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Research article

Morphoagronomic and industrial performance of cassava (*Manihot esculenta* Crantz) germplasm for the production of starch and solid byproducts

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Abstract: The objective of the present research work was to study the morphoagronomic and industrial performance of three cassava clones. The study was carried out in two stages: A) the MMEXV5, MMEXV40, and MMEXCH23 clones were established in a subhumid warm climate; B) the storage roots were processed. For the first essay, a randomized complete block design was used, while for the second essay, a completely randomized design was used. An ANOVA analysis was done, as well as a means comparison (Tukey, 0.05) and a Principal Component Analysis (PCA). The ANOVA showed differences in the evaluated traits ($p \leq 0.05$). The MMEXV5 clone showed high storage roots yield (RY = 41.24 t ha^{-1}), plant height (PH = 4.79 m), and pulp (PUL = 80.58%); MMEXCH23 achieved higher contents of bagasse and peel; while, MMEXV40 had the highest total starch extraction (STL = 12.57%). Additionally, the three clones reached high dry matter content (DM = 34.22 to 38%), trait considered a quality factor. The PCA showed that RY was associated with a higher number of storage roots generated, PH, PUL, and lobe dimensions; but, the clones with high RY and PH developed poor DM and yield of starch extraction. Finally, the valorization of the evaluated germplasm could make cassava into the basic raw material in a great variety of products with high added value for the food and non-food industry, even obtain

bioproducts and bioenergy through the conversion of bagasse and peel.

Keywords: *Manihot esculenta* Crantz; native germplasm; storage roots; byproducts

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of 100 species of trees, shrubs, and weeds of the *Manihot* genus, distributed in tropical and subtropical zones [1]. In these areas, its starch rich storage roots [2–4] are considered the main source of calories and part of food sovereignty for more than 800 million people around the world [5].

Furthermore, cassava could contribute to rural industrial development [6,7], since its starch shows potential for the development of products with high added value in alimentary and non-alimentary industrial applications like pharmaceutical products, paper, fabrics, sweeteners, animal feed, biofuels [1,5], and even the manufacture of bioplastics [8–11]. Regarding the aforementioned, the preference for cassava starch is because it is an inexpensive and easy to extract raw material. Moreover, its properties are equal or superior to those of starch from maize, wheat, and rice; these factors make it a choice ingredient for industry, especially for small businesses with little capital [12]. Another of its advantages is that the plants can be grown under adverse climatic and soil conditions [13,14]. Its yield can be potentialized [15–17] in places with adequate rainfall conditions, good soil fertility levels, optimum crop management [18], and use of highly productive clones [19,20]. Regarding this last aspect, it is important to learn their agronomical, technological, and even culinary attributes, as these influence their selection and adoption by farmers [16,21,22]. In the evaluation of brazilian elite germplasm, clones less than 3 m high, height at the first branch >0.6 m, high productivity of fresh roots (>30 t ha^{-1}) and starch (24.17 to 30.94%) were found; these are considered important characteristics for the selection of outstanding materials [23]. Meanwhile, Peprah et al. [24], found a positive association between the fresh roots yield with the number of total roots, biomass, and harvest index in elite and local germplasm of Ghana; also, the dry matter content with starch yield. In this regard, the content of dry matter and starch are considered quality factors, which tend to affect the yield in the industrial manufacture of cassava [25,26].

Nevertheless, industries based on cassava generate a high amount of lignocellulosic byproducts [27–29] with incorrect disposal creates severe public health issues, while their disuse results in economic losses and depletion of natural resources [30]. For example, in the production of 1 t of cassava starch, 2.5 t of bagasse are produced [31], a lignocellulosic byproduct [32] with a high amount of carbohydrates (82.6%), essentially from residual starch [33,29]; in addition to 100 to 200 kg of peels per ton of processed cassava [34], and a high amount of wastewater [35]. Hence, the assessment of byproducts has raised a lot of interest [36], since thanks to advancements in industrial biotechnology, these agro-industrial byproducts have potential for economic use [37].

In Mexico, cassava has a yield of 12.58 t ha^{-1} [38], being mainly used as a food source. Moreover, it is underexploited, being grown in a traditional fashion and mostly in family orchards [39–42]. Also, given the great diversity of traditional clones in the country [43,44], with specific characteristics such as plant height, stem diameter, leaf dimensions, weight of roots per plant, field yield of fresh roots, and starch content [45], it is relevant to explore native germplasm to evidence its morphoagronomic and industrial performance. This work would help to protect, value, boost, and foster the incorporation of cassava into production systems.

2. Materials and method

2.1. Description of the study site

This research was carried out in the Cotaxtla Experimental Field of the National Institute of Forestry, Agricultural, and Livestock Research (NIFALR), Veracruz, Mexico (18°56'11.28" N; 96°11'49.53" W, altitude 14 masl). Its historical (1986–2018) climatic data are mean annual maximum, mean, and minimum temperatures of 31.15 °C, 25.11 °C, and 19.07 °C, respectively; rainfall of 1,453.91 mm [46]; and subhumid warm climate (Aw_2), according to the Köppen climate classification modified by García [47]. The soil representing the experimental area has sandy loam texture, having moderately acidic pH (5.8), moderately low organic matter content (12.0 g kg^{-1}), low N content (11.0 mg kg^{-1}), high P and K contents available (132.8 and 139.0 mg kg^{-1}, respectively), very high exchangeable Ca (1,800 mg kg^{-1}) with suitable Mg content (191.0 mg kg^{-1}), and small amounts of micronutrient [48].

2.2. Climatic conditions

The climatic conditions such as monthly mean temperature, maximum, minimum; and accumulated monthly rainfall of the cultivation cycle (300 days) were recorded through the El Tejar meteorological station, Medellin de Bravo, Veracruz (19°2'30.00" N; 96°8'26.99" W), located 15.45 km from the experimental site [46].

2.3. Plant material

Three clones were evaluated: MMEXV5, MMEXV40 and MMEXCH23, safeguarded in the Cassava Germplasm Bank of NIFALR, based on their high starch and dry matter content, according to previous characterization data [44,49] obtained through the gravimetric method [50]. According to the phenotype of the clones, they showed a mean of seven lobes per leaf and two main stems; two branching levels in MMEXV40 (open shaped and tetrachotomic) and MMEXCH23 (umbrella shaped and trichotomic), while MMEXV5 showed erect growth (cylindrical shaped).

2.4. Clone planting

To prepare the soil, weeds were removed by hand; it was ploughed once, crossed and rowed mechanically. Planting was done on February 13th, 2018; consisting of 20 cm long cuttings. Also, to ensure the phytosanity of the agronomic seed, the cuttings were submerged in a chemical treatment consisting of a Benomyl solution at a ratio of 2 g L^{-1} water ((Benomyl: Methyl-1-(butyl carbamoyl) benzimidazole-2-ylcarbamate)) and Cypermethrin with 4 mL L^{-1} water ((Cypermethrin: alpha-Cyano (3-phenoxybenzyl (+) Cis trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate)). Field-trials were established in a randomized complete block design with three replicates, each consisting of five rows of six plants, giving a plot size of 30 plants. Planting was done at a spacing of 1 × 1 m. Weeds were controlled manually every month during the crop growth cycle. The plants were monitored every 15 days to detect the presence of pests and diseases. An amino acid, Ca, B, and Zn based foliar fertilizer was applied at a dose of 2.2 mL L^{-1} water at 30, 60, and 150 days after planting (dap), while soil fertilization was applied with 20-20-20 (NPK) fertilizer at 75 dap.

2.5. Evaluated traits

Morphoagronomic and industrial traits were recorded at 300 dap, time when the roots are usually harvested [1,26,51].

2.5.1. Morphoagronomic traits (stage 1)

Three healthy plants were randomly selected from the central part of each experimental unit. For each plant, we registered: length and width of the central lobe of the leaf (LLL and LLW, cm), this located in the middle part of the plant; the diameter of the stem (STDIA, cm), at 2 cm from the soil surface; height, from the soil surface to the last leaf developed (PH, m), as well as the number of total roots (NTR) and commercial roots (NCR) were counted [50]. Lastly, fresh storage roots with no peduncle were washed, dried, and weighed (Torrey L-PCR 40 scale) to report root yield (RY, t ha^{-1}).

2.5.2. Agro-industrial traits (stage 2)

The fresh storage roots were immediately transported to the Laboratory of General Uses of the School of Biological and Agriculture-Livestock Sciences, from the Veracruz University. To register the yield of starch extraction and related byproducts (completely randomized design), we used the method proposed by López et al. [52] and Vargas et al. [53] with some modifications. First, the storage roots were submerged in a NaClO solution (250 ppm L^{-1}) for 10 minutes. To determine dry matter (DM), 100 g of thin slices were cut from the midlle section of two random storage roots per plant and dried at 60 ℃ for 72 hours in an oven (Ecoshell PCD 2000 serials), measuring until it reached a constant weight. The remaining storage roots were peeled and both the pulp (PUL) and peels (PE) were weighed. Subsequently, 1 kg of pulp was taken, cut into small pieces, and ground in a juice extractor (Haus, model: 74.20304) until a white viscous liquid was obtained, with a fibrous fraction or bagasse as byproduct. The liquid was filtered through cloth and 500 mL distilled water was added and shaken. Then, it was left to settle for three hours and finally, it was decanted to obtain a starch paste (this procedure was repeated three times). The fresh bagasse (FBAG) was weighed and then, to avoid starch loss, it was wrung to later be subjected to the previously described procedure. Both samples, having been decanted separately, were placed in an oven (Ecoshell PCD 2000 serials) at 50 ℃ for 24 h. Subsequently, we determined the starch extraction yield from the pulp (SPUL), the bagasse (SBAG), and total starch (STL = SPUL + SBAG). Finally, the fresh bagasse was dried at 50 ℃ for 72 h [54] to register its weight (DBAG). All the traits were weighed on a scale (Brainweigh B® 3000D). The values of traits DM, PUL, and PE were registered as a percentage of the fresh storage root weight; and the values of FBAG, DBAG, SPUL, SBAG, and STL were registered as percentages based on 1 kg of pulp.

2.6. Statistical analysis

The traits were subjected to verification of assumptions of normality and homogeneity through the Shapiro-Wills and Leven tests, respectively. An analysis of variance was done as well as a Tukey mean comparison test ($p \leq 0.05$). To learn the correspondence between morphoagronomic and agro-industrial traits and the clones, a PCA was done through the correlation matrix of the original traits. The eigenvalues, eigenvectors, and total accumulated variance were considered. Subsequently, the values were graphed in a two-dimensional plane using the R Studio v3.5.2 software [55].

3. Results and discussion

3.1. Climatic conditions

The climatic conditions were favorable for the development of the crop (Figure 1), since there was an average maximum, mean, and minimum temperature of 33.12, 26.68, and 20.24 °C, respectively; and a total accumulated rainfall of 1283.20 mm [1,56]. The location was characterized for a dry period, with a constant increase in temperature and decrease in rainfall from the time of planting until May, followed by a wet period from June to October. It is worth mentioning that cassava plants subjected to water stress decrease their physiological activity, consequently decreasing yield [57], especially when the drought period coincides with root tuberization and thickening [58], particularly between 60 and 90 dap [26,59,60]. Therefore, surface irrigation was performed 60 and 75 dap. Although cassava can withstand dry periods [17], these foster the appearance of economically important pests [61,62]. With regard to this, we detected the presence of thrips (*Frankliniella* spp.); this pest was controlled through the application of Abamectin (0.55 mL L^{-1} water ((Abamectin (avermectin)) and Cypermethrin.

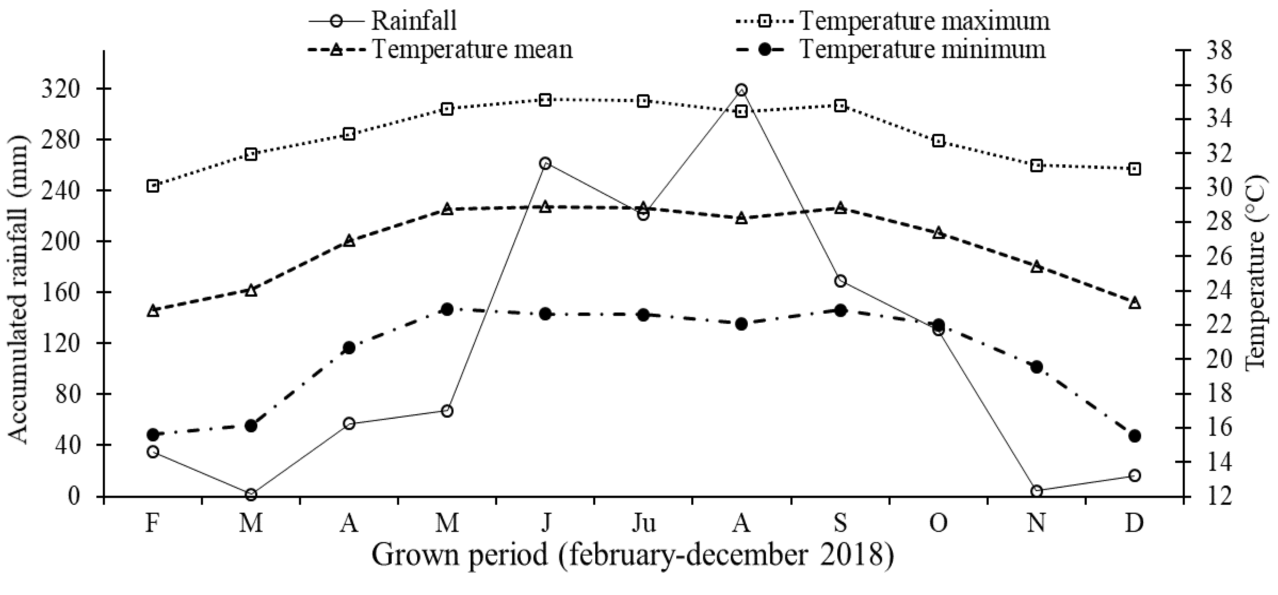


Figure 1. Climatic conditions during the crop cycle.

3.2. Morphoagronomic performance

Statistical assumptions were fulfilled. On the other hand, the ANOVA showed differences in the morphoagronomic traits STDIA, PH, and RY ($p \leq 0.01$) (Figure 2). STDIA values of 4.55 to 5.57 cm were recorded, with the largest dimension for the MMEXV40 clone (Figure 2a). These values are considered high, since stem diameters of between 2 and 6 cm have been reported [63]. The notable development of STDIA is attributed partly to the adecuate edaphoclimatic conditions present at the study site. In this regard, the optimal conditions for the development of cassava are between 20 and 30 ℃, while areas with <500 mm cause drought stress. Cassava plants prefer soils with medium and light texture, features that facilitate drainage. They tolerate acidic soils, high levels of interchangeable Al and low levels of P [56,64]. In addition, they respond well to the application of external nutrients, as low soil fertility is one of the limitations in the production of cassava [15,65–67]. In turn, low rainfall (281.51 to 872 mm) negatively affects growth [68,69], as a result of stomatal closure, which decreases the CO_2 concentration in leaves and consequently affects the photosynthesis process and translocation of photo-assimilates to growth organs [57]. In turn, the low availability of water decreases nutrient transportation and cell growth and multiplication [70].

In the case of PH, clone MMEXV5, with an erect branching habit, registered the highest value at 4.79 m, compared with the branching clones (Figure 2b). These plants of erect habit are preferred for their ease of agronomic management, seed production, ease of harvest, and the possibility to be associated with other crops [26,71]. One advantage to the use of erect branching clones is to increase planting density, decreasing the planting framework from 90 × 90 to 75 × 75 cm [16,72]; however, an efficient study of this agronomic practice is necessary, since an increase in density could decrease the number of storage roots due to competition between plants [73,74]. To this regard, a decrease of 67 and 73% in the total storage roots production was caused when the population density increased from 5,000 to 20,000 plants per ha^{-1} [51]. Furthermore, environmental conditions and crop management affect this feature. Tall plants have been observed in zones with a mean temperature of 27 ℃ [75] and optimum water management to supply water demand [76,77], as well as the use of fertilizers [18,78]. On the other hand, shorter plants, like MMEXV40 and MMEXCH23 (<3.00 m), are useful in regions with strong winds [73].

Fresh storage roots yield (Figure 2c) is considered the most important trait for selection, empowerment, and conservation of traditional clones by farmers [16,19,79]. In this regard, the MMEXV5 clone reached the greatest value with 41.24 t ha^{-1}, while the MMEXCH23 clone achieved lower production (15.85 t ha^{-1}). The MMEXV5 clone performance was similar to the Tambou clone with 46.23 t ha^{-1} under the same period of growth and timely weed management [80], even higher than the yield of 25 cassava clones (1.79 to 31.79 t ha^{-1}) grown on the same soil texture, fertilized with low and high N levels (0 and 125 kg N ha^{-1}, respectively) in two growth cycles [81]; likewise, to the yield of 8 to 24.8 t ha^{-1}, reached by 12 genotypes (native, improved, and new genotypes originated by line crosses) subjected to a density of 10,000 plants per ha^{-1}, and harvested at 270 dap [82]. Therefore, there is highly productive germplasm in Mexico, since the national average is 12.58 t ha^{-1} [38].

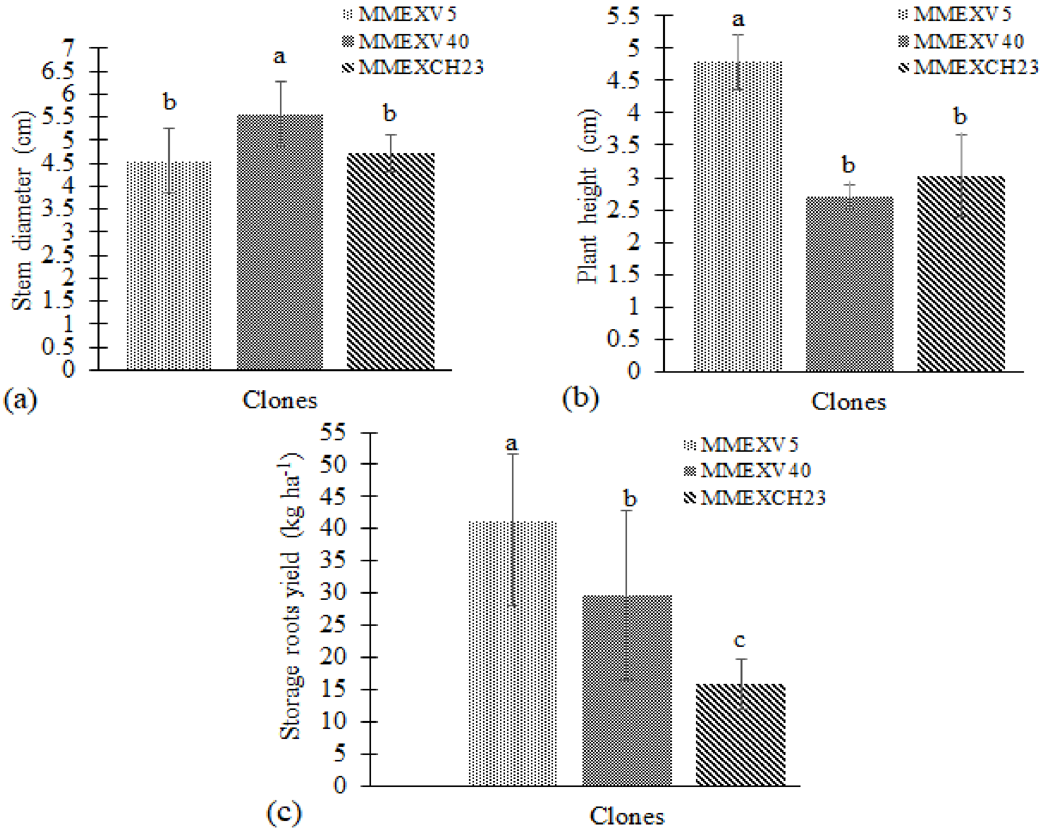


Figure 2. Stem diameter (a), plant height (b), and storage roots yield (c). Data ± standard deviation.

3.3. Agro-industrial performance

The analysis of variance showed differences ($p \leq 0.05$) in the agro-industrial traits: PE, PUL, FBAG, DBAG, SPUL, SBAG, and STL (Table 1). In cassava crops, pulp is considered the most economically relevant issue. In this research work, high PUL percentages were found in the MMEXV5 and MMEXV40 clones, with 80.58 and 80.12%, respectively, which is considered a characteristic value for the species [63,83].

Notwithstanding both clones having equal percentages of pulp in their storage roots, the MMEXV40 clone stood out for its higher yield in pulp extraction starch (10.59%) and total starch (12.57%), compared against MMEXV5, which had a lower amount, even than MMEXCH23. Thus, the results show that the amount of pulp is not related with starch yield. Similar STL amounts were reached by the improved Mexican cassava varieties Esmeralda and Sabanera, with 10 and 12%, respectively [53], also by varieties from Trinidad and Tobago, Palo Rojo (11.8%), although lower than M Col22 (16.1%) and Maracas (20.3%) [12]. Furthermore, starch yields ranging from 8.4 to 34% have been reported for native and improved clones with high carotenoid content [3,4], which indicates great variation with respect to the yield of starch extraction. This response could be due to the genotype, harvest age, environmental conditions during growing [1,84] or harvest, plant density, growing season, soil type [3], even to the extraction conditions [85].

The greatest presence of the byproduct bagasse in the MMEXCH23 clone (64.39% fresh and 21.96% dry) was related with the high extraction of SBAG (2.79%), this suggests that a high number of starch granules (15.75 to 22.64%) could be caught among the bagasse fiber net [86]. Thus, good byproduct management should be considered during the industrial extraction process in order to minimize losses. Various authors believe that the presence of bagasse depends on factor such as age and the clone [12], even at ratios greater than 30% of dry bagasse [54].

On the other hand, although no significant differences were found for DM, it is reported that the MMEXCH23 clone reached 38% (6.02 t DM ha^{-1}), followed by MMEXV40 (35.91% = 10.66) and MMEXV5 (34.22% = 14.11). Values of 4 to 45% of DM have been reported in the literature [87–89], while the values found can be considered high. In this regard, clones with a high dry matter content, a trait closely related to the starch content [24,26,90], are preferred by farmers, breeders, and processors; however, the low fresh storage roots yield of MMEXCH23 may limit its potential [82,89].

Table 1. Means comparison for agro-industrial traits of starch extraction in MMEXV5, MMEXV40, and MMEXCH23 cassava clones.

Source of variation	Clones		
	MMEXV5	MMEXV40	MMEXCH23
Pulp (%)	80.58 ± 1.96a†	80.12 ± 0.99a	75.47 ± 3.31b
Peel (%)	19.41 ± 1.96b	19.88 ± 0.99b	24.52 ± 3.29a
Pulp starch (%)	8.96 ± 1.14b	10.59 ± 1.04a	9.53 ± 0.94ab
Bagasse starch (%)	1.91 ± 0.69b	1.98 ± 0.49b	2.79 ± 0.78a
Total starch (%)	10.87 ± 1.40b	12.57 ± 1.27a	12.32 ± 1.02ab
Fresh bagasse yield (%)	60.18 ± 2.32b	57.36 ± 2.51b	64.39 ± 3.00a
Dry bagasse yield (%)	20.19 ± 2.00ab	19.06 ± 1.66b	21.96 ± 2.62a

Note: † Means with the same letter in a row are not significantly different (Tukey, 0.05).

3.4. Principal Component Analysis

The general purpose of the PCA is to summarize the information from a series of response traits into a lower number of dimensions (Principal Components) whose graphs allow us to do an exploratory analysis [91–93]. The PCA evidences that with that, the general performance of the traits is explained with a mere two components (Table 2), where PC1 is related with components of storage roots yield and the generation of byproducts, while PC2 explains the yield of starch extraction. The PCA graph (Figure 3) shows the association between morphoagronomic and agro-industrial traits, as well as the differences between the cassava clones.

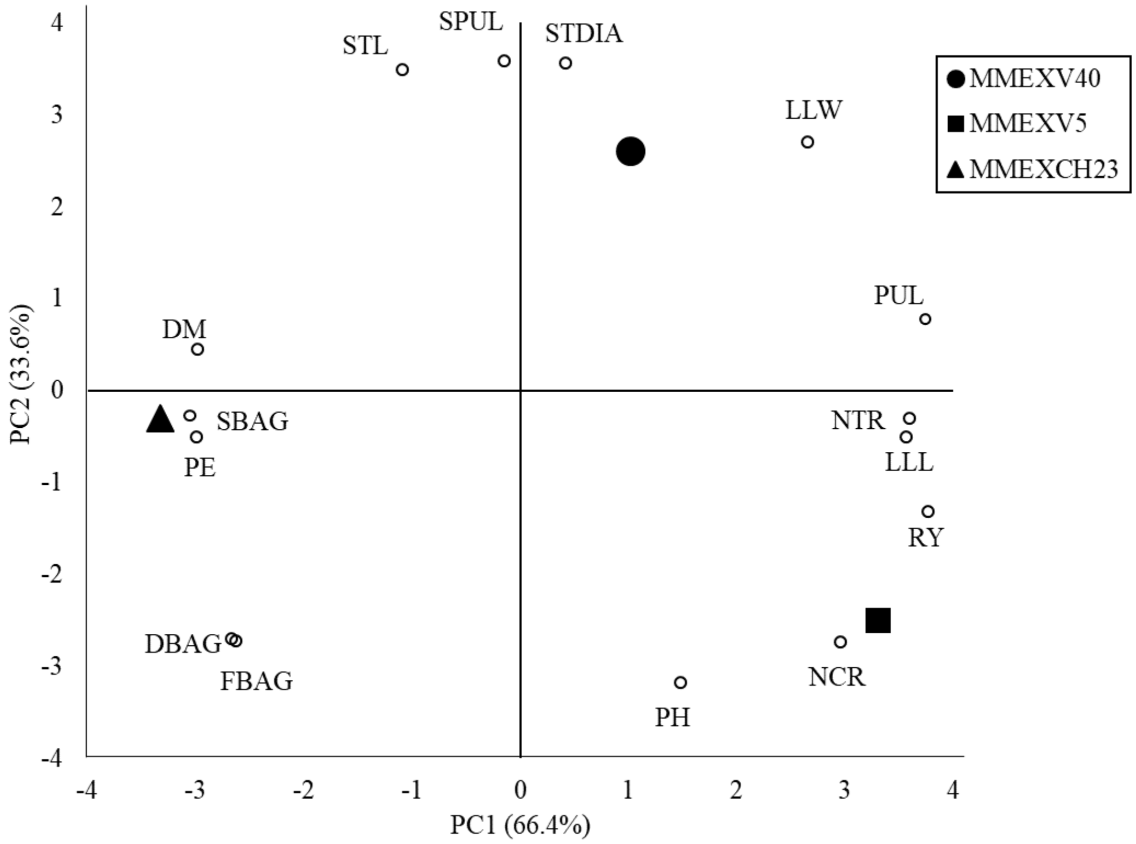
The particular traits of the MMEXV5 clone were related with high productivity in the field. A high fresh storage roots yield was correlated with a greater number of storage roots, plant height, leaf lobe dimensions, and pulp, similar to the reports by Agre et al. [21], Nadjiam et al. [71], Silva et al. [73], and Temegne et al. [94]. It is important to mention that there was a negative association between NTR and DM; PH with SPUL and STL; as well as RY with STL. Hence, the clones with a higher number of fresh roots and height, developed poor dry matter and yield of starch extraction. Because of this, MMEXV5 can be considered a multipurpose clone given its high productivity of storage roots, since besides its capacity to be grown for human consumption [95] or cattle feed [96,97], which requires a removal process to decrease or eliminate the presence of cyanogenic glucosides, precursors of cyanhydric acid [63,98], its leaves can also be harvested and used as a vegetable or forage (fresh or dehydrated) given their high amount of proteins, vitamins (B1, B2, C, and carotenoids), and minerals (K, P, Mg, and Ca) [7,22], given that their collection does not significantly affect storage roots yield [18].

The MMEXCH23 clone was characterized for its high percentage of solid byproducts (bagasse and peels) and total starch, factors which cause a better proportion of dry matter in the storage root [99]. On the other hand, although bagasse and peels are low-value resources, they can be used as organic fertilizer [100], animal feed [101,102], or cultivation medium for fungi [103,104]. Moreover, they have the potential to be adapted adapt to the biorefinery model, useful to obtain bioenergetic benefits and bioproducts [105], such as the conversion to biofuels of second generation: biobutanol, biodiesel, bioethanol, bio-oil, charcoal [28,106–109], biogas [31,110]; or bioproducts: cyclodextrins [111], organic acids, lactic acid, α-amylase, biodegradable packaging [112], and even fibers from cellulose [113], nanofibers [32,114], and nanocellulose [115] with potential to be used as reinforcement and improvement of the properties of biodegradable films. These alternatives of use can decrease the volume of bagasse and peels, on the whole, offer added value [116,117].

On the other hand, the traits of MMEXV40 characterize it for its high yield of starch extraction (SPUL and STL), which in turn was associated with STDIA; this morphoagronomic trait can be used as an indicator for the indirect selection of clones with high starch yield. In this regard, the identification of clones with high starch extraction is important for their processing [20], as this helps to decrease production costs, plan the extraction, and provide greater industrial yield [20,25]. Among the potential uses for the MMEXV40 clone stands out thickener for broths, baby food, sauces, cold meats, and processed meats, as well as making cookies, dextrose and glucose syrup as sweeteners in confectionery, monosodium glutamate as a flavor enhancer, adhesives, pills and tablets [5], bioethanol production [118–120], and even replace maize as an energy source in animal diets [101,121].

Table 2. Contribution of the traits for the formation of the principal components.

Feature	PC1	PC2
Plant height	0.55	−0.84
Leaf lobe length	0.99	−0.13
Leaf lobe width	0.83	0.56
Stem diameter	0.18	0.98
Total number of storage roots	1.00	−0.06
Number of commercial storage roots	0.77	−0.63
Storage root yield	0.97	−0.25
Peel	−0.99	−0.13
Pulp	0.99	0.13
Pulp starch	−0.05	1.00
Bagasse starch	−1.00	−0.06
Total starch	−0.41	0.91
Dry matter	−0.99	0.12
Fresh bagasse yield	−0.81	−0.59
Dry bagasse yield	−0.82	−0.58
Eigenvalues	9.97	5.03
Explained variance (%)	0.66	0.34
Accumulated variance (%)	0.66	1.00



Note: LLL = leaf lobe length; LLW = leaf lobe width; STDIA = stem diameter; PH = plant height; NTR = number of storage roots; NCR = number of commercial storage roots; RY = yield; DM = dry matter; PUL = pulp; PE = peel; SPUL = pulp starch; SBAG = bagasse starch; STL = total starch; FBAG = fresh bagasse yield; DBAG = dry bagasse yield.

Figure 3. Association between morphoagronomic and industrial traits in starch extraction through principal component analysis.

4. Conclusion

This study successfully determined several morphoagronomic and industrial traits that may be of value in production of byproducts to multiples purposes. The results suggested that the clones MMEXV5, MMEXV40 and MMEXCH23 were highly productive (15.85 to 41.24 t ha^{-1}), with yields higher than the national average (12.58 t ha^{-1}). During the process starch extraction, a greater presence of bagasse in MMEXCH23 was related to a high extraction of bagasse starch (2.79%), which suggested that a high amount of starch granules (15.75 to 22.64%) can be trapped between the fibrous network of bagasse. Based on the PCA, it was found that high productivity of the cassava clones in the field, presented low dry matter and starch extraction; these morphoagronomic traits, must be taken into account in cassava improvement programs. Likewise, this study reveals that the local cassava clones have not been fully exploited in tropics regions for its improvement and further uses.

Finally, the valorization of the evaluated germplasm could make cassava into the basic raw material in a great variety of products with high added value for the food and non-food industry, even obtain bioproducts and bioenergy through the conversion of bagasse and peel.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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