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Chemical composition and morphophysiological responses of *Manihot* plants

Composição química e respostas morfofisiológicas de plantas do gênero *Manihot*

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Highlights

Maniçoba and Pornunça presented similar photosynthesis compared to Cassava plants.
Manihot plants had similar dry matter levels and *in situ* degradability of plant shoot.
K and Ca are the main macro minerals in the shoot of *Manihot plants.*

Abstract

Cassava (*Manihot esculenta* Crantz) is an important forage source for livestock, while wild cassavas (maniçoba and pornunça – Manihot sp.), native from Brazilian semi-arid have the potential to feed ruminants in drylands. We hypothesized that maniçoba and pornunça have a chemical composition and morphophysiological responses similar to cassava cultivars. Nine *Manihot* plants were evaluated, six wild cassava accessions (BGMS 20, BGMS 21, BGMS 22, BGMS 26, BGMS 79, and BGMS 102), pornunça (BGMS 24) and two cassava cultivars (gema-de-ovo [GO] and engana-ladrão [EL]). We evaluated two 6-month crop cycles under a completely randomized design with four replicates. The genotype BGMS 20 had higher shoot biomass than BGMS 24, BGMS 79, EL, and GO, as well as higher leaf mass than EL and GO. Photosynthesis, leaf temperature, stomatal conductance, and transpiration were similar among the genotypes (13.83 $\mu mol.m^{-2}.s^{-1}$, 29.90 ºC, 0.12 $mol.m^{-2}.s^{-1}$ and 2.75 $mmol.m^{-2}.s^{-1}$, respectively). EL had

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a shoot crude protein content higher than the others, except for BGMS 21 and BGMS 24. Genotypes did not differ concerning *in situ* and *in vitro* (dry matter (DM)) degradability and mineral composition. BGMS 24 had a higher number of leaves than the others, and BGMS 20 had taller plants than the other genotypes, except for BGMS 21 and BGMS 22. *In vitro* gas production was similar among the genotypes considering total carbohydrates. Wild cassava accessions showed shoot biomass, leaf mass physiological responses, and chemical composition compatible with cassava cultivars; therefore, they show potential as alternative forages for livestock.

Key words: Cassava. *Manihot esculenta* Crantz. Maniçoba. Mineral composition. Pornunça.

Resumo

A mandioca (*Manihot esculenta Crantz*) é um importante recurso forrageiro para a pecuária, enquanto a maniçoba e a pornunça (*Manihot* sp.), nativas do semiárido brasileiro, tem potencial para alimentar ruminantes em terras secas. Foi hipotetizado que os genótipos de maniçoba e pornunça têm composição química e respostas morfofisiológicas semelhantes às cultivares de mandioca. Nove acessos de plantas do gênero *Manihot* foram avaliados, seis maniçobas (BGMS 20, BGMS 21, BGMS 22, BGMS 26, BGMS 79, BGMS 102), pornunça (BGMS 24) e duas cultivares de mandioca (gema-de-ovo (GO) e engana-ladrão (EL). A avaliação foi realizada em dois ciclos de cultivo de seis meses cada em delineamento experimental inteiramente casualizado com quatro repetições. O BGMS 20 apresentou maior biomassa da parte aérea em comparação maior com 24, 79, EL e GO, e maior massa foliar que EL e GO. A fotossíntese, temperatura foliar, condutância estomática e transpiração foram semelhantes entre os genótipos, apresentando em média 13,83 μmol.m^{-2}.s^{-1}, 29,90 ºC, 0,12 mol.m^{-2}.s^{-1} e 2,75 mmol.m^{-2}.s^{-1}. Proteína bruta da biomassa da parte aérea foi maior para EL em comparação com os demais, exceto para 21 e 24. Os genótipos não diferiram em relação à degradabilidade *in situ* e *in vitro* (matéria seca (MS) e composição mineral. O acesso 24 teve maior número de folhas que os demais e o BGMS 20 apresentou plantas mais altas, comparado aos genótipos avaliados, exceto 21 e 22. A produção de gás *in vitro* foi semelhante para os genótipos considerando os carboidratos totais. Os acessos de maniçoba exibem biomassa da parte aérea, massa de folhas, composição química e respostas fisiológicas compatíveis com as cultivares de mandioca, demonstrando potencial como forrageiras alternativas para a pecuária.

Palavras-chave: Mandioca. *Manihot esculenta* Crantz. Maniçoba. Composição mineral. Pornunça.

Introduction

Drylands cover about 40% of the global terrestrial area and encompass large areas of livestock systems and smallholder farmers (Chillo, Ojeda, Anand, & Reynolds, 2015). In these regions, animal production contributes economically (Schulze et al., 2016) and socially. However, in arid and semi-arid areas, animal feed is one major challenge, requiring forage alternatives adapted to environmental conditions or native forages.

Cassava (*Manihot esculenta* Crantz) is a usual feed resource for ruminants and has considerable root and shoot yields (Guimarães et al., 2017), with its shoot being rich in crude protein (CP) compared to other tropical forages (Pereira et al., 2017). Additionally, Maniçoba *(Manihot glaziovii* Mull. Arg., *Manihot catingae* Ule, and *Manihot* *carthaginensis* (Jacq.) Mull. Arg.) and Pornunça (*Manihot* sp.) are wild cassavas native to Brazilian semi-arid areas. Maniçoba is a perennial plant tolerant to water deficit (Lima Júnior et al., 2015; Maciel et al., 2019) and high temperatures (Oliveira et al., 2022), and pornunça is a natural hybrid between cassava and wild cassava (Amorim et al., 2022).

Wild cassavas may represent alternative feed materials for livestock, especially where environmental conditions are less favorable for cassava cultivation, such as in drier locations. It occurs because these plants have higher forage production (Gomes et al., 2022) and promote good animal performance (Guimarães et al., 2017; Gomes et al., 2022). Maniçoba, pornunça, and some cassava genotypes have high contents of cyanogenic glycosides and must be provided to animals as conserved forage (silage and hay) to reduce toxic levels (Amorim et al., 2022).

Ferreira et al. (2009) reported that cassava, maniçoba, and pornunça have similar forage yields and chemical compositions; however, these authors disregarded genotype differences. On the other hand, Beltrão et al. (2015) evaluated 14 accessions of Manihot and observed differences in their chemical composition. This genetic variability is essential for prospective studies on forage yield potential, physiological responses, and chemical composition. Based on these, plants can be used in breeding programs or recommended as feed for ruminant production systems.

Given the above, we hypothesized that the genotypes maniçoba and pornunça have chemical compositions, forage yields, and morphophysiological responses similar to those of cassava cultivars. Therefore, our goal was to evaluate these characteristics (productivity, morphophysiological aspects, and chemical composition) for *Manihot* plants and compare them to cassava cultivars.

Materials and Methods

The experiment was performed at the experimental field of Caatinga (09º 09’ S, 40º 22’ W, 376-m altitude), EMBRAPA Semi-arid, Petrolina-PE, Brazil, from April 2018 to March 2019. It was approved by the Ethics Committee on Animal Use of the Brazilian Agricultural Research Corporation - Embrapa Semi-arid, under protocol number 06/2018.

Nine *Manihot* plants were evaluated, six maniçoba genotypes (BGMS 20, BGMS 21, BGMS 22, BGMS 26 BGMS 79, and BGMS 102), one pornunça (BGMS 24), and two cassava cultivars (gema-de-ovo [GO], engana-ladrão [EL]), during two 6-month crop cycles. In the first cycle (from April to September 2018), rainfall was 215.10 mm, relative humidity 59.35%, while average, maximum, and minimum temperatures were 25.5 ºC, 31.3 ºC, and 20.5 ºC, respectively. During this cycle, an irrigation depth of 147 mm was applied, totaling 362.10 mm, considering rainfall and irrigation.

In the second cycle (from September 2018 to March 2019), rainfall was 161.00 mm; relative humidity 58.17%; and average, maximum, and minimum temperatures were 27.8 ºC, 33.4 ºC, and 22.8 ºC, respectively. An irrigation depth of 126 mm was applied via drip irrigation (three times a week), totaling 287 mm. It was irrigated for one hour in the morning time during weeks without rain.

The experimental design was completely randomized with nine *Manihot* plants and four replicates, totaling 36 experimental units. Four plants of each plot were evaluated for morphophysiological responses and chemical composition.

Soil chemical and physical compositions in the 0-20 cm depth layer were as follows: pH = 5.80; K = 0.01 $cmol_c$ dm^{-3}; Na = 0.01 $cmol_c$ dm^{-3}; Ca = 2.5 $cmol_c$ dm^{-3}; Mg = 1,00 $cmol_c$ dm^{-3}; Al = 0.00 $cmol_c$ dm^{-3}; CEC = 10.5 $cmol_c$ dm^{-3}; SB = 3.5 $cmol_c$ dm^{-3}; H + Al = 6.9 $cmol_c$ dm^{-3}; P = 1.00 mg dm^{-3}; electric conductivity = 0.70 mS cm^{-1}; V (%) = 33.5; sand = 715.0 g kg^{-1}; silt = 178.3 g kg^{-1}; clay = 106.6 g kg^{-1}. Plants were spaced 3.0 m between rows and 1.0 m between plants within the row, and fertilization was carried out with 1.0 L of manure per plant.

Five agronomic descriptors were measured: plant height (PH), stem diameter (SD), number of axillary gems (NAG), number of branches (NB), and leaf number per plant (LN). PH (cm) was measured using a measuring tape from the ground level to the leaf apex; SD (mm) was measured using a digital caliper at 20 cm from the ground level.

Shoot biomass (g dry matter; DM) was measured after plant harvest, leaving 30 cm of residue above ground. Forage material was weighed freshly and separated (leaves and stems). Leaf samples comprised leaf blades, while stem samples were stems, branches, and petioles. Shoot biomass, stem, and leaf samples were dried at 55 ºC for 72 hours in a forced air ventilation oven. Dry weights of leaves and stems were used to calculate the leaf: stem ratio.

Physiological responses were evaluated using measurements from the Li-6400 portable infrared gas analyzer (IRGA), with artificial light set at 2,500 μmol m^{-2} s^{-1}. The variables analyzed were photosynthesis rate (A), stomatal conductance (Gs), transpiration (E), and leaf temperature (Tf), which were performed on leaves exposed to the sunlight between 9h00 and 11h00 on non-cloudy days, performing one evaluation per cycle.

Forage material was pre-dried in a forced air circulation oven at 55 ºC for 72 hours and then weighed. After drying, the material was ground in a *Willey* mill fitted with a 1-mm screen. For *in vitro* gas production, forage material was ground using 3-mm sieves.

Dry matter content (DM; method 967.03), ash (method 942.05), and crude protein (CP; method 981.10) were determined according to AOAC (2016). Neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest, Robertson, & Lewis 1991), and lignin contents were determined according to Van Soest and Wine (1967).

The nylon bag technique (6 x 10 cm, 50-μ porosity with 0.5 g forage sample) was used to determine *in situ* DM degradability. The bags were incubated in the rumen of fistulated adult bovines for 288 hours. After removing the nylon bags, they were placed into a recipient containing water and ice for five minutes and then washed. The nylon bags were dried in an oven at 55 °C for 72 hours, then in an oven at 105 °C for eight hours, and subsequently weighed for estimation of DM degradability according to Orskov and McDonald (1979).

In vitro gas production was measured to determine gas yield from fibrous (FC), non-fibrous (NFC), and total carbohydrates (TC), calculating the degradation rates of FC, NFC, and TC, as well as lag phase and DM degradability at 48h. Five genotypes were evaluated, three maniçoba genotypes (accessions 22, 79, and 102), pornunça (accession 24), and one cassava (gema-de-ovo - GO) using the semi-automatic *in vitro* gas production technique.

The ruminal fluid used was extracted from two sheep through a ruminal cannula. It was kept under continuous CO_2 injection in a water bath at 39 ºC, then filtered and inoculated into vials. The vials were sealed, placed in Styrofoam boxes, manually shaken, and kept in a room at 39 ºC. Pressure, in psi (pound per square inch), was measured by a transducer (type GE Druck DPI 705 Series) connected at its end to a needle (0.6 mm). The readings were taken at 0, 2, 4, 6, 8, 9, 11, 12, 14, 17, 20, 24, 28, 34, 48, and 72h. The measures were converted from *psi* to volume (mL) through the equation: $V = 4.4392P+0.8943$, $R^2=0.98$; wherein V: volume (mL) and P: pressure (psi). Cumulative gas production was estimated by the bicompartmental model proposed by Schofield et al. (1994), as follows: $V (t) = Vf1/[1+e(2-4m1(L-T))] + Vf2/[1+e(2-4m2(L-T))]$; wherein: V(t) is the total volume of gas produced, Vf1 is the maximum gas volume from the rapid-digestion fraction (NFC), Vf2 represents the maximum volume of gas from the slow-digestion fraction (FC), m1 is the specific growth rate from the slow-digestion fraction, L is the duration of the initial digestion events, and T is the fermentation time.

In vitro dry matter degradability was determined by removing the bags after 48 hours of incubation, immersing them in ice water immediately to stop microbial fermentation, washed with running water, and weighed after drying them in an oven at 105 ºC for 12 hours. It was calculated according to Orskov and McDonald (1979). The effective degradability (ED,% total) was determined according to Menezes et al. (2015).

Statistical analysis was performed by analysis of variance and Tukey’s test, considering a significant probability value lower than 5% ($p<0.05$), using the software Statistical Analysis System - SAS.

Results and Discussion

Shoot and stem dry weights (g DM plant^{-1}) were greater ($p< 0.0001$; $p= 0.0001$) for genotype 20 than for EL, GO, 24, and 79. The leaf: stem ratio was not different among genotypes ($p> 0.05$), which was, on average, 0.66 for *Manihot* plants. The genotype BGMS 20 also had a greater leaf mass when compared to cassavas (EL and GO) ($p= 0.0065$) (Table 1).

Table 1
Shoot biomass, leaf, and stem mass and leaf: stem ratio (L:S) of *Manihot* plants in Petrolina-PE.

Genotype	Variable			
	Shoot biomass (g DM plant^{-1})	Stem mass (g DM)	Leaf mass (g DM)	L:S (g DM)
102	214.87^{ab}	117.54^{ab}	97.33^{ab}	0.83
20	321.87^{a}	212.84^{a}	109.03^{a}	0.51
21	244.40^{ab}	155.79^{ab}	88.62^{ab}	0.57
22	252.07^{ab}	173.55^{ab}	78.51^{ab}	0.45
24	160.60^{b}	85.14^{b}	75.45^{ab}	0.89
26	256.19^{ab}	167.99^{ab}	88.20^{ab}	0.53
79	183.28^{b}	99.64^{b}	83.64^{ab}	0.84
EL	132.13^{c}	74.95^{b}	57.18^{b}	0.76
GO	149.98^{c}	95.76^{b}	54.22^{b}	0.57
SEM	23.92	20.53	10.28	0.27
P-value	<.0001	0.0001	0.0065	0.072

In the column, means followed by different lowercase letters differ from each other by the Tukey test ($p< 0.05$). SEM = standard error of the mean. GO = gema de ovo, EL = engana-ladrão. DM = dry matter.

Shoot biomass provided by the *Manihot* genotypes ranged from 132.13 to 321.87 g DM plant^{-1}, which corroborates the report by Ferreira et al. (2009). These authors evaluated Manihot plants in a semi-arid environment and obtained a range from 43 to 461 g DM plant^{-1}. On average, leaf corresponded to 39% of shoot biomass, representing significant participation.

Wild cassava (maniçoba and pornunça) proportioned greater shoot biomass than EL and GO genotypes, thus having yields potentially similar to those of cassavas. Lower biomass in cassava shoots can be attributed to the evaluated cultivars. It can also be specific to root production, storing nutrients in plant shoots. Wild cassavas may have directed them to the shoot part. After two crop cycles, the amount of shoot mass observed for maniçoba and pornunça genotypes characterizes them as more tolerant to successive harvests. Such characteristic may be associated with the number of sprouts per plant. Moreira Filho et al. (2008) reported that maniçoba has a high regrowth capacity due to its root system and regulatory mechanisms of storage and use of plant reserves, and its greater number of sprouts lead to higher forage yields.

The genotypes evaluated had different responses in shoot biomass and leaf proportions. Among them, maniçoba genotypes provided higher forage mass, especially BGMS 20, while pornunça (BGMS 24) promoted a higher leaf mass. This result may be due to the more significant number of leaves of pornunça genotypes, which had more than 150 leaves per plant. Great leaf numbers and proportions are crucial for forage production, as leaves have a higher nutritional value than stems, which may be associated with a higher lignification (França et al., 2010). The genotype BGMS 20 had taller plants than EL, GO, 79, and 24, which increased stem mass.

The *Manihot* genotypes had a higher proportion of stems than leaves in shoot biomass (Table 1) when harvested at six months of regrowth, except for accessions 24, 102, and 79, which had between 53 and 54% of leaves.

Plant physiological responses (photosynthesis, stomatal conductance, transpiration, and leaf temperature) were not affected by genotypes (Table 2, p> 0.05). Moreover, plant shoot, leaf, and stem biomasses had no relationship with physiological responses. The averages of photosynthesis rate, stomatal conductance, transpiration, and leaf temperature were 13.83 μmol.m^{-2}.s^{-1}, 0.12 mol.m^{-2}.s^{-1}, 2.75 mmol. m^{-2}.s^{-1}, and 29.90 ºC, respectively. Therefore, these results denote a similar gas exchange between cassavas and wild cassavas.

Table 2
Physiological responses of *Manihot* plants in Petrolina-PE.

Genotype	Variable			
	Photosynthesis (μmol.m^{-2}.s^{-1})	Stomatal conductance (mol.m^{-2}.s^{-1})	Transpiration (mmol.m^{-2}.s^{-1})	Leaf temperature (ºC)
102	13.40	0.13	3,14	30.10
20	13.09	0.12	2.82	29.32
21	12.76	0.10	2.46	29.90
22	13.97	0.09	2.37	30.15
24	16.04	0.13	3.14	29.96
26	10.74	0.15	3.07	30.05
79	17.08	0.11	2.98	29.80
EL	13.63	0.11	2.70	29.91
GO	13.72	0.11	2.43	29.93
SEM	3.50	0.05	0.98	0.42
P value	0.97	0.99	0.99	0.97

In the column, means followed by different lowercase letters differ from each other by the Tukey test (p< 0.05). SEM = standard error of the mean. GO = gema de ovo, EL = engana-ladrão.

Genotypes also had a significant effect on leaf DM (p= 0.01), ash (p< 0.0001), and CP (p< 0.0001) contents (Table 3). BGMS 20 and 79 had higher DM than GO, while BGMS 22, 20, 21, 26, and GO showed higher ash levels than the others. Average dry matter levels of *Manihot* genotypes were 25.27 and 25.50% for shoot and leaves, respectively. This result shows an interesting characteristic for forage ensiling, thus promoting a proper fermentation process. Maciel et al. (2019) ensiled maniçoba containing 34.7% DM and reported the production of silages of adequate quality. In our study, most of the shoot biomass from the genotypes evaluated ranged from 25.44% to 28.26% DM. The higher leaf DM contents of BGMS 20 and 79 compared to GO may be due to the faster regrowth capacity of maniçoba genotypes, leading to an advanced leaf physiological maturity stage and hence higher DM concentrations at forage harvest.

CP contents were higher in engana-ladrão than in the other genotypes, except for BGMS 24 and 79, which was due to its less advanced maturity stage. Leaf NDF contents were higher (p= 0.01) for the accessions BGMS 102 and 22 compared to BGMS 26, which was due to a greater participation of dead leaves in leaf mass; however, there were no differences between the accessions BGMS 102 and 22. Leaf and shoot CP contents in Manihot plants may be considered high for roughage sources. In general, cassava and wild cassava genotypes demonstrated desirable traits for roughage production, such as low NDF and high CP contents.

Table 3
Chemical composition of the leaf of *Manihot* plants in Petrolina-PE.

Genotype	Variable, % dry matter						
	DM^1	Ash	CP	NDF	ADF	Lignin	Deg
102	27.57^{ab}	7.96^{b}	19.36^{b}	43.84^{a}	32.62	10.45	48.69
20	29.82^{a}	8.91^{a}	17.28^{c}	39.80^{ab}	29.42	10.57	46.13
21	24.02^{ab}	8.73^{a}	18.76^{bc}	39.30^{ab}	27.11	8.49	41.99
22	25.25^{ab}	9.11^{a}	18.02^{b}	42.81^{a}	29.82	10.06	44.87
24	26.46^{ab}	6.90^{b}	22.92^{ab}	39.65^{ab}	21.74	10.76	55.96
26	25.27^{ab}	8.47^{a}	19.52^{b}	35.11^{b}	21.14	9.64	49.71
79	28.93^{a}	6.77^{b}	23.46^{ab}	41.31^{ab}	24.18	11.80	53.08
EL	22.77^{ab}	7.58^{b}	24.65^{a}	36.38^{ab}	26.84	12.06	53.76
GO	19.43^{b}	8.99^{a}	20.08^{b}	37.69^{ab}	21.77	8.66	47.01
SEM	1.79	0.14	0.95	1.60	4.09	1.19	6.07
P-value	0.01	<0.0001	<0.0001	0.01	0.39	0.48	0.84

In the column, means followed by different lowercase letters differ from each other by the Tukey test ($p < 0.05$). SEM = standard error of the mean. GO = gema-de-ovo, EL = engana-ladrão. DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; Deg = *In situ* dry matter degradability. 1 -% as food.

Contents of NDF varied between 35.11% and 43.84% in leaves and between 36.48% and 49.86% in shoot biomass. These ranges are low compared to those reported by Ramos et al. (2015) (61.78%), who evaluated a longer harvest interval. The higher NDF content in shoot biomass for BGMS 20 compared to 21, 26, 79, 102, GO, and EL may be due to its greater stem biomass. The genotype BGMS 20 had taller plants, which may require stem support and larger stem diameters. Shoot biomass NDF reached 49.86%, while leaf content was up to 43.84%. This difference is due to the presence of stems in plant shoots. Furthermore, stems have greater cell wall components, which may reduce DM degradability compared to leaves. According to França et al. (2010), NDF contents are higher in stems than in leaves of maniçoba due to the highly lignified secondary walls of conducting vessels and fibers. The cassava genotype (EL) had a lower NDF content in shoot biomass than the wild cassavas genotypes 102, 20, 22, and 24 because it showed shorter plants hence less stem.

There were no differences in leaf ADF, lignin, and DM degradability ($p > 0.05$). Lignin levels ranged from 10.36 to 12.39 % DM, while ADF level and DM degradability were 26.07% and 49.02%, respectively (Table 3). Levels of ADF and lignin were also low for the leaf and shoot biomass of Manihot plants. Backes et al. (2014) reported 46.65% and 14.12% for ADF and lignin in maniçoba plants, respectively.

Shoot biomass contents of DM, ADF, lignin, and in situ DM degradability had no significant differences among the Manihot genotypes ($P > 0.05$). Still, ash levels were higher for BGMS 20 and EL ($p < 0.0001$) compared to 102, 22, and 24. Moreover, EL showed higher CP contents ($p=0.03$) in comparison to 20, 22, 26, and 79, while BGMS 20 had more NDF ($p= 0.004$) than 21, 26, 79, EL and GO (Table 4). Differences in CP contents are attributed to the advanced physiological stage of the genotypes 20, 22, 26, and 79, as they had a faster regrowth and taller plants (102.75 to 172.50 cm) than EL (91.50 cm) at harvest.

Table 4
Chemical composition of shoot biomass of *Manihot* plants in Petrolina-PE.

Genotype	Variable, % dry matter						
	DM^{1}	Ash	CP	NDF	ADF	Lignin	Deg
102	27.85	6.97^{c}	16.45^{ab}	49.01^{ab}	40.12	11.64	42.64
20	27.35	9.73^{a}	14.98^{b}	49.86^{a}	35.16	12.39	49.28
21	28.26	9.59^{ab}	16.53^{ab}	44.07^{bc}	31.31	10.92	42.98
22	25.44	8.08^{bc}	15.08^{b}	46.97^{ab}	37.47	10.69	50.79
24	27.15	7.97^{bc}	16.49^{ab}	49.46^{ab}	33.94	11.71	44.51
26	22.78	9.17^{ab}	15.64^{b}	42.86^{bc}	29.70	10.36	43.70
79	28.09	8.49^{ab}	15.99^{b}	46.51^{bc}	32.20	10.53	50.38
EL	18.06	9.84^{a}	21.72^{a}	36.48^{c}	30.94	12.00	45.01
GO	22.42	9.31^{ab}	20.44^{ab}	44.47^{bc}	-*	-*	32.23
SEM	2.52	0.30	1.19	1.87	2.80	0.66	6.84
P-value	0.10	<0.0001	0.003	0.004	0.15	0.27	0.70

In the column, means followed by distinct lowercase letters differ from each other by the Tukey test ($p < 0.05$). SEM = standard error of the mean. GO = gema-de-ovo; EL = engana-ladrão. DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; DEG = *In situ* dry matter degradability; ^{1} -% of food. *-non-determined.

The accession BGMS 20 had taller plants than the others (p< 0.0001), except BGMS 21 and 22. On the other hand, BGMS 22 showed larger stem diameters than the others (p< 0.0001), except for BGMS 24. EL had the highest number of gems (p< 0.0001), while BGMS 24 reached higher numbers of branches and leaves (p<0.0001); however, the latter did not differ from BGMS 102 and 79 in the number of branches (Table 5).

Table 5
Morphological characteristics of *Manihot* plants evaluated in Petrolina-PE.

Genotype	Variable				
	Plant height, cm	Stem diameter, cm	Number of gems, n	Number of branches, n	Leaf number, n
102	114.40^{de}	1.23^{cde}	9.50^{c}	9.00^{ab}	101.40^{b}
20	172.50a	1.14^{e}	16.60^{c}	2.40^{c}	55.50^{bc}
21	162.60^{ab}	0.87^{e}	12.60^{c}	1.10^{c}	42.70^{c}
22	156.20^{abc}	2.50^{a}	8.10^{c}	4.90^{bc}	73.50^{bc}
24	123.80^{cde}	2.09^{ab}	13.90^{c}	14.40^{a}	156.50^{a}
26	136.50^{bcd}	0.83^{e}	9.90^{c}	1.70^{c}	45.50^{c}
79	102.75^{e}	1.75^{bcd}	13.50^{c}	8.80^{ab}	82.50^{bc}
EL	91.50^{e}	1.80^{bc}	72.00^{a}	5.10^{bc}	78.90^{bc}
GO	106.50^{de}	1.21^{de}	46.00^{b}	5.10^{bc}	72.40^{bc}
SEM	1.79	0.14	0.95	1.60	5.22
P-value	<.0001	<.0001	<.0001	<.0001	<.0001

In the column, means followed by distinct lowercase letters differ from each other by Tukey test (p< 0.05). SEM = standard error of the mean. GO = gema-de-ovo, EL = engana-ladrão.

Both cassava genotypes (EL and GO) showed a great number of gems, pornunça presented a great number of leaves and branches, and three maniçoba genotypes (20, 21, and 22) had taller plants. These results demonstrate the variability among the *Manihot* genotypes evaluated, and morphological characteristics are important and useful to perform a primary evaluation as indicators to distinguish species.

Macro-mineral composition (P, K, Ca, Mg, and S) did not influence genotypes (p> 0.05) (Table 6). Ca and K showed the highest concentrations (27.11 g kg^{-1} and 22.36 g kg^{-1}, respectively). The averages of P, Mg, and S contents were 2.0, 4.19, and 3.08 mg kg^{-1}, respectively (Table 7). Likewise, micro-mineral composition (B, Cu, Fe, Mn, Zn, and Na) had no genotype effect either (p> 0.05). Na and Fe had the highest concentrations (Table 7).

Table 6
Macromineral composition of *Manihot* plants in Petrolina-PE.

Genotype	Macromineral (g kg^{-1})				
	P	K	Ca	Mg	S
102	1.78	31.60	20.52	2.23	2.72
20	2.24	22.25	34.45	4.22	2.74
21	1.70	24.00	30.51	4.76	3.00
22	1.89	21.80	22.83	4.06	3.06
24	1.75	18.22	25.06	4.21	3.53
26	2.21	20.02	28.54	4.85	2.14
79	1.59	19.42	29.19	4.62	3.14
EL	2.64	20.90	30.75	4.62	3.39
GO	2.27	23.07	22.21	4.13	4.01
SEM	0.61	4.88	2.75	1.06	1.14
P-value	0.94	0.73	0.07	0.79	0.97

In the column, means followed by distinct lowercase letters differ from each other by Tukey test ($p < 0.05$). SEM = standard error of the mean. GO = gema-de-ovo, EL = engana-ladrão.

Table 7
Micromineral composition of *Manihot* plants harvested in Petrolina-PE.

Genotype	Micromineral (mg kg^{-1})					
	B	Cu	Fe	Mn	Zn	Na
102	10.41	21.13	157.84	146.97	106.15	1,970
20	13.90	21.71	149.41	101.37	112.58	3,255
21	18.16	20.31	176.45	91.48	114.82	1,665
22	17.99	17.72	181.77	92.28	108.60	2,730
24	9.80	19.33	144.13	105.03	121.39	2,005
26	16.49	19.46	125.19	98.47	106.76	2,390
79	9.46	22.04	144.10	135.43	228.75	3,280
EL	19.15	23.04	152.26	93.74	118.36	2,280
GO	10.68	24.75	118.58	97.86	102.62	2,565
SEM	10.84	6.46	171.55	19.65	66.81	1,636
P-value	0.99	0.99	0.53	0.46	0.92	0.99

In the column, means followed by distinct lowercase letters differ from each other by the Tukey test ($p < 0.05$). SEM = standard error of the mean. GO = gema-de-ovo, EL = engana-ladrão.

BGMS 20 and EL had higher ash contents compared to the others. Forages are essential sources of minerals for grazing or forage feed livestock, performing several functions in the animal organism. Or maniçoba genotypes, Ca and K were the main macro-minerals. Andrade et al. (2014) observed an average P content of 2.91 g kg^{-1}, while we recorded 2.0 g kg^{-1}. This difference was due to the chemical composition of the soil. In this sense, forage mineral concentration can be affected by several factors, such as plant species, soil type, and soil chemical composition.

Among the main micro-minerals found in Manihot shoot biomass were Na and Fe. Overall, the compositions of macro- and micro-minerals in plant shoots were similar between cassavas and wild cassavas. Besides the role of minerals in animal nutrition, our findings may help determine soil extraction and export values, as well as nutrient input needs.

BMGS 79 had a higher in vitro gas production than GO, 24, and 102, suggesting the highest (p= 0.006) NFC fraction in that accession. For fibrous carbohydrates (FC), GO had a higher (p= 0.03) gas production than did 79 and 102 (Table 8), which might be related to its fibrous composition and quality (21.77% ADF and 8.66% lignin in leaf mass). Gas production rates of BGMS 79 from NFC (p= 0.01) and FC (p= 0.002) were higher than in the other genotypes (Table 8). In vitro gas production from total carbohydrates (TC) and degradability at 48h were not different among the genotypes (p> 0.05). In vitro degradability was consistent with in situ measurement, i.e., no differences among the genotypes (p> 0.05). The gas production rate from TC (p=0.006) and colonization time (p= 0.0002) were higher for BGMS 79 than the other genotypes.

Table 8
***In vitro* gas production (mL g DM^{-1}) and gas production rates (mL g DM^{-1} h^{-1}) from non-fibrous (NFC, Knfc), fibrous carbohydrates (FC, Kfc) and total carbohydrates (TC, Ktc), colonization time, and degradability at 48 hours (Deg 48h) of Manihot plants in Petrolina-PE.**

Genotype	Variable							
	NFC	FC	Knfc	Kfc	TC	Ktc	Lag time	Deg 48h
102	100.75^{b}	57.79^{b}	0.10^{b}	0.03^{ab}	158.54	0.14^{b}	7.58^{b}	54.33
22	103.35^{ab}	61.26^{ab}	0.11^{b}	0.03^{b}	164.61	0.14^{b}	6.70^{c}	55.56
24	93.61^{c}	61.84^{ab}	0.11^{b}	0.03^{b}	155.45	0.14^{b}	7.86^{b}	51.42
79	108.93^{a}	39.68^{c}	0.15^{a}	0.04^{a}	148.61	0.19^{a}	8.50^{a}	55.23
GO	94.66^{c}	65.84^{a}	0.11^{b}	0.03^{b}	160.50	0.14^{b}	7.48^{b}	55.06
SEM	2.70	5.47	0.008	0.0007	4.39	0.008	0.19	5.22
P-value	0.006	0.03	0.01	0.002	0.17	0.006	0.0002	0.98

Means followed by distinct lowercase letters differ from each other by the Tukey test (p< 0.05). SEM = standard error of the mean. EL = engana-ladrão. GO = gema-de-ovo. NFC = non-fibrous carbohydrate; FC = fibrous carbohydrate; Knfc = non-fibrous carbohydrate rate; Kfc = fibrous carbohydrate rate.

Stem participation can negatively affect plant degradability due to increased levels of indigestible fibers and lignin and lower contents of cellular components, such as non-fibrous carbohydrates (NFC). Accessions 20, 21, and 22 had taller plants at six months, which suggests larger stems, with BGMS 22 also showing large stem diameters. Between-harvest intervals may influence stem development. In this sense, Andrade et al. (2014) indicated that maniçoba must be harvested before fruiting. In our study, harvest was made at six months of regrowth, i.e., before the fruiting phase.

In vitro gas production from total carbohydrates was not different among the genotypes. However, some genotypes showed higher in vitro gas production from FC or NFC fractions. This result indicates a similar use of carbohydrates between cassavas and wild cassavas in the ruminal environment, thus promoting similar in vitro degradability. The highest gas production rate from TC of BGMS 79 may be due to its greater gas production rate from NFC, suggesting a higher NFC content in its shoot biomass. The total gas produced from NFC by BGMS 79 was 108.93 mL g DM^{-1}, which increased colonization time, whereas total gas production from FC was 39.68 mL g DM^{-1}. FC are digested more slowly by microorganisms than NFC; therefore, the FC gas production observed suggests a lower NDF degradation. Accession 79 had 46.51% NDF, 10.53% lignin, and an NDF/lignin ratio of 4.42.

Conclusion

Wild cassavas (maniçoba and pornunça) show shoot biomass, leaf mass, chemical composition, and physiological characteristics compatible with cassava cultivars. Accessions 20, 21, 22, and 102 have great potential as forage resource, considering their shoot biomass.

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Declaration of interest statement

The authors do not have any conflict of interest to declare.

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