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Plant Breeding | Article

Genetic characterization of a germplasm collection of cagaiteira, a species native to the cerrado

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Abstract

Cagaiteira is a common fruit species in the Cerrado. The plant produces a berry fruit that is consumed fresh or processed in several ways. Concerning the yield potential of the species, there is interest in its domestication for large-scale production. To this aim, the evaluation of genetic variability is essential to support breeding and domestication programs for the species. In this context, this study aimed to estimate genetic parameters of quantitative traits of the germplasm collection of *Eugenia dysenterica* DC. to provide information that may be useful in breeding programs. The experiment was set up in 1998 in a randomized block design, with 110 treatments and four replications. We evaluated the following variables of plants, which include height (*AP*), height of the first bifurcation (*AB*), the stem circumference (*CC*), average crown projection (*DC*) and variables of leaves, which include leaf length (*CL*), leaf width (*LL*), leaf format *(FF*) and petiole length (*CP*). The heritability coefficients ranged from 87.66 % to 25.16% and the coefficients of genetic variation ranged from 45% and 7%, similar to other wild species of the Cerrado. Parameter estimates suggest modest responses to selection for plant variables and expressive responses for leaf variables.

Key words: *Eugenia dysenterica* DC., genetic variability, conservation, domestication of wild species, fruit species.

Caracterização genética de uma coleção de germoplasma de cagaiteira, uma espécie nativa do cerrado

Resumo

A cagaiteira é uma espécie frutífera comum no bioma Cerrado. A planta produz um fruto do tipo baga que é consumido ***in natura*** ou processado de várias formas. Em função do potencial produtivo da espécie, existe interesse na sua domesticação, para produção em larga escala. Para atender esse objetivo, a avaliação da variabilidade genética é fundamental para subsidiar programas de melhoramento e domesticação da espécie. Nesse sentido, o objetivo do trabalho foi estimar parâmetros genéticos quantitativos da coleção de germoplasma de *Eugenia dysenterica* DC., para disponibilizar informações que possam ser úteis em programas de melhoramento da espécie. O experimento foi montado no ano de 1998 em delineamento experimental de blocos completos ao acaso com 110 tratamentos e quatro repetições. Foram avaliadas as variáveis das plantas, que incluem a altura (*AP*), altura da primeira bifurcação (*AB*), circunferência do caule a 10 cm do solo (*CC*), projeção média da copa (*DC*) e variáveis das folhas, que incluem o comprimento do limbo (*CL*), largura do limbo (*LL*), formato das folhas (*FF*) e comprimento do pecíolo (*CP*). Os coeficientes de herdabilidade ao nível das médias variaram entre 87,66% e 25,16% e os coeficientes de variação genética entre 45% e 7%, valores próximos aos obtidos para outras espécies silvestres do Cerrado. As estimativas dos parâmetros sugerem respostas modestas à seleção para as variáveis das plantas e respostas expressivas, para as variáveis das folhas.

Palavras-chave: *Eugenia dysenterica* DC., variabilidade genética, conservação, domesticação de espécies nativas, espécies frutíferas.

1. INTRODUCTION

Cagaiteira – *Eugenia dysenterica* DC. (Myrtaceae) is a fruit species native to the Cerrado, with a wide distribution in the biome. Its natural subpopulations are found more frequently in the states of Goiás, Minas Gerais and Bahia; in some regions, it forms dense aggregations of individuals (Martinotto et al., 2008). Adult plants of this species are arboreal, ranging from 4 to 10 m height, long and dense canopy. The fruit is a globose berry, flat, pale yellow, 2 to 3 cm in diameter (Donádio, 2002). In the Cerrado, the flowering of cagaiteira takes place from August to September and fruiting occurs about a month after flowering (Proença and Gibbs, 1994). The species has a mixed mating system, with a prevalence of allogamy (Proença and Gibbs, 1994). The pollination of flowers is by *Bombus* bees, the species *B. atratus* and *B. morio* (Proença and Gibbs, 1994).

Cagaiteira fruit can be consumed fresh or processed in the form of liqueurs, ice creams, juices and jams, which constitutes an important source of food and an increase in income of small farmers in Central-West Brazil. Concerning the yield potential and marketing of fruit, there is interest in the domestication of the species, with the aim of making it a target for orchards, which aim to meet the consumer market. Cagaiteira therefore is an important genetic resource of the Cerrado (Chaves and Telles, 2006).

The exploitation and management of resources for breeding purposes depend on the knowledge about the genetic diversity and the level of differentiation between subpopulations. The breeder should consider the variability as essential for achieving genetic gains through selection of superior genotypes (Cruz, 2005). This knowledge is also important to elucidate the biology and to obtain information about the evolution of the species (Brammer, 2002). In this sense, the characterization of germplasm collections can assist in the domestication of the species, while providing important information on levels of genetic variation available for breeding. The characterization seeks to understand the genetic diversity of accessions to enable their identification and promote their use in plant breeding programs. This step is crucial in the management and efficient use of materials preserved in the collections (Ferreira et al., 2007).

Previous studies on the species tried to understand the pattern of organization of genetic variation in natural subpopulations based on morphometric traits (Silva et al., 2001), isoenzymatic markers and soil conditions (Telles et al., 2001), RAPD markers (Trindade and Chaves, 2005; Zucchi et al., 2005) and microsatellites (Zucchi et al., 2003). Silva et al. (2001) evaluated phenotypic data of cagaiteira trees and fruits from 10 areas of southeastern Goiás State. The authors observed that, for all traits of the fruits, the differentiation between plants within subpopulations and between subpopulations was significant. Telles et al. (2001) examined 112 progenies (maternal families) from the collections made in the 10 natural subpopulations evaluated phenotypically by Silva et al. (2001). The plants were evaluated genetically from isoenzymatic markers and soil conditions. The authors found that the distribution pattern of genetic variability as measured by neutral markers is correlated with phenotype, through an indirect effect of the spatial pattern. It was also observed that the plant phenotype is influenced by soil type. Studies using RAPD markers (Zucchi et al., 2005) and microsatellites (Zucchi et al., 2003) indicated that natural subpopulations of *Eugenia dysenterica* DC. are strongly structured. However, for a contrasting result see Trinidad and Keys (2005), who observed low structuring of genetic variability using RAPD markers.

Knowledge of the magnitude of genetic variability as well as its distribution in subpopulations is a guiding for strategies of collection, management and use of germplasm. In this sense, this study aimed to characterize, through quantitative traits, plants from the germplasm collection of *E. dysenterica* DC. of the Agronomy College, Federal University of Goiás, Goiânia, Goiás State, to provide information that may be useful in breeding programs of this species.

2. MATERIAL AND METHODS

The study was conducted in the germplasm collection of *E. dysenterica* DC. The collection was made up of seeds collected in October 1996, in the natural range of the species. At the time, ten sites of southeastern Goiás State (Figure 1) were selected. The points for each area were GPS marked (Global Positioning System) and the altitude was obtained by an altimeter, accurate to 10 m (Table 1).

Seeds were sown in the greenhouse, and in January 1998, four plants of each progeny were transplanted to the field to represent the variability of subpopulations. The germplasm collection was initially composed of 440 plants from 110 mother trees, belonging 10 to natural subpopulations. On average, eleven progenies (maternal families) per subpopulation (from eight to twelve) were used. Individuals were transplanted to the field in a randomized block design, comprising 110 treatments, four replications and one plant per plot, totaling 440 plants spaced 6 x 6 meters. The original vegetation of the area where the in vivo germplasm collection of *E. dysenterica* was installed is interfluvial forest and the soil is classified as Dark Red Latosol.

Quantitative data were analyzed in two steps. With the aid of measuring tapes, four variables were evaluated: plant height (*AP*), height of the first bifurcation (*AB*), stem circumference at 10 cm from the ground (*CC*), average crown projection (*DC*) which was measured in two standardized directions and averaged. In the next step, we collected the metric data of leaves. These data were obtained by measuring leaves collected in four standard quadrants, in each plant. From each quadrant, we measured three leaves, therefore, twelve leaves per plant. With a digital caliper Mitutoyo©, accurate to 0.01 mm, we measured: length (*CL*) and width (*LL*) of the blade and petiole length (*CP*). Finally, *LL* measurements were divided by the corresponding values of *CL*, of each leaf, creating a format index (*FF*) of the leaves. The smaller the FF value, more spear-shaped is the leaf. Data were subjected to analysis of variance in a hierarchical random model, in which the total variance is divided into components of variance associated with the sources of variation in two levels: subpopulations and progenies within

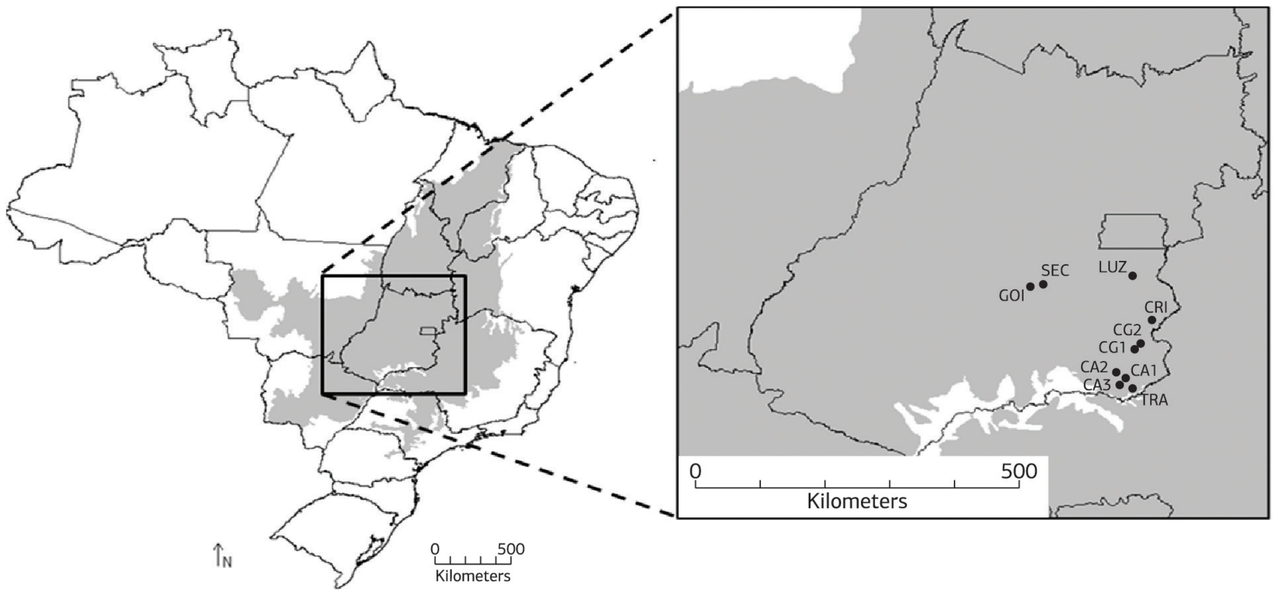

Figure 1. Sites of collection of fruit in the ten natural subpopulations of *Eugenia dysenterica* DC., where CA1, CA2, CA3 represent the municipality of Catalão, TRA - Três Ranchos, CG1, CG2 - Campo Alegre de Goiás, CRI - Cristalina, LUZ - Luziânia, GOI - Goiânia and SEC - Senador Canedo, located in southeastern Goiás State.

Table 1. Locations with geographical coordinates of the collection sites of fruit of *Eugenia dysenterica* DC. in southeastern Goiás State

Subpopulations	Number of mother plants	Location	GPS points		
			Latitude	Longitude	Altitude (m)
1	11	Catalão	18° 07' 35"	47° 54' 20"	880
2	11	Catalão	18° 02' 0,3"	48° 02' 31"	860
3	12	Catalão	18° 13' 39"	47° 58" 12"	800
4	12	Três Ranchos	18° 17' 15"	47° 48' 41"	820
5	12	Campo Alegre de Goiás	17° 39' 11"	47° 46' 37"	930
6	12	Campo Alegre de Goiás	17° 34' 24"	47° 42' 12"	780
7	8	Cristalina	17° 10' 47"	47° 31' 07"	890
8	12	Luziânia	16° 28' 48''	47° 48' 40''	900
9	8	Goiânia	16° 40' 30"	49° 14' 42"	740
10	12	Senador Canedo	16° 37' 13''	49° 04' 29''	840

subpopulations (Table 2). From the variance components, we obtained the mean estimates of phenotypic variance (σ^2_p) and genetic variance (σ^2_g). On the basis of these estimates, the mean coefficient of heritability was calculated (h^2_m) and the coefficient of genetic variation (CV_g). Statistical analyses were performed manually, using Excel© for Windows only for calculations.

The indices k_1, k_2, k_3 and k_4 in unbalanced hierarchical model correspond to the coefficients of variance between blocks ($\hat{\sigma}^2_b$), between progenies ($\hat{\sigma}^2_p$) and between subpopulations ($\hat{\sigma}^2_s$). The coefficients of variance were calculated based on Anderson et al. (1952). The values of degrees of freedom, considering the unbalance were:

$$n_s = \sum_i (n_i - 1), n_r = (b-1)\left(\sum_i n_i - 1\right) \text{ and } n_T = \sum_{ij} n_{ij} - 1 \quad (1)$$

where n_{ij} is the number of plants of the progeny *j* in the subpopulation *i* and n_i is the number of progenies of the subpopulation *i*.

3. RESULTS AND DISCUSSION

Thirteen years after being transplanted to the field (1998-2011), 402 plants had survived, which equates to a loss of 8.63% of the collection. The replacement of some dead plants was performed only at transplanting. Sano and Fonseca

(2003) reported a survival rate of 77% for *E. dysenterica* DC., 10 years after planting in a soil classified as Haplustox. In the present study, a survival rate of 91.37% was achieved after 13 years in Dark Red Latosol. The observed survival rate can be considered high for non-domesticated species. Aguiar et al. (2009) investigated the same plants of the collection until four years of age and found a loss rate of 13% of the collection. The reduction in the loss rate with the passage of time can be explained by regrowth of some plants, which had been regarded as lost until the age of four. The regrowth is fairly common in plants belonging to the germplasm collection of *E. dysenterica* DC.

As for the estimates of phenotypic means ($\bar{X}$) (Table 3), it was obtained the value of 4.7 m (sd: 0.21 m) for mean height (*AP*) of plants at 13 years of age. Aguiar et al. (2009) observed that progenies reached, at four years of age, on average, 1.68 m height, with annual average growth of 0.38 m. Based on this growth rate, it would be expected, after 13 years, that the progenies had an average height of 4.94 m, assuming a linear growth. The expected value is very close to that registered in this study, which indicates that the growth rate observed in the first four years of age can be used to predict the plant height until at least the first 13 years. The data for this trait also reinforce the current statement in the literature about the slow growth of *E. dysenterica* DC. (Sano and Fonseca, 2003).

Regarding the average crown projection (*DC*) and stem circumference (*CC*), the average phenotypic values observed (2.74 m, sd: 0.17 m and 0.40 m, sd: 0.02 m) correspond less than half of those obtained by Silva et al. (2001). The authors evaluated the mother plants from which the seeds were collected to form the collection of this work. It was obtained the phenotypic mean for the average crown projection of 5.95 m and 0.86 m for the stem circumference. These differences may be related to the age of the plants, because in natural subpopulations the plants measured by the authors were probably older than those in the collection. As to leaf variables, the mean values obtained were 69.71 mm (s.d: 4.35 mm) for leaf length (*CL*), 39.12 mm (s.d: 2.23 mm) for leaf width (*LL*), 5.52 mm (s.d: 2.67 mm) for petiole length (*CP*) and 0.57 mm (s.d: 0.04 mm) for leaf shape (*FF*) (Tabela 3). Among these variables, it is interesting to note that the subpopulations of Goiânia and Senador Canedo present the petiole length (9.57 mm and 11.39 mm respectively), on average, 59% higher than the other subpopulations.

Table 2. Scheme of the hierarchical analysis of variance with two levels of structure: subpopulations and progenies within subpopulations and the expected mean squares

Source of variation	Degrees of freedom	Mean squares	E(QM)
Blocks	b-1	Q_1	$\sigma_e^2 + k_1\sigma_b^2$
Subpopulations	q-1	Q_2	$\sigma_e^2 + k_2\sigma_p^2 + k_3\sigma_s^2$
Progeny/ Subpopulations	n_s	Q_3	$\sigma_e^2 + k_4\sigma_p^2$
Residual	n_r	Q_4	σ_e^2
Total	n_T	Q_5	

Table 3. Estimates of mean ($\bar{X}$) and standard deviation of (*s.d*) of the quantitative genetic parameters for plant variables, including plant height (*AP*), height of the first bifurcation (*AB*), stem circumference (*CC*), average crown projection (*DC*) and leaf variables, including leaf length (*CL*), leaf width (*LL*), petiole length (*CP*) and leaf shape (*FF*) referring to plants of *Eugenia dysenterica* DC.

Subpopulations	Location	Tree variables				Leaf variables			
		H (m)	*AB (m)*	*CC (cm)*	*DC (m)*	*CL (mm)*	*LL (mm)*	*TP (mm)*	*FF (mm)*
1	Catalão	4.93	1.08	0.41	3.01	69.64	39.69	4.27	0.57
2	Catalão	4.73	1.10	0.40	2.81	72.57	40.28	4.17	0.56
3	Catalão	4.87	1.08	0.41	2.94	69.41	41.73	4.56	0.61
4	Três Ranchos	4.69	1.12	0.41	2.87	70.84	42.35	4.67	0.60
5	Campo Alegre de Goiás	4.73	1.14	0.42	2.62	69.41	39.73	4.27	0.57
6	Campo Alegre de Goiás	4.61	1.22	0.38	2.45	70.99	40.47	3.82	0.57
7	Cristalina	5.05	1.09	0.39	2.62	65.31	37.25	3.84	0.57
8	Luziânia	5.04	1.37	0.38	2.66	60.21	35.94	4.61	0.60
9	Goiânia	4.36	1.10	0.41	2.62	72.69	36.74	9.57	0.51
10	Senador Canedo	4.75	1.09	0.43	2.76	76.05	37.02	11.39	0.49
	$\bar{X}$	4,78	1.14	0.40	2.74	69.71	39.12	5.52	0.57
	s.d	0,21	0.09	0.02	0.17	4.35	2.23	2.67	0.04

The analysis of variance pointed out no significant differences between subpopulations, for the variables plant height (*AP*), height of the first bifurcation (*AB*), stem circumference (*CC*) and average crown projection (*DC*) (Table 4). This indicates that the variables are not structured at the subpopulation level, because the magnitude of variation in this hierarchical level is not significant at 5% probability. In this way, the subpopulations can be considered homogeneous, as to these variables.

The height of the first bifurcation (AB) showed no significance in any of the hierarchical levels tested. This indicates that the difference between the bifurcation pattern between individuals is caused simply by random environmental variables that are not structured in any hierarchical level. This result corroborates Aguiar et al. (2009), who worked with quantitative traits from the same individuals of the germplasm collection. On the occasion, the authors found that the plant height and stem diameter at 30 cm from the ground only had significance for the proportion of variation between subpopulations in the first year of assessment. From the second to fourth year, the differentiation between was not significant for these variables.

Moreover, for leaf traits, the results of the analysis of variance indicated that, unlike the plant traits, there is a significant distinction between the subpopulations of cagaiteira, at a significance level of 1%. These results suggest that the subpopulations are heterogeneous and are structured as for the traits: length (*CL*) and width of the blade (*LL*), petiole length (*CP*) and leaf shape (*FF*) (Table 4).

The mean heritability level of ranged from 25.16% for plant height to 87.66% for petiole length (Table 5). Aguiar et al. (2009) evaluated the height of the progenies in the initial four years and obtained a mean coefficient of heritability of 33%. This difference can be explained in terms of time. Over the years, the differences between plant height should be further enhanced according to environmental differences, rather than genetic. This fact justifies a higher heritability of height in the first years. The same was observed for stem circumference. The study by Aguiar et al. (2009) reported a mean coefficient of heritability of 39% for the stem diameter. In the present study, we obtained 26% for stem circumference. Usually the characteristics that develop over a longer period are more susceptible to environment and thus have lower heritability (Borém and Miranda, 2009).

Leaf variables: length (*CL*), width (*LL*), shape (*FF*) and petiole length (*CP*) showed the highest mean coefficient of heritability of progenies, ranging from 47.3% to 87.6% (Table 5). These results are interesting since leaf traits usually present greater phenotypic plasticity and their differences are much more associated with differential response of the phenotype to an environmental condition, than with some genetic pattern. In this study, high values for this coefficient indicates that the variation in leaf characteristics are mostly related to high levels of genetic variation.

The coefficients of genetic variation (CV_g) obtained in this work were between 7% and 45% (Table 5). These estimates for both plant height and stem circumference were lower than those verified by Aguiar et al. (2009) for plant height and stem diameter. This lower value may be partially due to a greater increase in the average trait in relation to genetic variance among progenies. A study on the species native to the Cerrado *Caryocar brasiliense* Cambess (known locally as *pequizeiro*) reported coefficients of genetic variation for seedling height at the same magnitude of those estimated in

Table 4. Summary of analysis of variance for plant height (*AP*), height of the first bifurcation (*AB*), stem circumference (*CC*), average crown projection (*DC*), leaf length (*CL*), leaf width (*LL*), petiole length (*CP*) and leaf shape (*FF*) referring to plants of *Eugenia dysenterica* DC.

SV	Mean squares								
	GL	AP	AB	CC	DC	CL	LL	CP	FF
Block	3	15.684**	1.159*	0.194**	13.628**	321.128*	48.258	6.012	0.006
Prog.	109	1.996*	0.416	0.021**	1.672**	183.350**	54.205**	30.127**	0.011**
Pop.	9	1.607	0.382	0.013	1.336	852.442**	221.155**	305.255**	0.068**
Prog./Pop.	100	2.032*	0.419	0.022**	1.702**	123.131*	39.179*	5.365*	0.006**
Residual	N	1.494	0.431	0.014	0.974	91.400	28.532	3.718	0.003

* Significant at 5% probability and ** 1%.

Table 5. Estimation of quantitative genetic parameters for plant height (*AP*), height of the first bifurcation (*AB*), stem circumference (*CC*), average crown projection (*DC*), leaf length (*CL*), leaf width (*LL*), petiole length (*CP*) and leaf shape (*FF*) relative to plants of *Eugenia dysenterica* DC.

Coefficient	AP	AB	CC	DC	CL	LL	CP	FF
CV_{RES} (%)	25.574	57.456	29.708	35.982	13.708	13.605	32.286	10.004
CV_g (%)	7.758	---	10.817	15.936	7.193	6.752	45.019	8.036
h_m^2 (%)	25.163	---	32.633	41.748	50.150	47.363	87.660	70.216

this study (between 5.19% and 13.96%) (Giordani et al., 2012). The coefficient of genetic variation of the petiole size was far superior to the other traits (Table 5). This variable showed very high levels of variability within the collection, due to the characteristic size of the petiole in subpopulations of Goiânia and Senador Canedo.

The phenotypic characterization of plants native to the Cerrado has been carried out by several authors. For example with baru tree – *Dipteryx alata* Vogel. (Fabaceae) by Correa et al. (2008), pequi tree – *Caryocar brasiliense* Camb. (Caryocaraceae) by Moura et al. (2013) and jatobá tree – *Hymenaea stigonocarpa* Mart. (Fabaceae) by Matuda and Maria Netto (2005). Nevertheless, these studies are more focused on the physical and chemical characterization of the fruit, the most widely used resource of the species. In the current work, however, it has not been possible to characterize the fruit, because many trees are still young and have not reached reproductive age.

4. CONCLUSION

Subpopulations of *E dysenterica* DC. are not structured for the traits: planta height (*AP*), height of the first bifurcation (*AB*), stem circumference (*CC*) and average crown projection (*DC*). Thus, the subpopulations evaluated show no phenotypic differentiation in the characteristics of the trees.

Subpopulations of *E dysenterica* DC. are structured for the traits: leaf length (*CL*), leaf width (*LL*), leaf shape (*FF*) and petiole length (*CP*). In this way, the subpopulations analyzed show phenotypic differentiation in the characteristics of the leaves.

Heritability and genetic variation coefficients suggest the possibility of modest responses to selection of plant height (*AP*), stem circumference (*CC*), average crown projection (*DC*) and expressive response to leaf length (*CL*), leaf width (*LL*), leaf shape (*FF*) and petiole length (*CP*).

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