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Genetic divergence among *Psidium* accessions based on biochemical and agronomic variables

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ABSTRACT - *Sixty-nine* Psidium *accessions collected in six Brazilian states were analyzed by two non-hierarchical clustering methods and principal components (PC), to provide orientation for breeding programs. The variables ascorbic acid, β-carotene, lycopene, total phenols, total flavonoids, antioxidant activity, titrable acidity, soluble solids, total soluble sugars, moisture content, lateral and transversal fruit diameter, fruit pulp and seed weighs, and plant fruit number and weight were analyzed. Specific groups were observed for the araçazeiros accessions, by the Tocher and the k-means methods, as well as by the three-dimensional dispersion of the four PCs. The clustering separated accessions of araçazeiros from the guava. There was no specific grouping in terms of States of origin, indicating the absence of barriers in the guava propagation accessions. Analyses suggested the collection of a greater number of guava germplasm samples from a smaller number of regions and divergent accessions with high nutritional compound levels to develop new cultivars.*

Key words: Psidium guajava*; antioxidants; nutritional compounds; multivariate analysis.*

INTRODUCTION

In Brazil, in 2007 guava ranked 11^{th} in cultivated area and production value, 10^{th} in yield and 5^{th} in productivity among other important fruits such as orange, banana, grapes, papaya, apple, mango, tangerine, passion fruit, lemon, peach, persimmon, avocado, fig, pear, and quince. The main guava-producing states in 2007 were Pernambuco, São Paulo, Goiás, and Bahia, which together accounted for almost 75 % of the national production (http://www.sidra.ibge.gov.br).

Guava (*Psidium guajava*) belongs to the Myrtaceae family, with approximately 130 genera and 3000 species of trees and shrubs distributed mainly in the tropics and subtropics (http://delta-intkey.com). Guava is native to northern South America and widely distributed in the tropical regions of America (Risterucci et al. 2005). Other fruits of the genus *Psidium*, with about 150 species, are the Brazilian guava called araçazeiros, a term that refers to native *Psidium* species, with *P. cattleyanum* Sat., *P. incanesces Martius*, *P. gradiflorum Martius*, and *P. arboretum* Vell (Raseira and Raseira 1996). Although not as interesting as guava, Brazilian guava species have some important attributes such as earliness, tolerance to certain pests and diseases and exotic fruit flavor (Manica 2000).

Despite being regarded as one of the diversity centers of guava and araçazeiros (Soares-Silva and Proença 2008), Brazil has few research institutions with gene banks for this *Psidium* species which, in total, have compiled 310 accessions and 174 accessions of Brazilian guava. Important cultivars for the Brazilian guava agribusiness are Paluma, Rich, Sassaoka and Pedro Sato, which were developed

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from selections of producers or research institutions in open-pollinated seed orchards (Pommer et al., 2006).

The available collections of guava and araçazeiros in Brazil have been almost exclusively characterized for agronomic traits and only few studies focused on the chemical characterization, e.g., the paper of Lima et al. (2002). The importance of studies of functional compounds in guava can be exemplified by lycopene, the carotenoid responsible for the red color of the guava pulp. According to Yan et al. (2006), lycopene is considered an excellent antioxidant, with a high resistance potential to diseases such as prostate cancer and arteriosclerosis. According to these authors, the lycopene level found in the guava cultivar Paluma is the double found in tomatoes.

For Pereira and Nachtigal (2003) the aim of the improvement of guava characteristics would be an average fruit weight > 100 g, a red pulp and pulp yield > 70 %, soluble solids > 10 °Brix, sugar/acidity ratio > 11, vitamin C content of around 100 mg of ascorbic acid per 100 g of pulp and a minimum production of 30 t ha^{-1}.

The agronomic descriptions of guava collections in Brazil (Souza Junior et al. 2002, Gonzaga Neto 2002, Santos et al. 2008, Silva Junior et al. 2008) did not establish estimates of genetic distances between accessions, to support controlled crossings and the development of cultivars with improved traits, including higher levels of nutritional compounds. In the international literature divergence studies have been published based on morphological traits for collections of Mexican (Hernandez-Delgado et al. 2007) and Cuban accessions (Rodriguez et al. 2004).

The purpose of this study was to group accessions belonging to the *Psidium* gene bank of Embrapa Semiarid by multivariate techniques based on biochemical and agronomic variables, to provide orientation for genetic breeding programs of the genus *Psidium*.

MATERIAL AND METHODS

Fruits of 59 guava (*P. guajava*) and 10 Brazilian guava or araçazeiros (*Psidium* spp.) accessions of the field germplasm bank of *Psidium* (Table 1) installed in the experimental field Bebedouro of Embrapa Semiarid were collected. The accessions were sampled at physiological maturity in six different Brazilian states, as described by Santos et al. (2008). Each accession was established in the field from four-month-old seed-grown seedlings, in January 2007. The data of six plants were evaluated, from two replications with three plants each. The accessions were managed according to the regional practices for commercial guava production, using a drip irrigation system in a plant spacing of 4.0 m x 4.0 m. To induce fruiting, the plants were pruned in August 2008 and harvest began in December 2009.

Psidium accessions were characterized for 16 variables. Antioxidant variables: ascorbic acid (ASA), mg g^{-1} MF, determined by the method proposed by Carvalho et al. (1990); β-carotene(BCT), mg 100g^{-1} MF, and lycopene (LYC), mg 100 g^{-1} MF, according to the methodology used by Nagata and Yamashita (1992); total phenols (PHEN), mg g^{-1}EAG MF, determined as proposed by Alothman et al. (2009), total flavonoids (FLV), rutin mg 100g^{-1} MF, based on the method of Lombard et al. (2002), antioxidant activity (AOX), AAS mg g^{-1} MF, determined as proposed by Mensor et al. (2001).

Biochemical variables: acidity, % citric acid was determined by titration method proposed by the Instituto Adolfo Lutz (1985); soluble solids (%), ºBrix, determined using a digital refractometer, according to standard techniques of the Instituto Adolfo Lutz (1985), total soluble sugars, % M, quantification by the anthrone method (McCready et al. 1950); moisture content, % H_2O determined as weight of oven-dried (under forced air circulation at 65 °C to constant weight) fresh fruit.

Variables of agronomic characters: lateral fruit diameter, mm; transversal fruit diameter, mm; yield per plant, g; seed weight per fruit, g; number of fruits per plant and pulp weight per fruit, g, were obtained from samples taken for these variables every two or three days, during the production cycle.

The data were statistically analyzed by: 1. analysis of variance, 2. principal component analysis, after standardizing the data to exclude the effect of different measurement scales of variables, using SAS (SAS 1989), 3. cluster analysis by the modified Tocher method (Vasconcelos et al. 2007), based on Mahalanobis´ generalized distances, supported by software Genes (Cruz 2008), 4. cluster analysis with standardized data, according to the (SAS) FASTCLUS Procedure (1989), with the formation of six similar groups corresponding to the number of states sampled. The FASTCLUS Procedure uses a method called nearest centroid sorting, where the dissimilarity measure is the mean Euclidean distance, which is a k-means clustering method (SAS 1989).

RESULTS AND DISCUSSION

In the analysis of variance a significant effect ($P < 0.01$) was observed for each of the 16 variables by test F,

Table 1. Identification of 69 accessions of guava (G) and Brazilian guava or araçazeiro (A) of the *Psidium* genebank

Accession	Origin	State	Accession	Origin	State
G01MA	Caxias	MA	A45PE	Escada	PE
G02MA	Caxias	MA	G46PE	Escada	PE
G03MA	Coelho Neto	MA	G47PE	Riacho das almas	PE
G05MA	Buriti	MA	G48SE	Nossa Senhora da Glória	SE
G07MA	Mata Roma	MA	G49SE	Dores	SE
A08MA	Mata Roma	MA	G50SE	Capela	SE
G10MA	Presidente Vargas	MA	G51SE	Capela	SE
G11MA	Presidente Vargas	MA	G52SE	Capela	SE
G12MA	Cajari	MA	G53SE	Japoratuba	SE
G13MA	Viana	MA	G54SE	Japoratuba	SE
G14MA	Pindarí	MA	G55SE	Pirambu	SE
G15MA	Bom Jardim	MA	G58SE	SantaLuzia	SE
G16MA	Bom Jardim	MA	G59SE	Umbamba	SE
G17MA	Santa Luzia	MA	G60SE	Umbamba	SE
G18MA	Santa Luzia	MA	G61SE	Riachão dos Dantas	SE
G19MA	Graiaú	MA	G65RO	Ji-paraná	RO
G20MA	Tuntum	MA	G66RO	Ouro Preto do Oeste	RO
G21MA	Tuntum	MA	G67RO	Jaru	RO
G22MA	Presidente Dutra	MA	G68RO	Buritis	RO
G23MA	Presidente Dutra	MA	G69RO	Buritis	RO
G24MA	Colinas	MA	G70RO	Buritis	RO
G25MA	Colinas	MA	G73RO	Ariquemes	RO
G26MA	Paibano	MA	A78RO	Candeias do Jamarí	RO
G28PI	Colônia Gurqueia	PI	A79RO	PortoVelho	RO
A29PI	Eliseu Martins	PI	A80RO	PortoVelho	RO
G30PI	Canto do Buriti	PI	G81RO	PortoVelho	RO
G31PI	Brejo do Piauí	PI	G83AM	ItacoatiA	AM
G32PE	Ibimirim	PE	G87AM	Iranduba	AM
G33PE	Ibimirim	PE	G92AM	Manacapuru	AM
G34PE	Ibimirim	PE	G94AM	Autazes	AM
G35PE	Ibimirim	PE	G95AM	Autazes	AM
G38PE	Pesqueira	PE	G96AM	Autazes	AM
A42PE	Escada	PE	G98AM	Autazes	AM
A43PE	Escada	PE	A100AM	Careiro	AM
A44PE	Escada	PE			

indicating the existence of phenotypic variability in the 69 *Psidium* accessions studied. In the analysis of variance performed only with the guava accessions the effect was also significant ($P < 0.01$) by the F test for all variables, except moisture content ($p < 0.05$), indicating the existence of phenotypic variability among the 59 *Psidium* guava accessions under study.

The 10 araçazeiros accessions were grouped in a specific cluster when analyzed by the k-means (Table 2), while in the Tocher analysis, these same 10 accessions were grouped into two clusters, one with seven and the other with three accessions (Table 3). Results of this study indicate that the Brazilian guava showed biochemical and agronomic traits that distinguish them from guava accessions, which may be the result of the intense artificial selection in guava for the purpose of agronomic cultivation and fruit quality-related traits. Analyzing the araçazeiros and guava accessions from different Brazilian states for morphological traits, Santos et al. (2008) concluded that the fruit traits e.g., pulp color and fruit size, were the most influenced by the artificial selection in guava.

As discussed by Raseira and Raseira (1996), the term araçazeiro refers to several *Psidium* species with wide distribution in Brazil (Soares-Silva and Proença 2008). In the analysis by the k-means clustering method, all araçazeiros were grouped into a single cluster (Table 2), suggesting that the differences among araçazeiros ecotypes were smaller than of araçazeiros compared to guava accessions.

Table 2. Grouping of accessions of the *Psidium* gene bank of Embrapa Semiarid, analyzed for 16 biochemical and agronomic traits, distributed in six groups by the k-means clustering method based on the Euclidean mean distance

Group	Number of accessions per group	Accessions
Group1	14	G02MA, G05MA, G07MA, G11MA, G14MA, G16MA, G17MA, G18MA, G22MA, G23MA, G31PI, G55SE, G73RO, G98AM
Group2	10	A08MA, A100AM, A29PI, A42PE, A43PE, A44PE, A45PE, A78RO, A79RO, A80RO
Group3	05	G01MA, G03MA, G10MA, G38PE, G47PE
Group4	13	G12MA, G34PE, G51SE, G60SE, G66RO, G67RO, G68RO, G69RO, G81RO, G83AM, G92AM, G95AM, G96AM
Group5	08	G13MA, G21MA, G24MA, G49SE, G50SE, G54SE, G58SE, G94AM
Group6	19	G15MA, G19MA, G20MA, G25MA, G26MA, G28PI, G30PI, G32PE, G33PE, G35PE, G46PE, G48SE, G52SE, G53SE, G59SE, G61SE, G65RO, G70RO, G87AM

Table 3. Grouping of accessions of the *Psidium* gene bank of Embrapa Semiarid, based on 16 biochemical and agronomic traits, distributed in 10 groups by the modified clustering method of Tocher based on Mahalanobis' distance

Group	Total accessions per group	Accessions
Group1	34	G26MA, G30PI, G25MA, G19MA, G50SE, G05MA, G58SE, G32PE, G55SE, G70RO, G20MA, G87AM, G65RO, G69R0, G67RO, G46PE, G92AM, G61SE, G83AM, G98AM, G12MA, G54SE, G73RO, G15MA, G18MA, G13MA, G21MA, G48SE, G16MA, G59SE, G94AM, G52SE, G51SE, G66RO,
Group2	07	A44PE, A45PE, A29PI, A43PE, A79RO, A78RO, A43PE
Group3	06	G95AM, G96AM, G68RO, G60SE, G34PE, G81RO
Group4	07	G07MA, G22MA, G11MA, G31PI, G14MA, G01MA, G10MA
Group5	03	G28PI, G53SE, G35PE
Group6	04	G38PE, G47PE, G03MA, G23MA
Group7	03	A80RO, AO8MA, A100AM
Group8	02	G24MA, G49SE
Group9	02	G02MA, G17MA
Group 10	01	G33PE

The formation of two araçazeiros groups in the Tocher cluster analysis (Table 3) suggests that there were probably different species or even variability within a single species, considering that the total number of Brazilian guava species was not determined in this study. In view of the presence of different araçazeiros species in Brazil, this analysis indicates that the biological significance of the Tocher analysis was greater than of the k-means. In both analyses, results indicated that the differences in agronomic and biochemical variables were sufficient to form specific groups of araçazeiros and guava accessions.

The groups of *Psidium* accessions formed by the two clustering methods showed different results, with no coincidence: the 13 accessions clustered in group 4 by the k-means method were distributed in groups 4 and 1 by the Tocher method (Tables 2 and 3). Many clustering methods are biased in relation to group size, shape and dispersion; the k-means method, for example, tends to find groups with an approximate number of elements (SAS 1989), as observed in this study. The Tocher method clustered a first group with 34 accessions, in other words, 50 % of the accessions formed a single group and group 10 contained only one plant, indicating limitations to identify differences between accessions. As concluded in the review of Fonseca et al (2004), the application of the discriminant function technique can minimize the probability of genotype misclassification, in the case of a preexisting classification study.

There were no specific clusters for sampling states of *P. guajava* accessions by the two grouping methods (Tables 2 and 3). Not even by the k-means method, where the number of six clusters had been defined in reference to the number of sampled Brazilian states. The absence of relationships between clusters and germplasm sampling regions has been reported for traits influenced by environmental conditions, such as phenotypic, for *Stipa lagascae* and *Artocarpus heterophyllus* (Visser and Reheul 2001, Jagadeesh et al. 2007) or for species exposed to long-term selection, such as *Solanum tuberosum* (Rio et al. 2004). It should be noted that the guava genotypes were subject to selection for multiple traits and may have been vegetatively dispersed from one location to another, for various reasons, resulting in the formation of groups independent from the collection site. Although Brazil is considered an area of guava diversity (Risterucci et al. 2005), no naturally occurring guava plants were found among the accessions of the collection studied; guava was always associated with human presence, growing in backyards, on road sides or beside old houses, among other sites.

The accumulated variation of the three principal components was 69 %, indicating a restricted suitability for a three-dimensional dispersion model of *Psidium* accessions (Table 4). When the four principal components are considered, accumulated variation was 76 %, indicating good suitability, without major distortion of accessions in three-dimensional diagrams, as discussed by Cruz and Regazzi (1994). The 10 araçazeiros accessions formed a specific group in the three-dimensional diagrams (Figures 1 A and B), suggesting a smaller differentiation among araçazeiros than among guava accessions, probably due to the process of artificial selection with the latter, as observed in the analysis of non-hierarchical grouping methods (Tables 2 and 3).

In the principal component analysis, the variables with highest weight of eigenvalues which explained most of the variation among *Psidium* accessions were transversal fruit diameter, total antioxidant activity, number of fruits per plant and total phenols. The variables with highest weight of eigenvalues, which explain least of the variation among *Psidium* accessions, were pulp weight per fruit and total flavonoids, and are therefore dispensable (Table 4). Cruz and Regazzi (1994) reported that traits that are relatively invariant among accessions and/or redundant due to their high correlation with other characters are dispensable or less important in divergence studies.

This is the first study to apply multivariate techniques to analyze genetic diversity in guava and araçazeiros accessions in Brazil. Hernandez-Delgado et al. (2007) analyzed 52 *Psidium* accessions in Mexico with 50 qualitative and quantitative characters and reported that the analysis of 14 principal components explained less than 30 % of the total variation in the characterization of the accessions, which is well below the value observed in this study. Sanabria et al. (2005) reported that the first three principal components explained 72 % of the total

Table 4. Eigenvalues, accumulated percentage and variables associated to the eigenvectors with highest absolute value for 16 principal components in 69 accessions of the *Psidium*, analyzed for 16 biochemical and agronomic traits

Principal component	Eigenvalues	Accumulated	Variables associated to the eigenvectors with highest absolute value
1	5.83	0.364	Transversal fruit diameter
2	3.51	0.584	Total antioxidant activity
3	1.73	0.692	Number of fruits per plant
4	1.13	0.763	Total phenols
5	0.83	0.815	Lycopene
6	0.75	0.862	Ascorbic acid
7	0.60	0.900	Total soluble sugars
8	0.43	0.927	Titrable acidity
9	0.34	0.949	Soluble solids
10	0.26	0.965	Ascorbic acid
11	0.20	0.978	Soluble solids
12	0.12	0.986	B-carotene
13	0.08	0.991	Total flavonoids
14	0.06	0.995	Pulp weight per fruit
15	0.04	0.998	Pulp weight per fruit
16	0.03	1.000	Pulp weight per fruit

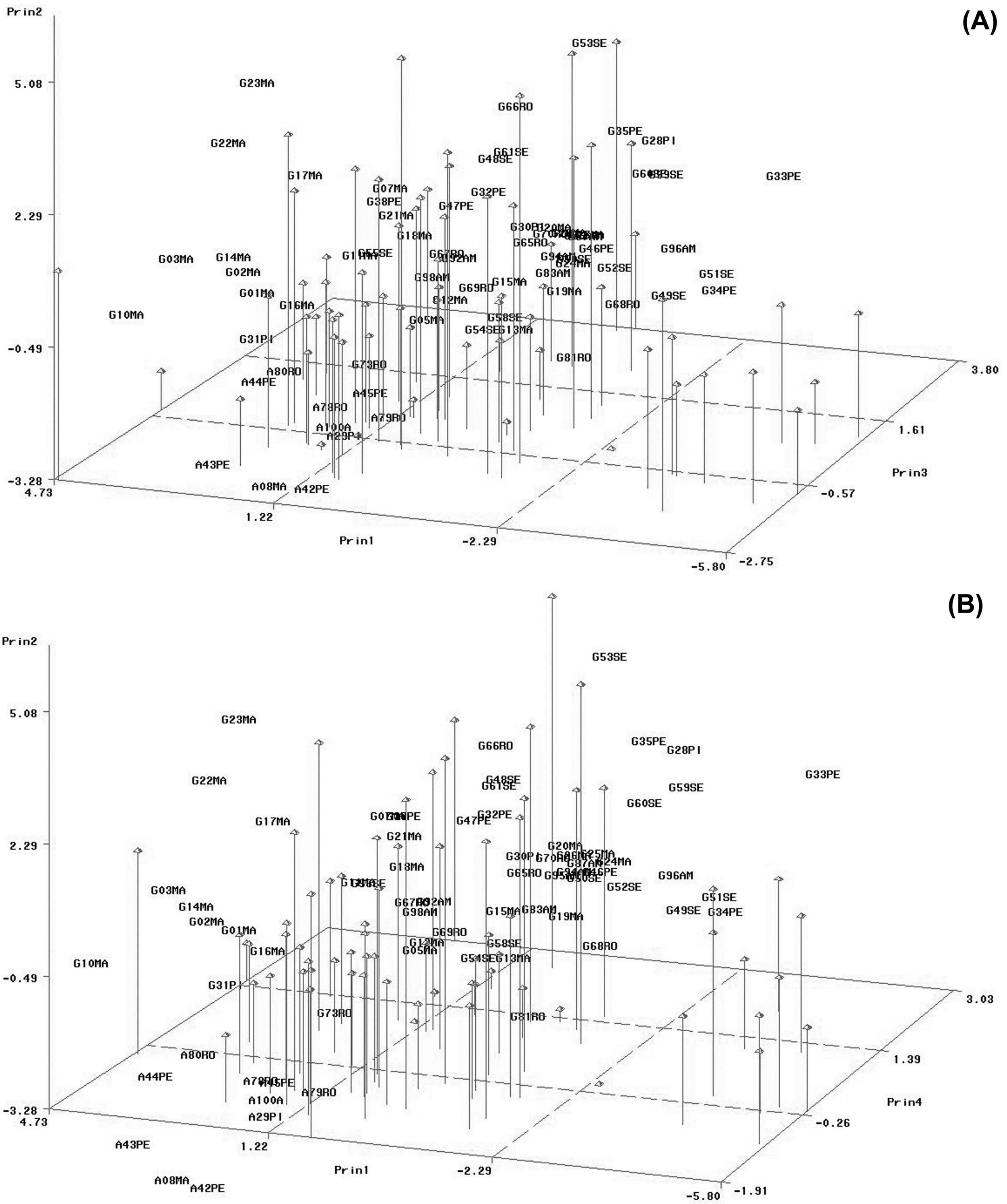

Figure 1. Dispersion diagram based on the principal components prin1, prin2 and prin3 (A) and prin1, prin2 and prin4 (B) of 69 accessions of the *Psidium*, analyzed for 16 biochemical and agronomic traits.

variation in 53 Colombian accessions analyzed with 17 qualitative characters and 9 quantitative traits. In the same paper, the authors reported the formation of three groups, with 24, 12 and 17 accessions per group, and reported no correlation between sampling locations and grouping of accessions.

The multivariate analysis techniques used to separate 69 *Psidium* accessions were generally effective for the discrimination of araçazeiros from guava accessions, indicating that the selections for guava, the most important species of the genus, were efficient to induce changes in the biochemical and agronomic traits evaluated (Tables 2 and 3; Figures 1A and 1B). The groups formed by the two methods of non-hierarchical clustering as well as the dispersion of the four principal components indicated no relationship with the sampling location of both araçazeiros as well as guava accessions. For *Psidium* germplasm collection activities, the analysis of biochemical and agronomic variables suggests restricted collections of seed germplasm, in a smaller number of regions, with a larger number of samples per region.

The information compiled in this study of genetic divergence along with the levels of antioxidant compounds found in the tested accessions, can be used as a reference in guava breeding programs for cultivars with high levels of antioxidant activity. This property has been reported as important for human health because antioxidant compounds can reduce the damaging effects of free radicals in cells (Leong and Shui 2002) and consequently the predisposition to mutagenic diseases.

For the controlled crosses accessions should be selected that performed consistently different by the two clustering methods, as well as in the dispersion of the first four principal components, associated with higher values for the traits of agronomic and biochemical interest. For example, accessions G03MA, G10MA and G01MA, with highest values for antioxidant activity (AOX), may be suitable for crossings with accessions G73RO, G20MA and G32PE, with highest lycopene contents.

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Divergência genética em acessos de *Psidium* com base em variáveis bioquímicas e agronômicas

RESUMO - *Sessenta e nove acessos de* Psidium*, coletados em seis estados brasileiros, foram analisados para dois métodos não hierárquicos de agrupamento e por componentes principais (CP), visando orientar programas de melhoramento. Foram analisadas as variáveis ácido ascórbico, β-caroteno, licopeno, fenóis totais, flavonóides totais, atividade antioxidante, acidez titulável, sólidos solúveis, açúcares solúveis totais, teor de umidade, diâmetro lateral e transversal do fruto, peso da polpa e das sementes/fruto, número e produção de frutos/planta. Foram observados agrupamentos específicos para os acessos de araçazeiros no método de Tocher e do k-means e na dispersão tridimensional dos quatro CPs. Os acessos de araçazeiros foram separados dos de goiabeira. Não foi observado nenhum agrupamento específico por estado de coleta, indicando a inexistência de barreiras na propagação dos acessos de goiabeira. As análises sugerem a prospecção de maior número de amostras de germoplasma num menor número de regiões, bem como acessos divergentes com alto teor de compostos nutricionais.*

Palavras-chave: Psidium guajava*, antioxidantes, compostos nutricionais, análise multivariada.*

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