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Artigo Científico

Physical-chemical and biochemical characterization in biribazeiro fruits through multivariate analysis

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Abstract

The present study aimed to select matrices of biribazeiro based on physical, physicochemical and biochemical characteristics of its fruits, in order to establish criteria for the genetic improvement program of this species, through the use of multivariate techniques. The UPGMA and main component cluster analysis using biplot were performed in Statística 7.7 software. The characteristics evaluated were: fruit weight, longitudinal and transverse diameter, peel thickness, seed number, spike size, pulp weight, fruit firmness, soluble solids, titratable acidity, SS / AT ratio, pH, starch, compounds total phenolics, antioxidant activity and vitamin C. The analysis of clusters brought together the matrices in four heterotic groups on the physical and chemical attributes of the fruits. The analysis of the first two main components represented 58.88% of the total data variation and allowed to correlate matrices according to the angle and length of their vectors to the most relevant characters. Based on the results obtained, it can be concluded that the G33, L89, D15 and K24 matrices are characterized by the high soluble solids content, while the matrix E18 presents high potential due to the higher content of bioactive compounds. Regarding the physical characters, highlight materials E23, J08 and C27 due to the higher weight of the pulp. The techniques of multivariate analysis used are consolidated as fundamental tools for the visualization and selection of the promising matrices, aiming at the genetic improvement of the biribazeiro.

Keywords: *Rollinia mucosa* [Jacq.] Baill., exotic fruit, biplot, dendrogran.

Caracterização física, físico-química e Bioquímica em frutos de biribazeiro através através de análise multivariada

Resumo

Este estudo teve como objetivo selecionar matrizes biribazeiro com base nas características bioquímicas de frutas física, físico-química e, a fim de estabelecer critérios para programa de melhoramento genético da espécie, utilizando características técnicas multivariadas. A análise de cluster UPGMA e o componente principal usando biplot foram realizados no software estatístico 7.7. As características analisadas foram o peso do fruto, longitudinal e transversal de diâmetro, a espessura da casca, o número de sementes de tamanho espiga polpa peso firmeza de sólidos solúveis de frutas, SS / AT, o pH, compostos de amido relação acidez titulável Fenólicos totais, atividade antioxidante e vitamina C. A análise de agrupamento reuniu as matrizes em quatro grupos heteróticos sobre os atributos físicos e químicos dos frutos. A análise dos dois primeiros componentes principais representou 58,88% da variação total dos dados e permitiu correlacionar as matrizes de acordo com o ângulo e comprimento de seus vetores com os caracteres mais relevantes. Com base nos resultados obtidos, pode concluir-se que G33, matrizes L89, D15 e K24 são caracterizados por um elevado teor de sólidos solúveis, enquanto que a matriz E18 tem um elevado potencial devido ao maior teor de compostos bioactivos. Quanto aos caracteres físicos, destacar os materiais E23, J08 e C27 devido ao maior peso da polpa. As técnicas de análise multivariada utilizadas consolidam-se como ferramentas fundamentais para a visualização e seleção de matrizes promissoras, com o objetivo de melhorar a genética do biribazeiro.

Palavras-chave: *Rollinia mucosa* [Jacq.] Baill., frutas exóticas, biplot, dendrograma.

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Caracterización física, físico-química y bioquímica en frutos de biribazeiro através del análisis multivariada

Resumen

Este estudio tuvo como objetivo seleccionar matrices biribazario con base en las características bioquímicas de frutas física, físico-química y, a fin de establecer criterios para programa de mejoramiento genético de la especie, utilizando características técnicas multivariadas. El análisis de cluster UPGMA y el componente principal utilizando biplot se realizaron en el software estadístico 7.7. Las características analizadas fueron el peso del fruto, diámetro longitudinal y transversal, el espesor de la cáscara, el número de semillas, tamaño de espiga, peso de la pulpa, firmeza, sólidos solubles, SS / AT, el pH, compuestos de almidón de acidez titulable, fenólicos totales, actividad antioxidante y vitamina C. El análisis de agrupamiento reunió las matrices en cuatro grupos heteróticos sobre los atributos físicos y químicos de los frutos. El análisis de los dos primeros componentes principales representó el 58,88% de la variación total de los datos y permitió correlacionar las matrices de acuerdo con el ángulo y longitud de sus vectores con los caracteres más relevantes. Con base en los resultados obtenidos, se concluyó que G33, matrices L89, D15 y K24 se caracterizan por un alto contenido de sólidos solubles, mientras que la matriz E18 tiene un elevado potencial debido al mayor contenido de compuestos bioactivos. En cuanto a los caracteres físicos, se destacaron los materiales E23, J08 y C27 debido al mayor peso de la pulpa. Las técnicas de análisis multivariadas utilizadas se consolidan como herramientas fundamentales para la visualización y selección de matrices promisorias, con el objetivo de mejorar la genética del biribazeiro.

Palabras clave: *Rollinia mucosa* [Jacq.] Baill., frutas exóticas, biplot, dendrograma.

Introduction

Fruticulture is an area in constant development, especially as regards the new cultivation options, both for the search of the producers and for the demand of new varieties of fruit by the consumers, contributing for the expansion of production and market of the exotic fruits (SEREJO et al., 2009). In addition, biriba has a high market potential.

The biribazeiro (*Rollinia mucosa* [Jacq.] Baill), is a fruit belonging to the family of Annonaceae with about 8-10 m high, native to the Amazon region and Atlantic Forest (FERREIRA and RIBEIRO, 2006). It is mainly consumed, in the in natura form, in the form of pulps, ice creams and presents good perspectives to be explored economically (LORENZI, 1998; FERREIRA et al., 2010).

The quality of the fruit against the sweet taste and exotic aroma, the rusticity of the plants in establishing themselves in any type of soil, the fast growth and the fruiting around the 4 years (CAVALCANTE, 1996) have aroused the interest of producers by this culture in the most diverse regions of Brazil (FERREIRA et al., 2009).

Knowledge about diversity and genetic relationships between accesses is a fundamental task in breeding programs (CARDONA, 2010), through the creation of new genotypes based on techniques that aid in the identification and selection of superior genotypes (MACHADO, 2014).

The multivariate statistics is one of the techniques that has propelled the increase in the studies on genetic divergence in several areas of the agrarian sciences (Alencar et al., 2013). According to Mingoti (2007), multivariate techniques allow the integration of multiple information from a set of data, offering greater choice of divergent parents and the characters that contribute most to genetic variability in breeding programs. Among the multivariate statistical techniques available to analyze plant genetic diversity, we highlight clustering methods (RODRIGUES et al., 2010) and principal component analysis (MIGUEL et al., 2011).

The identification and selection of genetic material that produces fruits with good physical and physico-chemical characteristics, appropriate to the commercialization and adapted to the local conditions, is of great importance for the culture of the biribazeiro given its market potentiality. Therefore, the present study aims at the physical, physicochemical and biochemical characterization of biribazeiro fruits collected in different matrices in order to establish criteria for the genetic improvement program of this species, using the multivariate method of clustering and principal components.

Material and methods

The work was carried out in a commercial orchard of biribazeiro located under the geographic coordinates 03º 44 '00 "south latitude and 52º 88' 00" west, located in the municipality of Tomé-Açu, in the northeastern region of the state of Pará. According to Koppen's classification, the Aw3 type is characterized as hot and humid, and rainfall is determined by two distinct periods of rainfall, one from December to May, with rates above 170 mm / month, where about 80 % of the total annual precipitation, and another, from June to November, with index ranging from 61 mm to 115 mm (IDESP, 2011).

Orchard irrigation is performed by drip irrigation and receives annual fertilization with NPK in the 10:10:10 formulation by means of soil and leaf fertility analysis, with the plants distributed in a spacing of 7x7 meters.

The fruits were collected at the peak of the 2016/2017 crop, on the same day and at the physiological maturation stage of 16 maize bioregulary plants, aged 08 years old, phenotypically selected and coded according to their distribution in the orchard: A09, A23, B06, B13, C27, D15, D29, E18, E23, F61, G33, H42, I17, J08, K24 and L89.

In the analyzes, the experimental design was completely random, and 12 mature fruits of each plant were collected (4 replicates with 3 fruits each) and transported in thermal boxes to the Post Harvest Technology Laboratory of the Federal University of Pará, Altamira Campus, PA and evaluated the following physical, physicochemical and biochemical variables:

The fruits and pulp weight were determined using a semi-analytical balance of 0.1 g and the results were expressed in grams (g). Measurements related to longitudinal and transverse diameter, bark thickness and spike size were made using a digital caliper and the results were expressed in millimeters (mm). The number of seeds was quantified by direct counting inside the fruits being determined in units (und).

The firmness of the fruit was carried out with the aid of the Texture Analyzer CT3 (Brookfield) apparatus, at a penetration rate of 6.9 mm s-1 at a depth of 7 mm. The analyzes were performed in the equatorial region and on opposite sides of each fruit, the values being expressed in Newtons (N).

Soluble solids content was measured by direct refratometric reading under the fruit pulp previously macerated and homogenized in a refractometer Quimis brand digital refractometer, according to AOAC recommendations (2012) and results expressed in degrees Brix (° Brix).

The titratable acidity content, expressed as grams of citric acid per 100 g of pulp, was determined by the known mass titration of homogenized pulp and diluted with distilled water with a standard solution of 0.1 M sodium hydroxide, to 1% phenolphthalein, following the AOAC recommendation (2012).

The ratio determined by the ratio between the soluble solids and the acid content present in the fruit pulp (SS/AT) and the results expressed in whole numbers with two decimal places.

The pH was measured using a digital potentiometer (DMOH 2) calibrated with solutions of pH 4.0 and 7.0 and the reading was carried out directly on the sample juice, as recommended by AOAC (2012).

The starch content was determined enzymatically as described by (CORDENUNSI and LAJOLO, 1995). The starch was extracted with 0.5N sodium hydroxide, neutralized with acetic acid (5N), precipitated with 80% ethanol, hydrolyzed with amyloglucosidase (14 U/ml) and the released glucose determined by the glucose oxidase / peroxidase / ABTS system, according to the method of Bergmayer (1974), the results being expressed as mg starch/gw fruit weight.

The total phenolic compounds were determined using the Follin-Ciocalteau spectrophotometric method (XU and CHANG, 2009). 1 ml aliquots of extract were transferred to 10 ml flasks containing 5 ml of distilled water. 1 ml of 25% sodium carbonate and 1 ml of the Follin-Ciocalteou reagent were added, making up to volume with distilled water. After 30 minutes, the absorbance at 760 nm was determined in a spectrophotometer. The results were expressed as mg of gallic acid equivalents kg^{-1}MF.

The total antioxidant capacity of the fruits was determined by the methodology of DPPH according to AOAC (2012) with spectrophotometric reading of the aliquots at the wavelength of 734 nm and the results expressed in EC50 (µg/mL).

The content of vitamin C was determined by the method proposed by Chen and Wang (2002) in a spectrophotometer at 525 nm, the results being expressed in the calibration curve in $g.100g^{-1}$ of ascorbic acid.

Data were submitted to cluster analysis, using the hierarchical method of linkage between groups (UPGMA), using Euclidean distance and principal component analysis. The analyzes were obtained using Statistica software (STATSOFT, 2004).

Results and discussion

The cluster analysis by the agglomerative mean unweighted linkage method (UPGMA) based on the mean euclidean distance is represented by the dendrogram (Figure 1), where it is verified that the 16 matrices of biribazeiro were grouped in four heterotrophic groups, evidencing the presence of genetic variability in the fruits of this species.

The cut in the dendrogram (Figure 1) was performed at 30%, at which point abrupt level changes were observed, as recommended by Cruz et al. (2004).

The matrix E23 (Figure 1) was isolated, indicating that it possesses specific characteristics, being evidenced mainly by the smaller thickness of the house and the firmness of the fruits (Table 1). For the commercialization, there is a preference for nuts (Fig. 1). The results obtained in this work are presented in Fig. However, the lower firmness of the fruit may compromise its storage during commercialization, not being a desirable post-harvest character.

The matrices C27 and J08 (Figure 1) are characterized by the greater weight of the fruits (>1,200 kg) and consequently of the pulp weight and the

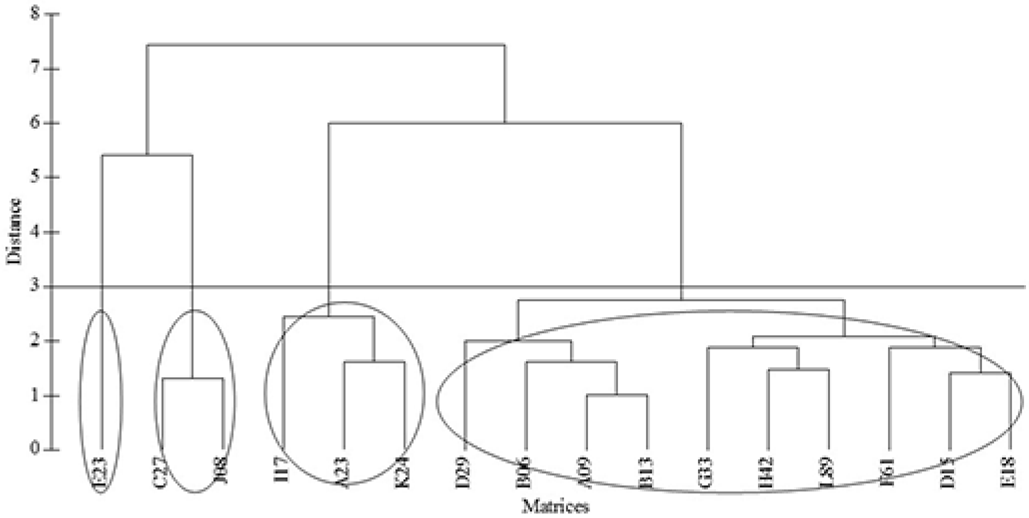

Figure 1. Genetic distance dendrogram generated by the UPGMA grouping method among 16 matrixes of biribazeiro, obtained by physical, physical-chemical and biochemical characteristics of the fruits. Tomé-Açu, PA.

longitudinal transversal diameters of the fruits (Table 1).

The absence of spicules on the bark, seeds inside the fruits and the average weight ranging from 400 to 600 g (Table 1) were the most similar characters in the grouping of the I17, A23 and K24 matrices (Figure 1).

According to Santos et al. (2014), the fruits of the biribazeiro present excellent organoleptic characteristics of consumption, be it in natura or processed form for the elaboration of ice cream, juices, etc. Thus, in the breeding program of this species it is possible to opt for fruits with greater weight and size aiming at the market of pulp or fruits with average weight thus attending the in natura consumption, being that fruits without seeds, may be more attractive to the commercialization.

Also according to Figure 1, it is observed that the largest grouping combined the matrices D29, B06, A09, B13, G33, H42, L89, F61, D15 and E18 through the physical, physicochemical and biochemical characteristics. Thus, the matrices A09, B13, B06, D29 and F61 were characterized mainly by the lower weight of the fruit, larger spike size and greater firmness of the fruit, and the higher acidity was observed mainly in matrices D29 and B06, H42 matrix presented higher shell thickness (Table 1).

The G33, L89, D15 and E18 matrices were more associated with chemical and biochemical characterization, with a higher accumulation of soluble solids (sugars), a lower acidity and a higher flavor ratio between SS / AT in fruit pulp, concentration of total phenolic compounds, antioxidant activity and vitamin C (Table 1).

Andrade et al. (2008) and Santos et al. (2011) also identified heterogeneous groupings by the UPGMA method in the physical and physicochemical characterization of rambutanzeiros and cupuaçuzeiros accesses, evidencing that these characteristics allow to distinguish promising materials for the improvement of these broad-cultivated species in Amazonia.

Table 1. Original means on the physical, physical-chemical and biochemical characters obtained in 16 matrix plants of biribazeiro. Tomé-Açu, PA.

Matrix	PF	DL	DT	EC	NS	PP	TE	FF	SS	AT	SS/AT	pH	AM	FT	AAT	VC
A09	*246.46*	4.24	5.14	0.21	47	79.73	*2.04*	*115.24*	*13.46*	*0.65*	*15.26*	4.44	13.76	240.90	16.20	19.63
A23	*537.23*	6.12	7.34	0.16	*0*	263.72	*0*	103.29	9.47	1.21	12.92	4.65	*16.87*	291.88	21.49	24.82
B06	*278.87*	4.32	5.21	0.43	51	88.19	*2.21*	*129.72*	10.22	*1.03*	12.86	4.50	14.68	205.93	11.13	13.78
B13	*352.30*	5.44	5.28	0.23	54	93.88	*1.91*	*127.03*	*15.87*	*0.22*	*19.20*	4.52	9.17	245.88	16.21	20.02
C27	*1,216.43*	*11.59*	*12.88*	0.19	23	*373.93*	0.59	106.02	10.81	1.11	12.42	4.44	14.26	150.50	7.96	11.21
D15	391.67	6.35	7.54	0.22	75	118.58	0.74	95.11	*18.29*	*0.09*	*25.62*	3.33	7.68	310.45	23.86	25.19
D29	*342.44*	5.26	5.62	0.33	75	191.18	*2.11*	*125.82*	10.49	*0.99*	11.40	4.41	14.5	205.27	13.34	17.61
E18	325.84	4.26	5.68	0.41	62	107.18	1.23	124.68	*16.83*	*0.13*	*24.91*	4.33	7.37	*786.37*	*52.84*	*57.05*
E23	*1,189.21*	10.32	*11.61*	*0.13*	19	355.18	0.33	*80.06*	13.42	0.67	14.98	4.61	13.96	224.16	11.18	13.90
F61	358.30	5.14	5.48	0.65	71	178.16	*2.12*	*121.26*	12.27	0.84	14.35	4.61	12.89	352.73	28.14	31.64
G33	357.10	4.38	5.27	0.28	45	116.24	0.28	97.96	*18.12*	*0.07*	*25.90*	3.74	5.09	*518.85*	*41.74*	*44.60*
H42	546.51	5.11	5.92	*0.84*	27	114.88	0.35	103.68	10.37	0.99	11.91	4.51	12.84	*506.62*	*39.06*	*43.69*
I17	*583.83*	7.56	8.39	0.61	0	177.16	0	127.74	11.24	0.91	13.47	4.40	15.60	282.07	29.22	33.31
J08	*1,262.75*	*11.88*	*12.32*	0.19	64	*389.96*	0.41	89.56	11.86	0.89	13.69	3.94	15.37	207.63	10.82	15.01
K24	425.84	6.22	7.62	0.17	*0*	168.12	*0*	108.30	*18.08*	*0.08*	*25.72*	4.71	5.01	398.51	33.74	37.50
L89	395.68	4.77	5.28	0.19	23	98.78	0.47	94.64	*18.17*	*0.18*	*25.76*	3.85	6.89	326.59	25.88	29.21

PF = weight of the fruit (g), DL = longitudinal diameter (cm), DT = transverse diameter (cm), EC = peel thickness (cm), NS = number of seeds (und), PP = pulp weight (g), TE = spike size (cm), FF = fruit firmness (N), SS = soluble solids (° Brix), AT = titratable acidity (% citric acid), SS/AT = solids solubles/titratable acidity (und), pH = hydrogenation potential (H +), AM = starch (mg amido.g^{-1} peso fruit), FT = total phenolics (mg of gallic acid (EAG).kg^{-1} MF), ATT = total antioxidant activity (EC50 (µg mL), VC = vitamin C (g.$100g^{-1}$ ascorbic acid).

Table 2 presents the main component analysis obtained for the 16 evaluated characters. It is observed that the total variation in the fruits of the matrix of biribazeiro is explained in 58,88%, by two main components. Major component 1 (CP1) accounts for 37,15% of variability and main component 2 (CP2) accounts for 21,73%.

According to Silva and Benin (2012), minimum estimates of 70% of the total variation, among the initial components, allow to explain satisfactorily the manifested variability among the evaluated characters. In this work, the sum of the accumulated variance between CP1 and CP2 is 58,88%, this low magnitude of the correlation estimates between the two initial components reflects the complexity of the relationships between the characteristics analyzed. In addition to that, the biplot representation using only the first two main components, facilitates the visualization of the associations among characteristics and the identification of the prominent matrices for each of them.

In general, this percentage is still higher than that obtained by Maciel et al. (2010), that in the physical-chemical characterization of fruits belonging to 18 genotypes of cherry trees, where the total variation observed in the first two components was responsible for retaining 53,87% of the data. Carvalho Júnior (2015), when characterizing physical and physical-Chemically fruits of 26 genotypes of pink mango obtained 50,67% of the total variation of the data in the first two main components.

According to Cruz et al. (2011), using the analysis of main components can identify the characters that contribute more or less to the accumulated variation, considering as characteristics those with values greater or close to 0,600. For Villelaet al. (2014), the largest value of the variables, in module, also represents a strong contribution of the characteristic acting on that main component. Thus, the characters with the greatest contribution to the variability in main component 1 (CP1) were: longitudinal and transverse diameter, spike size, soluble solids, SS/AT ratio, starch, total phenolics and antioxidant activity. The second component (CP2) presented the characteristics of pulp weight, firmness of the fruit, soluble solids and titratable acidity (Table 2).

Table 2: Eigenvectors and eigenvalues associated with the characters and the first two main components (CP1 and CP2), estimated in fruits of 16 matrices of biribazeiro. Tomé-Açu, Pará.

Characters	CP1	CP2
Weight of the fruit (g)	-0,21	-0,44
Longitudinal diameter (cm)	0,79	0,39
Transverse diameter (cm)	0,74	0,52
Shell thickness (mm)	-0,5	-0,28
Number of seeds (und)	-0,2	-0,17
Spiral size (cm)	0,73	0,56
Weight of pulp (g)	0,04	-0,72
Firmness of fruit (N)	-0,01	-0,77
Soluble solids (° Brix)	-0,62	0,60
Titratable acidity (% citric acid)	0,59	-0,68
Ratio SS/AT	-0,78	0,46
pH (H $^{+}$)	0,51	-0,4
Starch (mg starch.g^{-1} fruit weight)	0,84	-0,2
Total phenolics (mg gallic acid. Kg^{-1}MF)	-0,76	0,00
Antioxidant activity (EC50 µg / mL)	-0,77	-0,07
Vitamin C (g.100g^{-1} ascorbic acid)	-0,58	-0,15
Eigenvalue	5,94	3,47
Cumulative variance (%)	*37,15*	*21,73*

Figure 2 shows the biplot plot, in which the main components 1 and 2 were plotted.

In general, it is observed that the matrices E23, J08 and C27 are strongly correlated to the characteristics of pulp, lateral diameter and transversal diameter (Figure 2). These characteristics are interesting for the agroindustry of pulp and its derivatives, given the size of these fruits (Table 1). In a close comparison with the fruits of the soursop, a species belonging to the same family of the biribazeiro, Araújo Filho et al. (2008) report that graviola with an average weight ranging from 1,5 to 2,0 kg present market potential to be used in processing, since fruits with these dimensions present irregular production volumes, making it difficult to export.

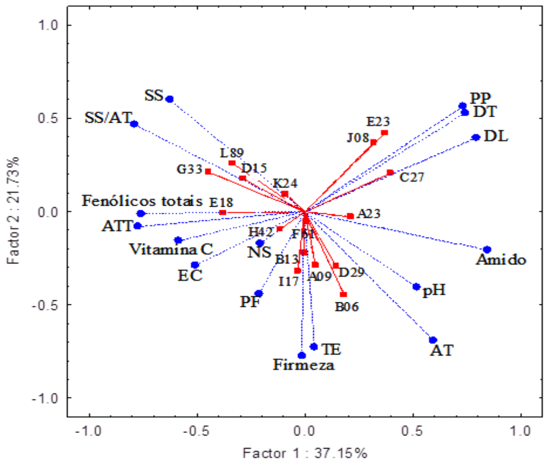

Figure 2. Biplot graph with 16 physical, physical-chemical and biochemical characters analyzed in 16 matrices of biribazeiro. Tomé-Açu, PA. matrices ■ of biribazeiro, variables ●. SS = soluble solids, AT = titratable acidity, SS/ AT = soluble solids ratio / titratable acidity ATT = total antioxidant activity, PF = fruit weight, DL = longitudinal diameter, DT = transverse diameter, EC = number of seeds, PP = pulp weight, TE = spike size.

The G33, L89, D15 and K24 matrices are characterized by a high correlation with the content of soluble solids (sugars) dissolved in their pulp, as well as the ratio (SS/AT), through the highest concentration of sugars (SS) and less than (TA), which is on the opposite side of the biplot projection (Figure 2), indicating low correlation with the fruit flavor, such characteristics allow such matrices to be exploited, either for the development of fruits for the natural consumption or processing. In summary, these matrices had soluble solids content higher than 18.0 ° Brix (Table 1), which is higher than that verified by Ribeiro et al. (2016) in analysis with fruits of biriba whose maximum verified level was of 17.0 ° Brix.

The E18 matrix showed an expressive correlation as a function of the angle and length of the vector associated with the characters, total phenolic compounds, antioxidant activity and vitamin C content in the fruit pulp (Figure 2).

According to Dembitsky et al. (2011), fruits are generally considered important sources of macro and micronutrients, but also have other compounds with bioactive properties, called phenolic substances, and are represented by their antioxidant role on the cells and vitamins in general, among which the vitamin

C is highlighted.

According to Kim et al. (2008), these phenolic compounds have numerous benefits for plants (growth, seed germination, systemic defense against pests, increase of sensorial properties, among others). In humans, these substances inhibit the oxidative stress of cells protecting the human body against different chronic diseases, such as obesity, diabetes, premature aging, heart problems, cancers, among others (MALTA et al., 2012; NIKI, 2010).

In this context, the selection of this matrix for breeding programs is fundamental, since the benefits associated with phenolic substances provide innumerable benefits both for the plant itself and for the human being.

The matrices A09, B06, B13, D29, F61, H42 and I17 correlation angle with physical and chemical characters, such as lower fruit weight and higher firmness index, spike size and titratable acidity (Figure 2). With the exception of the spike size, which is a peculiar characteristic of the fruits of the biribazeiro, fruits with small size (weight), very firm and acid may not be desirable characteristics for the improvement of this species that seeks its insertion in the market, mainly for the consumption in natura, like others of the same family as the pine cone, atemoia and cherimoia.

The correlation angle between the A23 matrix and the starch content character (Figure 2) shows that the fruits present a high content of pectic substances that delay the hydrolysis of starch in sugars during maturation, thus, the fruits of this matrix do not present potential to be consumed in natura form.

Conclusions

The clustering analysis by the UPGMA method and the biplot analysis are presented as fundamental tools for the visualization of the interrelations between the matrices of biribazeiro and the characters evaluated.

Through the obtained results, it is possible to discriminate the matrices with specific characteristics. If the interest of the improvement is for sweet fruits, the matrices G33, L89, D15 and K24 deserve attention. For the increment of bioactive compounds, such as total phenolics and vitamin C, the matrix E18 presents high potential. The matrices E23, J08, C27 important requirements regarding the weight of the pulp and the fruits aiming at the market in natura and pulps. Thus, these materials are promising for the practice of selection for the installation of new crop plantations.

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