

Structure of the phenotypic variability of fruit and seed traits in natural populations of *Eugenia dysenterica* DC. (Myrtaceae)¹

Carolina Ribeiro Diniz Boaventura Novaes², Elias Emanuel Silva Mota³, Evandro Novaes⁴, Mariana Pires de Campos Telles⁵, Lázaro José Chaves⁶

Abstract - *Eugenia dysenterica* DC. (cagaita tree) is a fruit tree native to the Brazilian Cerrado. It is a promising species for cultivation, but little basic information exists on the phenotypic diversity and quantitative variation of its fruits and seeds at a population scale. Thus, the present study proposes to estimate the phenotypic parameters of the species' fruits and seeds based on the variability among mother plants and among subpopulations, thereby aiming to increase knowledge for breeding and conservation of the species. For this, 25 natural subpopulations (local populations) were sampled in five Brazilian states. Within each subpopulation, 20 fruits were collected from each of six sampled mother plants. Data for biomass, transverse and longitudinal lengths of the fruits and seeds were subjected to estimates of descriptive parameters, correlation and hierarchical analysis of variance (ANOVA). Significant variation, including high levels of phenotypic variation, was observed among mother plants within the subpopulations and between the subpopulations. The high variation and the formation of phenotypically divergent groups are important elements for the breeding of cagaita tree, whose mother plants can now be selected for the traits studied. Phenotypic divergence between populations (P_{ST}) can be used as an indicator of the structuring of the phenotypic variation of the species in its natural area of occurrence.

Index terms: cagaita, quantitative variation, Cerrado, morphometric characterization, plant genetic resources.

Estrutura da variabilidade fenotípica de caracteres de frutos e sementes em populações naturais de *Eugenia dysenterica* DC. (Myrtaceae)

Resumo - *Eugenia dysenterica* DC. (cagaiteira) é uma árvore frutífera nativa do Cerrado brasileiro. É uma espécie promissora para cultivo, mas que carece de informações básicas sobre diversidade fenotípica e variação quantitativa de seus frutos e sementes em escala populacional. Neste contexto, o presente estudo propõe estimar parâmetros fenotípicos de frutos e de sementes da espécie, a partir da variabilidade entre matrizes e subpopulações, visando a ampliar o conhecimento para o melhoramento e a conservação da espécie. Para isso, foram amostradas 25 subpopulações (populações locais) naturais, em cinco estados brasileiros. Dentro de cada subpopulação, foram coletados 20 frutos de cada uma das seis matrizes amostradas. Dados de massa, comprimento transversal e longitudinal dos frutos e sementes foram submetidos à análise descritiva, correlações e análise de variância, por um modelo hierárquico. Houve variação significativa entre matrizes dentro de subpopulações e entre subpopulações, com altos níveis de variação fenotípica. As altas variações e a formação de grupos divergentes fenotipicamente são importantes elementos para o pré-melhoramento da cagaiteira, cujas matrizes já podem ser selecionadas para os caracteres estudados. A divergência fenotípica entre populações (P_{ST}) pode ser utilizada como um indicador da estruturação da variação fenotípica da espécie em sua área natural de ocorrência.

Termos para indexação: cagaita, variação quantitativa, Cerrado, caracterização morfométrica, recursos genéticos vegetais.

Corresponding author:
cboaventura@gmail.com

Received: June 02, 2017.
Accepted: October 02, 2017.

Copyright: All the contents of this journal, except where otherwise noted, is licensed under a Creative Commons Attribution License.



¹Suporte Financeiro: CAPES, CNPq (GENPAC 10 - Proc. 563727/2010-1) e FAPEG.

²Bióloga Doutora, Pós doutoranda bolsista PNPd/CAPES do Programa de Pós-Graduação em Genética e Melhoramento de Plantas da Universidade Federal de Goiás. E-mail: cboaventura@gmail.com

³Biólogo Mestre, Doutorando do Programa de Pós-Graduação em Genética e Melhoramento de Plantas da Universidade Federal de Goiás. E-mail: elias_emanuel@hotmail.com

⁴Engenheiro Florestal Doutor, Professor Adjunto da Escola de Agronomia da Universidade Federal de Goiás. E-mail: evnovaes@gmail.com

⁵Bióloga Doutora, Professora do Departamento de Genética da Universidade Federal de Goiás e da Escola de Ciências Agrárias e Biológicas da Pontifícia Universidade Católica de Goiás. E-mail: tellesmpc@gmail.com

⁶Engenheiro Agrônomo Doutor, Professor Titular da Escola de Agronomia da Universidade Federal de Goiás. E-mail: lchaves@ufg.br

Introduction

Eugenia dysenterica DC. (cagaita tree) is a species of the family Myrtaceae, which includes genera such as *Plinia* (jaboticaba) and *Syzygium* (clove) and other *Eugenia* species (“araçá-boi,” Surinam cherry, rose apple, and uvalha) with fleshy and edible fruits. The distribution of *E. dysenterica* is predominantly discontinuous and gregarious and is present in most of the Cerrado (Brazilian savanna) region (NAVES et al., 2002). *Eugenia dysenterica* has a big-bang flowering strategy, with synchronous and abundant flowering (SOUZA et al., 2008). The flowers are pollinated mainly by bees of the genus *Bombus*, and reproduction is characterized by pollen self-compatibility (PROENÇA and GIBBS, 1994) and predominant cross-fertilization (RODRIGUES et al., 2016).

The fruits are phenotypically heterogeneous between plants (CAMILO et al., 2014), and their development coincides with the beginning of the rainy season in the Cerrado region, with full ripening occurring 37 days after flower anthesis (SILVA et al., 2017). Observations made between 2003 and 2008 in 5- to 10-year-old plants (SOUZA et al., 2013) and in 2011 in 13-year-old plants (CAMILO et al., 2013) showed lack of uniformity in *E. dysenterica* fruit production.

Cagaita tree fruits have several different applications (CAMILO et al., 2014). The pulp has low calories and is rich in vitamin C, vitamin A, folates, and minerals (CARDOSO et al., 2011; GUEDES et al., 2017), and it has good microbiological quality. The seeds are a source of potassium, phosphorus, sodium, and magnesium (RIBEIRO et al., 2013) and unsaturated fatty acids (CAMILO et al., 2016). In regional cuisine, the fruits are used in the production of ice creams, jams, jellies, and “cachaça curtida” (a natural and artisanal alcoholic drink made from the infusion of cachaça with fruit). The microbiological and sensorial characteristics have encouraged the commercialization of cagaita jelly (ARRUDA et al., 2016) and cagaita wine (OLIVEIRA et al., 2011). As one of the Brazilian Cerrado native plants, the cagaita tree has bioactive compounds with great potential to be exploited (BOLZANI et al., 2012; NOVAES et al., 2013; CORREIA et al., 2016), namely due to its antioxidant effects (ROCHA et al., 2013; MOREIRA et al., 2017). A peptide extracted from the pulp (NCBI accession P86708.1) can be used as a new compound in laxatives (LIMA et al., 2010). In addition, its phenolic compounds are effective for preventing obesity (DONADO-PESTANA et al., 2015).

To better recommend and encourage its use, conservation, and commercial exploitation, while also avoiding predatory extraction of the resource, the development of cultivation strategies of the species is fundamental. The fruits and seeds of *E. dysenterica* have shown variation in their characteristics in some

studies (e.g., SILVA et al., 2001; ANDRADE et al., 2003; TRINDADE and CHAVES, 2005; CARDOSO et al., 2011; SILVA et al., 2017). However, these studies sampled local populations or restricted geographic regions relative to the area of occurrence of the species. Thus, a broader characterization of this genetic resource is necessary.

The Federal University of Goiás has an *in vivo* cagaita tree collection, established with seedlings collected since 1996, from populations of Goiás state, Brazil (TRINDADE and CHAVES, 2005; CHAVES and TELLES, 2006; RODRIGUES et al., 2016). This study presents the results of the fruits collected for augmenting this collection with a geographically comprehensive sample of *E. dysenterica*, from 25 populations in the Cerrado region. The objective of the present study was to estimate the phenotypic parameters of cagaita fruits and seeds, partitioning the phenotypic variation that occur between and within the native subpopulations sampled to establish a geographically comprehensive germplasm collection. Knowledge about the phenotypic divergence is important for the management, prebreeding, and conservation of the species.

Materials and methods

Fruits of *E. dysenterica* were collected in five states of the region of occurrence of the species in the Brazilian Cerrado (Figure 1), in October and November 2011. At least 20 fruits were collected from each mother plant. Collections were performed in 25 subpopulations, from which six mother plants per subpopulation were sampled to obtain the fruits. A subpopulation, corresponding to local populations, was defined as a set of plants sampled at a minimum distance of 40 km and meeting the other assumptions of SILVA et al. (2001).

Fruits with different degrees of ripening were collected. Collection of fruits with the same maturation pattern was not possible for the 25 populations sampled because fruit maturation do not occur uniformly in the regions of occurrence of the cagaita tree (SOUZA et al., 2008). Therefore, in those sites where the plants were at the end of the maturation process, fruits that had already fallen were also collected for some mother plants to obtain a comprehensive sample for the establishment of a germplasm collection.

The fruits were transported from the field in plastic containers in a cooler with ice and later stored in a refrigerator until they could be physically characterized. The sampling of all populations was carried out in three sampling campaigns within a period of 30 days, covering approximately 10,000 km. The physical characterization of the fruits was conducted within 5 days after the last collection. The subpopulations were numbered 1-25 according to the order of collection of the fruits.

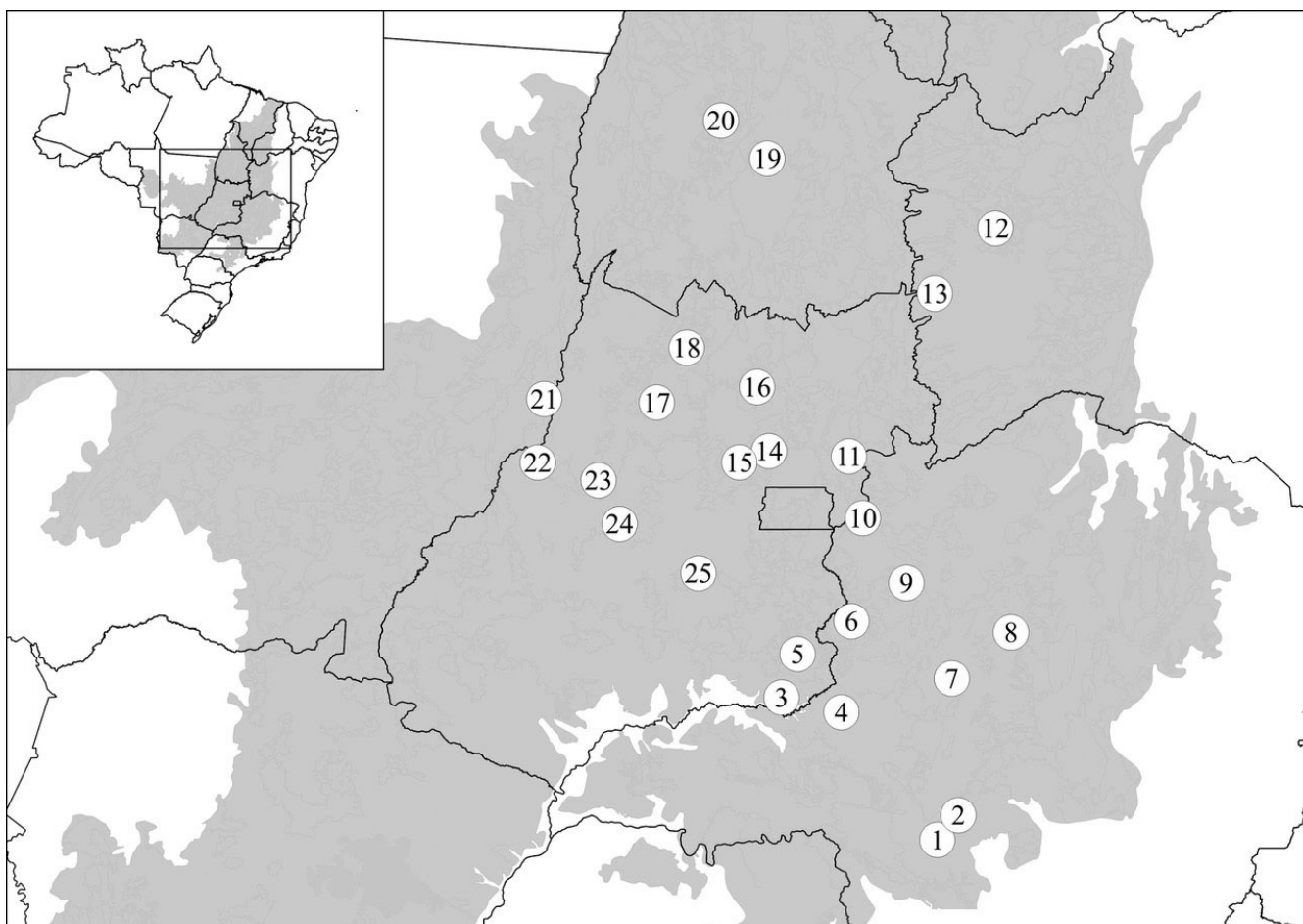


Figure 1. Map of the 25 local populations of *Eugenia dysenterica* sampled. Detail: Brazil and Brazilian states; in gray is the Cerrado Biome.

A random sample of five ripe and healthy fruits per mother plant, totaling 750 fruits, was analyzed for the following traits: number of seeds per fruit (NSF), fruit longitudinal (FLD) and transverse (FTD) diameters, seed longitudinal and transverse diameters (STD), fruit mass (FM), and seed mass (SM). The mass traits were measured with a semi-analytical digital scale, and the results were expressed in grams (g). The size measurements were obtained with a digital caliper and recorded in millimeters (mm). The fruit shape (FS) was obtained by the ratio between the fruit longitudinal and transverse diameters (FLD/FTD) as indicated: oblong fruit (FLD/FTD > 1), globose fruit (FLD/FTD ≈ 1), and flat fruit (< 1).

The pulp and peel yield of the fruit was calculated as a percentage according to the following formula:

$$YIELD = \frac{(FM - SM)}{FM} \times 100$$

YIELD: percentage yield of pulp and peel

FM: fruit mass

SM: seed mass

Descriptive statistical analysis was performed together with hierarchical analysis of variance (ANOVA) with the effects of the subpopulations, mother plants within the subpopulations, and fruits or seeds within the mother plants (residuals), according to the following model:

$$Y_{ijk} = \mu + s_i + m_{j(i)} + e_{k(ij)}$$

Y_{ijk} : phenotypic value of the fruit (or seed) k of the mother plant j of subpopulation i

μ : overall average of all observations

s_i : effect of subpopulation i , $i = 1, 2, \dots, S$

$m_{j(i)}$: effect of mother plant j within the subpopulation i , $j = 1, 2, \dots, m_i$

$e_{k(ij)}$: residual, which is the effect of the fruit (or seed) k of the mother plant j of subpopulation i

The analysis was performed with unbalanced data because of the 750 fruits analyzed, 115 could not be measured due to high level of decomposition. From these 750 fruits, 964 seeds were obtained for evaluation.

The variance components associated with the effects of the model, the proportion of total phenotypic variation among the subpopulations (P_s), among the mother plants within the subpopulations (P_{MS}), and among

the fruits or seeds within the mother plants within the subpopulations (P_{NM}) were estimated. The expected mean squares of the variance components are shown in Table 1. The coefficients of the phenotypic correlation between the averages of the traits for the 25 subpopulations were estimated by Pearson’s method.

Phenotypic divergence between populations was estimated by the parameter P_{ST} :

$$\hat{P}_{ST} = \frac{\hat{\sigma}_p^2}{\hat{\sigma}_p^2 + 2\hat{\sigma}_m^2}$$

σ^2 : phenotypic variance between subpopulations
 σ_m^2 : phenotypic variance between mother plants within subpopulations.

To compare the differences among the subpopulations using the quantitative traits evaluated, the generalized Mahalanobis distance (MAHALANOBIS, 1936) was adopted as a measure of dissimilarity for the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis. To estimate the Mahalanobis distances, multivariate analysis of variance (MANOVA) was performed with all quantitative traits to obtain the residual covariance matrix. The dendrogram node consistency was evaluated with 10,000 bootstraps, and the Mantel test was used to evaluate the significance of the correlation between the cophenetic distances and the Mahalanobis distances. The Mantel test was also used to assess the correlation between the Mahalanobis and geographic distances among the subpopulations. All analyses were performed using the R software (R Core Development Team, 2013), with the following packages: adegenet, ape, geosphere, MASS, and StatMatch.

Table 1. Sources of variation (SV) and expected mean squares (MS) according to the hierarchical statistical model.

SV	DF	MS	E(MS)
Subpopulations	$S - 1$	Q_1	$\sigma^2 + k_2\sigma_m^2 + k_3\sigma_p^2$
Mother plants/Subpopulation	$M - S$	Q_2	$\sigma^2 + k_1\sigma_m^2$
Residual	$N - M$	Q_3	σ^2
Total	$N - 1$		

* - S : number of populations ($S = 25$); M : total number of mother plants ($M = 150$); and N : total number of fruits ($N = 635$) or seeds ($N = 964$). Values of k , adapted from Anderson and Bancroft (1952).

Results and discussion

The traits evaluated for the fruits and seeds showed high phenotypic variation and population structuring in the area where the cagaita tree occurs naturally. The largest variations among the evaluated traits, based on the coefficient of variation, were observed for fruit mass and seed mass (Table 2). Although the coefficient of variation obtained was slightly higher, as expected given the heterogeneity and comprehensiveness of the sample, it was similar to those found in other studies with *E. dysenterica* (Table 3).

The fruits presented rapid post-harvest decomposition. After 7 days, although they were stored under refrigeration, the riper fruits were not firm enough to be measured. The use of packaging and low storage temperatures is not sufficient to promote fruit preservation for longer times (CARNEIRO et al., 2015). The high percentage of water, greater than 90% (Silva et al., 2008), the rapid physiological maturation cycle (SILVA et al., 2017), and the fragility of the peel (FERREIRA and PEREIRA, 2016) contribute to the fruit’s susceptibility to

enzymatic and microbial degradation (CARDOSO et al., 2011). As a result of degradation, 15.33% of fruits were not measured. On the other hand, all seed measurements were obtained successfully.

The estimate of the average fruit mass obtained in the present study (15.43 g) is between the values of the two studies cited (Table 3). Subpopulation 14, from the city of Mimoso/GO (state of Goiás), presented the highest mean fruit mass: 21.66 g (Table 2), but this value was still lower than the highest value (25.11 g) found in the literature (CARDOSO et al., 2011). The authors worked with a subpopulation of Felixlândia/MG (state of Minas Gerais) and analyzed a sample of only 30 fruits. The study did not report the number of trees from which the fruits were collected. Half of the fruits evaluated in the present study presented mass between 11.07 g and 18.80 g, but notably, fruits up to 40.40 g were observed. The shape of the sampled fruits comprises globose and flat fruits, but oblong fruits with a mean ratio of the longitudinal to transverse diameter of 1.26, not yet described in the literature, were found in subpopulation 17 of Santa Terezinha/GO (Table 2).

For the variables related to seed diameter and mass, the values found agree with those reported by Silva et al. (2001) for 1,344 fruits from 10 locations in the southeast region of Goiás. As with the fruit mass, the seed mass of 3.39 g, observed by Cardoso et al. (2011), was higher than the average of all the subpopulations studied here, although subpopulation 22 (Santa Terezinha/GO) had seeds with a similar mass (3.36 g).

No fruit with five or six seeds was found in the present study. Naves et al. (1995) and Camilo et al. (2014) found between one and three seeds, with most fruits showing only one seed. Silva et al. (2001) observed that 97% of the fruits had one to three seeds, but fruits with five and six seeds were described. In the present study, 76.40% of the fruits presented only one seed. Subpopulation 20 (Porto Nacional/TO) showed no variation in the number of seeds, with all sampled fruits showing only one seed. On the other hand, subpopulation 2 (Luz/MG) presented from one to four seeds, with the highest average number of seeds (1.73) per fruit (Table 2).

The fruit yield and the percentage mass of pulp and peel are of interest for the genetic improvement of the species when the aim is to produce fruits with higher pulp content. The phenotypic variation for such traits, also found by Silva et al. (2001), is influenced by environmental components but is expected also to be caused by genetic

differences. With this existing variability, the selection of fruits with higher pulp yield, which is a desirable trait for the commercial exploitation of the species, becomes possible. Subpopulations 05 (Campo Alegre/GO) and 04 (Coromandel/MG) had the highest average pulp percentages (92.52% and 90.97%, respectively). Progenies of subpopulation 05, from another collection (CAMILO et al., 2014), were also selected among the most promising mother plants for genetic improvement of pulp content, reinforcing the region's contribution for future breeding actions.

All traits evaluated showed significant variation among the mother plants within populations ($P_{M/S}$) and among populations (P_S). High levels of phenotypic variation in the fruits and seeds were observed for both the cagaita tree subpopulations and the mother plants (Table 4). The proportion of the total variance explained by differences among the mother plants within the populations reached 62.1% for fruit shape. The lowest proportion was found for the seed longitudinal diameter, in which mother plants within subpopulations accounted for 29.67% of the variance found. The broad phenotypic variations found can be used to support future breeding programs of the cagaita tree. The study of these subpopulations is useful not only for the trait-based selection of the fruit but also for the conservation of germplasm and to guide future seed-sampling programs.

Table 2. Estimates of average, minimum and maximum, and coefficients of phenotypic variation (CV) of physical variables of fruits and seeds from 25 natural populations of *Eugenia dysenterica* DC.

Population	N	Traits									
		FLD	SLD	FTD	STD	NSF	MF	MS	F_S	YIE	FS
01	30	25,34	10,70	33,75	15,69	1,37	16,66	1,59	10,63	90,46	0,76
02	25	25,77	10,29	30,67	14,42	1,73	13,92	1,34	10,37	90,37	0,85
03	28	27,25	10,68	33,99	15,85	1,47	18,15	1,78	10,52	90,19	0,80
04	27	25,35	9,95	31,92	14,90	1,60	15,50	1,40	11,17	90,97	0,80
05	20	28,19	9,66	33,27	14,70	1,24	17,78	1,33	13,84	92,52	0,85
06	29	25,32	9,90	31,93	14,46	1,33	14,50	1,37	11,60	90,55	0,80
07	16	23,82	9,92	31,15	15,29	1,33	14,11	1,56	10,22	88,94	0,77
08	22	24,54	9,55	30,92	14,24	1,20	13,85	1,31	12,68	90,54	0,80
09	25	26,21	10,67	31,49	15,75	1,10	15,39	1,92	8,22	87,52	0,84
10	30	23,84	9,73	30,63	14,44	1,40	12,18	1,25	9,96	89,74	0,78
11	26	26,46	10,29	32,89	15,12	1,33	16,02	1,50	11,27	90,64	0,82
12	18	21,23	10,51	26,91	15,75	1,13	9,28	1,67	6,79	82,00	0,79
13	30	29,03	10,98	34,38	16,51	1,37	19,43	1,88	10,57	90,32	0,85
14	30	29,71	11,07	36,18	17,82	1,30	21,66	2,14	10,76	90,12	0,82
15	30	28,48	11,32	33,37	18,64	1,30	17,56	2,28	7,81	87,02	0,85
16	30	24,86	10,68	30,64	16,49	1,07	13,94	1,91	8,11	86,30	0,82
17	25	29,24	12,38	23,19	18,16	1,23	14,61	2,52	6,08	82,75	1,61
18	29	25,56	11,53	31,45	17,32	1,53	14,33	2,10	7,24	85,35	0,82
19	29	27,11	11,60	31,61	16,40	1,13	14,99	1,91	8,35	87,26	0,86
20	11	22,88	11,24	27,97	17,26	1,00	10,97	2,24	6,92	79,58	0,82
21	27	28,14	13,09	34,57	19,09	1,22	18,16	2,82	6,65	84,47	0,81
22	22	26,04	13,94	29,94	19,28	1,08	12,71	3,36	4,15	73,56	0,87
23	23	27,62	11,44	33,56	17,18	1,30	17,93	2,10	9,55	88,29	0,83
24	28	24,81	11,03	30,22	16,41	1,30	13,68	1,89	7,36	86,18	0,83
25	30	23,61	9,73	30,26	14,98	1,13	12,42	1,43	9,23	88,49	0,78
Average	25,60	26,02	10,88	31,47	16,25	1,29	15,19	1,86	9,20	87,69	0,85
Minimum	11	14,93	3,77	10,74	5,76	1	1,00	0,11	0,81	69,74	0,56
Maximum	30	38,96	17,79	49,07	24,86	4	40,40	6,48	31,46	94,98	2,79
CV	19,76	15,38	16,14	16,56	16,42	0,45	39,66	45,62	40,60	5,04	28,75

N: number of fruits evaluated by subpopulation; FLD: fruit longitudinal diameter (mm); FTD: fruit transverse diameter (mm); SLD: seed longitudinal diameter (mm); STD: seed transverse diameter (mm); NSF: number of seeds per fruit; FM: fruit mass (g); SM: seed mass (g); F_S: ratio of fruit mass to seed mass; YIELD: percentage of pulp and peel in fruit; FS: fruit shape.

Trindade and Chaves (2005) found high phenotypic and genotypic structuring in 13 subpopulations in the northeast of Goiás, with most of the variation occurring within the subpopulations. However, in the present study, the phenotypic structure of the population, calculated by P_{ST} , shows that the magnitude of the phenotypic divergence among the subpopulations varied from low to high for the quantitative traits evaluated (Table 4). The lowest P_{ST} estimate was observed for the fruit mass, with a value of 0.0840, and the highest was observed for the seed longitudinal diameter ($P_{ST} = 0.3231$). Although the phenotypic divergence was of low magnitude among the subpopulations for the fruit mass, the population structuring for this trait was significant. Thus, the population is structured for all evaluated traits.

The differentiation of the populations analyzed, evaluated by P_{ST} , may be influenced by both the genetic component and the environmental component, as well as by the interaction of these factors. The molecular genetic structure has been reported in some studies (TELES et al., 2003; ZUCCHI et al., 2003; ZUCCHI et al., 2005; TRINDADE and CHAVES, 2005) but may not reflect the effect of the same evolutionary factors of the quantitative genetic structure. While the molecular genetic structure provides tools for discussing the evolutionary and conservation issues of neutral processes, quantitative genetic structure also explores local selection and adaptation (MERILÄ and CRNOKRAK, 2001). Phenotypic variation, besides providing the essential raw material for evolutionary changes, is an estimation of the adaptation of individuals within a population (HALAMA and REZNICK, 2001). The P_{ST} parameter, although containing environmental effects, can be considered as a first approximation of the analogous parameter Q_{ST} which measures the genotypic divergence of quantitative traits (LEINONEN et al., 2013; RAEYMAEKERS et al., 2007).

The ratio between the proportions of the additive variances among and within the populations is essential for validating the estimated population structure calculated by P_{ST} (BROMMER, 2011). In the present study, this ratio was not calculated because the additive variance could not be estimated according to the sampling performed, and as such, the cause of the phenotypic structure was not discussed. In addition, phenotypic plasticity may be the nongenetic component responsible for such diversity in *E. dysenterica* fruits and seeds. It represents a way to adjust phenotypes to the environmental heterogeneity, which generates morphological diversity in a population (HALAMA and REZNICK, 2001). Because the fruits were collected where the species occurs naturally, possibly, the environmental factors are influencing the morphological variability of the fruits and seeds.

All the fruits' and seed's phenotypic traits were significantly correlated, except for the peel and pulp yield vs. the fruit longitudinal diameter. Naves et al. (1995) also found positive correlations at the 1% probability level between fruit weight, diameter, and volume. The present

study did not correlate the number of seeds with the other variables because the data did not meet the assumption of normality. In the studies of Naves et al. (1995) and Silva et al. (2001), who analyzed the correlation between the number of seeds per fruit and the other traits, such correlation was not significant (Table 5).

Negative and significant correlations were found between the pulp and peel yield with the fruit shape, fruit diameters, and seed mass. Thus, the larger the seeds, the less pulp the fruit will have. The fruit shape, its transverse diameter, and its mass were also negatively correlated. Although of low magnitude, the correlation between fruit shape and mass indicates that round fruits have higher mass (Table 5).

According to the Mahalanobis distance, subpopulation 22 (Britânia/GO) was the one that most diverged from the others, mainly due to the larger mass and size of the evaluated seeds (Figure 2). Subpopulation 22 was the one with the highest seed mass and, consequently, the lowest ratio between the fruit mass and the seed yield and the lowest pulp yield. Another subpopulation diverging greatly from the others was subpopulation 12 (Barreiras/BA [state of Bahia]), whose fruits presented the smallest mass and the smallest diameters (longitudinal and transverse) among all subpopulations. For these reasons, subpopulations 12 and 22 are less likely to generate promising genotypes in terms of pulp yield. The nodes that separate these two populations from the others show high consistency in the bootstrap analyses. The dendrogram represents well the Mahalanobis distances (cophenetic correlation of 0.89, with $p < 0.001$ by the Mantel test). On the other hand, the other subpopulations formed a large cluster with low bootstrap consistency in their nodes (Figure 2). This result indicates that, except for subpopulations 12 and 22, the others do not form apparently consistent clusters, showing a random distribution of phenotypic divergence.

The phenotypic divergences observed among the subpopulations are not associated with their geographical distances ($r = 0.04$, with p value = 0.34 by the Mantel test). Other studies with *E. dysenterica* have observed significant correlation between geographic and genetic distances (TELLES et al., 2001a; TELLES et al., 2003; ZUCCHI et al., 2003; BARBOSA et al., 2015). Therefore, possibly, local environmental variation influences the fruit phenotype more than genetic variation does, as found by Telles et al. (2001b) for soil patterns.

The fruit and seed traits evaluated indicate broad phenotypic variability among the cagaita subpopulations sampled. The characterization and differentiation of these subpopulations were successful and will be important guides for future studies on the breeding and conservation of this genetic resource.

Table 3. Comparison of mean estimates among four studies on the physical variables of fruits and seeds of *Eugenia dysenterica* DC.

Sampling Area	FLD	FTD	FM	SM	YIE	N	References
Sen. Canedo, Bonfinópolis e L. de Bulhões, GO	2,82 (12,98)	3,40 (10,75)	20,32 (26,41)	-	-	99 [24]	Naves et al., (1995)
10 areas southeastern Goiás	2,40 (13,70)	2,89 (13,73)	12,67 (39,25)	1,31 (34,15)	89,66%	1344 [112]	Silva et al., (2001)
13 areas northeastern Goiás	-	-	15,34 (25,78)	2,19 (39,32)	85,72%	- [156]	Trindade e Chaves (2005)
Felixlândia, MG	3,47 (10,09)	3,16 (10,76)	25,11 (23,94)	3,39 (35,99)	86,4%	30 [-]	Cardoso et al., (2011)
UFG Germplasm collection, GO	2,74 (10,54)	3,25 (10,66)	18,08 (27,32)	2,80 (28,23)	84,51%	480 [40]	Camilo et al., (2014)
Abadia, GO	3,6 (-)	3,8 (-)	25,40 (-)	-	-	320 [30]	Silva et al., (2017)
25 Cerrado populations	2,62 (15,38)	3,17 (16,56)	15,43 (39,66)	1,86 (45,62)	87,94%	635 [150]	This paper

FLD: fruit longitudinal diameter (cm); FTD: fruit transverse diameter (cm); FM: fruit mass (g); SM: seed mass (g); Yield: yield = (FM-SM/FM) × 100; N: number of fruits analyzed, showing between brackets the number of trees sampled; between parentheses (coefficient of variation).

Table 4. Analysis of variance and estimates of phenotypic parameters for fruit longitudinal diameter (FLD), fruit transverse diameter (FTD), fruit mass (FM), seed longitudinal diameter (SLD), seed transverse diameter (STD), seed mass (SM), pulp and peel yield (YIE), and fruit shape (FS) in 25 natural populations of *Eugenia dysenterica* DC.

SV	DF	Mean Square							
		FLD	FTD	FM	SLD	STD	SM	YIE	FS
Subp	24	106.520***	176.687**	187.193*	42.912***	96.041***	9.825***	310.498***	0.651***
Mat/Subp	112	43.460***	74.377***	98.972***	7.456***	19.789***	1.843***	60.426***	0.176***
Residual	482	5.643	9.656	15.859	1.355	3.086	0.236	8.628	0.004
$\hat{\sigma}_s^2$		2.419	3.92	3.334	0.913	1.96	0.205	9.759	0.018
$\hat{\sigma}_M^2$		8.269	14.152	18.174	0.957	2.619	0.252	11.327	0.037
\hat{P}_S		0.148	0.141	0.089	0.283	0.256	0.296	0.328	0.306
\hat{P}_{MS}		0.506	0.510	0.486	0.297	0.342	0.363	0.381	0.621
\hat{P}_{NM}		0.346	0.348	0.424	0.420	0.403	0.341	0.290	0.074
\hat{P}_{ST}		0.128	0.122	0.084	0.323	0.272	0.290	0.301	0.198

*, **, *** significant F-test at the 5%, 1%, and 0.1% probability level, respectively. DF: degrees of freedom; σ_s^2 : estimation of population variance; σ_M^2 : estimation of parent plant variance; P_S : proportion of the total variance explained by differences between populations; P_{MS} : proportion of the total variance explained by differences between mother plants within the same population; P_{NM} : proportion of total variance explained by differences between fruits or seeds within mother plants. P_{ST} : quantitative phenotypic differentiation among the populations.

Table 5. Correlation among the physical variables, fruit longitudinal diameter (FLD), fruit transverse diameter (FTD), fruit mass (FM), fruit shape (FS), seed mass (SM), peel and pulp yield (YIE), seed longitudinal diameter (SLD), and seed transverse diameter (STD) of 25 natural populations of *Eugenia dysenterica* DC.

	FLD	FTD	FM	FS	SM	YIE	SLD	STD
FLD	1.000	-	-	-	-	-	-	-
FTD	0.578***	1.000	-	-	-	-	-	-
FM	0.845***	0.948***	1.000	-	-	-	-	-
FS	0.293***	-0.533***	-0.088*	1.000	-	-	-	-
SM	0.598***	0.515***	0.668***	0.078*	1.000	-	-	-
YIE	0.077 ^{NS}	0.129**	0.141***	-0.079*	-0.593***	1.000	-	-
SLD	0.529***	0.326***	0.444***	0.147***	0.683***	-0.474***	1.000	-
STD	0.560***	0.416***	0.535***	0.105**	0.677***	-0.359***	0.841***	1.000

*, **, *** Significant at 5%, 1%, and 0.1% probability. ^{NS} Not significant.

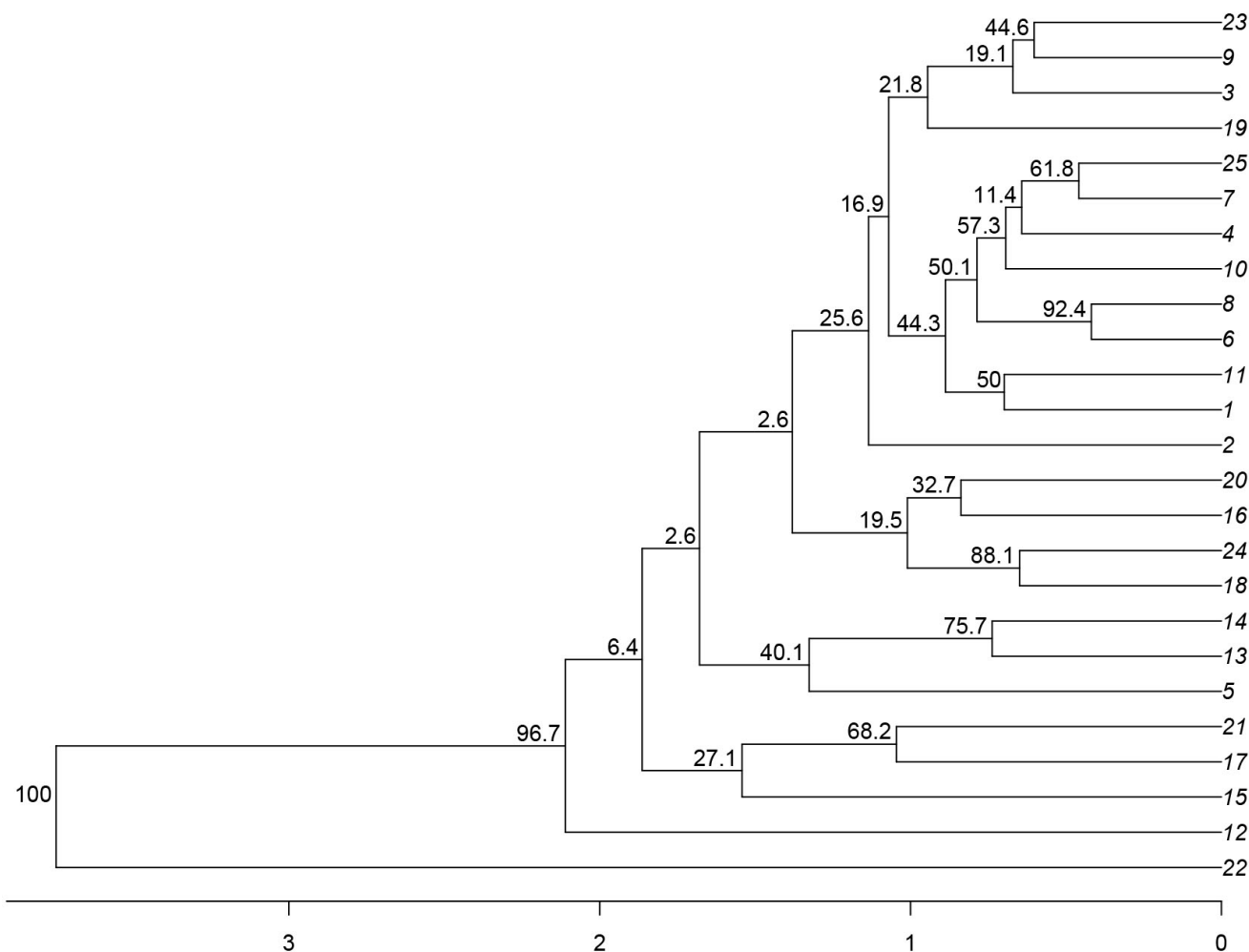


Figure 2. Dendrogram of the generalized Mahalanobis distances (x-axis) among the 25 subpopulations (y-axis) of *Eugenia dysenterica* DC, using the UPGMA cluster method. The dendrogram node consistency was obtained from 10,000 bootstraps. Cophenetic correlation: 0.89 ($p < 0.001$ by the Mantel test).

Conclusion

In general, *E. dysenterica* fruits have a flat globose shape. However, an oblong shape, not yet described, has also been found.

The *E. dysenterica* subpopulations are phenotypically structured for the traits evaluated. However, the subpopulations do not form consistent clusters based on phenotypic dissimilarity, and no geographical structuring is present.

There is broad phenotypic variation for fruit and seed traits, both between and within subpopulations. Therefore, *E. dysenterica* has high potential for breeding and selection of fruits with higher pulp yield and uniformity.

References

- ANDERSON, R.L.; BANCROFT, T.A. **Statistical theory in research**. New York: McGraw-Hill Book Company, 1952. 399 p.
- ANDRADE, A.C.S.; CUNHA, R.; SOUZA, A.F.; REIS, R.B.; ALMEIDA, K.J. Physiological and morphological aspects of seed viability of a neotropical savannah tree, *Eugenia dysenterica* DC. **Seed Science and Technology**, Bassersdorf, v.31, p.125-137, 2003.
- ARRUDA, H.S.; BOTREL, D.A.; FERNANDES, R.V.D.B.; FERREIRA de ALMEIDA, M.E. Development and sensory evaluation of products containing the Brazilian Savannah fruits araticum (*Annona crassiflora* Mart.) and cagaita (*Eugenia dysenterica* Mart.). **Brazilian Journal of Food Technology**, Campinas, v.19, 2016.

- BARBOSA, A.C.D.O.F.; COLLEVATTI, R.G.; CHAVES, L.J.; GUEDES, L.B.S.; DINIZ-FILHO, J.A.F.; TELLES, M.P.C. Range-wide genetic differentiation of *Eugenia dysenterica* (Myrtaceae) populations in Brazilian cerrado. **Biochemical Systematics and Ecology**, Amsterdam, v.59, p.288-296, 2015.
- BOLZANI, V.D.S.; PIVATTO, M.; VALLI, M.; VIEGAS, C., JR. Natural products from Brazilian biodiversity as a source of new models for medicinal chemistry. **Pure and Applied Chemistry**, Oxford, v.84, n.9, p.1837-1846, 2012.
- BROMMER, J.E. Whither *Pst*? The approximation of *Qst* by *Pst* in evolutionary and conservation biology. **Journal of Evolutionary Biology**, Ede, v.24, p.1160–1168, 2011.
- CAMILO, Y.M.V.; SOUZA, E.R.; NAVES, R.V.; VERA, R.; VIEIRA, M.; CARMO, D. Determination of the fatty acid profile in *Eugenia dysenterica* DC. seeds. **Revista Brasileira de Fruticultura**, Jaboticabal, v.38, n.4, p.1-8, 2016.
- CAMILO, Y.M.V.; SOUZA, E.R.B.; VERA, R.; NAVES, R.V. Fenologia, produção e precocidade de plantas de *Eugenia dysenterica* visando melhoramento genético. **Revista de Ciências Agrárias**, Recife, v.36, n.2, p.192-198, 2013.
- CAMILO, Y.M.V.; SOUZA, E.R.B.; VERA, R.; NAVES, R.V. Fruit characterization and progeny selection of cagaita (*Eugenia dysenterica* DC.). **Científica**, Jaboticabal, v.42, n.1, p.1-10, 2014.
- CARDOSO, L.M.; MARTINO, H.S.D.; MOREIRA, A.V.B.; RIBEIRO, S.M.R.; PINHEIRO-SANT'ANA, H.M. Cagaita (*Eugenia dysenterica* DC.) of the cerrado of Minas Gerais, Brazil: Physical and chemical characterization, carotenoids and vitamins. **Food Research International**, Burlington, v.44, n.7, p.2151-2154, 2011.
- CARNEIRO, J.D.O.; SOUZA, M.D.A.; RODRIGUES, Y.; MAPELI, A. Efeito da temperatura e do uso de embalagem na conservação pós-colheita de frutos de cagaita (*Eugenia dysenterica* DC.). **Revista Brasileira de Fruticultura**, Jaboticabal, v.37, n.3, p.568-577, 2015.
- CHAVES, L.J.; TELLES, M.P.C. Cagaita. In: VIEIRA, R.F.; COSTA, T.S.A.; SILVA, D.B.; FERREIRA, F.R.; SANO, S.M. (Ed.). **Frutas nativas da região centro-oeste do Brasil**. Brasília, DF: Embrapa Recursos Genéticos e Biotecnologia, 2006. cap.7, p.120-135.
- CORREIA, A.F.; SILVEIRA, D.; FONSECA-BAZZO, Y.M.; MAGALHÃES, P.O.; FAGG, C.W.; SILVA, E.C.; MEDEIROS NÓBREGA, Y.K. Activity of crude extracts from Brazilian cerrado plants against clinically relevant *Candida* species. **BMC Complementary and Alternative Medicine**, London, v.16, p.203, 2016.
- DONADO-PESTANA, C.M.; BELCHIOR, T.; GENOVESE, M.I. Phenolic compounds from cagaita (*Eugenia dysenterica* DC.) fruit prevent body weight and fat mass gain induced by a high-fat, high-sucrose diet. **Food Research International**, Burlington, v.77, p.177-185, 2015.
- FERREIRA, L.C.; PEREIRA, W.R. Aspectos microbiológicos e físico-químicos da conservação de cagaita (*Eugenia dysenterica* DC) com aplicação de biofilme comestível. **Caderno de Ciências Agrárias**, Belo Horizonte, v.8, n.2, p.9-13, 2016.
- GUEDES, M.N.S.; RUFINI, J.C.M.; MARQUES, T.R.; MELO, J.O.F.; RAMOS, M.C.P.; VIOL, R.E. Minerals and phenolic compounds of cagaita fruits at different maturation stages (*Eugenia dysenterica*). **Revista Brasileira de Fruticultura**, Jaboticabal, v.39, n.1, p.e360, 2017.
- HALAMA, K.J.; REZNICK, D.N. Adaptation, optimality, and the meaning of phenotypic variation in natural populations. In: ORZACK, S.; SOBER, E. **Adaptationism and optimality**. Cambridge: Cambridge University Press, 2001. cap.8, p.242-272.
- LEINONEN, T.; MCCAIRNS, R. S.; O'HARA, R. B.; MERILÄ, J. QST–FST comparisons: evolutionary and ecological insights from genomic heterogeneity. **Nature Reviews Genetics**, v. 14, n. 3, p. 179-190, 2013.
- LIMA, T.B.; SILVA, O.N.; OLIVEIRA, J.T.A.; VASCONCELOS, I.M.; SCALABRIN, F.B.; ROCHA, T.L.; GROSSI-DE-SÁ, M.F.; SILVA, L.P.; GUADAGNIN, R.V.; QUIRINO, B.F.; CASTRO, C.F.S.; LEONARDECZ, E.; FRANCO, O.L. Identification of *E. dysenterica* laxative peptide: A novel strategy in the treatment of chronic constipation and irritable bowel syndrome. **Peptides**, New York, v.31, n.8, p.1426-1433, 2010.
- MAHALANOBIS, P.C. On the generalised distance in statistics. **Proceedings of the National Institute of Sciences of India**, New Delhi, v.2, p.49-55, 1936.

- MERILÄ, J., CRNOKRAK, P. Comparison of genetic differentiation at marker loci and quantitative traits. **Journal of Evolutionary Biology**, Ede, v.14, p.892-903, 2001.
- MOREIRA, L.C.; ÁVILA, R.I.; VELOSO, D.F.M.C.; PEDROSA, T.N.; LIMA, E.S.; COUTO, R.O.; VALADARES, M.C. In vitro safety and efficacy evaluations of a complex botanical mixture of *Eugenia dysenterica* DC.(Myrtaceae): orospects for developing a new dermocosmetic product. **Toxicology in Vitro**, Oxford, v.45, n.3, p.397-408, 2017.
- NAVES, R.V.; ALMEIDA NETO, J.X.; ROCHA, M.R.; BORGES, J.D.; CARVALHO, G.C.; CHAVES, L.J.; SILVA, V.A. Determinação de características físicas em frutos e teor de nutrientes, em folhas e no solo, de três espécies frutíferas de ocorrência natural nos cerrados de Goiás. **Anais das Escolas de Agronomia e Veterinária**, Goiânia, v.25, n.2, p.107-114, 1995.
- NAVES, R.V.; BORGES, J.D.; CHAVES, L.J. A cagaiteira. **Revista Brasileira de Fruticultura**, Jaboticabal, v.24, n.2, 2002. Capa.
- NOVAES, P.; MOLINILLO, J.M.J.; VARELA, R.M.; MACÍAS, F.A. Ecological phytochemistry of Cerrado (Brazilian savanna) plants. **Phytochemistry Reviews**, Cádiz, v.12, n.4, p.839-855, 2013.
- OLIVEIRA, M.E.S.; PANTOJA, L.; DUARTE, W.F.; COLLELA, C.F.; VALARELLI, L.T.; SCHWAN, R.F.; DIAS, D.R. Fruit wine produced from cagaiteira (*Eugenia dysenterica* DC) by both free and immobilised yeast cell fermentation. **Food Research International**, Burlington, v.44, n.7, p.2391-2400, 2011.
- PROENÇA, C.E.B.; GIBBS, P.E. Reproductive biology of eight sympatric myrtaceae from central Brazil. **New Phytologist**, Lancaster, v.126, n.2, p.343-354, 1994.
- R CORE DEVELOPMENT TEAM. **R: A language and environment for statistical computing**. Vienna: R Foundation for Statistical Computing, 2013. Disponível em: <<http://www.R-project.org/>>. Acesso em: 13 mar. 2013.
- RAEYMAEKERS, J.A.M.; VAN HOUTT, J.K.J.; LARMUSEAU, M.H.D.; GELDOLF, S.; VOLCKAERT, F.A.M. Divergent selection as revealed by P_{ST} and QTL-based F_{ST} in three-spined stickleback (*Gasterosteus aculeatus*) populations along a coastal-inland gradient. **Molecular Ecology**, London, v.16, p.891-905, 2007. Disponível em: <<http://doi:10.1111/j.1365-294X.2006.03190.x>>
- RIBEIRO, E.M.G.; CARVALHO, L.M.J.; ORTIZ, G.M.D.; CARDOSO, F.S.N.; VIANA, D.S.; CARVALHO, J.L.V.; GOMES, P.B.; TEBALDI, N.M. An overview on cagaiteira (*Eugenia dysenterica* DC) macro and micro components and a technological approach. In: MUZZALUPO, I. (Ed.). **Food industry**. Croatia: InTech, 2013, p.3-22.
- ROCHA, M.S.; FIGUEIREDO, R.W.; ARAÚJO, M.A.M.; MOREIRA-ARAÚJO, R.S.R. Caracterização físico-química e atividade antioxidante (in vitro) de frutos do cerrado Piauiense. **Revista Brasileira de Fruticultura**, Jaboticabal, v.35, n.4, p.933-941, 2013.
- RODRIGUES, E.B.; COLLEVATTI, R.G.; CHAVES, L.J.; MOREIRA, L.R.; TELLES, M.P. Mating system and pollen dispersal in *Eugenia dysenterica* (Myrtaceae) germplasm collection: tools for conservation and domestication. **Genetica**, the Hague, v.144, n.2, p.139-146, 2016.
- SILVA, M.M.M.; DA SILVA, E.P.; DA SILVA, F.A.; OGANDO, F.I.B.; DE AGUIAR, C.L.; DAMIANI, C. Physiological development of cagaiteira (*Eugenia dysenterica*). **Food Chemistry**, London, v.217, p.74-80, 2017.
- SILVA, M.R.; LACERDA, D.B.C.L.; SANTOS, G.G.; MARTINS, D.M.O. Caracterização química de frutos nativos do cerrado. **Ciência Rural**, Santa Maria, v.38, n.6, p.1790-1793, 2008.
- SILVA, R.S.M.; CHAVES, L.J.; NAVES, R.V. Caracterização de frutos e árvores de cagaiteira (*Eugenia dysenterica* DC.) no sudeste do estado de Goiás, Brasil. **Revista Brasileira de Fruticultura**, Jaboticabal, v.23, n.2, p.330-334, 2001.
- SOUZA, E.R.B.; NAVES, R.V.; BORGES, J.D.; VERA, R.; FERNANDES, E.P.; SILVA, L.B.; TRINDADE, M.G. Fenologia de cagaiteira (*Eugenia dysenterica* DC.) no estado de Goiás. **Revista Brasileira de Fruticultura**, Jaboticabal, v.30, n.4, p.1009-1014, 2008.
- SOUZA, E.R.B.; NAVES, R.V.; OLIVEIRA, M.F. Início da produção de frutos de cagaiteira (*Eugenia dysenterica* DC) implantada em Goiânia, Goiás. **Revista Brasileira de Fruticultura**, Jaboticabal, v.35, n.3, p.906-909, 2013.

- TELLES, M.P.C.; DINIZ-FILHO, J.A.F.; COELHO, A.S.G.; CHAVES, L.J. Autocorrelação espacial das frequências alélicas em subpopulações de cagaiteira (*Eugenia dysenterica* DC., Myrtaceae) no sudeste de Goiás. **Revista Brasileira de Botânica**, São Paulo, v.24, p.145-154, 2001a.
- TELLES, M.P.C.; COELHO, A.S.G.; CHAVES, L.J.; DINIZ-FILHO, J.A.F.; VALVA, F.D. Genetic diversity and population structure of *Eugenia dysenterica* DC. (“cagaiteira”- Myrtaceae) in Central Brazil: spatial analysis and implications for conservation and management. **Conservation Genetics**, München, v.4, p.685–695, 2003.
- TELLES, M.P.C.; SILVA, R.S.M.; CHAVES, L.J.; COELHO, A.S.G.; DINIZ FILHO, J.A.F. Divergência entre subpopulações de cagaiteira (*Eugenia dysenterica*) em resposta a padrões edáficos e distribuição espacial. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.36, p.1387-1394, 2001b.
- TRINDADE, M.G.; CHAVES, L.J. Genetic structure of natural *Eugenia dysenterica* DC (Myrtaceae) populations in northeastern Goiás, Brazil, accessed by morphological traits and RAPD markers. **Genetics and Molecular Biology**, Ribeirão Preto, v.28, n.3, p.407-413, 2005.
- ZUCCHI, M.I.; BRONDANI, R.P.V.; PINHEIRO, J.B.; CHAVES, L.J.; COELHO, A.S.G.; VENCOVSKY, R. Genetic structure and gene flow in *Eugenia dysenterica* DC.in the Brazilian Cerrado utilizing SSR markers. **Genetics and Molecular Biology**, Ribeirão Preto, v.26, n.4, p.449-457, 2003.
- ZUCCHI, M.I.; PINHEIRO, J.B.; CHAVES, L.J.; COELHO, A.S.G.; COUTO, M.A.; MORAIS, L.K.; VENCOVSKY, R. Genetic structure and gene flow of *Eugenia dysenterica* natural populations. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.40, p.975–980, 2005.