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Parents choice and genetic divergence between cambuci fruit tree accessions

Flávio Gabriel Bianchini[1], Rodrigo Vieira Balbi[1], Rafael Pio[1*], Adriano Teodoro Bruzi[1] and Daniel Fernandes da Silva[2]

Abstract: *Fifty-eight cambuci fruit accessions were collected and propagated by seeds. Forty fruits of each accession were collected and evaluated for longitudinal and transverse diameter, fruit weight, number of seeds, seeds weight, total soluble solids, % citric acid, ratio, pH, firmness, vitamin C, and color. The phenotypic correlation between the characters and the relative contribution of the characters for the divergence among accessions were estimated and quantified by the Euclidean genetic distance, and cluster analysis was carried out according to the Neighbour Joining Tree. The significant correlations between the variables allowed the use of indirect selection as an auxiliary tool in the process of domestication and breeding of this species. Weight of 1000 seeds presented the greatest variation and contributed the most with genetic diversity. The expansion of the variability and the association of characters of interest can be promoted by the hybridization of the most divergent accessions, 14 and 43.*

Key words*: Campomanesiaphaea (Berg) Landr.,characters association, variability.*

INTRODUCTION

Campomanesia genus belongs to the Myrtaceae family. Native fruit species of the genus *Campomanesia* have potential for commercial cultivation, according to their desirable agronomic characters, such as high yield and high soluble solids (Oliveira et al. 2011). *Campomanesia phaea* (Berg) Landr. is popularly known as cambuci or Cambuci fruit tree (Vallilo et al. 2005), and it spontaneously occurs in the states of São Paulo, Rio de Janeiro and Minas Gerais, at the slope of Serra do Mar, denominated Coastal Atlantic Forest, an endangered vegetation, in small and grouped populations (Maluf and Pisciottano-Ereio 2005). Its *in natura* consumption is limited due to the low carbohydrate content and high acidity. Although its shape is not uniform, it has potential for industrialization for the production of juices, jams and fermented foods, due to its quality attributes, such as high-yield pulp, high acidity, and reasonable concentrations of ascorbic acid (Vallilo et al. 2005).

The large industrial and commercial potential of cambuci fruit tree is the amount of pectin in the pulp. This polysaccharide has high gelling power, a very important property of some proteins used in many industrial foods, such as gelatin gels, candy, textured vegetable protein, jellies, etc (Andrade et al. 2011). In 2006, the Cooperative of Producers of Cambuci and Derivates of Rio Grande da Serra (Cooper Cambucy da Serra) was founded, which brings together 21 members, in

***Corresponding author:**
E-mail: rafaelpio@dag.ufla.br

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[1] Universidade Federal de Lavras (UFLA), Departamento de Agricultura, 37.200-000, Lavras, MG, Brazil
[2] UFLA, Departamento de Biologia,

order to spread cambuci fruit and demonstrate its cultivation potential and different ways of processing. The cooperative also works together with the Coordination of Integral Technical Assistance (CATI) for the production of new seedlings (Andrade et al. 2011). Much information on the cultivation and management of cambuci fruit tree, and also few reports on the fruit pattern and on the main characters (Bianchini et al. 2015) are available in the literature (Santos et al. 2016).

The dimensions of cambuci fruits can vary, due to their occurrence extension, which goes from mountain regions to areas close to sea level. This fact can cause not only morphological variation, but also variation in the chemical composition of the fruits. Studies on the morphology of fruits and seeds, and chemical characterization of the pulp are common to several species and are carried out to assist in pre-breeding programs of undomesticated species (Moura et al. 2013) and detect the genetic variability between individuals or accessions in a population (Almeida Júnior et al. 2014). In this sense, in order to expand the genetic variability, works that focus on the introduction, exchange, collection, evaluation, documentation and conservation of germplasm have gained attention.

The collection of accessions is essential for the formation of an active germplasm bank at the beginning of a breeding program that aims to increase the variability of a species under study (Chagas et al. 2015). Four types or groups are found in the germplasm collection: a) cultivated species, or obsolete cultivars of varieties derivative from breeding programs or not, which are outdated; b) primitive cultivars or landraces; c) wild relatives of cultivated plants; d) wild species with potential for domestication (Nass 2007).

Studies on the genetic divergence are carried out for the evaluation of the variability between the accessions (Silva et al. 2017). Several methods have been employed to study the diversity, such as the use of molecular markers (Almeida et al. 2011), kinship coefficient (Costa e Silva et al. 2014) and multivariate analysis (Assis et al. 2014). Studies on the genetic diversity provide information on the identification of parents that allow the exploitation of heterotic effect and the obtainment of segregating populations with greater variability in crosses. Moreover, such studies enable the identification of duplicates, and thus reduce costs with the maintenance of germplasm banks.

Two ways of inferring genetic diversity are used: the quantitative and predictive. Diallel analysis is a quantitative way to infer genetic diversity, in which crosses between the parents and their evaluation are necessary. The predictive way is based on morphological differences of nutritional, physiological or molecular nature, quantified in a dissimilarity measure that can express the degree of genetic diversity among parents (Cruz and Carneiro 2003).

The evaluation of Cambuci fruit trees accessions by means of studies on the genetic distance allows obtaining information on the divergence, relating them to possible promising crosses. The accessions are separated into different subgroups, in order to obtain homogeneity and heterogeneity within the subgroups (Cruz et al. 2012).

No studies on the genetics and plant breeding of cambuci fruits are found in the literature. Works related to genetic diversity can assist in the identification of parents for crosses, as well as in pre-breeding programs. The aims of this study were: i) to evaluate the divergence between cambuci fruit tree accessions by means of quantitative and morpho-agronomical characters, and ii) to identify the accessions forcrosses,aiming at the expansion of genetic variability.

MATERIAL AND METHODS

Ripe Cambuci fruitscollected from native plants in different locations of the Atlantic Forest and Serra do Mar, in the states of São Paulo, Minas Gerais and Rio de Janeiro, had their seeds extracted and identified. After drying, seeds were separately sown in 3 liter plastic bags filled with substrate consisting of organic matter. Eight months later, 58 accessions were planted in 2006, spaced 5 x 4 m apart, in an area belonging to the Seedling Production Center of São Bento do Sapucai SP (lat 22° 41' S, long 45°44' W, alt 874 m asl), in a randomized block design, with three blocks. The climate is Cwb type, mesothermal, or high-altitude tropical, with dry winter and rainy summer, according to Köppen.

A sample of 40 ripe fruits was collected in 2014 from the 58 accessions. Fruits were packed in transparent plastic bags, stored in expanded polypropylene box containing ice, and transported to the Federal University of Lavras (UFLA), Lavras-MG. In the Pomology Laboratory of the Fruticulture Sector of UFLA, the dimensions were measured (length and diameter), and mean fruit weight, number of seeds, weight of 1000 seeds, total soluble solids (TSS), percentage of ascorbic acid (acidity), pH, TSS and acidity ratio (ratio), firmness, vitamin C content and color were determined.

The evaluations are described as follows:

- Longitudinal and transverse mean fruit diameter: measured with the aid of a digital caliper (150 mm);

- Mean fruit weight: determined by individual weighing in semianalytical scale;

- Number of seeds: determined by removing and counting the seeds of each fruit of the plot;

- Weight of 1000 seeds: performed by counting the seeds of 10 fruits and weighing them on semianalytical scale, with subsequent ratio for 1000 seeds;

- Total Soluble Solids content (TSS): determined by maceration of fruit pulp samples of each accession in porcelain crucibles, with two readings per sample. The TSS content was determined with the aid of a portable refractometer at 20 ° C, and reading was expressed in ° Brix;

- Percentage of ascorbic acid (titratable acidity - TA): determined by transferring the weight of approximately 10 g to Erlenmeyer flasks, completing the volume of100 mL with distilled water. Three drops of 1% phenolphthalein indicator were added to the solution, following with thetitrations by manual shaking, with 0.05 N NaOHsolution, previously standardized with potassium biphthalate. Results were expressed in g citric acid per 100 g pulp;

- Total soluble solids and titratable acidity (ratio): obtained by the ratio between total soluble solids (TSS) and titratable acidity (TA);

- PH: determined in a phmetrer;

- Firmness: measured by the strength necessary for a 3 mm probe coupled with a digital penetrometer to penetrate the fruit, in order to obtain the firmness value;

- Vitamin C: determined by the colorimetric method, using 2,4-dinitrophenylhydrazine. The reading was carried out on a Beckman 640 B spectrophotometer, using a computerized system, and results were expressed in mg of ascorbic acid per 100 g pulp;

- Color: determined at two different points of the fruit, using the colorimeter Minolta CR-400, with determination in the mode CIE L * a * b *. The L* coordinate refers to the luminosity level representing how bright or dark the sample is, with values ranging from 0 (totally black) to 100 (totally white). The a* coordinate may assume values from -80 to +100, in which the extremes correspond to green and red, respectively. Finally, the b* coordinate, with intensity from blue to yellow, can vary from -50 (totally blue) to +70 (totally yellow). Measurements were obtained at two diametrically opposite points in the equatorial zone of the fruit. The color was expressed by the luminosity (L *), which determines the brightness, by the chromaticity (chroma), which determines the intensity of the color, and by the hue angle (^{o}hue), which determines the tonality.

Afterwards, analysis of variance was performed, the genetic parameters and the coefficient of genetic correlation were estimated, and diversity was determined by the Euclidean distance and cluster analysis by the Neighbour Joining Tree (NJT) method (Saitou and Nei 1987). Cluster analyses were carried out using the package "ape" of the R software (R Core Team 2013), while the others were carried out using the Genes software (Cruz 2006).

RESULTS AND DISCUSSION

Analysis of variance of the evaluated fruit characters revealed the existence of genetic variability among the accessions (Table 1). This fact corroborated the estimate of the total variation due to treatments. Most of the observed variation is owing to genetic factors (Table 2).

In general, accessions showed considerable genetic variability values for fruit characters, since the lowest coefficient of genetic variation was 11.47% for diameter. According to Vencovsky (1969) and Resende (2002), this estimate is not considered of low magnitude for this parameter, although the authors state that values greater than 20% should be considered as representative. The study showed coefficient of genetic variation that ranged from 11.47% to 46.86%, which evidences the possibility of selections of contrasting genotypes among the accessions.

Table 1. Summary of the analysis of variance for the characters fruit length, fruit diameter, weight, soluble solids, pH, acidity, firmness, number of seeds per fruit, weight of 1000 seeds, vitamin C, and ratio for 58 cambuci fruit tree accessions

		MS										
S.V.	**df**	**LEN**	**DIA**	**WEI**	**SS**	**PH**	**ACI**	**FIR**	**NSF**	**WTS**	**VTC**	**RAT**
Accessions	57	55.07**	105.11**	479.34**	6.16**	0.30**	0.89**	28.60**	34.34**	3647.65**	1394.76**	6.82**
Blocks	2	8.97	31.36	142.05	0.06	0.15	0.01	0.30	27.92	19.23	31.25	0.27
Error	114	2.83	5.97	27.18	0.14	0.03	0.06	0.40	1.40	3.82	3.69	0.57

** Significantly at 1% level. LEN (fruit length), DIA (fruit diameter), WEI (weight), SS (soluble solids), PH (pH), ACI (acidity), FIR (firmness), NSF (number of seeds per fruit), WTS (weight of 1000 seeds), VTC (vitamin C) and RAT (soluble solids/acidity).

Table 2. Estimates of genetic and phenotypic parameters obtained in the evaluation of 58 Cambuci fruit trees accessions

Traits	CVg(%)	CVg/CVe
Fruit length	11.79	2.48
Fruit Diameter	11.47	2.35
Weight	29.06	2.36
Soluble solids	13.64	3.77
Ph	15.98	1.82
Acidity	22.00	2.20
Firmness	37.81	4.83
Number of seeds per fruit	26.60	2.80
Weight of 1000 seeds	46.86	17.82
Vitamin C	27.00	11.21
Ratio	31.03	1.91

Genetic variance for all characters was superior to the observed environmental variance. This fact is fundamental to this work, since it allows clustering different accessions in more genetic terms. The variation that exists among the accessions can be observed by the variance and by the amplitude of fruit characters (Table 3).

Weight of 1000 seeds presented the greatest variation, with high observed amplitude (166.40). This fact allows inferring that the evaluated accessions present great difference regarding this attribute. Moreover, the percentage of citric acid and the pH were the characters of lower variation amplitude. According to Cruz and Carneiro (2003) and Alexandre et al. (2015), characters that express lower variability are considered of minor importance.

Table 4 shows the estimates of the relative contribution of characters for the divergence. Again, weight of 1000 seeds was the character, which contributed the most to the divergence among the accessions. The second greatest contribution was obtained by vitamin C. Costa e Silva et al. (2014) studied the contribution of different characters for

Table 3. Descriptive statistics obtained for the evaluated cambuci fruit tree accessions

	Estimates				
Traits	**Variance**	**Mean**	**Maximum**	**Minimum**	**Rank**
Fruit length	18.36	35.39	46.85	27.24	19.61
Fruit diameter	35.04	50.10	64.94	38.24	26.70
Weight	159.78	42.25	82.33	21.20	61.13
Soluble solids	2.06	10.39	13.14	7.38	5.76
Ph	0.10	1.88	2.87	1.36	1.51
Acidity	0.30	2.39	3.43	0.95	2.48
Firmness	9.53	8.11	19.76	3.33	16.43
Number of seeds per fruit	11.45	12.46	18.43	4.93	13.50
Weight of 1000 seeds	1215.88	74.37	187.38	20.98	166.40
Vitamin C	464.92	79.76	128.8	24.55	104.25
Ratio	2.28	4.65	11.99	2.65	9.34

genetic dissimilarity between peach trees populations and found distribution with lower discrepant values among them, ranging from 3.7% to 12.13%.

Table 5 shows the estimates of the coefficient of phenotypic correlation between the pairs of characters combination. Variation in the magnitude of the estimates was observed, and they were positive or negative. Among the characters, fruit length showed significant high correlations with fruit diameter (0.75) and fruit weight (0.76), and diameter presented significant correlation with fruit weight (0.94), indicating strong relationship between the increase in fruit size with theweight. Good correlation was also observed between the weight of one thousand seeds and length (0.51), diameter (0.43), and fruit weight (0.43), indicating that seed weight influences the increase in fruit size and fruit weight . On the other hand, negative correlation was observed between the number of seeds per fruit with length (-0.19), diameter (-0.29), and fruit weight (-0.25), indicating that fruits with less seeds have smaller dimensions (length and diameter) and lower weight.

Among the chemical characters, soluble solids in the fruits presented significant moderate correlations with pH (0.37) and ratio (0.33), which was expected, since high percentages of soluble solids are found in fruits with higher pH, and in the case of the ratio, the greater the percentage of soluble solids, expressed in ° Brix, the higher is the total soluble solids/acidity ratio. Another expected correlation occurred between acidity and firmness (0.44), since the fruits that did not reach physiological ripe stage have higher acidity contents, and therefore greater firmness. This explains the negative correlation between ratio and firmness (-0.33) and acidity and ratio (-0.82).

Correlations can be useful when the phenotypic evaluations of a particular character are difficult to be obtained. If this character presents significant phenotypic and genotypic correlations with other character of easier measurement, indirect

Table 4. Relative contribution of the traits for the divergence among the evaluated Cambuci fruit tree accessions

Traits[1]	**S.j**	**Value (%)**
Fruit length	2883.28	0.15
Fruit diameter	33160.00	1.72
Weight	2152.42	0.11
Soluble solids	42600.52	2.20
Ph	20596.07	1.07
Acidity	18241.25	0.94
Firmness	96658.35	5.00
Number of seeds per fruit	40050.43	2.07
Weight of 1000 seeds	1237836.55	64.00
Vitamin C	435065.13	22.49
Ratio	4917.95	0.25

[1] Calculations were performed using non-standard means.

Table 5. Phenotypic correlation (above) and significance level (below) obtained by the t test among biometric and chemical characters of Cambuci fruit tree fruits

	ACI	**SS**	**LEN**	**DIA**	**WEI**	**NSF**	**WTS**	**PH**	**RAT**	**FIR**	**VTC**
ACI	-	0.11	0.04	0.13	0.07	0.06	-0.15	0.05	-0.82	0.44	-0.04
SS	0.429	-	-0.51	-0.46	-0.43	0.30	-0.48	0.37	0.33	0.04	-0.14
LEN	0.771	0.001	-	0.75	0.76	-0.19	0.51	-0.07	-0.29	0.07	0.01
DIA	0.328	0.001	0.001	-	0.94	-0.29	0.43	-0.22	-0.29	0.30	-0.29
WEI	0.547	0.001	0.001	0	-	-0.25	0.43	-0.22	-0.24	0.16	-0.01
NSF	0.658	0.024	0.164	0.033	0.066	-	-0.37	0.10	0.14	-0.18	-0.07
WTS	0.276	0.001	0.001	0.001	0.001	0.005	-	-0.38	-0.04	0.08	-0.05
PH	0.669	0.007	0.551	0.114	0.106	0.486	0.004	-	0.07	-0.12	0.19
RAT	7.993	0.014	0.035	0.031	0.086	0.315	0.760	0.581	-	-0.33	-0.06
FIR	0.001	0.770	0.583	0.026	0.235	0.168	0.557	0.366	0.016	-	-0.04
VTC	0.763	0.300	0.458	0.963	0.920	0.611	0.692	0.173	0.649	0.781	-

See code in Table 1.

selection based on the character of easy measurement can be obtained. When two characters present positive and significant correlations, selection in one of these characters results in the improvement of the other. Difficulties arise when two characters have positive and significant correlation and one of them is undesirable, or when the two characters are desirable, but correlation is negative and significant (Nascimento et al. 2014).

In this work, variability among the accessions of the active germplasm bank (BAG) of cambuci fruit tree was observed. The significant correlations between variables allow performing indirect selection as an auxiliary tool in the process of domestication and breeding of this species.

The last step in a study on divergence consists in the cluster analysis, in order to obtain groups of accessions in function of the greater similarity among them. Figure 1 shows that accessions 14 and 43 are the most dissimilar. On the other hand, accessions 14 and 21 are the most similar.

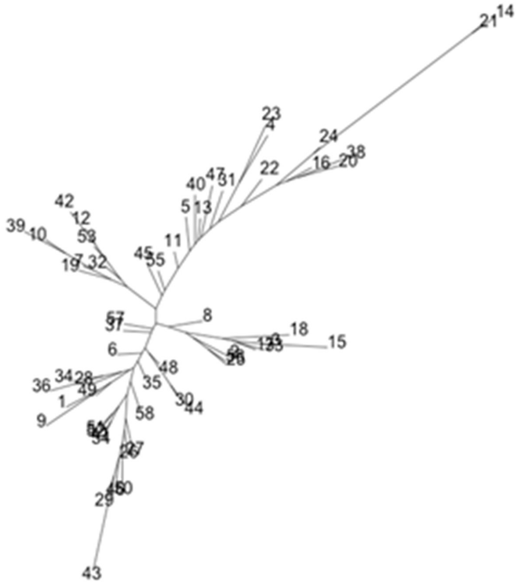

Figure 1. Dispersion diagram according to the Neibour Joining Tree (NJT) method, based on biometric and chemical traits between 58 Cambuci fruit trees accessions.

CONCLUSION

Variability for fruit characters among the evaluated cambuci fruit tree accessions was observed. The expansion of genetic variability can be obtained by hybridization among the accessions 14 and 43.

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REFERENCES

Alexandre RS, Chagas K, Marques H IP, Costa P R and Filho JC (2015) Caracterização de frutos de clones de cacaueiros na região litorânea de São Mateus, ES. Revista Brasileira de Engenharia Agrícola e Ambienta l8: 785-790.

Almeida Júnior EB, Chaves LJ and Soares TN (2014) Genetic characterization of a germplasm collection of cagaiteira, a species native to the cerrado. Bragantia 73: 246-252.

Almeida MCC, Chiari L, Jank L and Valle CB (2011) Diversidade genética molecular entre cultivares e híbridos de *Brachiaria* spp. e *Panicum maximum.* Ciência Rural 11: 1998-2003.

Andrade BAGF, Fonseca PYG and Lemos F (2011) Cambuci: o fruto, o bairro, a rota: história, cultura, sustentabilidade e gastronomia. Ourivesaria da Palavra, São Paulo, 175p.

Assis GML, Santos CF, Flores OS and Valle CB (2014) Genetic divergence among *Brachiara humidicola* (Rendle) Schweick hybrids evaluated in the Western Brazilian Amazon. Crop Breeding and Applied Biotechnology 14: 224-231.

Bianchini FG, Balbi RV, Pio R, Silva DF, Pasqual M and Vilas Boas EVB (2015) Caracterização morfológica e química de frutos de cambucizeiro. Bragantia 75: 10-18.

Chagas EA, Lozano RMB, Chagas PC, Bacelar-Lima CG, Garcia MIR, Oliveira JV, Souza OM, Morais BS and Araújo MCR (2015) Intraspecific variability of camu-camu fruit in native populations of northern Amazonia. Crop Breeding and Applied Biotechnology 15: 265-271.

Costa e Silva JO, Cremasco JPG, Matias RGP, Silva DFB, Salazar AH and Bruckner CH (2014) Divergência genética entre populações de pessegueiro baseada em características da planta e do fruto. Ciência Rural 10: 1770-1775.

Cruz CD (2006) Programa GENES: biometria. Editora UFV, Viçosa, 382p.

Cruz CD and Carneiro PCS (2003) Modelos biométricos aplicados ao melhoramento. Editora UFV, Viçosa, 585p.

Cruz CD, Regazzi AJ and Carneiro PCS (2012) **Modelos Biométricos Aplicados ao Melhoramento Genético**. Editora UFV, Viçosa, 516p.

Maluf AM and Pisciottano-Ereio WA (2005) Secagem e armazenamento de sementes de cambuci. **Pesquisa Agropecuária Brasileira 40:** 707-714.

Moura NF, Chaves LJ and Naves RV (2013) Caracterização física de frutos de pequizeiro (*CaryocarbrasilienseCamb.*) do cerrado. **Revista Árvore**

37: 905-912.

Nascimento WMO, Gurgel FL, Bhering LL and Ribeiro OD (2014) Pré-melhoramento do camucamuzeiro: estudo de parâmetros genéticos e dissimilaridade. **Revista Ceres 61**: 538-543.

Nass LL (2007) **Recursos genéticos vegetais**. Embrapa Recursos Genéticos e Biotecnologia, Brasília, 858p.

Oliveira MC, Santana DG and Santos CM (2011) Biometria de frutos e sementes e emergência de plântulas de duas espécies frutíferas do gênero *Campomanesia*. **Revista Brasileira de Fruticultura 33**: 446-455.

R Core Team (2013) **R: A language and environment for statistical computing**. R Foundation for StatisticalComputing, Vienna.

Resende MDV (2002) **Genética biométrica e estatística no melhoramento de plantas perenes**. Embrapa Informação Tecnológica, Brasília, 975p.

Saitou N and Nei M (1987) The Neighbor-joining method: A new method for reconstructing phylogenetic trees. **Molecular Biology and Evolution 4**: 406-425.

Santos DN, Nunes CF, Setotaw TA, Pio R, Pasqual M and Cançado GMA (2016) Molecular characterization and population structure study of cambuci: strategy for conservation and genetic improvement. **Genetics and Molecular Research15**: 1-13.

Silva VA, Machado JL, Resende JC, Oliveira AL, Figueiredo UJ, Carvalho GR, Ferrão MAG and Guimarães RJ (2017) Adaptability, stability, and genetic divergence of conilon coffee in Alto Suaçuí, Minas Gerais, Brazil. **Crop Breeding and Applied Biotechnology 17**: 25-31.

Vallilo MI, Garbelott ML, Oliveira E and Lamardo LCA (2005) Características físicas e químicas dos frutos do cambucizeiro (*Campomanesiaphaea*). **Revista Brasileira de Fruticultura 27**: 241-244.

Vencovsky R (1969) Genética quantitativa, I. In Kerr WE **Melhoramento e genética**. Edições Melhoramento, São Paulo, p.17-38.