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Parâmetros morfo-agronômicos, composição química e divergência genética entre acessos de *Manihot* sp

Morpho-agronomic parameters, chemical composition and genetic divergence among *Manihot* sp. access

Parámetros morfoagronómicos, composición química y divergencia genética entre *Manihot* sp. accesos

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Resumo

Objetivou-se avaliar a caracterização morfo-agronômica composição química e divergência genética entre acessos de maniçoba (*Manihot* sp) coletados nas microrregiões do Curimataú e Cariri do Estado da Paraíba. O experimento foi conduzido em um delineamento inteiramente casualizado com 10 acessos e 8 repetições. As caracterizações morfo-agronômicas foram avaliadas através de 16 caracteres agronômicos e 12 caracteres morfológicos. Para a avaliação química foram discriminados 11 caracteres. De acordo com os resultados, o acesso 2 (Monteiro-PB) foi considerado uma planta alta, com maior área de copa com folhas de menor tamanho. Os acessos apresentam-se como plantas cilíndricas com folha apical verde escuro e lóbulo central obovada-lanceolada, crescimento reto ou zig zag, nervura verde e com a presença de cinco lóbulos e ramos terminais nas plantas adultas de cor verde. Os níveis de ramificação variam de 1 a 4, o pecíolo inclinado para cima ou horizontal e cor do pecíolo verde avermelhado. As estípulas são curtas e a sinuosidade de lóbulo foliar liso. Das variáveis químicas avaliadas nos acessos de maniçoba as que mais contribuíram para diferenciações entre genótipos foram os teores de fibra em detergente neutro, carboidratos não-fibrosos e carboidratos totais. As características morfo-agronômicas juntamente com os dados de composição química servem de parâmetro para indicar os possíveis acessos a serem utilizados nos processos de beneficiamento da forragem, com melhor valor nutricional.

Palavras-chave: *Manihot sp*.; Morfologia; Valor nutricional.

Abstract

The objective of the present study was to evaluate the morpho-agronomic parameters, chemical composition and genetic divergence among *Manihot* sp. access collected in the Curimataú and Cariri micro regions of the State of Paraíba - Brazil. The experiment was conducted in a completely randomized design with 10 access and 8 replicates. Morpho-agronomic characterization was evaluated through 16 agronomic traits and 12 morphological traits. For the chemical evaluation, 11 traits were discriminated. According to the results, access 2 (Monteiro-PB) was considered a tall plant, with larger canopy area with smaller leaves. The access are cylindrical plants with dark green apical leaf and obovate-lanceolate central lobe, straight or zig zag growth, green vein and with the presence of five lobes and terminal branches in the green plants. The branching levels range from 1 to 4, upward or horizontal petiole and reddish green petiole color. The stipules are short and the sinuosity of leaf lobe is smooth. From the chemical variables evaluated in the access of *Manihot*, the ones that contributed the most to distinguish genotypes were the content of neutral detergent fiber, non-fiber carbohydrates and total carbohydrates. The morpho-agronomic traits together with the chemical composition data serve as a parameter to indicate the possible access to be used in the forage processing, with better nutritional value.

Keywords: *Manihot sp*.; Morphology; Nutritional value.

Resumen

El objetivo de este estudio fue evaluar la caracterización morfoagronómica de la composición química y la divergencia genética entre los accesiones de *Manihot* sp recogido en las micro regiones de Curimataú y Cariri en el estado de Paraíba - Brazil. El experimento se realizó en un diseño completamente al azar con 10 accesiones y 8 repeticiones. Las caracterizaciones morfoagronómicas se evaluaron utilizando 16 caracteres agronómicos y 12 caracteres morfológicos. Para la evaluación química, se desglosaron 11 caracteres. Según los resultados, el acceso 2 (Monteiro-PB) se consideró una planta alta, con un área de dosel más grande con hojas más pequeñas. Las accesiones aparecen como plantas cilíndricas con hojas apicales de color verde oscuro y lóbulo central lanceolado obovado, crecimiento recto o en zig zag, costilla verde y con la presencia de cinco lóbulos y ramas terminales en plantas verdes adultas. Los niveles de ramificación varían de 1 a 4, el pecíolo inclinado hacia arriba u horizontal y el color del pecíolo verde rojizo. Las estípulas son cortas y la sinuosidad del lóbulo de la hoja es suave. De las variables químicas evaluadas en las accesiones de maniçoba, las que más contribuyeron a las diferencias entre genotipos fueron los niveles de fibra detergente neutra, carbohidratos no fibrosos y carbohidratos totales. Las características morfoagronómicas junto con los datos de composición química sirven como parámetro para indicar los posibles accesos que se utilizarán en el procesamiento del forraje, con un mejor valor nutricional.

Palabras clave: *Manihot* sp.; Morfología; Valor nutricional.

1. Introduction

The morpho-agronomic characterization of plant species consists in providing an identity for each access through the knowledge of a series of data that allow to study its genetic variability. Morphological variability is characteristic of the genus *Manihot* due to the ability of its species to interbreed and produce fertile hybrids, which makes species identification difficult. Plant breeders work with variations of a genetic nature. However, the greatest difficulty usually faced by researchers is the ability to distinguish and quantify genetic and environmental effects in the phenotypic response of genotypes (Carmona et al. 2015).

To determine how genetically distant a genotype is from another, biometric and molecular methods are used, which are analyzed by multivariate statistics, allowing to summarize a set of data extracted from the experiment, offering more objective information about the study population. The study of genetic diversity through biometric and molecular analyses aims at: grouping similar genetic materials and characterizing the variability of genetic resources in in situ and ex situ germplasm banks (Machado et al. 2016).

Manihot plays a key role in the northeastern scenario, especially in the semi-arid region, where it is used as an alternative for feeding herds of domestic animals during prolonged droughts (Costa et al. 2015). It can be considered as a forage of high palatability, because it is highly sought by goats, sheep, horses and cattle.

The genetic potential of *Manihot* has still been little studied, it needs to be collected, characterized and preserved in a germplasm bank, so that it can be used in breeding programs. In this sense, the objective of the present study was to evaluate the morpho-agronomic parameters, chemical composition and genetic divergence among *Manihot* sp. access collected in the Curimataú and Cariri micro regions of the State of Paraíba - Brazil.

2. Methods

Study site

The experiment was carried out under field conditions, at the Germplasm Bank of the maniçoba of the Agricultural Sciences Center of the Federal University of Paraíba (CCA/UFPB), Campus II, located in the municipality of Areia in the Brejo Paraibano microregion, with latitude 6° 58’ 12 ” South and longitude 35° 42’ 15’’ West, at an altitude of of the Greenwich meridian, with an altitude of 684 m above sea level. The region's presenting a humid tropical climate, with dry summer, rainy season beginning in January or February and end in September, being able to advance until October. The average annual rainfall in the municipality of Areia is 1400 mm, with an average temperature of 25.5 ° C, with the months of November, December and January being considered the warmest and, June, July and August the coldest. The annual average of relative humidity is 80%, according to data from the Agrometeorological Station of the CCA/UFPB.

Seedling production

Ten access of *Manihot* sp. (Table 1) of the UFPB active germplasm bank (AGB) collected in the State of Paraíba, in the municipalities of Barra de Santana, Monteiro, Junco, Juazeirinho, Barra de Santa Rosa, Pocinhos, Cubatí, Pedra Lavrada, Boa Vista, and Sumé, all georeferenced with the aid of a GPS (Global Positioning System).

From each access, stakes measuring 30 cm in length, with three nodes and bevel cut at the bottom, were taken for seedling production. The stakes were immersed in indole-butyric acid (IBA) solution (100 mg L^{-1}) for 10 minutes, and then transferred to black polyethylene bags (23 x 13 cm) under standard conditions of organic substrate (1:1:1 sand/cattle manure/soil) and irrigation, to have a homogenous exteriorization of each genotype. The seedlings remained for 2 months in the greenhouse and, after this period, were transplanted to the field, forming the germplasm bank.

Transplanting was carried out in pits (30 x 30 x 30 cm) at a spacing of 1.5m between plants and 1.5m between rows. In the field, seedlings were irrigated to ensure their complete establishment, as well as weeding and ant control were carried out regularly. The experiment was conducted in a completely randomized design with 10 access of *Manihot* and 8 replicates.

Table 1 - Origin location of *Manihot* sp access of the active germplasm bank.

Access	Collection Sites (PB)	Specie	Degree of Domestication	Elevation (m)	Latitude (S)	Longitude (W)
1	Barra de Santana	*Manihot* sp.	Wild	381	7° 42.6’’	36°57.2’’
2	Monteiro	*Manihot* sp.	Wild	608	7° 10.6’’	37° 41.9’’
3	Junco	*Manihot* sp.	Wild	590	6° 59’49’’	36° 42’46’’
4	Juazeirinho	*Manihot* sp.	Wild	554	7° 4’ 4’’	36°34’ 40’’
5	Barra de Santa Rosa	*Manihot* sp.	Wild	473	6° 12.6’’	36° 41’’
6	Pocinhos	*Manihot* sp.	Wild	456	7° 58’’	36° 27.9’’
7	Cubatí	*Manihot* sp.	Wild	475	6°52’4’’	36° 21’6’’
8	Pedra Lavrada	*Manihot* sp.	Wild	596	6° 42.1’’	36° 59.6’’
9	Boa Vista	*Manihot* sp.	Wild	544	7° 24.5’’	36° 35.8’’
10	Sumé	*Manihot* sp.	Wild	562	7° 40′ 19″	36° 52′ 48″

Source: Authors.

The experimental soil was classified as Eutrophic Red-Yellow Latosol (Santos et al. 2018), presenting the following chemical characteristics: pH= 5.0, P (mg/dm^3) = 5.47, K (mg/dm^3) = 42.08, Al^{+3} (cmolc/dm^3) = 0.25, Ca^{+2} (cmolc/dm^3) = 0.90, Mg+ (cmolc/dm^3) = 0.90, CEC (cmolc/dm^3) = 4.23 and OM (g/dm^3) = 10.39.

Agronomic characteristics evaluated

Sixteen agronomic traits were evaluated at each access: Plant height (PH - cm): vertical distance from the apex to ground level; Number of axillary buds (NAB); Stem diameter (SD - mm): measured at 20 cm above ground level with the aid of a digital caliper; Number of plant branches (NPB); Number of leaves per plant (NLP); Leaf petiole length (LPL - cm): measured from the insertion of the petiole in the stem to the insertion of the leaf; Diameter of leaf petiole basis (DLPB - mm): measured at the base of the leaf petiole with the aid of a digital caliper; Mean diameter of leaf petiole (MDLP - mm): measured in half of the leaf petiole with the aid of a digital caliper; Upper diameter of leaf petiole (UDLP - mm): measured in the proximal part of the leaf petiole with the aid of a digital caliper; Length of

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central lobe (LCL - cm): measured from the insertion of the petiole in the main stem to the apex of the median lobe of the leaf; Width of central lobe (WCL - mm); Upper width of median lobe (UWML - cm): measured with the aid of a ruler; Length between the basal lobes of the leaf (LBLL - cm): comprises the distance between the basal lobes of the leaf; Length between the median lateral lobes of the leaf (LMLL - cm): comprises the distance between the median lobes; Number of fruits (NFR); Number of senescent leaves (NSL): comprises the total of senescent leaves in the evaluation period (Fukuda et al. 2010). The measurements were taken with the aid of a digital caliper, graduated ruler and a measuring tape.

Morphological characteristics evaluated

Twelve morphological traits were evaluated according to the methodology used by Fukuda et al. (2010) with adaptations for *Manihot* sp.:

Type of plant (TP), evaluated using a scale of grades, from 1 to 4, where: 1-compact; 2-open; 3-umbrella; 4-cylindrical.

Apical leaf color (ALF), evaluated the predominant color of leaves using a color scale, with values ranging from 3 to 9, where: 3-light green; 5-dark green; 7- purplish green; and 9-purple.

Central lobe shape (CLS), observed in intact leaves, a scale of values from 1 to 10, where: 1-oval; 2- elliptical lanceolate; 3- obovada-lanceolate; 4- oblong-lanceolate; 5-lanceolate; 6-straight or linear; 7- hanging; 8-linear-pyramidal; 9-linear-patterned; and 10- linear-hostatilobed.

Vein Color (VC), observed in the lower part of the central lobe of the leaves of the middle third of the plant, evaluated using a color scale, in which: 3-green; 5-green with red in less than half of the lobe; 7- green with red in more than half of the lobe; 9-all red.

Lobe number (LN), obtained by counting the lobes of each leaf evaluated in the middle third of the plant, using a value scale numbered from 1 to 9, in which: 1-three lobes; 3-five lobes; 5-seven lobes; 7-nine lobes; and 9-eleven lobes.

Growth habit (GH), evaluated according to the scale of grades, from 1 to 2, where 1- straight and 2- zig-zag.

Color of terminal branches on adult plants (CTBPA), observed in the upper 20 cm of the plant, using a color scale, with values ranging from 3 to 7, in which: 3- green; 5- purplish green; and 7- purple.

Branching levels (BL), evaluated by a scale of values numbered from 0 to ≥5, where: 0- no branches; 1- a branch; 2- two branches; 3- three branches; 4- four branches; ≥5- greater than or equal to 5 branches.

Petiole position (PP), evaluated by a scale of values, numbered from 1 to 7, observed in the middle third of the plant in young leaves, in which: 1- tilted Up; 3-horizontal; 5-tilted down; and 7-irregular.

Stipule length (SL), evaluated in 1-short or 2-long.

Leaf lobe sinuosity (LLS), evaluated according to the format. 1- smooth; 2- winding.

Green leaf petiole color (GLPC), evaluated on a scale of values numbered 1 to 6, in which: 1-green-yellow; 2-green; 3-reddish-green; 4- greenish-green; 5- red; 6- purple. Figure 1 shows the measurement of agronomic markers.

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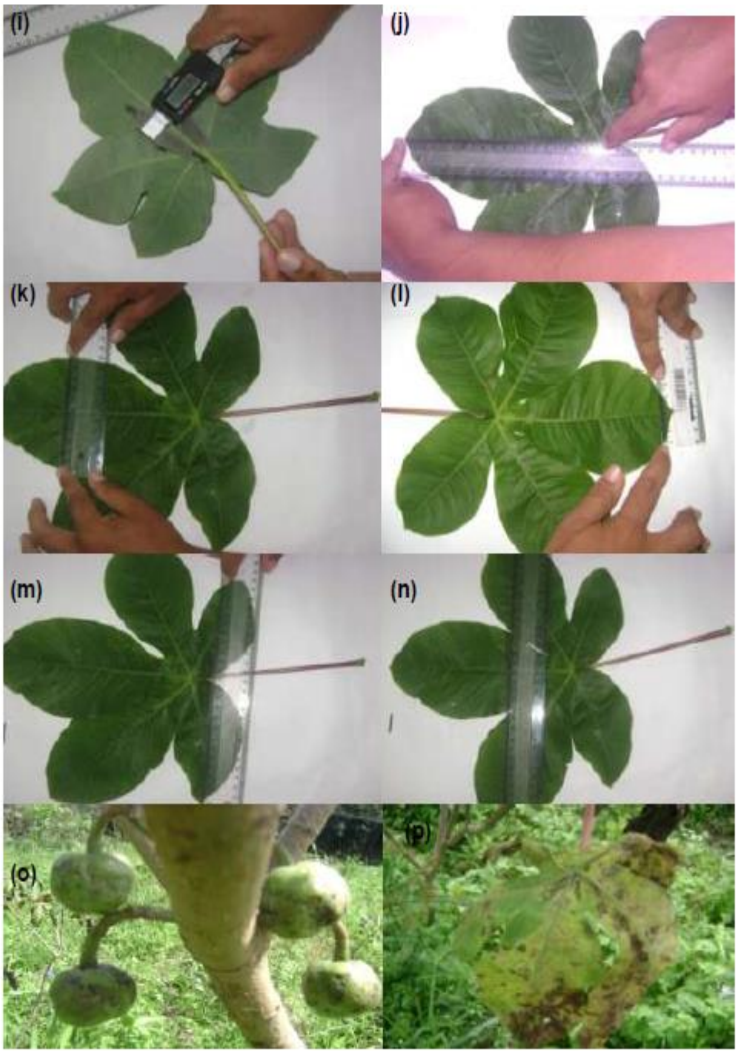
Figure 1. Measurement of the agronomic markers of *Manihot* sp. access.

Source: Personal archive of the first author.

a) Plant height (cm); (b) Number of axillary buds; (c) Stem diameter (mm); (d) Number of plant branches; (e) Number of leaves per plant; (f) Leaf petiole length (cm); (g) Diameter of leaf petiole basis (mm); (h) Mean diameter of leaf petiole (mm). Figure 2 shows another measurement of agronomic markers.

Figure 2. Measurement of the agronomic markers of *Manihot* sp. access.



Source: Personal archive of the first author.

(i) Upper diameter of leaf petiole (mm); (j) Length of central lobe (cm); (k) Width of central lobe (cm); (l) Upper width of median lobe (cm); (m) Length between the basal lobes of the leaf (cm); (n) Length between the median lateral lobes of the leaf (cm); (o) Number of fruits; (p) Number of senescent leaves.

Chemical composition

Three samples of each access were collected to determine the chemical composition. The samples were pre-dried in a forced-air oven at 55ºC for 7200 hours and ground to 1-mm particles in knife mills (Wiley Mill, Marconi, MA-580, Piracicaba, Brazil).

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Analyses were performed for percentage of dry matter (DM, method 967.03), mineral matter (MM, method 942.05), crude protein (CP, method 981.10) and ether extract (EE, method 920.29) (AOAC, 2016), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Silva & Queiroz, 2006), lignin and cellulose (Van Soest, 1994). Total carbohydrates (TC) were calculated using the equation proposed by Sniffen et al. (1992): TC (% DM) = 100 - (CP + EE + MM). The non-fiber carbohydrate (NFC) content was calculated as proposed by Hall (2003): NFC =% TC -% NDF. Hemicellulose (HEMI) was calculated by the equation: HEMI = NDF - ADF.

Statistical analysis

Data were submitted to Shapiro-Wilk and Levene's tests to verify the normality of the residues and homogeneity of the variances, respectively; once the assumptions were met, they were submitted to analysis of variance with a significance level of 5%, according to Tukey's test. The quantification of the relative contribution of morphometric and chemical characteristics to genetic divergence was estimated using the methodology proposed by Singh (1981). Statistical analyses were run in Genes Software.

3. Results

The analysis of the data referring to the agronomic traits showed that there was genetic variability ($P<0.05$) among *Manihot* access, as can be seen in Table 2. The height of the plants ranged from 180 cm (access 2) to 63.3 cm (access 8). Access 2 presented the largest number of leaves, 146.5 leaves.

The increase in the number of leaves in access 2 resulted in an increase in the number of axillary buds (232), number of branches (19), number of fruits (32.5) and number of senescent leaves (38). Higher results for the number of branches in access 2 directly assisted the increase in the number of leaves, because, with the increase in their number, there is a concomitant increase in the number of buds, providing a greater number of points of leaf emergence, increasing the canopy area of the plant. Although access 2 had a larger number of leaves, these presented smaller sizes for the diameter of leaf petiole base (2.8 mm), mean diameter of leaf petiole (2.3 mm) and secondary diameter of leaf petiole (2.1 mm).

The stem diameter provides the plant with the ability to support and sustain, mainly because *Manihot* is a shrub-tree species. Among the access, this characteristic ranged from 17.6 mm (access 3) to 34.8 mm (access 2).

Access 7 presented the highest values for diameter of leaf petiole base (6.9 mm), mean diameter of leaf petiole (20.8 mm), secondary diameter of leaf petiole (17.3), length between the medial lobes of the leaf (18.8 cm), width of the central lobe (11.7 cm), upper width of central lobe (7 cm), length of central lobe (26.7 cm) and length between the basal lobes of the leaf (24.7 cm), which can be selected as a possible parent, when considering the use of the species as forage.

Despite the high phenotypic variability detected for the great majority of the measured traits, some phenotypes, such as purplish green apical leaf, elliptic-lanceolate and lanceolate shape of the central lobe and seven lobes were detected at very low frequency (2.8%), indicating the difficulty of finding these access in the field or the possible genetic loss for future breeding programs due to their extinction. Nevertheless, 83.3% of the access have 5 lobes, with a green vein (Table 3).

A characteristic of important visualization and differentiation among species is the type of plant. According to Table 4, three phenotype classes were observed for this trait, being predominant the cylindrical plant with 63.9% of the individuals studied.

Among the 11 variables analyzed in the chemical analysis (Table 4), only mineral matter presented no significance at the 5% probability level, indicating genetic variability among the 10 access evaluated for most of the characteristics. DM and CP concentrations between access ranged from 16.90% (access 2) to 25.03% (access 10) and from 30.94% (access 4) to 18.34% (access 3), respectively for DM and CP (Table 4).

For the analysis of ether extract, the results varied from 3.56% (access 6) to 4.83% (access 4). Regarding non-fiber carbohydrates, access 7 presented the best result, 28.34%. For total carbohydrates, access 3 stood out with 69.96% content.

The NDF comprises the entire cell wall and therefore the larger the amount of fiber, the less the feed intake by the animal. *Manihot* access analyzed in this study presented NDF values ranging from 53.84 (access 3) to 37.84% (access 8). Among the access, ADF ranged from 30.12% (access 1) to 23.06% (access 3). For lignin and cellulose, access 3 presented the highest values (17.91 - cellulose, 17.95 - lignin), whereas for hemicellulose, access 1 presented the best result (16.02%).

The significant differences between agronomic and chemical characteristics (Table 5) indicate that there is a high degree of dissimilarity among the 10 access. Based on the

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criterion proposed by Singh (1981), the variables that contributed the most to the genetic divergence, based on the agronomic and chemical characteristics, were, in decreasing order, neutral detergent fiber with 49.52%, non-fiber carbohydrates with 31.99%, length of central lobe representing 30.34% of the variation, width of central lobe with 22.96%, total carbohydrates with 13.33% and number of axillary buds with 11.47% of genetic variation (Table 5).

The parameters that contributed least and could be discarded in future studies were the number of fruits per plant with 0.01% and hemicellulose with 0.07% of the genetic variability.

Table 2 - Agronomic characteristics evaluated in *Manihot* sp access.

Variables	*Manihot* sp access										
	1	2	3	4	5	6	7	8	9	10	CV (%)
Plant height	133.6 b	180 a	111 bc	153 ab	168 a	144.3 ab	169.3 a	63.3 c	93 bc	142.3 ab	21.6
Number of axillary buds	55 bc	232 a	29 cd	27.3 cd	104.5 b	94 b	48.7 bc	8.7 d	4 d	31.3 c	26.5
Stem diameter	26.9 ab	34.8 a	17.6 c	20.6 c	23.4 bc	28.5 ab	25.6 ab	19.6 c	23 bc	21.7 c	15.9
Number of plant branches	11 b	19 a	2.6 d	4.3 cd	8.5 bc	9 b	10 b	1.3 d	4 d	6.3 c	30.3
Number of leaves per plant	104.3 b	146.5 a	31.6 cd	40.3 cd	102 b	53.6 cd	61.6 bc	6.6 d	55 cd	60 bc	23.6
Leaf petiole length	18.3 a	8.5 bc	17 ab	17.4 ab	10.1 bc	13.8 abc	12.6 abc	9 bc	8.3 bc	9.5 bc	23.6
Diameter of leaf petiole base	4.5 abc	2.8 d	5.3 ab	5.6 ab	3.6 cd	4.6 abc	6.9 a	3.9 bc	5.4 ab	5.5 ab	24.5
Mean diameter of leaf petiole	3.6 b	2.3 b	3.5 b	4.3 b	4.1 b	3.6 b	20.8 a	2.9 b	4.4 b	4 b	25.4
Upper diameter of leaf petiole	4.8 b	2.1 b	4.3 b	4.8 b	3.2 b	4.5 b	17.3 a	2.5 b	4.8 b	4.3 b	38.8
Length of central lobe	15.7 ab	11.5 cd	14.3 ab	17.2 a	10.5 cd	14.2 ab	18.8 a	13.7 abc	16.8 ab	18.5 a	16.0
Width of central lobe	5.8 c	6.3 c	6 c	7.5 bc	4.8 cd	7 bc	11.7 a	11 a	8.7 b	7.3 bc	16.1
Upper width of median lobe	3.5 d	4.3 cd	4.8 bc	5.3 b	3.9 cd	5.9 b	7 a	5.2 b	6.3 a	4.9 bc	15.5
Length between the base lobes of the leaf	21.5 b	17.8 bc	19.3 b	19 b	9.4 cd	16.3 c	26.7 a	3.7 d	5.3 d	24.5 a	16.8
Length between the median lobes of the leaf	19.3 ab	15.7 abc	17.2 abc	17.1 abc	13.9 abc	20.5 ab	24.7 a	16 abc	13 bc	21 a	23.9
Number of fruits	3.7 bc	32.5 a	3.3 bc	12.3 abc	19.3 abc	23.7 abc	9 abc	3 bc	4 bc	17 abc	16.2
Number of senescent leaves	20.3abc	38 a	6.7 bcd	15 bc	24.7 ab	12.7 bc	11.7 bc	7.7 bcd	3 bcd	5.3 bcd	37.8

Coefficient of variation (CV); Means followed by the same letters do not differ statistically from each other by the Tukey test at the 5% probability level.

Fonte: Authors.

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Table 3 - Phenotypic characters (morphology) evaluated in *Manihot* sp access.

Variables	Phenotypic characters	Frequency (%)
Type of plant	Open	16.6
	Umbrella	19.5
	Cylindrical	63.9
Apical leaf color	Light green	5.6
	Dark green	91.6
	Purplish green	2.8
Central lobe shape	Elliptical-lanceolate	2.8
	Obovada-lanceolate	94.4
	Lanceolate	2.8
Vein Color	Green	83.3
	Green with red	16.7
Lobe number	Three lobes	13.9
	Five lobes	83.3
	Seven lobes	2.8
Growth habit	Straight	50
	Zig zag	50
Color of terminal branches on adult plants	Green	61.1
	Green-purplish	36.1
	Purple	2.8
Branching levels	One	11.1
	Two	5.55
	Three	55.5
	Four	16.7
Petiole position	Tilted Up	55.6
	Horizontal	44.4
Stipule length	Short	100
	Long	0
Leaf lobe sinuosity	Smooth	69.4
	Winding	30.6
Green leaf petiole color	Reddish Green	86.11
	Red	8.33
	Purple	5.56

Source: Authors.

Tabela 4 – Chemical composition in *Manihot* sp access.

Variables	*Manihot* sp access										CV(%)
	1	2	3	4	5	6	7	8	9	10	
Dry matter (%NM)	17.24 b	16.90 b	22.70ab	21.63 ab	22.67 ab	23.42 a	21.70 ab	24.90 a	22.38 ab	25.03 a	0.33
Mineral matter (%DM)	8.17 a	6.15 a	7.42 a	7.16 a	6.56 a	5.45 a	5.48 a	6.51 a	6.88 a	7.06 a	15.41
Crude protein (%DM)	21.99 bc	21.01 bc	18.32 c	30.94 a	23.14 b	21.75 bc	22.15 bc	24.70 b	22.70 b	20.84 c	6.32
Ether extract (%DM)	3.88 b	4.73 a	4.30 ab	4.83 a	3.80 b	3.56 b	4.03 b	4.54 ab	4.71 a	4.40 ab	9.59
Non-fiber carbohydrates (%DM)	18.34 c	22.56 b	16.13 c	16.17 c	22.31 b	26.15 ab	28.34 a	22.92 cd	27.86 a	28.30 a	9.77
Total carbohydrates (%DM)	65.97 ab	68.12 a	69.96 a	57.07 c	66.50 ab	69.24 a	68.33 a	64.26 b	65.70 ab	67.70 a	2.75
Neutral detergent fiber (%DM)	47.63 b	45.54 b	53.84 a	40.90 c	44.19 b	43.09 b	39.99 c	41.33 bc	37.84 c	39.40 c	2.56
Acid detergent fiber (%DM)	30.12 a	29.90 a	23.06 c	25.40 bc	27.28 b	26.27 b	24.88 bc	26.98 b	25.13 bc	24.94 bc	2.99
Cellulose (%DM)	9.86 c	11.26 bc	17.91 a	8.58 bc	12.56 b	11.64 bc	9.94 c	16.57 a	8.75 c	9.15 c	17.51
Hemicellulose (%DM)	16.02 a	11.87 b	12.39 ab	12.87 ab	15.08 a	8.95 bc	13.59 ab	4.31 c	9.82 b	13.18 ab	10.9
Lignin (%DM)	9.90 bc	11.30 ab	17.95 a	8.80 bc	12.60 ab	11.67 ab	9.98 bc	16.61 a	8.79 bc	9.19 bc	17.47

NM – Natural matter; DM – Dry matter; Coefficient of variation (CV); Means followed by the same letters do not differ statistically from each other by the Tukey test at the 5% probability level.

Source: Authors.

Table 5 - Relative contribution of 16 agronomic markers and 11 bromatological characters for genetic divergence among *Manihot* sp access using the method of SINGH (1981).

Variables	S.j	Value %
Agronomic markers		
Plant height	2469.82	3.33
Number of axillary buds	8500.17	11.47
Stem diameter	8148.22	2.51
Number of plant branches	2050.76	2.76
Number of leaves per plant	5034.39	6.79
Leaf petiole length	949.15	1.28
Diameter of leaf petiole base	1527.67	2.06
Mean diameter of leaf petiole	1486.22	2.00
Upper diameter of leaf petiole	4144.52	5.59
Length of central lobe	1149.18	1.55
Width of central lobe	17022.10	22.96
Upper width of median lobe	1360.28	1.83
Length between the base lobes of the leaf	22489.92	30.34
Length between the median lobes of the leaf	1527.01	2.06
Number of fruits	9.76	0.01
Number of senescent leaves	1175.07	1.58
Chemical characters		
Dry matter	1505.797	0.80
Ether extract	4593.096	0.24
Crude protein	66797.71	3.44
Acid detergent fiber	5724.445	0.29
Neutral detergent fiber	961722.7	49.51
Lignin	3413.309	0.17
Mineral matter	11485.51	0.59
Cellulose	3164.703	0.16
Hemicellulose	1427.775	0.07
Total carbohydrates	258946.3	13.33
Neutral detergent fiber	621336	31.99

S.j.: Relative contribution of each variable. Source: Authors.

4. Discussion

According to Ayetigbo et al. (2018), plant height is positively correlated with yield of tuberous roots, being an important characteristic for the adequacy of spacing, definition of the competition potential with weeds and use of the branches as propagation material, since taller and more branched plants species tend to have greater potential for foliage production. The leaves are the most photosynthetically active botanical components in the plant, accounting for the synthesis of organic compounds essential to the growth and development of the plant (Araújo Filho et al. 2013).

Marowa et al. (2016) report that the reduction of cell expansion in plants is possibly associated with the reduction in turgescence potential, which causes a decrease in water pressure on the cell wall, a pressure that causes cell expansion, or, with hormonal balance of cytokinins or abscisic acid, alterations that can cause reduction in the extensibility of the cell wall and, consequently, in the leaf growth.

A larger diameter of the stem can be due to the increase in nutrient flow between the roots and the shoots, thus requiring the thickening of conducting tissues, so that these nutrients efficiently reach and suppress the demands of the shoots (Araújo Filho et al. 2013).

The lobe length and the width of central lobe influence the photosynthetic rate and, consequently, the leaf mass production. Narrow leaf lobes allow less shading among the leaves of the same plant, which allows better distribution and use of the sun rays for photosynthesis (Haworth et al. 2018).

The traits evaluated in the phenotype classes, the most important for using the species as forage: plant type, leaf color, number of lobes, growth habit and branching levels. According to Derso & Mahamud (2018), the morphological characteristics are useful for the preliminary evaluation, as they offer an easy and fast approach to evaluate the extent of diversity.

The number of lobes serves as an indicator to distinguish the species *Manihot pseudoglaziovii* Pax & Hoffmann from *Manihot glaziovii* Muell. Arg., closely related species, since, while the former presents mainly pentalobated leaves, the latter presents trilobated leaves. In this study, most of the access had pentalobated leaves, corresponding to 83.3% access evaluated, possibly belonging to the species *Manihot pseudoglaziovii* Pax & Hoffmann. 13.9% of the access presented trilobated leaves (*Manihot glaziovii* Muell. Arg.), and only 2.8% leaves were heptalobated possibly belonging to the species *Maníhot heptaphila* Ule.

By means of the analysis of the results obtained for the phenotype classes, it can be affirmed that the access preserved in the germplasm bank of *Manihot* sp. presented a high level of genetic variability, with the following phenotypic characteristics: cylindrical plant with dark green apical leaf and obovate-lanceolate central lobe with straight or zig zag growth, green rib and with the presence of five lobes, the color of the terminal branches in the adult plants is also green. Branching levels range from one to four with upward or horizontal petiole and the petiole color in leaves is reddish green. Stipules are short and the sinuosity of leaf lobe is smooth.

Araújo Filho et al. (2013) analyzed *Manihot* subjected to two planting spacings and fertilization, and found levels of 22.72% DM and 23.16% CP, values corresponding to those found among the evaluated access, which makes the species with adequate nutritional characteristics to be used in animal feed. Medina et al. (2013) state that the higher the dry matter content, the higher the nutrient input and the material is more suitable for fresh intake, production of good quality silage and hay, values between 30 and 40%, depending on the maturity of the plant, are indicated as good quality foods, not impairing the composition of the other nutrients.

The access can be considered with high percentages of CP from the nutritional point of view, since ruminants require 7% CP to reach levels of intake and digestibility sufficient for their maintenance (Van Soest, 1994). It is verified that the concentration of ether extract for all the access studied (Table 4), are below the limit of 8% EE, recommended by Van Soest (1994), so that there is no reduction in food intake decreasing animal performance. Mendonça Junior et al. (2008) evaluated the chemical composition of *Manihot* leaves hay and reported values similar to the ones found in this study, being 22.79% for non-fiber carbohydrates and 63.17% for total carbohydrates, which is indicative of forage with high nutritional value.

According to Costa et al. (2015), NDF is positively correlated to rumination and chewing time, promoting adequate ruminal pH, and inversely related to DM intake. A reduction in fiber content in diets for ruminant may be detrimental to total digestibility of food, since fiber is critical to maintaining optimum rumen conditions. ADF indicates the amount of non-digestible fiber and is one of the qualitative indicators of forage. The lower its value, the higher the energy value of the food. Van Soest (1994) reported that a good amount of ADF in forage is around 30%. Slavov et al. (2013) reported that cell wall content is considered to be the main factor affecting forage utilization, since it comprises the highest dry matter fraction and is correlated with intake and digestibility.

Importantly, Length of central lobe and Width of central lobe variables are part of the leaf constitution, and these are very important considering a forage species and therefore it would be interesting to preserve these characteristics in genetic breeding due to their contribution to the production of good quality forage. Similarly, NGA has great importance for branching and thus contribute to the increase of green and dry mass yield, notably composed of the parts of the plant most used in animal feed (Fioreze et al. 2018).

The lower value of number of fruits per plant among the evaluated agronomic traits suggests that *Manihot* sp. tends to present a relatively similar number of fruits among the access, being the seed production more related to the soil and climatic conditions than the physiological requirements of the species. Nevertheless, it would be prudent to do more research to check the trend observed in this research.

5. Final Considerations

The morpho-agronomic traits together with the chemical attributes of *Manihot* access can be used as qualitative indicator in breeding programs, with focus on their use as forage, enabling the use of accesses of *Manihot* sp. with the characteristics of better production and nutritional value.

Among the evaluated agronomic traits of *Manihot*, those related to leaf morphology, Length of central lobe and Width of central lobe, presented the most variations among access.

From the chemical variables evaluated in the access of *Manihot*, the ones that contributed the most to distinguish genotypes were the content of neutral detergent fiber, non-fiber carbohydrates and total carbohydrates.

It is possible to obtain heterotic hybrids from the crossings between the accessions due to the divergence in chemical characters, to start a maniçoba improvement program, thus, we recommend further studies on genetic diversity in plants of the genus *Manihot*.

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