the *Mandioca Amarela* III (AF17) and *Mandioca do Ano* (AF09) are the ones that are the most distant in this group, and the ethnovarieties *Cacau Arara* (AF02), and *Mandioca Vassourinha* (AF07) were isolated in a single group (GII), which shows that they are the most divergent within all the evaluated cassava ethnovarieties (Figure 5).

The male flower of the *Mandioca do Ano* (AF09) showed sepals of a green color and the male flower of the *Mandioca Amarela* III (AF17) exhibited a disc of a purple color, and these characteristics caused the distancing of the two ethnovarieties within the GI group. While in the GII group, the ethnovarieties *Cacau Arara* (AF02) and *Mandioca Vassourinha* (AF07) were the only ones to present purple coloration for the sepals of the

female and male flowers. For the color of the disc, the female and male flower of *Cacau Arara* (AF02) exhibited a red color, while the female and male flower of *Mandioca Vassourinha* (AF07) the color of the disc was purple. In other words, the coloration of the sepals and discs were the floral characteristics that contributed to their dissimilarity in relation to the others.

CONCLUSION

The floral characteristics used in the work allowed us to observe phenotypic differentiation between the flowers of the 15 cassava ethnovarieties, and, therefore, can be used in the characterization of cassava ethnovarieties. The ethnovarieties *Cacau Arara* (AF02) and *Mandioca Vassourinha* (AF07) showed to be the most divergent among those evaluated, and the color of the sepals and disc were the characteristics that contributed to the divergence.

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Recebido em: 12/09/2022

Aprovado em: 15/10/2022

Publicado em: 21/10/2022