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Published online: 17 Mar 2021.

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Phenotypic characterisation of the germplasm bank of mangaba (*Hancornia speciosa* Gomes), a unique Brazilian native fruit, with emphasis on its high vitamin C content

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ABSTRACT

Mangaba (*Hancornia speciosa* Gomes) is a native fruit of Brazil that has high antioxidant activity and may benefit human health. The goal of this study was to discriminate between accessions in the germplasm bank of mangaba to improve its management. Eight morpho-agronomic traits were analysed in the ripe fruit of 36 genotypes taken from six accessions, and descriptive analyses were undertaken using Pearson correlation analysis, principal component analysis (PCA) and cluster analysis. The highest correlation coefficients were obtained between fruit size traits, and there was also a moderate correlation between the vitamin C content and number of seeds. PCA showed that 80.6% of the variability was explained by the first three principal components, to which all eight traits made significant contributions, while cluster analysis revealed considerable germplasm variation among the genotypes, which formed five distinct clusters: one cluster with a lower fresh mass (FM) and a mean vitamin C content of 410.09 mg·100 g^{-1} FM; one cluster with a higher FM and a mean vitamin C content of 363.85 mg·100 g^{-1} FM; and a further three clusters with a high FM, vitamin C content and soluble solids content, respectively. These findings on the vitamin C content and FM of these accessions will be useful for the agro-economic development of this native Brazilian species.

Introduction

The biodiversity of Brazil is one of the richest in the world and includes a wide range of native fruits with high potential for the food industry (Narain et al. 2018). Among these, *Hancornia speciosa* Gomes (Apocynaceae), which is commonly known as 'mangaba', meaning 'good thing to eat', in the Tupi-Guarani language, has notable economic importance and immense conservation appeal, as its exploitation is carried out exclusively by extractivism and its areas of occurrence of natural populations are undergoing intense urban development (Silva et al. 2012; Santos et al. 2017; Soares et al. 2017). This

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species is found in a variety of Brazilian biomes, from Cerrado (Brazilian savannas) in the north, southeast and midwest to coastal plateaus and plains in the northeast (Vieira Neto et al. 2009). However, it is most widely used in northeastern Brazil, which is also considered the largest provider of this fruit, with the States of Paraiba, Sergipe and Bahia producing an average of 733, 373 and 243 tons per year, respectively (IBGE 2020).

In the last 25 years, the food industry has increased research into the development of new potential foods (Khan et al. 2013). Native fruits with particular properties represent important opportunities due to their great potential for agroindustrial exploitation and the development of new processed products (Sganzerla et al. 2019). Mangaba fruit is considered a viable food due to its high nutritional value and has exceptional economic, social and cultural importance in the food sector due to its great versatility in derivable products, being used in the production of cookies, syrup, juice, wine, liquor, jam, fruit compote, alcohol and vinegar (Clerici and Carvalho-Silva 2011). However, few manufactured products are currently produced with this species (Assumpção et al. 2014). Therefore, mangaba can be considered a native fruit of great potential promise for the global market.

The first objective of any plan for the conservation, pre-breeding and breeding of a commercial fruit species should be to investigate which morpho-agronomic traits can provide information for the description and classification of a particular germplasm to assist with screening and improve decision making. Some of this information can be obtained through simple statistical techniques, but a large portion of such information requires multivariate approaches involving the simultaneous analysis of multiple measurements to convert the data into useful knowledge (Hair et al. 2009).

The main objective of the present study was to use multivariate analysis to evaluate and select accessions from a mangaba germplasm bank to improve development of the conservation plan and management of the pre-breeding and future breeding programmes for this species.

Materials and methods

Plant material

The plant material used in this study was obtained from the Active Germplasm Bank of Mangaba in the Embrapa Tabuleiros Costeiros Experimental Field Station, which is a Restinga area (plant formations established on sandy soils in the coastal plain region) in the municipality of Itaporanga d'Ajuda, State of Sergipe, Brazil (11°06′40″S, 37°11′15″W). The soil in this area is classified as Humiluvic Spodosol. This germplasm bank currently comprises 271 genotypes in 22 accessions of different origins and ages. A total of 36 genotypes from six accessions were used in the present study (Table 1). Since the chemical composition of mangaba fruit can change according to maturation stage, the fruit were harvested at the 'ripe' maturation stage to maintain analytical uniformity (Figure 1).

Trait evaluation

Twelve fruit of each genotype were harvested at the 'ripe' maturation stage, placed in labelled plastic boxes, and transported to the Fruit and Vegetable Processing Laboratory of the Food Technology Department of the Federal University of Sergipe, Brazil, where

Table 1. Places of origin of the 36 genotypes from six accessions of the germplasm bank of mangaba (*Hancornia speciosa* Gomes) analysed in the present study.

State	Municipality	Accession	Number of genotypes
Bahia	Jandaíra	Costa Azul (CA)	6
	Conde	Barra de Itariri (BI)	6
	Mata de São João	Lagoa Grande (LG)	6
Sergipe	Indiaroba	Terra Caída (TC)	6
		Preguiça (PR)	6
		Pontal (PT)	6
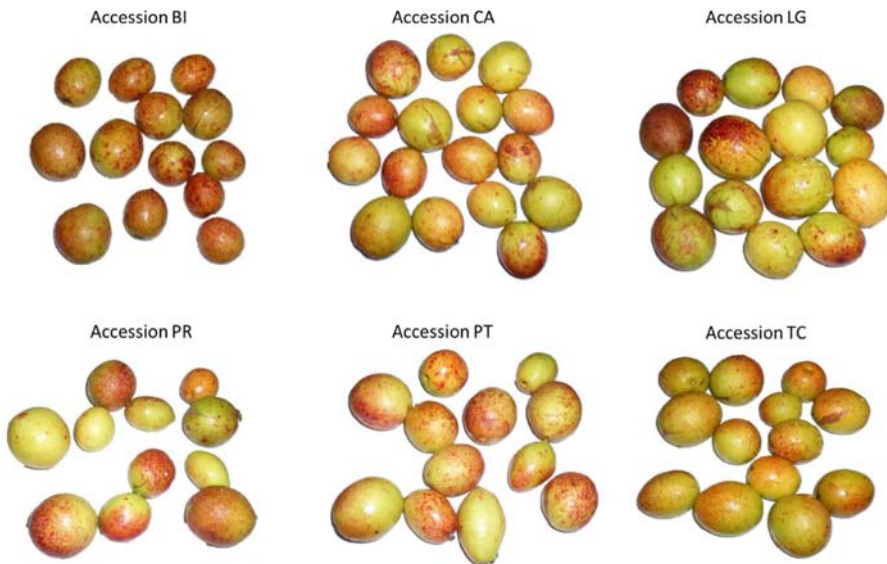
Figure 1. Photographs of the studied fruit from six accessions of *Hancornia speciosa* Gomes. Accessions: BI, Barra do Itariri; CA, Costa Azul; LG, Lagoa Grande; PR, Preguiça; PT, Pontal; TC, Terra Caída.

physical and physicochemical analyses were performed. The physical morpho-agronomic traits that were evaluated included fresh mass (FM; g), number of seeds per fruit (NS), and longitudinal (LD) and transverse (TD) diameters of the fruit (measured with calipers and expressed in mm). The physicochemical traits that were measured included pulp pH (measured with an electronic potentiometer using 5 g of pulp diluted in 50 mL of distilled water), total titratable acidity (TTA; determined by titration with 0.1 N NaOH solution and 1% phenolphthalein as an indicator and expressed as a percentage of citric acid), soluble solids [SS; measured using a PAL-1 Atago® digital refractometer following the standardised protocols of AOAC (1992) and expressed in ° Brix] and vitamin C content [measured according to the methodology of the Adolfo Lutz Institute (2008) and expressed in mg ascorbic acid·100 g^{-1} FM].

Statistical analysis

The mean, minimum and maximum values, standard deviation (SD), and coefficient of variation (CV%) were calculated for each of the measured traits, and average values were

used in the statistical analyses. For all traits, the variance was analysed using SPSS software. The suitability of the data for principal component analysis (PCA) was assessed using Bartlett's test and the Kaiser-Meyer-Olkin (KMO) test. The patterns of variability among the genotypes were examined using boxplots, Pearson correlation analysis, PCA and cluster analysis based on the unweighted pair group method with arithmetic mean (UPGMA method), which groups items with the shortest distances and recalculates new distances using the arithmetic means to produce new groupings. All multivariate analyses were performed using R software (R Core Team 2018).

Results and discussion

Morpho-agronomic traits

The studied genotypes showed significant variability in the analysed traits. Three of the eight traits measured had CVs greater than 20% (Table 2; Figure 2), indicating a high level of phenotypic diversity among the genotypes. These included the number of seeds (55.80%), FM (35.05%) and vitamin C content (21.46%). By contrast, the lowest CVs were recorded for the fruit pH (3.94%) and soluble solids content (8.80%), indicating that these traits were more homogeneous and repeatable between accessions and, therefore, can be considered stable characteristics.

High variability was detected in a number of physical traits, including TD (range = 23.49–36.55 mm), LD (29.19–42.87 mm), FM (8.30–32.84 g) and the number of seeds (3.22–28.56) (Table 2; Figure 2). The mean values for these traits were higher than were found in a previous study on mangaba germplasm in the Cerrado (Almeida et al. 2019). However, a study characterising the native plants of the State of Maranhão also found high average values for variation in the physical characteristics of mangaba fruit (Silva et al. 2017b).

In terms of the physicochemical parameters, the vitamin C content showed the greatest variation (210.93–639.10 mg ascorbic acid·100 g^{-1} FM; Table 2; Figure 2) and also had mean values that were 200–400% higher than were found in other studies on mangaba (Silva et al. 2013; Perfeito et al. 2015; Plácido et al. 2016), confirming that mangaba is a rich source of vitamin C. In addition, the total titratable acidity exhibited a moderate amount of variation (0.89–2.01% citric acid), while the pH and soluble solids content showed the lowest variation (3.19–3.90 and 14.03–20.67 °Brix, respectively)

Table 2. Descriptive statistics for eight morpho-agronomic traits among the 36 *Hancornia speciosa* Gomes genotypes studied.

Trait	Abbr.	Unit	Min	Max	Mean	SD	CV (%)
Total titratable acidity	TTA	%[a]	0.89	2.01	1.44	0.24	16.00
pH	pH	-	3.19	3.90	3.37	0.13	3.94
Soluble solids content	SS	°Brix	14.03	20.67	17.72	1.56	8.80
Vitamin C content	VC	mg[b]	210.93	639.10	398.08	83.11	21.46
Fresh mass	FM	g	8.30	32.84	18.52	6.74	35.05
Transverse diameter	TD	mm	23.49	36.55	30.36	3.74	12.40
Longitudinal diameter	LD	mm	29.19	42.87	35.54	4.17	11.75
Number of seeds	NS	-	3.22	28.56	10.39	6.34	55.80

[a]% citric acid; [b]mg·100 g^{-1} fresh mass; CV = coefficient of variation; SD = standard deviation. Total number of fruit evaluated = 432.
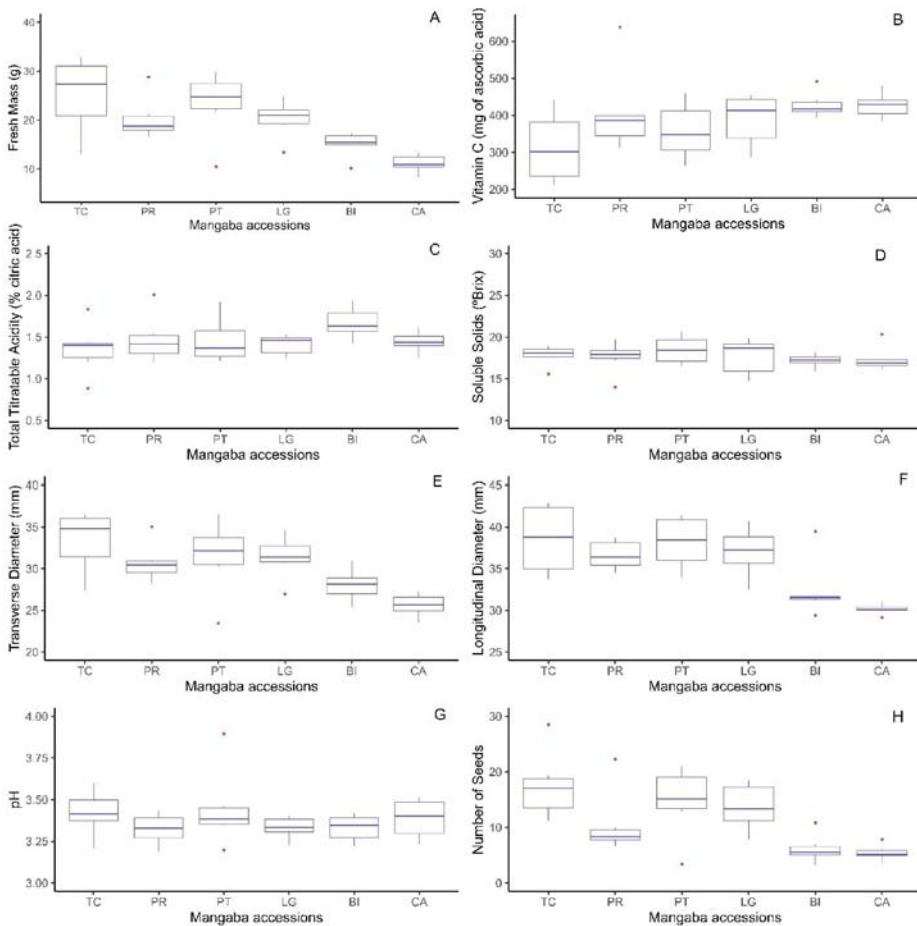
Figure 2. Boxplots of variation in eight morpho-agronomic traits among 36 genotypes from six accessions of *Hancornia speciosa* Gomes. (A) Fresh mass; (B) vitamin C content; (C) total titratable acidity; (D) soluble solids content; (E) transverse diameter; (F) longitudinal diameter; (G) pH; and (H) number of seeds. A total of 12 fruit were evaluated for each genotype and the mean value was calculated. Accessions: BI, Barra do Itariri; CA, Costa Azul; LG, Lagoa Grande; PR, Preguiça; PT, Pontal; TC, Terra Caída.

(Table 2). The addition of vitamin C to the diet is beneficial, as it is considered an important natural compound that has essential antioxidant action and so is useful in helping to prevent diseases (Almeida et al. 2011). Furthermore, the high soluble solids content and titratable acidity of mangaba are important for the artisanal production of jellies, juices, candies, ice cream and other products, as the artisanal market is more profitable when lower volumes of artificial additives are required to improve the taste and appearance of the products.

The extent of phenotypic variation in the mangaba germplasm can largely be explained by the lack of control for environmental factors and plant age, as well as genetic differences between individuals due to multiple cross-pollination events in this self-incompatible species (Ganga et al. 2010; Silva et al. 2017b; Maia et al. 2018). The observed variability in the physical traits in the present study indicates that the

environmental conditions in northeastern Brazil are highly favourable for the development of this species and the germplasm bank accessions are adapting well to the environmental conditions in Sergipe. The mean values of the studied traits also indicate that the genotypes in the mangaba germplasm bank show great potential for breeding programmes, as they can be selected for their desirable morphological and physicochemical parameters (Maia et al. 2018).

Correlation analyses

A correlation coefficient quantifies the degree to which the variation in one trait reflects the variation in another trait (i.e. provides a measure of the intensity of the biological or some other type of association between two traits). Correlation coefficients also provide extremely useful information for germplasm and cultivar evaluations in breeding programmes and the likely consequences of direct and indirect selection, particularly when measuring a large number of traits, which can be both costly and labor-intensive (Khadivi 2018).

Pearson correlation analysis showed that there were significant positive and negative correlations among the examined morpho-agronomic traits (Figure 3). In general, those traits that were associated with physical attributes showed high positive correlations, ranging from 0.75–0.97. For example, FM was positively correlated with TD ($r = 0.97$), the number of seeds ($r = 0.91$) and LD ($r = 0.85$); TD was positively correlated with the number of seeds ($r = 0.89$) and LD ($r = 0.81$); and LD was positively correlated with the number of seeds ($r = 0.75$). Thus, in general, the morphological traits strongly controlled the variability in phenotypic expression among the studied genotypes.
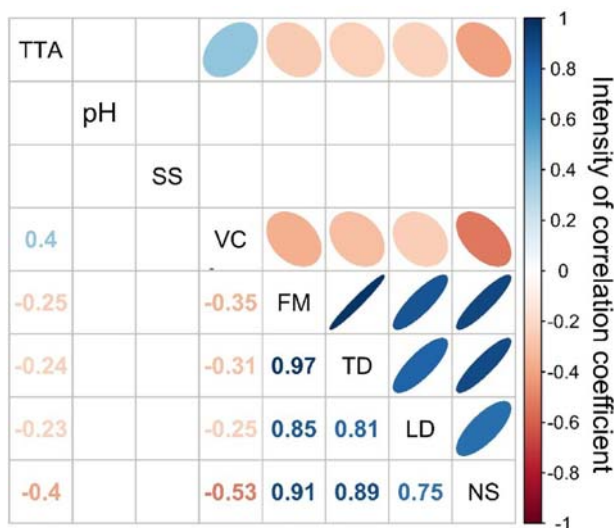

Figure 3. Pearson's correlation coefficients between the morpho-agronomic traits measured in the 36 studied genotypes of *Hancornia speciosa* Gomes. A total of 12 fruit were evaluated for each genotype and the mean value was calculated. TTA, total titratable acidity; SS, soluble solids content; VC, vitamin C content; FM, fresh mass; TD, transverse diameter; LD, longitudinal diameter; NS, number of seeds. Empty boxes indicate non-significant correlations ($P < 0.05$).

For the physicochemical traits, the total titratable acidity and vitamin C content were significantly positively correlated with each other ($r = 0.4$). In addition, only these traits showed significant correlations with other traits, with total titratable acidity being negatively correlated with FM ($r = -0.25$), TD ($r = -0.24$), LD ($r = -0.23$) and the number of seeds ($r = -0.4$), and the vitamin C content being negatively correlated with the number of seeds ($r = -0.53$), FM ($r = -0.35$), TD ($r = -0.31$) and LD ($r = -0.25$).

Positive correlations between physical traits were also observed in a previous biometric study on mangaba fruit from the Brazilian Cerrado using Spearman correlation analysis (Gonçalves et al. 2013) and in another biometric study of a mangaba germplasm collection containing different botanical varieties (Almeida et al. 2019). In addition, Silva et al. (2017a) reported a positive correlation among characteristics related to fruit weight, with emphasis given to the significant relationship between fruit weight and seed number.

Considering that the physical traits of mangaba fruit were significantly positively correlated with each other and significantly negatively correlated with the vitamin C content in the present study, it can be concluded that smaller fruit have a higher vitamin C content. Consequently, it should be possible to indirectly select for genotypes that produce fruit with a higher vitamin C content by selecting fruit with a lower fresh mass, which would facilitate the selection process in the field.

PCA

PCA allows different traits to be explained in the form of principal components (PCs) that include multiple series of correlated traits. The associations between traits and PCs that are emphasised by this method may be related to genetic linkage between the loci that control these traits or pleiotropy (Lezzoni and Pritts 1991; Rakonjac et al. 2010; Khadivi-Khub et al. 2014).

PCA indicated that the loadings of the eight measured traits made significant contributions to the PCs, highlighting their relevance in determining the extent of variability among the 36 evaluated mangaba genotypes. The first three PCs (PC1–PC3) explained 80.60% of the total variance in the data and were considered significant according to the Kaiser criterion, which requires the retention of only those PCs with eigenvalues greater than 1.0 (Kaiser 1958; Table 3).

Those traits with the highest loadings (> 0.56) were of greatest relevance for the respective PCs. PC1 was dominated by physical traits, indicating that these were responsible for most of the variation in the data and thus made the greatest contribution to genotype discrimination. Consequently, the effectiveness and accuracy of the selective process would be high because these phenotypic traits are less complex to measure (Ganopoulos et al. 2016; Maia et al. 2018). By contrast, the physicochemical traits total titratable acidity, pH and vitamin C were prominent in PC2, while the soluble solids content only made a significant contribution to PC3. Furthermore, in general, physical traits made the main contributions to all three PCs, with FM having the highest contribution and vitamin C content having the lowest (Figure 4). A scree plot of the variance explained by the eight PCs showed that there was a fairly large decrease in explained variance between PC1 and PC2 but a lower decrease in explained variance from PC3 onwards (Figure 5). These findings indicate that the measured traits are important and can be a useful tool when selecting for superior genotypes.

Table 3. Loadings, eigenvalues, variance and cumulative variance for the first three principal components (PC1–PC3) for the 36 *Hancornia speciosa* Gomes genotypes studied.

	Component		
Trait	PC1	PC2	PC3
Total titratable acidity	−0.42	0.62[a]	0.34
pH	0.36	−0.56[a]	0.50
Soluble solids content	0.36	0.12	0.80[a]
Vitamin C content	−0.53	0.60[a]	0.04
Fresh mass	0.95[a]	0.26	−0.03
Transverse diameter	0.93[a]	0.29	−0.07
Longitudinal diameter	0.85[a]	0.26	−0.08
Number of seeds	0.95[a]	0.00	−0.15
Eigenvalue	4.123[b]	1.282[b]	1.044[b]
% of variance	51.53	16.02	13.05
Cumulative %	51.532	67.552	80.601

[a]Loadings > 0.56 are significant; [b] Eigenvalues > 1 are significant.

Scores were calculated for each of the 36 genotypes to select the best genotypes for specific traits (Table 4). These scores can be interpreted geometrically as the projections of the observations onto the PCs, with the most stable genotypes being those with the highest values for more than one component. The indicated genotypes were 1, 2, 4, 6, 7, 8, 14, 16, 18, 19 and 21 for PC1; 4, 9, 12, 21, 26 and 30 for PC2; and 18, 20, 22, 27 and 35 for PC3. Thus, genotype 4 from the Terra Caída (TC) accession and genotype 18 from the Pontal (PT) accession were most stable for the traits appearing in all PCs, as they had the highest scores for physical and physicochemical traits. By contrast, genotype 31 from the Costa Azul (CA) accession had the lowest score in all three PCs.
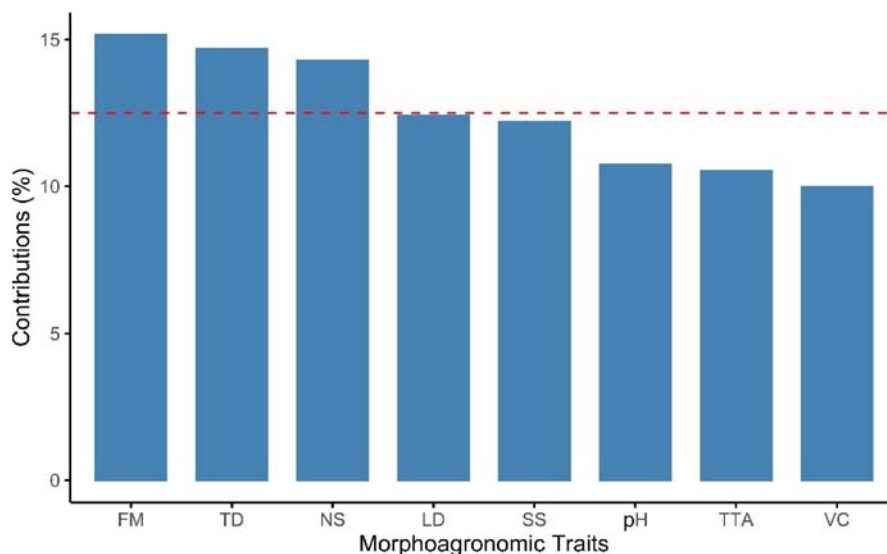

Figure 4. Percentage contributions of the eight measured morpho-agronomic traits to the first three components in the principal component analysis. FM, fresh mass; TD, transverse diameter; NS, number of seeds; LD, longitudinal diameter; SS, soluble solids content; TTA, total titratable acidity; VC, vitamin C content. The red line indicates the mean contribution of the eight morpho-agronomic traits.
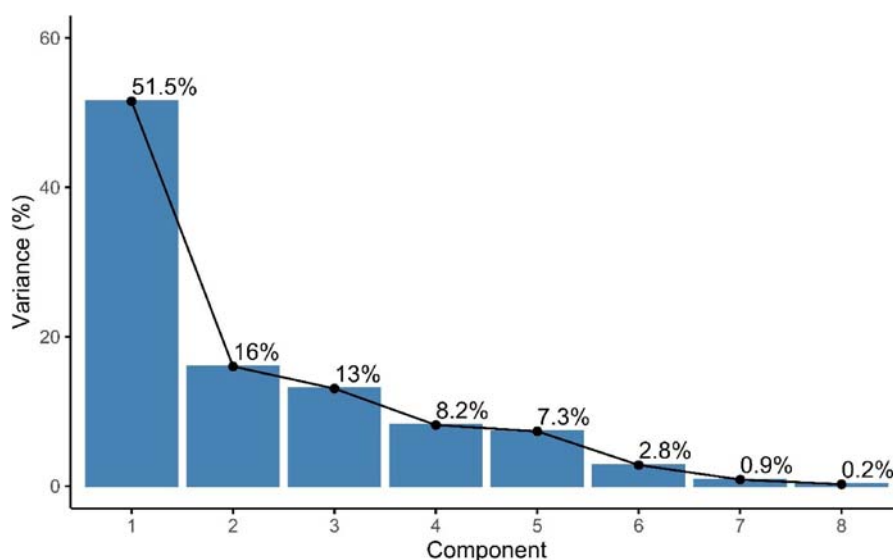
Figure 5. Scree plot of the percentage of variance explained by the eight principal components in the principal component analysis for the 36 studied genotypes of *Hancornia speciosa* Gomes.

Cluster analysis

UPGMA cluster analysis based on Euclidean distance was used to divide the available data into groups of increasing dissimilarity among the studied mangabeira germplasm (Figure 6). When the coefficient of cophenetic correlation reaches 80%, strong clustering consistency is indicated. Five clusters were identified in the dendrogram, with the two main clusters containing 17 and 16 genotypes, respectively, and the other three clusters containing only one genotype each.

The group that comprised genotype 2 showed the highest FM across all evaluated genotypes, while the group that comprised genotype 18 had the highest soluble solids content and the group that comprised genotype 12 had the highest vitamin C content. Thus, genotypes 2, 12 and 18 can be considered possible sources of favourable alleles and could be used in the formation of new populations in breeding programmes.

The group that comprised 16 genotypes included all genotypes from the CA accession and exhibited variation in FM of 8.80–17.26 g (mean = 13.19 g), vitamin C content of 292.66–492.04 mg·100 g^{-1} FM of ascorbic acid (mean = 410.09 mg·100 g^{-1} FM of ascorbic acid) and soluble solids content of 15.10–20.37 °Brix (mean = 17.05 °Brix). By contrast, the group comprising 17 genotypes showed variation in FM of 18.25–32.22 g (mean = 23.82 g), vitamin C content of 216.68–458.67 mg·100 g^{-1} FM of ascorbic acid (mean = 363.85 mg·100 g^{-1} FM of ascorbic acid) and soluble solids of 14.73–19.90 °Brix (mean = 18.32 °Brix). Genotype 4 was prominent in this group for having the highest values for total titratable acidity and FM.

A 3D scatter plot based on PC1, PC2 and PC3 supported the results of the UPGMA cluster analysis, with genotypes being distributed throughout the plot (Figure 7). Thus, it was broadly possible to show which genotypes had the most variant profiles across the range of evaluated accessions.

Table 4. Scores for the 36 genotypes of *Hancornia speciosa* Gomes studied in association with the first three principal components (PC1–PC3).

Genotype	Accession	Score[a]		
		PC1	PC2	PC3
1	TC	2.42	−0.22	0.48
2	TC	5.17	−2.03	−0.88
3	TC	0.90	−2.32	0.14
4	TC	2.17	3.14	−0.18
5	TC	−0.79	−1.27	−1.06
6	TC	2.29	0.93	0.01
7	PR	0.51	−0.29	0.71
8	PR	2.70	0.17	−0.65
9	PR	−1.61	1.78	0.38
10	PR	0.20	−1.21	0.36
11	PR	−0.12	0.43	−0.39
12	PR	−1.35	2.01	−1.93
13	PT	0.89	0.77	−0.02
14	PT	2.90	−0.76	−0.99
15	PT	−2.35	−0.51	−0.53
16	PT	2.91	0.18	−0.13
17	PT	0.79	0.16	0.65
18	PT	2.48	−0.21	3.83
19	LG	1.39	−0.90	−2.24
20	LG	0.39	0.64	0.84
21	LG	2.01	1.01	−0.16
22	LG	0.09	0.76	0.89
23	LG	−1.83	−0.41	−0.90
24	LG	1.29	0.35	−0.46
25	BI	−2.03	0.28	−1.03
26	BI	−1.53	1.14	0.36
27	BI	−2.93	0.08	0.91
28	BI	−0.31	−0.07	−0.25
29	BI	−1.71	−0.10	0.70
30	BI	−1.54	1.11	0.09
31	CA	−2.85	−1.76	−0.32
32	CA	−2.52	0.71	−0.50
33	CA	−2.13	−1.63	0.61
34	CA	−1.99	−1.19	0.58
35	CA	−1.59	−0.37	1.58
36	CA	−2.33	−0.44	−0.52

Accession: BI, Barra do Itariri; CA, Costa Azul; LG, Lagoa Grande; PR, Preguiça; PT, Pontal; TC, Terra Caída. [a] Scores are projections of each genotype onto this new coordinate system of principal components.
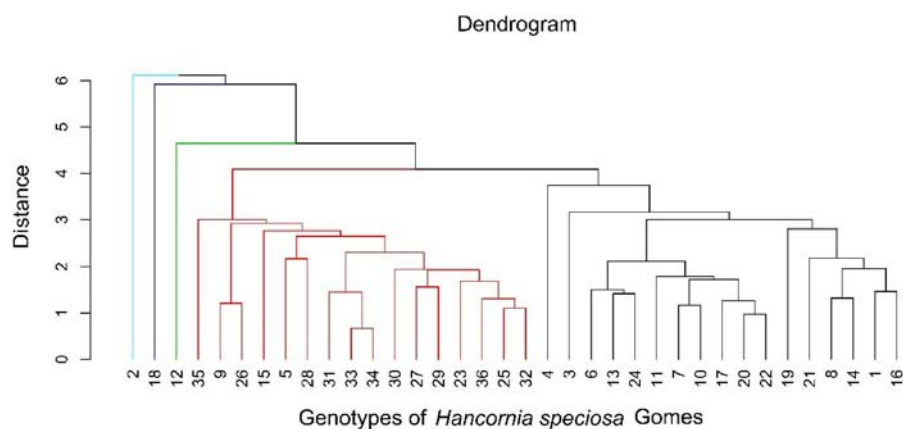
Figure 6. Dendrogram showing the clusters obtained by the unweighted pair group method with arithmetic mean (UPGMA method) based on Euclidean distances for the 36 studied genotypes of *Hancornia speciosa* Gomes.
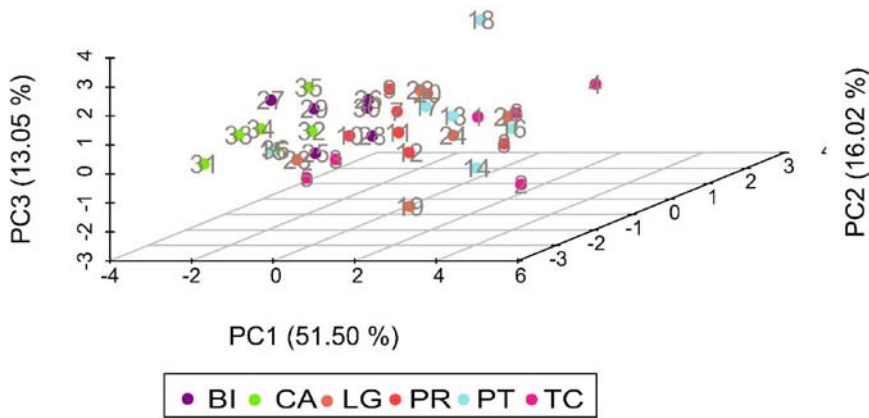
Figure 7. Dispersion of 36 *Hancornia speciosa* Gomes genotypes across the first three principal components (explaining 80.6% of the total variability) based on morpho-agronomic traits. Accessions: BI, Barra do Itariri; CA, Costa Azul; LG, Lagoa Grande; PR, Preguiça; PT, Pontal; TC, Terra Caída.

The recorded genetic divergences allow recommendations to be made for crosses between and within the groups formed by cluster analysis, which is a useful tool for breeders. Genetically different individuals should be used in crosses to obtain progeny that express transgressive segregation profiles (Rêgo et al. 2011; Maia et al. 2018). Thus, the best performing genotypes from the sampled germplasm can be prioritised in future crosses for traits that are of use for the production of processed mangaba fruit products.

Conclusion

This study provided a detailed analysis of a series of mangaba genotypes based on the morpho-agronomic traits of the fruit, which proved valuable for determining the level of genetic diversity. Phenotypic variability can be considered a potential germplasm resource that can be explored in appropriately structured pre-breeding and breeding programmes. The studied genotypes form an excellent resource with respect to high vitamin C content, and may be of particular value for cultivation and/or for developing cultivars with greater horticultural and commercial potential. Therefore, this investigation of variability in the mangaba germplasm is of great importance for the evaluation, management and utilisation of this native Brazilian species.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

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