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Phenotypic diversity of starch pasting properties in cassava for food industry

Cristiano Silva dos Santos[1], Massaine Bandeira e Sousa[1], Ana Carla Brito[2], Luciana Alves de Oliveira[2], Carlos Wanderlei Piler Carvalho[3] and Eder Jorge de Oliveira[2]*

[1]Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia, Rua Rui Barbosa, 710, 44380-000, Cruz das Almas, Bahia, Brazil. [2]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil. [3]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agroindústria de Alimentos, Guaratiba, Rio de Janeiro, Brazil. *Author for correspondence. E-mail: eder.oliveira@embrapa.br

ABSTRACT. The aim of this study was to evaluate the phenotypic variation and diversity of cassava for breeding purposes based on the pasting properties of starch, for food applications. The viscosities of the starches, extracted from 1031 accessions, were obtained using a Rapid Visco Analyzer. The best linear unbiased predictors were calculated for five critical points of the viscograms, which were then used to cluster the accessions based on the discriminant analysis of principal components. The wide phenotypic variation identified from the viscograms reveals the high potential for selection gains, especially for pasting temperature and setback. Certain strong correlations indicate that selection based on a specific viscogram trait can be used for indirect selection (e.g., the correlation between peak viscosity and breakdown [0.85]). The lowest Bayesian information criterion identified five different groups. Groups 3 and 4 exhibited high pasting temperatures, while Groups 3 and 5 exhibited low setbacks. Group 3 also exhibited low viscosity and breakdown. However, we focused on selecting cassava accessions with a high pasting temperature and low setback, as these are the most important traits for industrial applications. The predicted genetic gains from the selection of the top 15 cassava accessions for increasing pasting temperature and decreasing setback were 4.40% and 53.17%, respectively. The increased pasting temperature (~72.89°C) and high amplitude of setback (~600 cP) due to selection can guide the utilization of the cassava germplasm for breeding and provide a basis for further studies to develop varieties with added value, especially in the food industry.

Keywords: viscosity; characterization; germplasm; *Manihot esculenta* Crantz.

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Introduction

Cassava roots are composed of water (70%), starch (24%), fiber (2%), and other components such as proteins (1 – 3% of dry matter), lipids (0.1 – 0.3% of fresh weight), and minerals, especially calcium (19 – 176 mg), phosphorus (6 – 152 mg), and iron (0.3 – 14.0 mg) (Montagnac, Davis, & Tanumihardjo, 2009; Burns et al., 2012). The physicochemical properties of starches determine their suitability for certain end uses, while the relationships among their molecular structure, functional properties, and viscosity profiles (pasting curves) are powerful tools for representing the functional properties of starch. Each starch produces a different viscosity profile, even under identical processing conditions (Sulaiman & Dolan, 2013).

While the starch market demand has been supplied by wheat, corn, potato, and cassava crops, the differences in starch properties, amylose-amylopectin ratio, and non-amylose component contents generally define their applications (Alcazar-Alay & Meireles, 2015). As a commodity, cassava starch directly competes with the starches of other crops, especially maize; however, global corn starch production is far greater than cassava starch production (65 and 10 million tons, respectively) (Waterschoot, Gomand, Fierens, & Delcou, 2015). An important advantage of cassava starch as compared to corn starch is the absence of undesired cereal flavor (Demiate & Kotovicz, 2011). Therefore, although corn is the main source of starch production, cassava has a large potential market to conquer, which depends on the stability of raw material prices and the regularity of supply.

The performance or functionality of starch for industrial applications can be evaluated based on the apparent viscosity of the starch-water suspension (Ai & Jane, 2014). The curve or apparent viscosity graph provides several critical points, among which peak viscosity (146–1505 cP), breakdown (28-859 cP), and retrogradation tendency (-702–273 cP) are the most important (Sánchez et al., 2009). These properties affect the quality of starch products and are essential for determining their potential industrial applications. The pasting properties of starch are essential for determining its suitability for diverse industrial applications (Ai & Jane, 2014). A standard viscogram profile generally involves the following steps: i) the initial stage (temperature set at 50°C), ii) holding time at 50°C, iii) heating to 95°C, iv) holding at 95°C, v) cooling to 50°C, and vi) holding at 50°C. Therefore, the critical points include pasting temperature, peak viscosity, hot paste viscosity, cool paste viscosity, viscosity breakdown, final viscosity, and setback (Sánchez et al., 2009).

Cassava starch has several industrial applications, especially in the food industry, owing to its physicochemical properties when cooked in aqueous dispersion, which results in high-clarity and high-viscosity pastes while presenting a low gelatinization temperature and low retrogradation tendency when compared to cereal starches (Demiate & Kotovicz, 2011). It is necessary to determine the phenotypic diversity of cassava germplasm and the potential to exploit different types of starches (Sánchez et al., 2009).

However, information on the pasting properties of cassava starch is contradictory and insufficient for effective use in breeding programs because the few existing studies have used small numbers of genotypes and, often, with a restricted genetic basis (Kanagarasu, Sheela, Ganeshram, & Joel, 2014; Awoyale, Sanni, Shittu, & Adegunwa, 2015). The most complete report on the pasting properties of cassava starch was authored by Sánchez et al. (2009), who evaluated over 4,000 cassava genotypes from the International Center for Tropical Agriculture (CIAT) and reported wide variations in traits such as peak viscosity (146 – 1,505 cP), breakdown (28.1 – 859 cP), and setback (-702 – 273 cP). Another study, on a small set of cassava accessions from Nigeria (n = 40), also reported ranges for peak viscosity (261 – 593 cP), breakdown (141 – 329 cP), and setback (19 – 80 cP) (Onitilo, Sanni, Daniel, Maziya-Dixon, & Dixon, 2007). This information can be used to decide the traits that can be used for selection in genetic breeding.

The objective of this study was to evaluate the pasting properties of cassava starch from accessions of germplasm from Brazil and other Latin American countries, as a strategy to complement the characterization, organization, maintenance, and use of these genetic resources for several industrial applications. Accessions with different starch characteristics may be further used for quantitative trait loci mapping based on their starch pasting properties, to improve starch quality through cassava breeding.

Material and methods

Plant material

This study included 1,031 cassava accessions, representing 64% of the Cassava Germplasm Bank (CGB) at Embrapa Mandioca e Fruticultura (Embrapa Cassava and Fruits) in Cruz das Almas, Bahia State, Brazil (12°40′19″ S, 39°06′22″ W, and 226 m altitude). This subset of the germplasm bank comprised 170 local varieties identified by farmers or national research institutions and 861 improved varieties developed from artificial crosses of elite parents and subsequently selected based on root yield, dry matter content, disease resistance, or plant architecture. Most accessions originated from Brazil, while the others originated from other countries such as Colombia, Ecuador, Mexico, Nigeria, Panama, and Venezuela.

Planting was conducted at the beginning of the rainy season (May – July 2015 and 2016), during which 15 to 20 cm long stakes were planted with a spacing of 0.9 m between rows and 0.8 m between plants. The experimental design was an augmented block design with 12 replicates, and plots constituted of 16 plants each (two rows with eight plants each). The roots were harvested 12 months after planting, from June to July (2016 and 2017), which corresponds to the annual cultivation cycle in the Cruz das Almas region.

Starch extraction

The protocol of Sánchez et al. (2009) was followed for starch extraction. In brief, the roots from each accession were selected during the harvesting process based on specific characteristics such as the representative size and shape of the genotype under analysis, as well as the absence of pest and disease damage. White, yellow, and cream roots were evaluated while considering that the objective of this study was to promote the broad characterization of cassava accessions from Brazil. The roots were washed with running water to remove soil impurities and peel tissue. The root pulp was sectioned into cubes, and four 0.5 kg samples from each accession were processed individually. Thereafter, the roots were ground in a blender with non-cutting blades for 1 min at a 1:1 ratio of root pulp to distilled water at room temperature.

The mixture obtained from grinding was filtered through a voile-type fabric packed in a sieve (100 µm) within a 5-liter plastic bucket. Thereafter, the obtained slurry was washed with 3.5 L of distilled water at room temperature for starch extraction. The filtrate was then conditioned in a cold room at 5°C for 12h for decantation of starch. After this, the supernatant was discarded and the starch decanted at the bottom of the vessel was washed with 20 mL of 95% alcohol to accelerate the drying process. The alcohol was discarded, and the starch was then transferred to aluminum trays for drying in an oven with forced air circulation at 40°C until completely dry (~14% moisture). The dried starch was gently macerated using a mortar and pestle to obtain a fine powder.

Analysis of pasting properties

The pasting properties of starches, usually measured using a Rapid Visco Analyzer (RVA), provide a useful indication of their suitability for industrial applications. Despite the Brazilian origin of cassava and the large number of accessions stored in germplasm banks, little effort has been devoted to the characterization of the paste properties of cassava starch. Therefore, in an initial effort to understand these paste properties, we implemented the rapid assessment of a large panel of cassava germplasms using an RVA. Based on the results of this assessment, the most promising cassava accessions can be selected for crossings and other functional properties to optimize industrial applications.

The pasting properties of cassava starch were evaluated using an RVA (model RVA-4500, series 4; NewPort Scientific, Warriewood, Australia), according to the American Association of Cereal Chemists method 76-27.01 (1999), using the standard configuration of Thermocline software for Windows, version 3 (NewPort Scientific). For this evaluation, 3 g of starch from each cassava accession (~14% moisture, wet basis) and 25 g of distilled water were directly weighed into an aluminum vessel to yield a 10% suspension. The correction of samples and water weights to obtain 14% moisture was performed using the aforementioned software. Starch was added to the vessel with water and mixed with the blades attached to the RVA. The starch suspension was then subjected to the following regime (change in temperature/time): at 50°C for 1 min., heating from 50 to 95°C (6°C min.$^{-1}$), maintained at 95°C for 2.5 min. cooling from 95°C to 50°C (6°C min.$^{-1}$), and maintained at 50°C for 2 min. The suspension was subjected to 160 rpm throughout the analysis, which was performed twice.

Pasting viscosity was expressed in cP (centipoise units) and temperature (°C). The total duration of the analysis was 13 minutes for each replicate. During this period, the following traits were evaluated: pasting temperature (PastTemp), peak viscosity (PeakVisc), hot paste viscosity (Hot-PVisc), breakdown of viscosity: the difference between PeakVisc, and Hot-PVisc (BreDow), and retrogradation tendency: the difference between cool paste viscosity and Hot-PVisc (SetBack).

Data analysis

Historically, the selection of cassava germplasm for cultivation *per se* or in crossing blocks for recombination and development of new varieties has been performed based on the agronomic performance of the clones (i.e., high root yield, high starch yield, and disease resistance). Notably, no known selection has focused on clones with differentiated starch. Therefore, viscosity analysis in heating-cooling cycles is an important tool for identifying differentiated starches that exhibit physicochemical behaviors that could help to optimize many different food production processes. Therefore, the identification of a wide variation in viscogram parameters is essential for analyzing any potential genetic gains.

The best linear unbiased predictors (BLUPs) for each pasting property were estimated using the 'lme4' package from R (version 3.5.2; R Development Core Team 2018), based on the mixed linear model $y = Xu + Zg + e$, where y is the vector of phenotypic data, u is the general mean (fixed effect), g is the vector of the genotypic effects of the accessions (assumed to be random), and e is the vector of errors or residuals (random). X and Z represent the incidence matrices for the effects of u and g in the mixed model, respectively. Principal component analysis (PCA) was performed, and phenotypic correlations were calculated from the standardized BLUPs for each trait based on the scale function in R software according to $Z = \frac{X - \underline{X}}{S}$, where Z is the standardized value of X, $\underline{X}$ is the mean, and S is the standard deviation of the trait. PCA was performed using the 'factoextra' package implemented in R version 3.5.2 (R Development Core Team 2018).

To define the number of clusters in cassava germplasm based on starch pasting properties, we implemented successive K-means with an increasing number of clusters (k ranging from 2 to 15) after transforming the dataset by PCA using the *find.clusters* function of the 'adegenet' package in R version 3.5.2

(R Development Core Team, 2018). The number of clusters were compared using the Bayesian Information Criterion (BIC) goodness of fit, and the optimal clustering solution had the lowest BIC. After determining the most suitable number of groups, a cluster analysis was performed based on the discriminant analysis of principal components (DAPC).

Results and discussion

Phenotypic variation in the pasting properties of cassava starch

Estimating the phenotypic variation in plant germplasm is fundamental for determining genetic variability and evaluating potential gains from selection in breeding programs. Wide variations were observed for all pasting properties, which contributed to the selection of cassava germplasm for commercial use or even as parent material for crossing (Figure 1). This statement is supported by the analysis of starch from two cassava varieties widely grown in South-Central (Fécula Branca) and Northeast Brazil (BRS Novo Horizonte). These two varieties showed a PastTemp of ~70°C, which is very close to the germplasm average (69.82°C). The same trend was observed for PeakVisc (~4,900 cP). As for the other traits, Fécula Branca and BRS Novo Horizonte presented slightly more divergent averages (Hot-PVisc: 1,739 and 1,554 cP; BreDow: 3,171 and 3,351 cP; SetBack: 1,198 and 1,090 cP, respectively), although these averages were very close to the averages for these viscogram traits (Figure 1). However, these traits followed a normal distribution with high amplitude and discrepancy around the average, thereby indicating the possibility of selecting clones with different starch properties.

The PastTemp of the starches differed for each cassava germplasm (Figure 1). A difference of over 10°C in PastTemp between cassava accessions (range: 64.05 – 74.14°C) reveals an important variation that can be useful in the food industry, mainly because it shows a higher amplitude than that of conventional cultivars (63.4 – 66.9°C) and a high similarity with that of the cassava germplasms (58.8 – 71.2°C) evaluated in other countries (Sánchez et al., 2009). However, the identification of Brazilian cassava clones with high PastTemp is an important finding because a starch source with high pasting temperature shows high resistance to rupture and swelling, which is a property of interest when cassava starch is physically modified (Chatpapanom et al., 2019). The PastTemp data for Brazilian cassava accessions include the variations in that of potato cultivars (61.0 – 65.2°C), but not of other starchy species such as corn (80.8 – 89.0°C) and wheat (74.2 – 87.7°C) (Suh & Jane, 2003; Sánchez et al., 2009; Sánchez, Dufour, Moreno, & Ceballos, 2010).

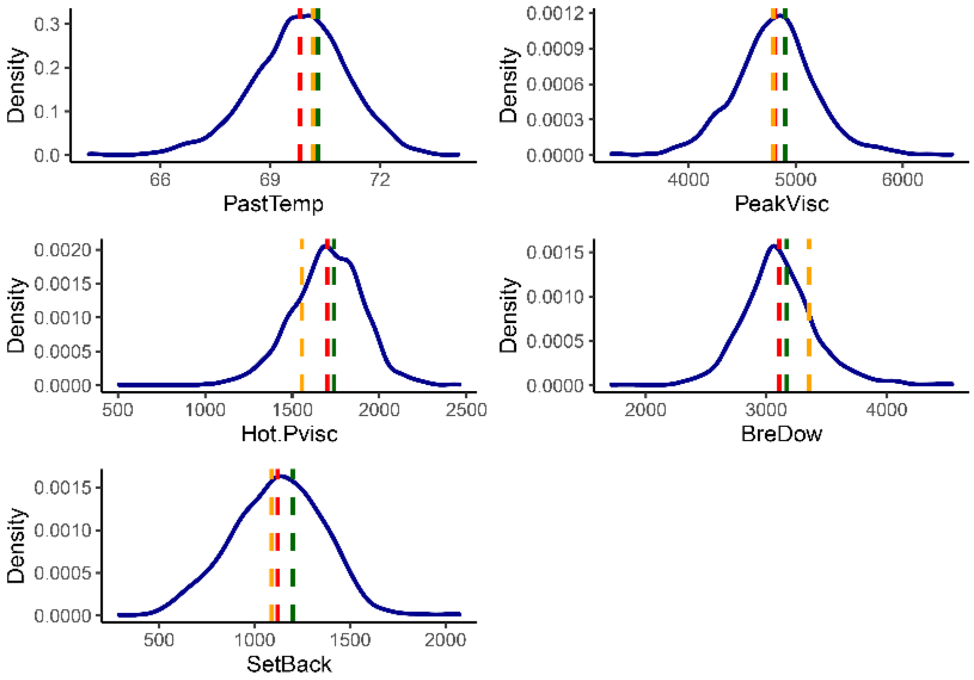

Figure 1. Density plots of the pasting properties of diverse cassava germplasm accessions constructed using a Rapid Visco Analyzer (RVA). The vertical dashed lines indicate the average of each trait (red), the Fécula Branca variety (orange), and the BRS Novo Horizonte variety (green).

The trait PeakVisc (range 3,279.11 – 6,459.50 cP, average 4,805.80 cP) exhibited the greatest variation, which was similar to the range of 3,555 – 6,377 cP reported by Dudu, Oyedeji, Oyeyinka, and Ma (2011). However, another study by Morante et al. (2016) reported values from 876 to 1,285 cP, with an average of 1,112 cP for PeakVisc. The differences in these values are potentially related to the lower starch content (5%) used by Morante et al. (2016) for RVA evaluations as compared to the 10% used in the present study.

Regarding BreDow, Kanagarasu et al. (2014) reported smaller variations (range: 134 – 848 cP, average: 336.1 cP) than those observed in the present study. Moreover, their observed variations for Hot-PVisc (range: 191 – 1051 cP) and SetBack (range: 64 – 476 cP) were also smaller than those observed in the present study (506.25 – 2,459.17 cP for Hot-PVisc and 289.69 – 3,681.29 cP for SetBack).

Clustering analysis

Wide variations among accessions and the groups formed by PCA were identified based on the pasting properties of starch (Table 1). The most important pasting properties associated with PC1 were PeakVis (36.38%) and BreDow (24.81%), while Hot-PVisc (39.90%), PastTemp (20.59%), and BreDow (20.63%) were the most important for PC2.

Based on the clustering procedure used in the DAPC, the lowest BIC was of k = 5. Therefore, five clusters were used in subsequent DAPC analyses because of the high homogeneity of accessions within groups and better partitioning between them. According to the representation of the first two discriminant functions of the DAPC, the cassava accessions were distributed among the groups in a relatively balanced manner, with 136, 376, 119, 263, and 137 accessions in Groups 1, 2, 3, 4, and 5, respectively (Table 2, Figure 2). All clusters were composed of both improved (varieties from breeding populations) and local varieties (varieties traditionally selected and cultivated by farmers, with no known breeding), which indicates that the specific pasting properties of the clusters were not based on the breeding level of the cassava germplasm (Figure 3B).

Table 1. Contributions of the pasting properties of 1031 diverse cassava accessions to the principal component analysis evaluated by a Rapid Visco Analyzer (RVA). PastTemp: pasting temperature; PeakVisc: peak viscosity; Hot-PVisc: hot paste viscosity; BreDow: breakdown; Setback: setback.

Trait	Contribution to the principal component (PC)			
	PC1	PC2	PC3	PC4
PastTemp	10.91	20.59	54.87	13.65
PeakVisc	36.38	0.12	11.26	0.79
Hot-PVisc	13.94	39.90	0.35	31.39
BreDow	24.81	20.63	13.91	6.52
SetBack	13.96	18.76	19.61	47.65
Eigenvalue	2.50	1.14	0.74	0.61
Variance (%)	50.01	22.95	14.87	12.15
Accumulated variance (%)	50.01	72.96	87.83	99.98

Table 2. List of cassava accessions per cluster based on the discriminant analysis of principal componentes.

Cluster	Number of accessions	Improved varieties		Local varieties	
		Number	Most known	Number	Most known
G1	136	22	BRS Jari, BRS Gema de Ovo	114	Manteiga, Cambadinha, Vassourinha, Pão da China, Vassoura Preta, Aipim Dendê
G2	376	70	BRS Novo Horizonte, BRS Caipira, BRS Dourada, BRS Verdinha	306	Aipim Manteiga, Cachimbo, Cigana Preta, Corrente, Cria Menino, Engana Ladrão, Eucalipto, Fécula Branca, Platina, Pretinha, Valência
G3	119	17	7734-7Wx, 7745-5Wx, BRS Kiriris, IAC90	102	Aipim Batata, Arrebenta Burro, Cacau, Cria Menino, Salangor Preta, Saracura, Sergipe
G4	263	37	BRS Formosa, BRS Poti Branca, BRS Tapioqueira	226	Aipim Abóbora, Cacauzinha, Cramuquem, Goela de Jacú, Pão do Chile, Paraguaiana, Riqueza, Sacai, Unha, Vassourinha
G5	137	24	IAC12	113	Arrebenta Burro, Batatinha, Cacau Rosa, Canela de Jacú, Jatobá, Manteiguinha, Nove Folhas, Pingo de Ouro, Recife, Seis Meses, Talo Roxo, Vassourinha Roxa

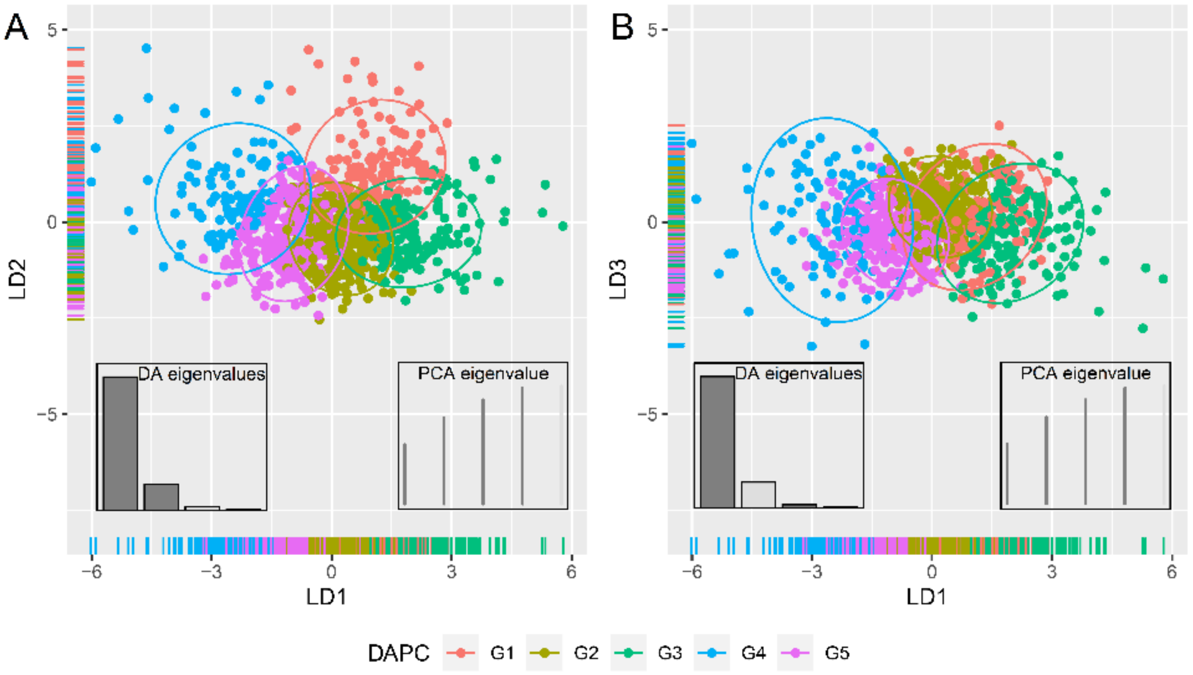

Figure 2. Scatter plots of A) the first and second principal components of the discriminant analysis of principal components (DAPC) and B) first and third principal components of the DAPC based on the pasting properties of 1031 cassava starch accessions.

The pasting characteristics of the different clusters are presented in Table 3 and Figure 3. The accessions from Group 1 were generally characterized by the lowest PastTemp (68.44 ± 1.30°C) and highest PeakVisc (5,397 ± 288 cP), Hot-PVisc (1,823 ± 224 cP), BreDow (3,579 ± 284 cP), and SetBack (1,346 ± 238 cP) values when compared to the other groups. Group 1 exhibited greater contrast among checks (Fécula Branca and BRS Novo Horizonte) than in other accessions, which exhibited high PastTemp and medium values for the other pasting properties. Based on the low PastTemp values in Group 1, these accessions can be used in the production of ready-to-eat foods, such as powdered soup (Schmitz et al., 2017). Notably, achieving gelatinization at low temperatures improves the quality of bakery products (Afoakwa, Budu, Asiedu, Karltun, & Nyirenda, 2012). Additionally, a high PeakVisc can be exploited in products that require high thickening power (Afoakwa et al., 2012). Conversely, the high BreDow values indicate that the starch of this group cannot be used to manufacture products that require high mechanical agitation because the paste can lose viscosity (Otegbayo, Oguniyan, & Akinwumi, 2013). Additionally, the high setback of Group 1 indicates that it is more susceptible to short-term retrogradation. Because setback viscosity is an indirect measurement of starch retrogradation, a reduction in retrogradation is desirable to increase starch stability during storage and use by the food industry (Bernardo, Ascheri, Chávez, & Carvalho, 2018).

Table 3. General characteristics of the different clusters based on the discriminant analysis of principal components using the viscoamylogram of 1031 cassava accessions.

Cluster	Pasting temperature	Peak viscosity	Hot paste viscosity	Breakdown	Setback
G1	Low	High	High	High	High
G2	Medium	Medium	High	Medium	Medium
G3	High	Low	Low	Low	Low
G4	High	Medium	Medium	Medium	Medium
G5	Medium	Medium	Low	Medium	Low
Checks	High	Medium	Medium	Medium	Medium

The main characteristics of Group 2 were high Hot-PVisc (1,792 ± 147 cP) and medium PastTemp (69.5 ± 0.85°C), PeakVisc (4,954 ± 162 cP), BreDow (3,164 ± 164 cP), and SetBack (1,215 ± 170 cP). The traits of Group 2 closely resembled those of commercial starches produced in Brazil, considering that Fécula Branca and BRS Novo Horizonte were allocated to this group. The traits of Group 2 would be useful in applications where high viscosity during heating is required because they would act as thickening agents that are desirable in soups (Marques, Pérégo, Le Meins, Borsali, & Soldi, 2006). Though Group 3 had the lowest number of accessions, it could be distinguished from the other groups because of its low PeakVisc (4,159 ± 217 cP), Hot-PVisc (1,508 ± 133 cP), BreDow (2,648 ± 209 cP), and SetBack (871 ± 113 cP), as well as its high PastTemp (70.5 ± 1.1°C). The high PastTemp of Group 3 indicates the potential for starch resistance against swelling, which may be correlated with the ratio of amylose to amylopectin. High PastTemp values can be considered a disadvantage for some industrial uses because more energy is required for starch pasting (Otegbayo et al., 2013).

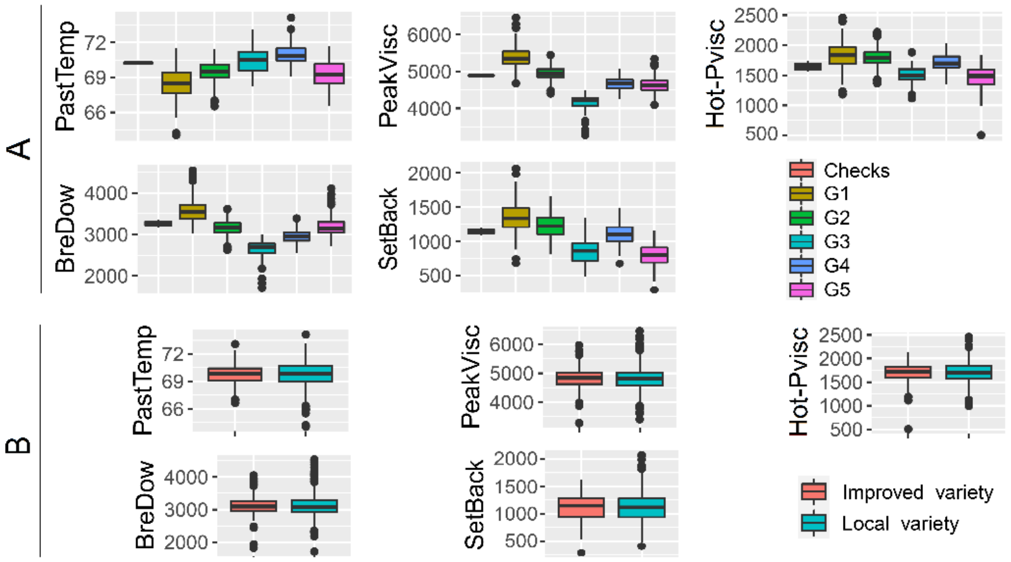

Figure 3. Box plot of the pasting property traits: pasting temperature (PastTemp), peak viscosity (PeakVisc), hot paste viscosity (Hot-PVisc), breakdown (BreDow), and setback of starch from 1031 cassava accessions considering A) the clustering identified by DAPC and B) the breeding level of accessions.

In addition to commercial varieties (BRS Kiriris and IAC90), Group 3 included two waxy cassava varieties (7734-7Wx and 7745-5Wx). Notably, waxy cassava showed similar PastTemp values to those of the normal cassava. Although clustered in Group 3, these accessions exhibited large differences in PeakVisc (~-876 cP), Hot-PVisc (~-102 cP), BreDow (~-765 cP), and SetBack (~-365 cP) when compared to the averages of Group 3. This could indicate that despite being in the same group, significant within-group differences exist.

Increased viscosity during gelatinization is related to the degree of swelling that occurs as water enters the granule until it ruptures with the simultaneous outflow of amylose (Afoakwa et al., 2012). Starches with lower peak viscosity generally exhibit greater resistance to swelling and thus require higher temperatures to break the granules (Kumar, Brennan, Mason, Zheng, & Brennan, 2017). In contrast, the BreDow trait is related to paste stability, where low values indicate starch resistance to agitation. This is important because granules are heated and subjected to mechanical agitation in various industrial processes, which can cause them to rupture with an associated reduction in paste viscosity. Thus, the higher the BreDow, the lower is the resistance to agitation (Otegbayo et al., 2013). Moreover, setback reflects the tendency of starch to retrograde after gelatinization and cooling, where molecular reassociation of amylose occurs. Low SetBack values indicate low retrogradation and low levels of amylose (Wang, Li, Copeland, Niu, & Wang, 2015), which is consistent with the presence of waxy accessions in Group 3 (7734-7WX and 7745-5WX).

Group 4 was characterized by high PastTemp (71 ± 0.8°C) and medium PeakVisc (4,665 ± 155 cP), Hot-PVisc (1,713 ± 126 cP), BreDow (2,951 ± 149 cP), and SetBack (1,107 ± 153 cP) values. The high value of the initial paste temperature is particularly interesting when starch undergoes modification that involves an intermediate heat process (either by annealing or a heat moisture process) in which thermal stability is important (Ashogbon, 2021).

In Group 5, the most significant characteristics were the low Hot-PVisc (1,463 ± 184 cP) and SetBack (798 ± 153 cP) values. The other traits exhibited medium values (PastTemp: 69 ± 1.2°C, PeakVisc: 4,641 ± 217 cP, and BreDow: 3,179 ± 244 cP). Setback reflects the tendency of starch to retrograde after gelatinization and cooling, where molecular reassociation of amylose occurs. Notably, the low setback of Group 5 indicates low retrogradation. Therefore, the starch from this group can be used in applications that require starch stability at low temperatures, for example, as thickeners in the bakery and frozen food industries (Afoakwa et al., 2012), as well as in foods that cannot lose water during storage (e.g., frozen and chilled products) (Schmitz et al., 2017). Additionally, the low Hot-PVisc of this group can be useful in manufacturing homemade products such as biscuits (Schmitz et al., 2017).

Because cassava starches generally have lower pasting temperatures than cereal starches, viscosity development begins at low temperatures. It has been reported that pasting temperatures in cassava starch range from 50.3 to 73°C (Sánchez et al., 2009; 2010; Uzomah & Ibe, 2011; Oladunmoye, Aworh, Maziya-Dixon, Erukainure, & Elemo, 2014). However, we identified accessions with PastTemp values above 73°C, especially in Groups 3 and 4 (Figure 3).

Furthermore, wide PeakVisc variations have been reported in the literature, ranging from 146 to 7,015 cP (Sánchez et al., 2009; Eke-Ejiofor, 2015). In addition to genotypic differences, methodological variations such as the starch suspension (%) and rate of heating (°C min.^{-1}) of the RVA cycles could explain the large variation in the dataset. Despite this, the variation covered by the five groups identified in the present study (3,279.11 – 6,459.50 cP) is within the range reported in the literature. According to Nuwamanya et al. (2010), cassava starches with low PeakVisc exhibit better culinary properties. Therefore, Group 3 clones are expected to have a greater potential for adoption in the food industry. Specifically, the waxy clones belonging to this group presented the lowest PeakVisc values (~3,200 cP). Moreover, a study from Colombia noted that the waxy cassava clones showed the smallest range of PeakVisc (Morante et al., 2016).

The BreDow and setback viscosity variations in the literature range from 28 to 3,843 cP and from -702 to 1,219 cP, respectively (Sánchez et al., 2009; 2010; Uzomah & Ibe, 2011; Oladunmoye et al., 2014; Eke-Ejiofor, 2015). The present study demonstrated that some Brazilian cassava clones had higher values for BreDow (1,714 – 4,542 cP) and setback (289 – 2,073 cP) than those reported in the literature. In general, a higher BreDow indicates lower resistance to high temperatures and sensitivity to shearing stress, whereas setback viscosity implies the degree of starch retrogradation. Therefore, the accessions from Group 3 should be further analyzed for their starch paste stability and lower retrogradation tendency.

Although Brazil is considered the center of diversity for *M. esculenta* (Carrasco et al., 2016), previous studies on the pasting properties of cassava germplasm did not include a large number of samples. Therefore, the characterization of pasting properties from Brazilian accessions is summarized in Figure 4, which represents an initial effort to select the most promising materials and emphasize refined studies on starch to improve the commercial exploitation of this germplasm. The Group 1 profile was generally well defined and distinct as compared to the others, with low PastTemp and high viscosities, while Group 3 exhibited high PastTemp and low PeakVisc, Hot-PVisc, and BreDow values. Groups 2 and 4 exhibited very similar PeakVisc, BreDow, and SetBack traits but differed in the other traits. Group 5 also exhibited similar values to Group 2 for PastTemp, PeakVisc, and BreDow.

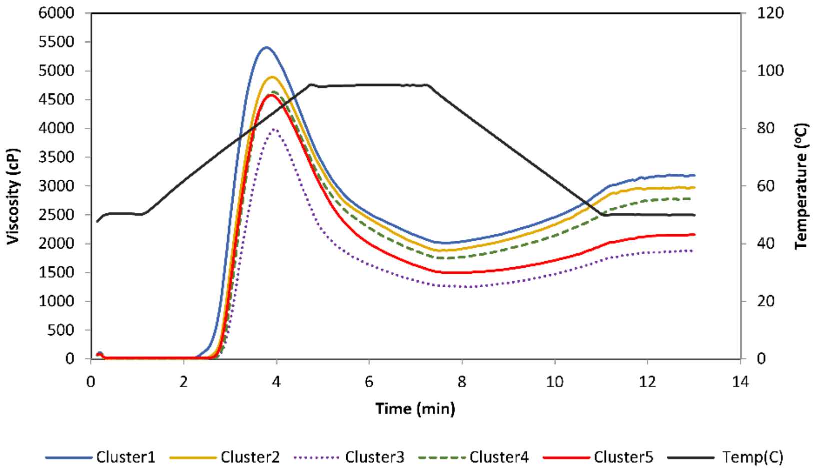

Figure 4. Average profiles for the five clusters of cassava starch accessions based on Rapid Visco Analyzer (RVA) data.

Phenotypic correlation between pasting properties

Positive correlations between the BLUPs of pasting properties plus the overall mean ranged from 0.07 to 0.85, while negative correlations ranged from -0.07 to -0.39 (Figure 5). Positive correlations of high magnitude (>0.80) were identified for Hot-PVisc vs. BreDow (0.85). Moreover, positive correlations of moderate magnitude (0.50 - 0.80) were identified for PeakVisc vs. Hot-PVisc (0.58). Negative correlations were generally of low magnitude for most pasting properties, and PastTemp was the only trait that correlated negatively with all the other traits. Such negative correlations were of high magnitude in certain cases, as was observed for PastTemp vs. BreDow (-0.39) and PeakVisc (-0.35).

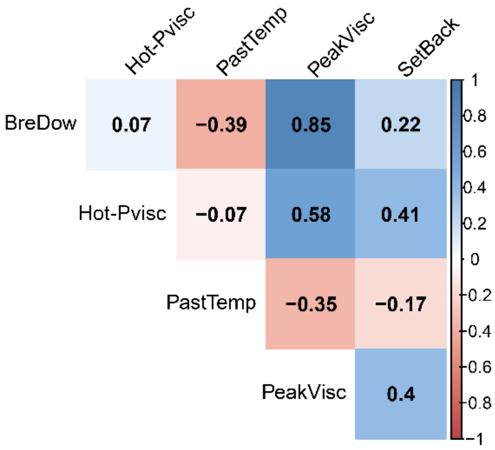

Figure 5. Pearson correlations between the best linear unbiased predictor (BLUP) plus the overall mean for each pasting property of cassava starch. PastTemp: pasting temperature; PeakVisc: peak viscosity; Hot-PVisc: hot paste viscosity; BreDow: Breakdown; SetBack: setback.

Similar correlations to those observed in this study were reported by Awoyale et al. (2015) on analyzing three biofortified cassava accessions in which negative correlations for PastTemp vs. PeakVisc and Hot-PVisc (-0.06 and -0.19, respectively), but with a positive correlation for PastTemp vs. BreDow (0.40).

Regardless of the botanical source, important correlations exist among starch pasting properties (Kanagarasu et al., 2014). In general, high correlations may be due to the nature of the analysis. For example, when the temperature reaches 95°C and begins to cool to 50°C (cold paste viscosity), the molecules tend to reorganize (SetBack) and that can increase the viscosity, which remains constant until the end of the analysis (FinalVis). Therefore, these traits generally have positive correlations with each other (Kanagarasu et al., 2014). Nuwamanya, Baguma, and Rubaihayo (2010) reported that the high PeakVisc observed in the parents used in their study was largely due to differences in the amylopectin structure because amylose content was not statistically different among the studied parents and their progenies. These correlations can be used by breeders to select genotypes of interest for crop breeding programs with specific objectives.

Expected gain with the selection of cassava accessions

The genetic variability of the 1,031 cassava accessions was high enough to allow the selection of useful accessions for breeding programs based on the starch pasting properties. Because the pasting properties PastTemp and SetBack have important implications for potential industrial applications of cassava starch (Ai & Jane, 2014), 15 of the most promising clones were selected independently with two goals: increasing PastTemp and decreasing SetBack (Figure 6). For the PastTemp trait, the selection of 15 accessions with values above the average of the original population (69.82°C) resulted in a predicted increase of 4.40%, thereby increasing the average of the selected population to 72.89°C. The set of selected clones included nine local varieties belonging to Group 4 (BGM0381, BGM0401, BGM0495, BGM0503, BGM0528, BGM0604, BGM0688, BGM1698, and BGM2071), five from Group 3 (BGM0597, BGM0690, BGM1327, BGM1957, and BGM2028), and an improved variety (IAC90) from Group 3.

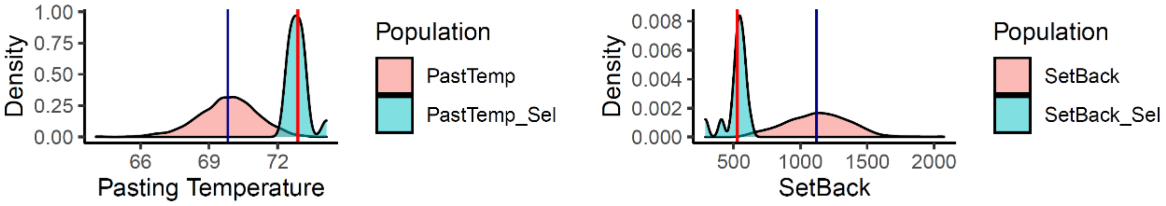

Figure 6. Expected genetic gains from the selection of 15 accessions with the highest average pasting temperature (PastTemp) and SetBack values as compared to the total panel of 1031 cassava germplasms evaluated for pasting properties. The vertical lines indicate the average for each trait.

For the SetBack trait, accessions with contrasting SetBack were selected in relation to the average of the population (1,121 cP). To reduce SetBack, the selection of 15 accessions with values below the average of the original population resulted in a predicted loss of 53.17%, thereby reducing the average to 525 cP (Figure 6). These promising accessions included a local variety (BGM-0975) and improved varieties (7734-7WX and 7745-5WX) from Group 3 and local varieties from Group 5 (BGM-0342, BGM-0350, BGM-0700, BGM-0729, BGM-0962, BGM-0981, BGM-0982, BGM-0983, BGM-1143, BGM-1359, BGM-1371, and BGM-1402).

The accessions selected from Groups 3, 4, and 5 can be crossed to obtain populations with high levels of segregation. Indeed, Nuwamanya et al. (2010) reported that F_1 progenies presented differentiated pasting properties in relation to those of the parents with different crosses, while contrasting for some starch branching enzymes. The potential gains predicted in the present study are much smaller than those obtained for some productive traits in cassava (44 – 182%) (Freitas, Santos, & Oliveira, 2016); however, they are close to those obtained in the selection of traits related to increasing or decreasing the size and number of starch granules in S_1 segregant populations (15 – 52%) (Oliveira, Barbosa, Diniz, Ferreira, & Oliveira, 2018).

Conclusion

Cassava has a high natural genetic diversity owing to its intense evolutionary and domestication processes (Carrasco et al., 2016). Studies that aim to estimate the diversity and characterize the genetic resources of cassava are of paramount importance. This study represents the first attempt to characterize cassava accessions from Brazil on their pasting properties, to guide germplasm conservation by identifying the core accessions, as well as the most contrasting ones for breeding. The accessions were clustered into five groups. Each cluster exhibited different properties in relation to starch traits and could thus be exploited for different industrial applications. The validation of these uses should be explored in future research. Additionally, our results indicate that it is possible to select superior accessions to increase PastTemp and reduce SetBack, which could allow breeding programs to meet the various demands of the cassava market regarding the pasting properties of cassava starch.

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