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Phenotypic diversity of starch granules in cassava germplasm

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ABSTRACT. Demand for the development of cassava varieties with different native starches has guided the search for these characteristics in the germplasm of *Manihot esculenta* Crantz. Therefore, the objective of this study was to estimate the genetic diversity of cassava accessions for root and starch granule characteristics to guide the future industrial application of this species. Starches from 56 accessions were evaluated for the number of granules in 1 g of starch (NTG), area (AG, mm^2), length (LG, mm), width (WG, mm), starch granule roundness (Round), dry matter content in the roots (DMC, %), pulp color (PulCo), and cyanogenic compounds (HCN). Images captured by light microscopy were used to determine the average phenotypic values, and these were further analyzed by principal component analysis (PCA) considering mixed data (quantitative and qualitative). Significant differences between the cassava accessions for all traits measured revealed wide variability in starch granule characteristics. Four diversity groups with better fitness for the classification of cassava accessions based on PulCo were identified, in comparison with HCN.

Accessions with differential starch characteristics were identified, and crossings for the generation of segregating populations in order to obtain table and industry varieties have been proposed.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important crops of the 21st century because of its high adaptability to adverse climate and soil conditions, and its low requirement for agricultural input during production in comparison with other crops. It is used widely for human and animal subsistence and for industrial applications in food, energy, and textiles (Howeler et al., 2013).

Cassava roots are comprised of water (70%), starch (24%), fiber (2%), protein (1%), and other substances, including minerals (3%) (Tonukari, 2004). Therefore, the most notable characteristic of cassava is its capacity to accumulate starch in its roots, the content of which varies from 70 to 90% dry matter content (Baguma, 2004). Furthermore, there is high diversity in the content, composition, physical, and chemical properties of cassava starch (Nuwamanya et al., 2010; Oliveira et al., 2015a; Sanoussi et al., 2015), resulting in its application in different industrial activities.

Starch is synthesized in amyloplasts of plant cells, and is formed by two types of polymers: amylopectin and amylose. Amylose is present as a predominantly linear chain composed of glucose residues linked by α-1,4 bonds. In contrast, amylopectin is a branched long chain molecule, consisting of hundreds of glucose chains, α-1,4 linked by α-1,6 linkages (Hoover, 2001). Most of the known cassava varieties present starch with approximately 20-30% amylose and 70-80% amylopectin content (Taggart and Mitchell, 2009). The organization of glucose molecules in starch, including the length of the glucose chain, and the relative amylose/amylopectin ratio influence the morphology and size of the starch granules (Copeland et al., 2009).

In general, cassava starch granules are rounded, oval, or truncated in form (circular with a flat surface on one face) and range in size from 5 to 40 µm (Ceballos et al., 2007). According to Lindeboom et al. (2004), starch granules can be grouped into four size classes: large (greater than 25 µm), average (10 to 25 µm), small (5 to 10 µm), and very small (less than 5 mm).

Compared with cereals, cassava starch has particular properties that confer enhanced resistance to acid treatments, permitting its use in the composition of unique pastes, which makes it suitable for the production of paper, textiles, sweeteners, alcohol, and monosodium glutamate (Taylor et al., 2012). The complexity of starch biosynthesis results in large natural variability in relation to the amylose/amylopectin ratio, reflecting the diversity in granule morphology (size and shape), which is associated with different functional properties in the food industry (Wani et al., 2012). Moreover, many cassava varieties are used for starch extraction, which allows a variety of starches to be obtained, and particle distribution can vary in size, morphology, and physicochemical properties. The distribution and granule size may result in distinct starch agglomerations, influence its behavior under certain processing conditions, and consequently affect the quality of the final product (Molenda et al., 2006).

In addition to the amylose/amylopectin ratio, molecular weight, and starch granule structure, some reports have shown that granule size can affect the composition, gelatinization paste properties, enzymatic susceptibility, crystallinity, swelling, and starch solubility (Wani et al.,

2012). All of these characteristics appear to be affected by environmental and agronomic crop conditions as well as by genetic factors, resulting in substantial changes in the functional properties of the starch (Lawal et al., 2011). Although cassava starch has been extensively studied in relation to its paste properties, and for its chemical and physical composition, few studies have investigated the genetic diversity of the distribution, shape, and size of cassava starch granules. Knowledge of the genetic diversity present in germplasm banks is essential when utilizing phenotypic variations in the development of new varieties that can bring functional properties to different industrial applications (Upadhyaya et al., 2007). The aim of this study was to characterize and evaluate the genetic diversity of cassava accessions for starch granule traits, which may contribute to future industrial application and, consequently, to the greater use of genetic resources of *M. esculenta*.

MATERIAL AND METHODS

Plant material

Fifty-six accessions of the Cassava Active Germplasm Bank at Embrapa Cassava & Fruits (Cruz das Almas, Bahia, Brazil) were collected during 11 months after planting (Table 1). The region has a hot and humid tropical climate, Aw to Am according to the Köppen classification, with average annual temperatures of 24.5°C, 80% relative humidity, and 1200 mm annual rainfall. The soil is classified as yellow Latosol dystrophic. The cassava accessions were selected based on their contrasting starch content, cyanogen compound content, and pulp color of the roots.

Starch extraction

Roots were harvested 11 months after planting. The selected roots were washed in water, following which 1 kg of pulp was sectioned to complete the extraction process. The selected pieces were ground in a blender using a non-cutting helix (to reduce the shearing of the starch grains and the consequent physical modification) for 90 s in a 1:1 root to cold water ratio. This process was repeated several times following 1-min pauses.

The crushed material was filtered in voile-type fabric, and through a sieve, to analyze particle size (220 mesh) in a plastic bucket (5 L). Next, the tritured mass was washed with 3.5 L cold water. The filtrate was placed in a cold chamber at 5°C for 12 h to enable the starch to settle. The supernatant was discarded and the decanted starch was washed with 20 mL 95% alcohol to accelerate drying. The alcohol was discarded, and the starch was stored in an oven with forced air circulation at 45°C until completely dry. The dried starch was then macerated using a mortar and pestle until a finely textured powder was obtained, which was then packed in sealed plastic vacuum bags for further analysis.

Morphological analysis of the starch granules

To visualize the starch granules, a solution of 1 g starch and 4 g water was used to simulate the concentration of starch present in the cassava roots. Subsequently, 0.8 mL of this solution was mixed with 1.8 mL 2% iodine solution (2 g potassium iodide [KI], 0.2 g iodide [I2], and 100 mL distilled water). A 0.2-mL volume of this solution was transferred to a Neubauer chamber for observation at 400X magnification under a LEICA optical microscope (DM500, Germany). Digital images were captured using LAS EZ software, which recorded

the edge quarters of the chamber and the center. Next, the images were processed and analyzed using ImageJ software (Schneider et al., 2012) to determine the following traits: number of granules in 1 g starch (NTG), granule area (GrAr, mm^2), length of granules (GrLe, µm), width of granules (GrWi, µm), granule roundness (GrRo), dry matter content in the roots (DMC, in %, measured by hydrostatic balance, as described by Kawano et al., 1978), pulp color (PuCo as described by Fukuda et al., 2010), and cyanogen compound classification (HCN, measured qualitatively by the picric acid method as described by Onwuka, 2005).

Table 1. Cassava accessions used for starch characterization.

Genotype	Origin	Obtaining form	Pulp color	Cyanide coumpound classification
98150-06	Cruz das Almas (BA)	Breeding	White	Sweet
BGM0250	Ilha dos Frades (BA)	Landrace	Cream	Sweet
BGM0394	Magé (RJ)	Landrace	White	Bitter
BGM0507	- (MT)	Landrace	Yellow	Sweet
BGM0540	União dos Palmares (AL)	Landrace	White	Bitter
BGM0620	Pentecoste (CE)	Landrace	White	Sweet
BGM0670	São Joao Da Barra (RJ)	Landrace	White	Bitter
BGM0889	Tocantinópolis (TO)	Landrace	Cream	Intermediate
BGM0982	- (AM)	Landrace	White	Bitter
BGM0989	Manaus (AM)	Landrace	White	Bitter
BGM1078	Sape (PB)	Landrace	Yellow	Bitter
BGM1081	Goiana (PE)	Landrace	White	Bitter
BGM1110	Araranguá (SC)	Landrace	White	Sweet
BGM1127	Conceição do Coite (BA)	Landrace	Cream	Intermediate
BGM1171	Bragança (PA)	Landrace	White	Bitter
BGM1174	-	Landrace	White	Bitter
BGM1178	-	Landrace	Yellow	Intermediate
BGM1180	-	Landrace	White	Sweet
BGM1202	Cruz das Almas (BA)	Landrace	White	Bitter
BGM1313	São Jose do Belmonte (PE)	Landrace	White	Sweet
BGM1327	Monteiro (PB)	Landrace	White	Bitter
BGM1370	Juazeirinho (PB)	Landrace	White	Sweet
BGM1376	São Vicente do Seridó (PB)	Landrace	White	Sweet
BGM1412	Cruzeta (RN)	Landrace	White	Intermediate
BGM1429	Caruaru (PE)	Landrace	White	Sweet
BGM1437	Altinho (PE)	Landrace	White	Bitter
BGM1458	Macapá (AP)	Landrace	White	Intermediate
BGM1495	Itiúba (BA)	Landrace	White	Intermediate
BGM1537	Paratinga (BA)	Landrace	White	Sweet
BGM1576	São João do Piauí (PI)	Landrace	White	Intermediate
BGM1590	Nazaré do Piauí (PI)	Landrace	White	Sweet
BGM1615	BR 407 (PI)	Landrace	White	Bitter
BGM1640	BR 135 (BA)	Landrace	White	Bitter
BGM1667	BR 316 (PA)	Landrace	White	Sweet
BGM1690	Araripina (PE)	Landrace	Cream	Sweet
BGM1698	BR 163 (MS)	Landrace	White	Sweet
BGM1704	BR 230 (AM)	Landrace	Yellow	Bitter
BGM1706	BR 230 (AM)	Landrace	Cream	Sweet
BGM1715	BR 177 (AM)	Landrace	White	Sweet
BGM1722	Santo Antônio Jesus (BA)	Landrace	Cream	Sweet
BGM1728	Cruz das Almas (BA)	Landrace	White	Sweet
BGM1772	Chapadinha (MA)	Landrace	White	Sweet
BGM1850	Belém (PA)	Landrace	White	Sweet
BGM1942	Faro (PA)	Landrace	White	Bitter
BGM1957	Juruti (AM)	Landrace	Cream	Bitter
BGM2028	Araripina (PE)	Landrace	White	Bitter
BGM2041	Cruz das Almas (BA)	Breeding	Cream	Sweet
BGM2052	Pelotas (RS)	Landrace	White	Intermediate
BRS Dourada	São Felipe (BA)	Breeding	Cream	Sweet
BRS Formosa	Não informado (BA)	Breeding	White	Sweet
BRS Jari	Cruz das Almas (BA)	Breeding	Yellow	Intermediate
BRS Kiriris	Cruz das Almas (BA)	Breeding	White	Sweet
BRS Poti Branca	Cruz das Almas (BA)	Breeding	White	Intermediate
Corrente	Laje (BA)	Landrace	White	Bitter
Fécula Branca	Marechal Cândido Rondon (PR)	Landrace	White	Sweet
IAC90	Marechal Cândido Rondon (PR)	Breeding	White	Sweet

Data analysis

A completely randomized design with three replications per accession (each repetition consists of three blades) was used, resulting in a total of 15 images per accession. Initially, the data were submitted to analysis of variance to investigate genetic variability among accessions. Because traits are measured using different units, resulting in different ranges and variances, the phenotypic means of quantitative traits NTG, GrAr, GrLe, GrWi, GrRo, and DMC were standardized, using the *scale* function $Z = \frac{X - \bar{X}}{S}$ (R Core Team, 2015), in which Z is the standardized value of X, is the mean value of the traits, and S is the standard deviation of the trait.

A principal component analysis (PCA), considering quantitative and qualitative data (pulp color of the root and classification of the cyanogenic compound content) was performed jointly using the *PCAmixdata* package (Chavent et al., 2014) implemented in the R program (R Core Team, 2015). In this case, the main algorithms were *PCAmix* (analysis of a mixture of numerical and categorical variables), *PCArot* (PCAmix rotation), and *MFAmix* (multiple factor analysis with mixed data within the data set).

The first four PCA axes were used to define the number of clusters by Euclidean distance, using the *NbClust* in R package (Charrad et al., 2014). Graphical analysis, and Hubert and D index statistics were used to determine the number of clusters based on the gain in the intra-cluster inertia group.

RESULTS AND DISCUSSION

Analysis of quantitative traits

The *F*-test was significant (P < 0.001), confirming there were significant differences between the means of all quantitative traits related to starch granules in cassava accessions (Table 2). Therefore, cassava accessions were clustered to identify different groups in order to maximize genetic gain when using this germplasm to develop new varieties. The experimental coefficient of variation (CV), which indicates the magnitude of experimental precision, was low (<10%) for all traits except NTG. The CV reveals the optimal experimental accuracy needed to make inferences on important phenotypic differences between different cassava accessions.

Table 2. Summary of the analysis of variance. Average values, and ranges, of starch content and granule traits evaluated in cassava germplasm.

Trait[1]	MS[2]	P value	CV[3]	Minimum	Maximum	Average
GrAr	14.07	<0.001	7.91	81.00	178.58	113.37
GrWi	11.11	<0.001	4.55	9.24	14.03	10.96
GrLe	11.10	<0.001	4.57	9.25	14.01	10.99
GrRo	10.75	<0.001	0.81	0.85	0.91	0.88
NTG	15.42	<0.001	12.1	7.10	45.69	27.13
DMC	10.80	<0.001	4.85	28.01	39.70	33.94

[1]GrAr: granule area; GrWi: width of the granules; GrLe: length of the granule; GrRo: granule roundness; NTG: number of granules in 1 g of starch; DMC: dry matter content in the roots. [2]MS: mean square of the treatment; [3]CV: coefficient of variation.

In other species, variation in starch granule size and shape has also been associated with germplasm, climatic conditions, and agricultural practices used by farmers (Singh et

al., 2012; Wani et al., 2012). In cassava, Kanagarasu et al. (2014) also reported significant morphological variability in the root quality of 52 landraces in India.

Positive and high-magnitude correlations were observed between the traits GrAr x GrWi (0.98), GrAr x GrLe (0.98), and GrWi x GrLe (0.99) (Figure 1). This indicates that the evaluation of only one of these traits may reflect variation in the others of the same direction and intensity. Moreover, a negative correlation of moderate magnitude was observed between PuCo x DMC (-0.43), indicating that yellowish accessions (related to total carotenoid content in cassava roots) tend to have lower dry matter content in their roots. This negative correlation is extremely undesirable in breeding programs, since cassava varieties with higher carotenoid content should also have greater DMC, considering the preference of farmers and industries.
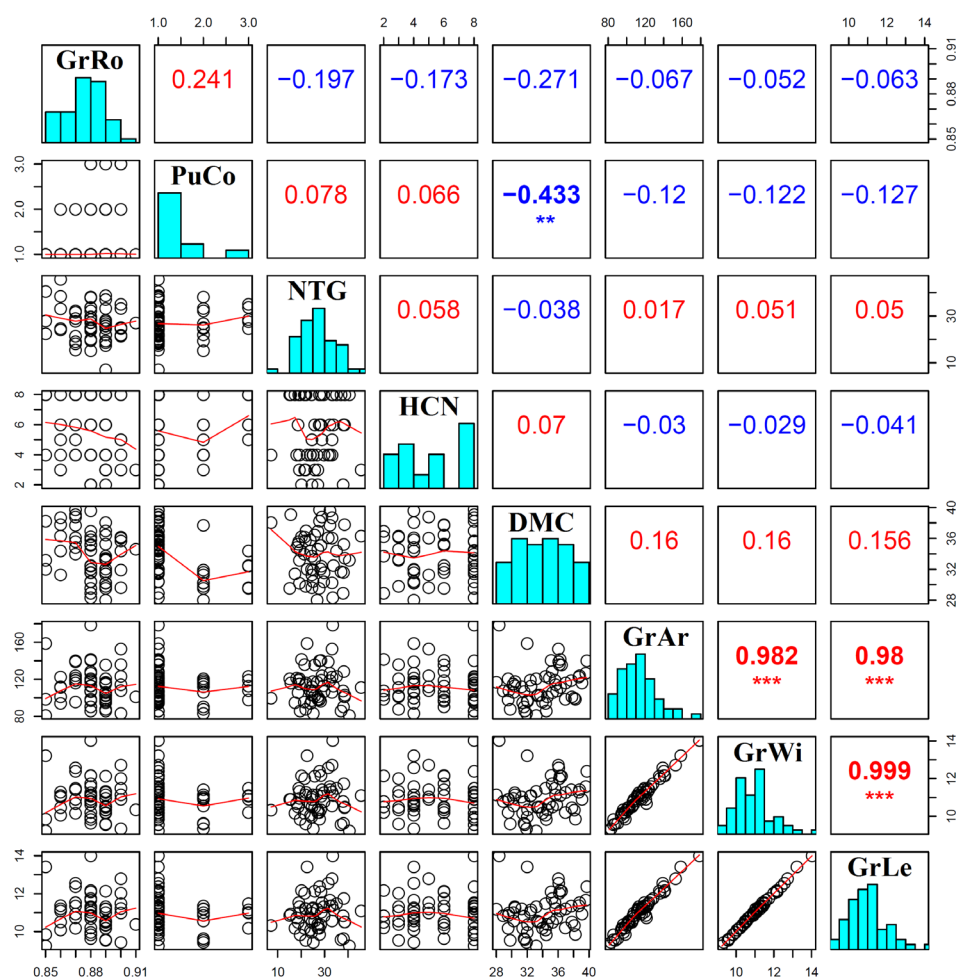

Figure 1. Pearson correlation between traits related to starch granule and root characteristics, based on an analysis of 56 cassava accessions. GrAr: granule area; GrWi: width of the granules; GrLe: length of the granule; GrRo: granule roundness; NTG: number of granules in 1 g of starch; DMC: dry matter content in the roots; HCN: cyanogen compound classification; PuCo: pulp color of the roots. Red text: positive correlations; Blue text: negative correlations; *** and **significant at $P < 0.001$ and $P < 0.01$, respectively.

Previous reports on African cassava breeding programs noted a negative correlation between PuCo x DMC, which ranged from -0.22 to -0.29 (Esuma et al., 2012; Njoku et al., 2015). Njoku et al. (2015) indicated the need to identify new sources of African or Latin germplasm with yellow colored roots in an attempt to generate segregating populations to break the possible genetic linkage between these traits.

Microscopy revealed homogeneity in the shapes of the starch granules from the different cassava genotypes, which were predominantly circular and truncated (Figure 2). Although oval shapes have been reported in cassava (Mweta et al., 2008), this shape of granule was not observed in the present study. The morphology of starch granules may vary among species, but in general, these shapes are oval, ellipsoid, spherical, angular, and lenticular (Hoover, 2001; Singh et al., 2003). Cereals such as wheat, barley, and rye have a lenticular shape and large size, or a spherical shape and small size (Vamadevan and Bertoft, 2015).
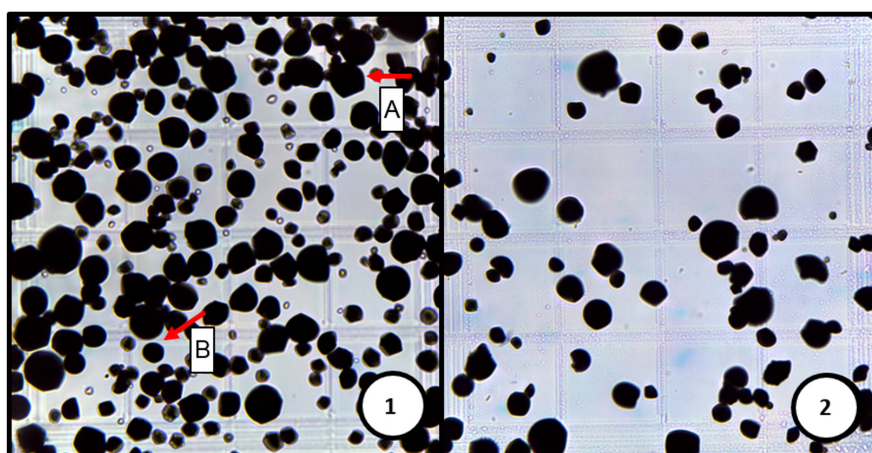
Figure 2. Microscopy of cassava starch granules at 400X magnification. (1) Accession BGM1537; (2) accession BGM1772. Arrows indicate the truncated (A) and circular (B) shapes.

Although the morphology of starch granules was similar between the evaluated cassava genotypes, significant differences in the number of granules and in granule size were identified (Table 2). The length and width of the starch granules exhibited similar ranges (9.24 to 14.03 µm), averaging 10.96 and 10.99 µm for width and length, respectively. This resulted in starch granule roundness that was close to 1 (range from 0.85 to 0.91; average 0.88). In addition, the length and width of the starch granules were reflected in granule area, which varied from 81.00 to 178.58 mm^2, averaging 113.37 mm^2. Granule size may vary with the developmental stage of the plant and the process of root tuberization from the central cambium (Leonel, 2007). However, the values obtained in the present study are consistent with those observed by other authors, with variation ranging from 10.0 to 17.0 µm (Mweta et al., 2008; Wickramasinghe et al., 2009; Nuwamanya et al., 2010; Rolland-Sabaté et al., 2012; Mtunguja et al., 2016). In contrast, Rolland-Sabaté et al. (2012) showed that cassava genotypes with waxy starch tend to present large starch granules (between 20 and 40 µm in size). Interestingly, various physicochemical properties, such as the amylose content by percentage of light transmittance, swelling power, and water binding capacity, are significantly correlated with the average size of starch granules in different plant species (Zhou et al., 1998).

The number of granules in 1 g of starch also varied widely (from 7.10 to 45.69 x 10^6) with an average of 27.13 x 10^6. Importantly, some starch granules were damaged during the extraction process. However, these granules were excluded from the analysis to avoid bias.

The dry matter content in the roots was also quite variable between accessions (28.01 to 39.70%), averaging 33.94% (Table 2). Other reports in the literature have shown that the dry matter content varies (16-53%) depending on the genotypes and environmental conditions of cultivation (Kawuki et al., 2011; Esuma et al., 2012; Oliveira et al., 2015a).

Cluster analysis

PCA was used to cluster and reduce redundancy between the starch content and granule traits. The first four principal components (PC) accounted for most of the observed variability (57.71%) in cassava germplasm for starch granule traits (Table 3). PC1 accounted for 24.2% of the phenotypic variation in the cassava germplasm collection and the quantitative traits GrAr, GrLe, and GrWi were the most important for explaining the variation in this PC. PC2 represented 12.87% of the total variation, and the quantitative traits GrRo and DMC, and qualitative trait (pulp color of the roots) were the most important for determining this variation. In contrast, PC3 explained 11.12% of the variation, primarily due to variation in the quantitative trait (NTG) and qualitative trait (HCN). PC4 accounted for 9.52% of the variation, and the most important variables were GrRo and HCN. The other PC presented low discriminatory power of cassava accessions and was therefore not considered in subsequent analyses.

Table 3. Absolute contributions of quantitative and qualitative traits (sum of the absolute contributions of qualitative variables), with eigenvalues and contribution to the total variation for the first four main principal components (PCs) from the principal component analysis with mixed data.

Traits	PC1	PC2	PC3	PC4
Cyanogen compounds classification	0.05	0.25	0.61	0.57
Pulp color	0.11	0.56	0.36	0.22
Granule area	0.97	0.15	-0.07	-0.01
Width of the granules	0.98	0.15	-0.04	0.01
Length of the granules	0.98	0.15	-0.05	-0.01
Granule roundness	-0.15	0.57	-0.12	0.61
Number of granules in 1 g of starch	0.07	-0.02	0.66	-0.24
Dry matter content	0.31	-0.68	0.05	0.11
Eigenvalues	3.15	1.67	1.45	1.24
Variation explained (%)	24.20	12.87	11.12	9.52

Macromolecular characteristics, such as crystallinity, grain size, and thermal properties of waxy and non-waxy cassava clones, as well as waxy starch from potato and corn, were used to classify the accessions with specific properties based on the PCA (Rolland-Sabaté et al., 2012). Rolland-Sabaté et al. (2012) observed that (a) the two PCs accounted for 55 and 27% of the total variance of the data, respectively; (b) the amylose content, the wavelength at maximum absorption of the iodine complexes with starch polymers (l_{max}), and the apparent particle density (dGappw) were grouped closely together; (c) there was a strong correlation between amylose content and l_{max}; and (d) amylose content and dGappw represent independent traits associated with PC1. Therefore, molecular characteristics associated with starch granules tend to reveal an important organization in global starch diversity.

Of the 23 criteria used for clustering in the *NbClust* package (Charrad et al., 2014), eight (35%) were noted for the formation of four clusters, based on the analysis of the four main components. Therefore, clustering of cassava accessions based on the root and starch granule traits was based on the criteria of Hartigan, TraceW, Rubin, Cindex, Silhouette, Ratkowsky, PtBiserial, and SDindex.

Cluster 1 consisted of 11 accessions: one improved clone (98150-06) and 10 landraces (BGM1171, BGM1942, BGM1412, BGM1495, BGM1576, BGM1429, BGM1376, BGM1772, BGM1590, and BGM1715). This cluster presented the highest average values for GrAr, GrWi, GrLe, and DMC (Figure 3). Cluster 2 was formed of three improved genotypes (BGM2041, BRS Dourada, and BRS Formosa) and 11 landraces (BGM1957, BGM0889, BGM1127, BGM1313, BGM1537, BGM1698, BGM1850, Fécula Branca, BGM1706, BGM1690, BGM1722, and BGM0250). The notable traits of this cluster were the low DMC and NTG (Figure 3).
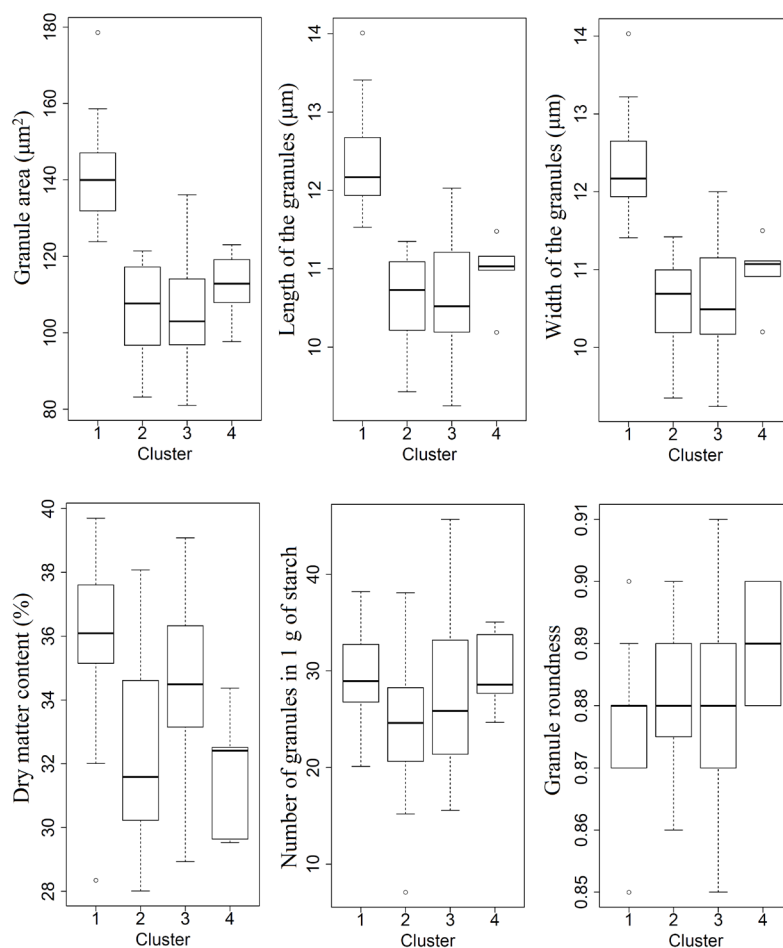
Figure 3. Box plot of traits related to starch granule and root quality in 56 cassava accessions, considering four clusters.

Three improved varieties (BRS Kiriris, BRS Poti Branca, and IAC90) and 22 landraces (BGM0394, BGM0540, BGM0670, BGM0982, BGM0989, BGM1081, BGM1174, BGM1202, BGM1327, BGM1437, BGM1615, BGM1640, BGM2028, Corrente, BGM1458, BGM2052, BGM1110, BGM1180, BGM1667, BGM0620, BGM1370, and BGM1728) were allocated on Cluster 3. On average, Cluster 3 presented small starch granule size (small GrAr, GrLe, and GrWi) and high DMC. Avijala et al. (2015) reported that the traits harvest index, production of commercial roots, and dry matter content in the roots were of low importance when studying the genetic diversity among 21 genotypes of cassava in Mozambique. However, DMC was important as it contributed to the differentiation of different clusters of cassava accessions evaluated in the present study, as noted by Oliveira et al. (2015a).

One improved variety (BRS Jari) and four germplasm accessions (BGM1078, BGM1704, BGM1178, and BGM0507) formed Cluster 4. The accessions of this cluster have lower DMC and medium-sized starch granules with a tendency to be more rounded compared with those of other accessions (Figure 3).

Genetic diversity clustering is extremely important for planning breeding programs in order to obtain hybrids and train populations for future uses. In general, accessions present in different clusters are promising in crosses performed to generate populations with a high level of segregation. However, as cassava is a highly heterozygous species, considerable diversity of genotypic combinations can be obtained even through the use of selfing. Therefore, accessions belonging to the same group can be crossed to achieve more rapid genetic gains focused on four main subjects in the development of varieties: biofortifying (increasing carotenoid content in roots), new sweet cassava varieties with white pulp color, and varieties for industrial purposes with large and small starch granules.

Considering a minimum of 30% DMC, low-to-intermediate cyanogen compound content, and cream-to-yellow pulp color, the accessions BGM1178, BGM1722, BGM1690, BGM0250, and BRS Dourada are considered the most promising. Conversely, considering the generation of segregating populations for sweet cassava with a minimum of 35% DMC, low cyanogenic compounds and white pulp color, the most promising accessions are BGM1590, BGM1850, BGM1180, BGM0620, BGM1429, BGM1728, BGM1376, BGM1715, and BGM1313.

For industrial purposes, and therefore independent of the cyanogenic compound content but with a minimum of 35% DMC, the cassava accessions most suitable for generating segregating populations with small starch granules, are BGM0540, BRS Dourada, BGM0982, BGM1127, BGM1437, BGM1174, and BGM0989. Accessions with these characteristics but with larger starch granules are BGM1495, BGM1715, BGM1376, BGM1728, BGM1429, BGM1412, and BGM1171. As small starch granules resemble those of lipids, cassava varieties producing small starch granules may be useful in the food industry as fat replacers (Leonel, 2007). Furthermore, according to Ceballos et al. (2008), genotypes with small starch granules have increased potential for use in the biofuel industry.

Considering paste properties using different starch sizes, Singh et al. (2012) reported that the small starch granules from rice beans (*Vigna umbellata*) were inversely related to starch swelling power, and therefore, inbred lines with a higher proportion of small starch granules were able to absorb water at higher temperatures. Additionally, those authors reported a positive correlation between peak viscosity, retrogradation, and setback when testing lines with large starch granules. Therefore, it is possible that the starch characteristics reported for rice bean can reflect different properties of the cassava accessions belonging to different groups identified by the PCA analysis.

The association of the clusters formed by the PCA and qualitative characterization regarding pulp color of the roots and the cyanogenic compound content are shown in Figures 4 and 5, respectively. The clustering formed by the PCA was very effective at classifying cassava accessions with yellow pulp in Cluster 4 and cream pulp in Cluster 2, although some accessions remained in the latter group with white roots (BGM1698, BGM1313, BGM1850, BGM1537, Fécula Branca, and BRS Formosa) (Figure 4). The accessions belonging to Clusters 1 and 3 presented a white pulp color.
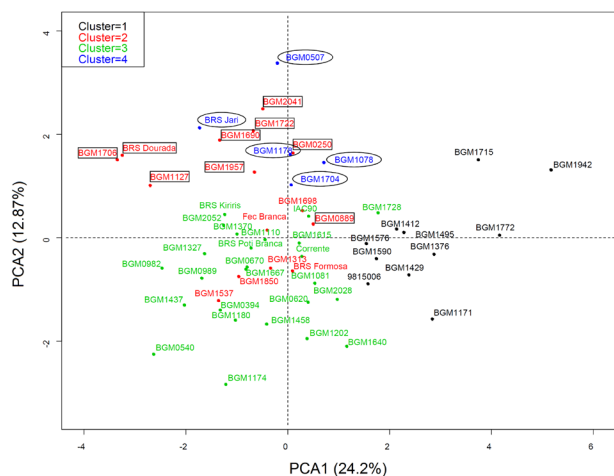
Figure 4. Two-dimensional clustering of the distribution of 56 cassava accessions based on root traits and starch granules, obtained by principal component analysis (PCA). The four identified clusters are represented by different colors. Accessions marked with rectangular and oval borders have roots with cream and yellow pulp color, respectively. The other accessions presented white pulp roots.
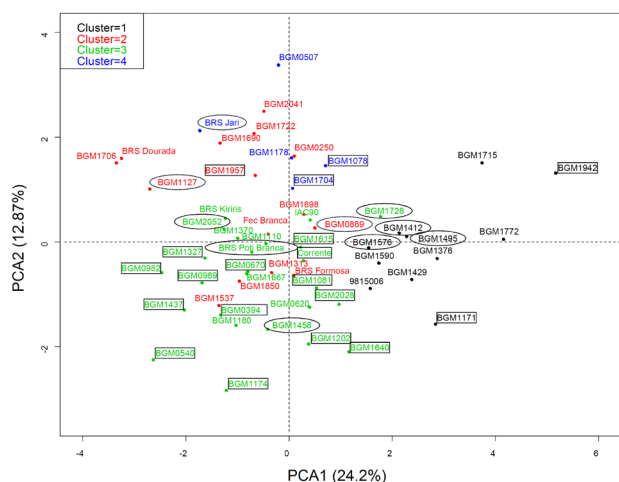
Figure 5. Two-dimensional clustering of the distribution of 56 cassava accessions based on root traits and starch granules, obtained by PCA. The four identified groups are represented by different colors. Accessions marked with rectangular and oval borders contain high (bitter cassava) and intermediate levels of cyanogenic compounds, respectively. The other accessions contain low levels of cyanogenic compounds in their roots (sweet cassava).

When estimating genetic variation between cassava accessions, Oliveira et al. (2015a) found a weak association (0.38) between the clusters formed with quantitative data and the clusters formed by the joint analysis (qualitative data: binomial and multicategoric, and quantitative data). This indicates that analysis of quantitative and qualitative data may consider genetic variations in distinct genomic regions. However, quantitative data related to cassava starch granule traits were accurate for clustering cassava accessions with yellow and cream pulp in distinct groups (Figure 4).

Regarding the classification of cyanogenic compound content, there was no consistency in the clustering proposed by the PCA, as bitter accessions (HCN > 100 ppm) were allocated in Cluster 1 (BGM1171 and BGM1942), Cluster 2 (BGM1957), Cluster 3 (BGM0394, BGM0540, BGM0670, BGM0982, BGM0989, BGM1081, BGM1174, BGM1202, BGM1327, BGM1437, BGM1615, BGM1640, BGM2028, and Corrente) and Cluster 4 (BGM1078 and BGM1704) (Figure 5). This was also observed with other classifications (low and intermediate levels of cyanogenic compounds). Oliveira et al. (2015b) used the affinity propagation algorithm to cluster 474 cassava accessions based on the following traits: starch yield, dry matter content, amylose, and cyanogenic compound content in the roots. These authors also reported difficulty in the classification of cassava accessions based exclusively on cyanogenic compound content, due to the large scale of this trait, especially in two groups, and the absence of an association between the genetic structure of accessions and the classification of bitter and sweet cassava.

The identification and use of accessions available in cassava germplasm banks may contribute to improvements in the productivity of fresh roots and starch (Table 4). However, despite the constant need to develop novel and more productive cassava varieties, it is necessary to consider current trends within the food industry, and farmers searching for native starches with special features that could replace chemically modified varieties or open new market opportunities (Leonel, 2007). Therefore, breeding programs should also seek to improve the starch quality produced by new varieties. In this regard, Brazil has a strategic advantage since it is considered the center of genetic diversity of *M. esculenta* (Olsen, 2004; Isendahl, 2011), and thus has sufficient genetic diversity to meet these demands. As demonstrated, the analysis of genetic relationships among cassava accessions based on root traits and starch granules is an important component of breeding programs, because it provides crucial information required for establishing segregated populations stratified by targeted goals for quality starch.

Conflicts of interest

The authors declare no conflict of interest.

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Table 4. Mean values of the starch traits analyzed in cassava accessions.

Genotype	DMC	HCN	GrAr	GrLe	GrWi	NTG	GrRo
98150-06	39.57	6	126.44	11.66	11.59	32.26	0.88
BGM0250	31.30	5	111.91	11.21	11.03	24.62	0.86
BGM0394	33.99	8	95.43	10.19	10.16	21.37	0.87
BGM0507	29.64	5	107.90	10.98	10.91	24.67	0.90
BGM0540	33.17	8	81.00	9.25	9.24	40.57	0.85
BGM0620	36.32	3	111.43	11.10	11.10	45.69	0.86
BGM0670	33.45	8	103.01	10.52	10.50	38.68	0.89
BGM0889	37.68	6	118.75	11.35	11.42	32.26	0.87
BGM0982	31.67	8	85.80	9.58	9.57	37.05	0.89
BGM0989	32.00	8	96.86	9.96	9.97	24.46	0.88
BGM1078	34.37	8	123.06	11.48	11.50	35.05	0.90
BGM1081	36.97	8	116.14	11.21	11.27	22.02	0.89
BGM1110	28.93	2	107.64	10.85	10.80	37.86	0.88
BGM1127	31.62	6	90.29	9.63	9.64	38.08	0.88
BGM1171	39.70	8	139.96	12.37	12.40	27.79	0.87
BGM1174	38.23	8	98.57	9.85	9.85	27.90	0.85
BGM1178	32.51	6	112.87	11.16	11.11	33.75	0.88
BGM1180	36.94	2	98.57	10.20	10.21	27.19	0.88
BGM1202	39.08	8	116.00	10.94	10.91	15.57	0.87
BGM1313	35.83	4	107.65	10.70	10.62	23.83	0.88
BGM1327	31.42	8	94.81	10.18	10.17	17.36	0.89
BGM1370	33.31	3	102.34	10.56	10.48	25.86	0.89
BGM1376	36.09	4	138.85	12.56	12.57	31.61	0.87
BGM1412	36.45	6	135.41	12.13	12.14	25.76	0.89
BGM1429	36.24	2	141.38	12.17	12.16	20.12	0.88
BGM1437	34.49	8	85.88	9.84	9.84	19.55	0.88
BGM1458	37.80	6	101.07	10.52	10.47	38.51	0.86
BGM1495	35.08	6	140.39	12.11	12.17	28.49	0.88
BGM1537	34.37	4	93.94	10.09	10.05	24.08	0.86
BGM1576	35.22	6	128.43	11.76	11.73	38.21	0.88
BGM1590	38.76	5	123.85	11.53	11.41	28.93	0.87
BGM1615	30.39	8	113.07	11.31	11.25	29.58	0.88
BGM1640	38.68	8	122.34	11.38	11.29	33.18	0.86
BGM1667	33.69	2	103.17	10.50	10.50	24.62	0.89
BGM1690	30.24	4	106.07	10.56	10.56	33.20	0.89
BGM1698	30.43	4	117.44	11.27	11.15	25.11	0.88
BGM1704	32.41	8	119.14	11.03	11.07	28.57	0.88
BGM1706	29.82	3	87.65	9.54	9.59	21.94	0.89
BGM1715	36.04	5	152.78	12.79	12.73	33.23	0.90
BGM1722	30.21	4	114.83	11.00	10.96	19.34	0.89
BGM1728	36.19	3	136.14	12.03	12.00	21.48	0.90
BGM1772	32.01	4	158.62	13.41	13.22	22.48	0.85
BGM1850	38.08	4	99.53	10.34	10.33	7.10	0.89
BGM1942	28.35	8	178.58	14.01	14.03	33.53	0.88
BGM1957	29.53	8	120.65	10.86	10.90	15.19	0.88
BGM2028	35.58	8	119.07	11.53	11.50	18.99	0.87
BGM2041	28.01	4	117.00	11.18	11.14	26.79	0.89
BGM2052	32.05	6	96.40	10.37	10.36	28.28	0.89
BRS Dourada	31.92	4	83.19	9.43	9.35	25.13	0.90
BRS Formosa	34.85	4	121.43	10.73	10.69	18.09	0.87
BRS Jari	29.53	6	97.70	10.19	10.20	27.68	0.89
BRS Kiriris	34.61	3	101.23	10.39	10.36	27.08	0.91
BRS Poti Branca	35.27	6	105.53	10.49	10.49	18.61	0.89
Corrente	35.68	8	114.07	11.22	11.15	21.86	0.90
Fécula Branca	31.58	4	106.87	10.82	10.77	29.71	0.88
IAC90	33.15	3	120.70	11.31	11.33	19.34	0.89

DMC: dry matter content in the roots; HCN: cyanide coumpound; GrAr: granule area; GrLe: length of the granule; GrWi: width of the granules; NTG: number of granules in 1 g of starch; GrRo: granule roundness.

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