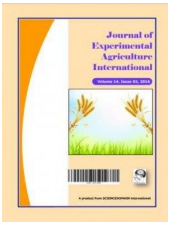

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Genetic Variability among Accessions of *Calotropis procera* Based on Agronomic Characters

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Authors' contributions

This work was carried out in collaboration between all authors. Author IVBA performed the data collection and scientific writing. Author MMR carried out the research planning and contributed to the writing of the manuscript; authors FRCB and ERR performed statistical analysis and author RLAB contributed with correction of the scientific writing. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the genetic diversity among 70 accessions of *Calotropis procera* based on agronomic characters. Seeds of *C. procera* were collected in the Northeast region of Brazil. The experiment was conducted in a greenhouse in Campina Grande, Paraíba State, between January and September 2016. The plants were grown for 240 days after sowing in plastic pots filled with soil. Data regarding 23 characteristics were recorded and analysed statistically (ANOVA and cluster analyses. The ANOVA has detected differences between accessions for all the characteristics

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indicating that all 23 agronomic characters were polymorphic. The coefficient of genetic variation ranged from 3.43% for average leaf length to 96.09% for the beginning of flowering, but were generally low (CVg < 15%) and moderate for leaf mass related parameters (CVg: 20.81-35.08). Heritability varied from 45.95 for seedling vigor index to 98.64 for total leaf mass. Globally, the high heritabilities of the various agronomic characters were explanatory of the low coefficients of genetic variation recorded. A total of 9 accessions over the 70 were found promising for use in the *C. procera* breeding program for final emergence percentage, speed of emergence index, beginning of flowering and number of flowers per inflorescence. They have also, presented forage potential (average total fresh mass: 1115.52 g and average leaf dry mass: 19.26 g), and should be preserved for posterity.

Keywords: Genetic improvement; xerophilous plant; genetic variability; Calotropis procera.

1. INTRODUCTION

Calotropis procera [Ait.] WT Aiton is a species native to Africa, Madagascar, the Arabian Peninsula, Southwest Asia and India as well as from China to Malaysia [1]. The species is a perennial, xerophilous plant that grows in arid to semiarid regions and stands out because of its drought resistance and salinity tolerance [2]. The economic applications of the species include its use as a medicinal plant due to its phytochemical and pharmacological properties [3] as well as in the production of bioenergy and biofuels in semiarid regions [4,5].

In this context, due to the economic potential and the need for new studies on the species, research has recently been conducted on its phenology [6], seed storage [7], oil and fatty acid contents [5], metabolomic response to changes in water availability [8] and genetic diversity [9,10,11], along with a literature review on the species [2].

In Brazil, research aimed at forage production has been conducted, due to the potential of this species for use as ruminant feed. Further studies are needed on the rational cultivation of the crop, especially genetic improvement studies [12], to evaluate the genetic divergence between various materials.

Genetic divergence is one of the most essential parameters evaluated by plant breeders during the initial phase of a breeding program [13], with the purpose of evaluating the genetic variability in the species to select promising materials. According to Cruz [14], the success of a plant breeding program is directly related to the existence of variability in the base population, with superior and divergent individuals recommended for crossbreeding.

Researchers have conducted genetic diversity studies with *C. procera* using molecular markers [10]; Yao et al. [11] and observed the occurrence of genetic variability. Nevertheless, Yao et al. [11] indicated that little is known regarding the genetic diversity in *C. procera*; thus, new studies are necessary to increase knowledge concerning this diversity.

Although the volume of genetic information from molecular markers has increased significantly in genetic diversity studies, emphasis is still placed on the study of diversity through phenotypic characteristics, mainly of a quantitative nature, due to their economic importance in plant breeding programs [15]. Almeida et al. [16] started a breeding program for *C. procera*, with the early selection of seedlings from estimates of genetic parameters. Recently, Almeida et al. [9] performed a study of genetic diversity based on seed physiological quality.

However, the literature contains no genetic diversity studies of the species using phenotypic characters, and research is needed to identify divergent accessions with potential for forage production. Thus, preliminary research involving the germplasm occurring naturally in the Brazilian territory becomes an important step in the *C. procera* breeding program. Therefore, considering the economic potential of the species, especially its forage potential, the present study aimed to evaluate the genetic diversity among *C. procera* accessions based on agronomic traits to support the selection of genotypes with forage potential.

2. MATERIALS AND METHODS

Seeds from 70 accessions of *C. procera* were collected between October and January 2016 in the Northeast region of Brazil within areas where the species occurs naturally (Fig. 1). Currently,

these accessions are included in the germplasm collection of the Instituto Nacional do Semiárido (National Institute for the Semiarid region, INSA). The experiment was installed and conducted in a greenhouse at INSA in Campina Grande, Paraíba State, Brazil (7°16'23.25"S, 35°58'17.06"W and an elevation of 531 m), between January and September 2016.

Physiologically mature *C. procera* fruit with open capsules and brown seeds were collected. The seeds were extracted manually and dried in the shade. After drying, the seeds were stored in paper bags at room temperature until sowing. Next, 20 of the seeds were sown per pot, and after an evaluation of emergence, thinning was performed, leaving only five seedlings per pot. Subsequently, the plants were collected for weighing throughout the experiment, leaving only one plant per pot until 240 days after sowing (DAS).

The plants were grown for 240 DAS in plastic 10-liter pots with the following dimensions: 25 cm in height, 27 cm in diameter at the opening and 18 cm in diameter at the base. A drainage system was installed at the base of the pots, with four holes to facilitate water drainage. The pots were filled with sandy soil of the following chemical composition: pH 4.9, 2.55 mg/dm^3 of P, 71.6 mg/dm^3 of K^+, 0.09 cmolc/dm^3 of Na^+, 1.82 cmolc/dm^3 of $H^+ + Al^{+3}$, 0.4 cmolc/dm^3 of Al^{+3}, 0.34 cmolc/dm^3 of Ca^{+2}, 0.8 cmolc/dm^3 of Mg^{+2}, 1.41 cmolc/dm^3 for the sum of exchangeable bases, 3.22 cmolc/dm^3 of cation exchange capacity and 1.79 g/kg of organic matter. The soil physical composition consisted of 900 g/kg of sand (0.05-2 mm), 60 g/kg of silt (0.002-0.05 mm), 40 g/kg of clay (<0.002 mm) and sand, as textural class. Soil fertilisation was performed as recommended by the chemical and physical analysis, and the plants were irrigated at 3-day intervals, based on water potential and soil field capacity.

A randomised block experimental design was used, with 70 accessions in three replicates. The number of seedlings emerged between the sixth and the tenth DAS were counted, and the following characteristics were evaluated: the final emergence percentage (FEP) [17]; speed of emergence index (SEI) [18]; and seedling vigor index (SVI) [19]. The plant height (HEI), stem diameter (SD), number of leaves (NL), average leaf length (ALL), average leaf width (ALW), leaf area (LA) and total leaf area (TLA) at 210 DAS were also measured, as were the stem fresh mass (SFM), leaf fresh mass (LFM), total fresh mass (TFM), leaf dry mass (LDM), stem dry mass (SDM) and total dry mass (TDM) at 120 DAS. The fresh mass and dry mass data were obtained in this evaluation period, when two plants were present per pot, after which only one plant remained in the pot for the analysis of the next phenological stages, that is, of the characteristics described previously, as well as the beginning of flowering (BF), the number of flowers per inflorescence (NFI) and the number of fruit per inflorescence (NFrI).

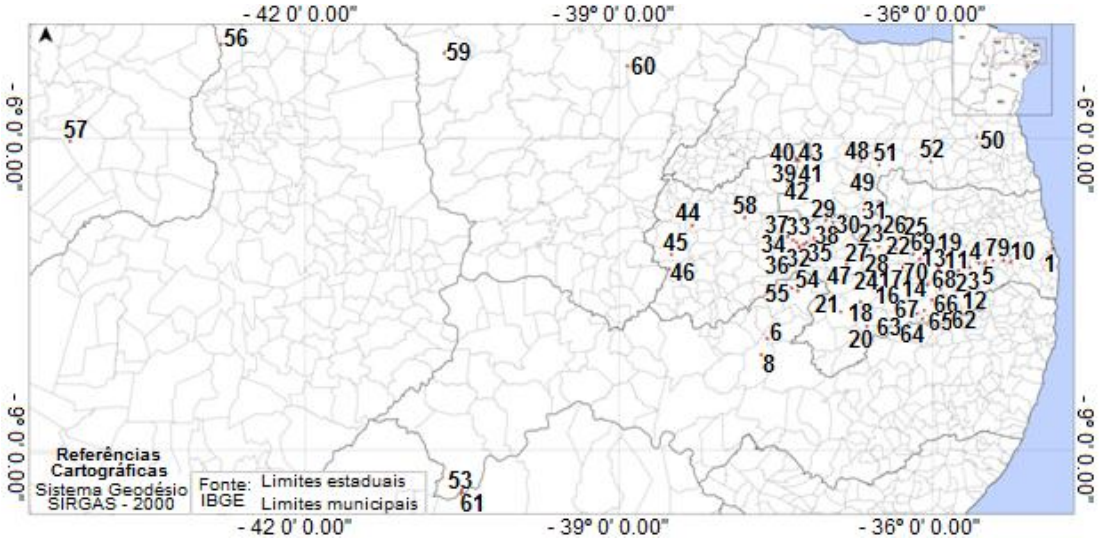

Fig. 1. Geographic location of the seed collection sites for the 70 *C. procera* accessions in the Northeast region of Brazil

The HEI was obtained by measuring the distance from the root crown to the apical bud with the aid of a graduated ruler (in centimeters). For the measurements of the SD (measured at the stem base, corresponding to the root crown of the plants), a digital caliper (in millimeters) was used. For the NL count, only fully expanded leaves were considered, and immature leaves were not counted; length (cm) and width (cm) were measured on three leaves obtained in the lower, middle and upper parts of the plant to generate the ALL and ALW values. The individual LA was obtained from the equation LA (cm²) = W x L x 0.75, proposed by [20] for *C. procera*; and the TLA was obtained by summing the individual LAs over all the leaves.

For the phytomass analysis, the plants were divided into leaves and stems for the determination of LFM, SFM and TFM on a precision scale (e = 0.0001 g). The material was then allowed to dry in a forced-air oven at 65 °C for 48 hours until reaching a constant mass, at which point the LDM, SDM and TDM were obtained, using the same scale.

In addition, chlorophyll a and b fluorescence was measured on base leaves (CAB and CBB) and on apex leaves (CAA and CBA) at 210 DAS, using a chlorophyll meter (Clorofilog Falker CFL 1030, manufactuter, country).

The data on the agronomic characteristics were subjected to analysis of variance (ANOVA) via the F test ($p < 0.05$). The genetic parameters were estimated based on the expected mean squares of the ANOVA. In addition, canonical variables analysis and cluster analysis by the Tocher method and a dendogram using the unweighted-pair-group method with arithmetic mean (UPGMA) were performed with the GENES software [21].

3. RESULTS AND DISCUSSION

The analysis of variance and estimates of the genetic parameters for the 23 agronomic characters are described in Table 1. The F test showed a significant difference (P = 0.01) among the 70 *C. procera* accessions for all the evaluated characters. Detecting differences between accessions at each of the agronomic characters indicated that the said characters were polymorphic. Thus, the genetic parameters were estimated to support the selection of promising materials. According to [22], estimates of genetic parameters assume an important predictive role for directing breeding programs during the process of selecting the most promising genotypes.

As a result, genetic variability was observed in this study, as the genetic parameter estimates demonstrate a genetic effect on the expression of the characters, with the CV*e* values indicative of good experimental precision. Additionally, the CV*g* values for most of the characters were high, higher than their CV*e* values, thus leading to a CV*g*/CV*e* ratio above 1—except for the characters SVI, CBA, CAB, CBB, SD, HEI, NL, ALL and TLA—a situation favorable to genetic gains in the selection of promising materials, according to [23].

However, these parameters should not be considered in isolation, with heritability being a better indicator of the success of a selection process in breeding programs. Because the h^2 estimates were high [24], the possibility exists for the selection of well performing accessions, with potentially significant genetic gains from the plant improvement process. [9] also observed genetic variability in *C. procera* genotypes, which supported an early selection of more productive seedlings. However, the study of genetic variability must account for information from all the phenological stages of the species, as a way of aiding the selection process.

Yao et al. [11] reported that little is known regarding genetic diversity in *C. procera*, and [5] added that no existing genetic studies have investigated the occurrence of different genotypes in the Brazilian territory. Therefore, knowing that genetic variability was present among the 70 *C. procera* accessions, a study of genetic structure was conducted through multivariate analysis, canonical variables analysis, and the measurement of dissimilarity between accessions as a way of generating a cluster structure and identifying similar and divergent materials to prevent inbreeding depression in the hybridisation programs [15].

Furthermore, Santos et al. [25] reported that studies involving the analysis of genetic diversity, based on multivariate analysis techniques, have offered effective contributions in the discrimination and indication of potential parents for use in breeding programs, besides providing a greater knowledge of the accessions in germplasm collections.

Table 1. Summary of analyses of variance and estimates of genetic parameters for the 23 agronomic characters of the 70 *C. procera* accessions

Source of variation	Mean squares								
	DF	**SEI**	**FEP**	**SVI**	**CAA**	**CBA**	**CAB**	**CBB**	**SD**
Blocks	2	39.05	124.64	0.351	11.32	6.63	0.052	0.252	16.28
Accessions	69	28.93**	677.4**	0.046**	9.27**	6.82**	16.96**	3.37**	9.26**
Residue	138	5.27	49.16	0.025	2.30	1.95	5.65	1.18	3.96
Genetic parameters									
Mean		14.73	92.92	0.37	38.74	10.69	32.97	6.75	27.41
CV*e* (%)		15.57	7.54	41.39	3.91	13.07	7.21	16.06	7.26
h²		81.78	92.74	45.95	75.15	71.32	66.67	65.07	57.17
CV*g* (%)		19.05	15.57	22.03	3.93	11.9	5.88	12.65	4.84
CV*g*/CV*e*		1.22	2.06	0.53	1.00	0.91	0.81	0.78	0.66
Source of variation	**Mean squares**								
	DF	**HEI**	**NL**	**ALW**	**ALL**	**LA**	**TLA**	**BF**	**NFI**
Blocks	2	615.24	8.93	1.96	3.74	1284.4	1124212.4	6104.16	1515.1
Accessions	69	354.57**	7.91**	1.26**	1.68**	583.6**	151842.4**	948.4**	132.0**
Residue	138	105.19	2.06	0.27	0.6	135.36	70671.51	203.35	24.1
Genetic parameters									
Mean		113.68	19.87	8.4	17.54	113.3	1678.8	168	8.73
CV*e* (%)		9.02	7.22	6.19	4.41	10.27	15.84	86.95	56.25
h²		70.33	73.96	78.47	64.5	76.81	52.73	78.56	81.75
CV*g* (%)		8.02	7.03	6.82	3.43	10.79	9.73	96.09	68.73
CV*g*/CV*e*		0.89	0.97	1.1	0.78	1.05	0.61	1.11	1.22
Source of variation	**Mean squares**								
	DF	**NFrl**	**SFM**	**LFM**	**TFM**	**SDM**	**LDM**	**TDM**	
Blocks	2	28.6	190.99	386.38	1115.52	9.9	1.71	19.26	
Accessions	69	3.50**	381.68**	685.93**	1866.43**	32.45**	14.36**	79.57**	
Residue	138	0.56	19.43	39.29	25.42	2.39	1.17	5.85	
Genetic parameters									
Mean		1.25	35.68	70.56	106.24	9.03	8.15	17.17	
CV*e* (%)		59.56	12.36	8.88	4.75	17.12	13.28	14.08	
h²		84.11	94.91	94.27	98.64	92.64	91.85	92.65	
CV*g* (%)		79.12	30.8	20.81	23.32	35.08	25.74	28.87	
CV*g*/CV*e*		1.33	2.49	2.34	4.91	2.05	1.94	2.05	

*DF - degrees of freedom; CVe - coefficient of environmental variation; h^2 - heritability; CVg - coefficient of genetic variation; ** significant ($p < 0.01$) by the F test*

In addition, the study of genetic diversity based on agronomic characters helps to distinguish the accessions, promoting the conservation of the genetic resources of the species; this protection occurs because the materials are conserved after their characterisation in the germplasm bank as a way to preserve the genetic variability of the species [26,27].

Thus, a cluster analysis of the 70 *C. procera* accessions by the Tocher optimisation method was performed, based on Mahalanobis' generalised distance (D^2). This analysis generated five distinct groups (Table 2), with three groups consisting of only one accession (groups 3, 4 and 5) and considered the most isolated among those evaluated accessions. The first group contained 88.57% of the accessions and was subdivided into three subgroups: subgroups 1, 2 and 3 formed by 25.72, 41.42 and 21.42% of the accessions, respectively.

During the collection of the seeds, a greater genetic divergence was assumed to occur with a greater geographic distance between the accessions. However, the results indicate that the clustering pattern was unrelated to the geographic origin of the materials, since accessions from distant regions showed relatively small genetic distances. Thus, future studies with molecular markers are needed to explain the observed clustering pattern.

The second group was formed by accessions 1, 50, 39, 70 and 48, which are differentiated by mean values above the overall means for all the characters and thus constitute materials with crop potential. These accessions were promising for species breeding programs and could be used in hybridisation programs, considering their production potential and genetic distance from the other groups. Cruz [14] noted that the success of a plant breeding program is directly related to the existence of variability in the base population, with superior and divergent individuals recommended for crossbreeding.

Group 3, composed of accession 20, also obtained mean values above the overall mean for all the characters, together with early flowering and fruiting, showing promise as a material for seed production. This group can also be used as a parent in hybridisation programs, given its genetic complementarity.

The fourth group, consisting of accession 53, stands out for its high phytomass production but reached a mean value below the overall mean for the SDM, LDM and TDM characters. Therefore, despite its genetic value, this genotype has no importance for the species breeding program because, according to Silva and Queiroz [28], dry matter is an important characteristic in forage plants, composing the feed portion that contains all the nutrients and corresponding to the total mass minus the moisture.

By contrast, group 5, comprising accession 28, represents the material with the greatest genetic divergence from the accessions of group I, with lower results obtained for all the examined characters. Despite the lower performance, access 28 should be preserved in the germplasm bank as a genetic resource.

In addition, cluster analysis via the UPGMA hierarchical method was performed to detail the dissimilarity between the accessions through a dendrogram (Fig. 2).

The UPGMA analysis was able to significantly represent the genetic diversity between the accessions, with a satisfactory cophenetic correlation coefficient (CCC) (0.7777). This coefficient indicates the degree of distortion regarding the representation of similarity between individuals in a dendrogram, where values close to unity represent low distortion. According to Rohlf [29], a CCC below 0.7 indicates inadequacy of the cluster method, that is, the actual genetic divergence is not reliably represented in the two-dimensional plane.

Table 2. Clustering of the 70 *C. procera* accessions by the Tocher optimisation method

Group		Accessions																	
I	I.1	25	64	4	15	7	63	43	33	5	55	16	34	54	2	26	9	37	35
	I.2	62	56	68	38	24	17	66	58	32	45	11	51	18	69	59	3	42	27
		41	44	65	61	49	46	40	14	8	52	36							
	I.3	19	29	10	31	6	13	30	23	60	47	22	57	67	12	21			
II	1	50	39	70	48														
III	20																		
IV	53																		
V	28																		

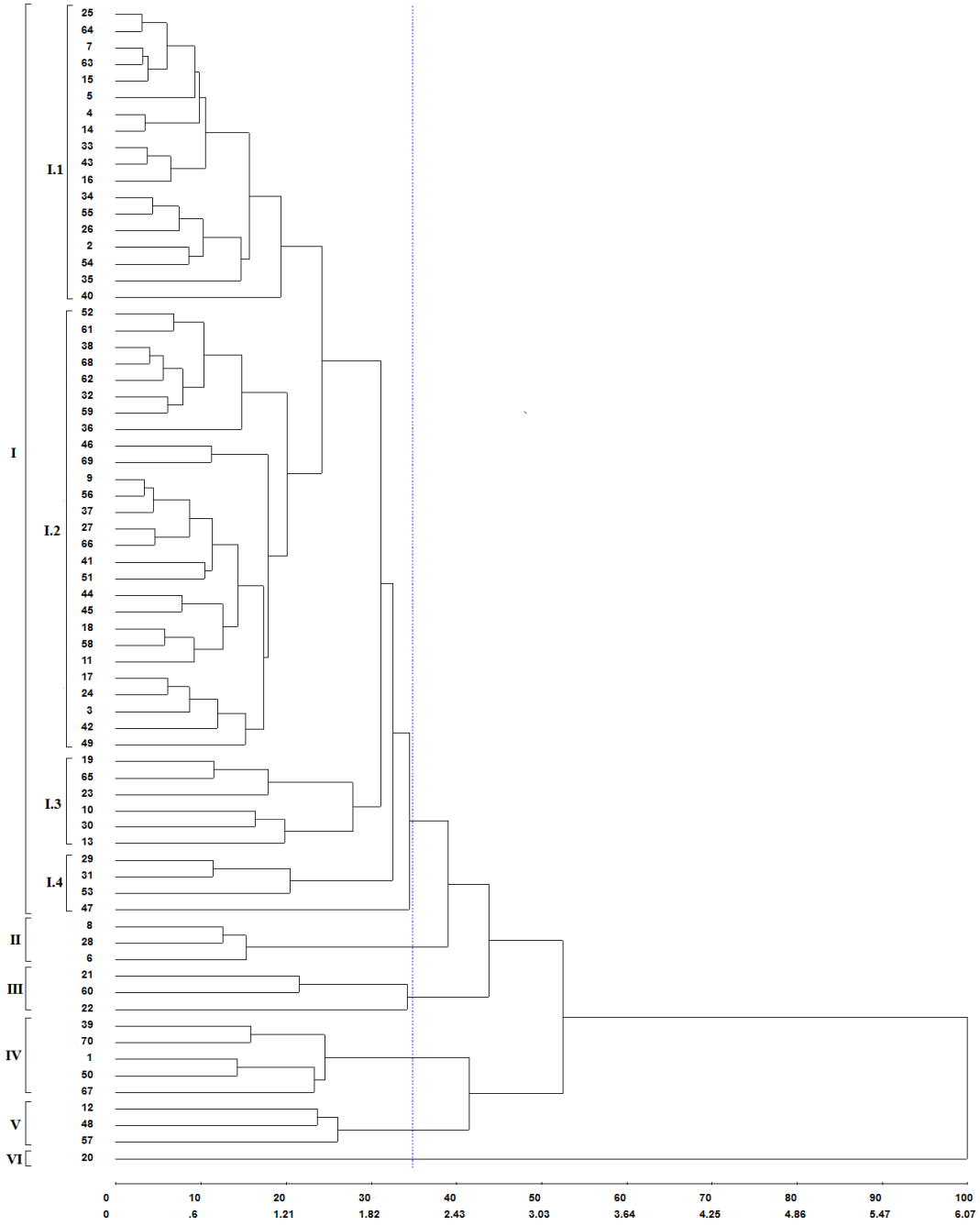

Fig. 2. UPGMA dendrogram obtained from the dissimilarity matrix (D^2) of the 70 *C. procera* accessions based on 23 agronomic characters. The cophenetic correlation coefficient was 0.777. The dotted line represents the cutoff point based on the methodology proposed by [30]

A cutoff point at 2.1268 for dissimilarity by the Mojena criterion [30] corresponds to a total dissimilarity of approximately 35%, which allowed the formation of six groups, with group 1 subdivided into four subgroups. The results observed by the Tocher method were similar to those obtained by the UPGMA analysis, differing mainly in the clustering of accessions 60, 22, 57, 67, 12, 48, 21, 6, 8 and 53, which were placed in different groups. In addition, three subgroups

were formed within group 1 with the Tocher method, whereas that same group was subdivided into four subgroups with the UPGMA analysis.

With the UPGMA analysis, accessions 12, 57 and 67 were placed in different groups, along with promising accessions in the collection, namely, 1, 39, 48, 50 and 70. However, the Tocher method clustered accessions 12, 57 and 67 within the last positions of group I.3. This positioning indicates that these are materials with a greater genetic distance from the other accessions in the same group and are therefore important from the agronomic point of view and can be recommended as potential progenitors in hybridisation programs.

As in the cluster analysis by the Tocher method, the UPGMA clustering pattern was unrelated to the geographic origin of the materials, since accessions from distant regions showed relatively small genetic distances.

In addition to the clustering methods, genetic diversity was evaluated by the canonical variables method, whose basic objective is to provide a structural simplification of the data in two or three-dimensional space. The viability of its interpretation is restricted to the concentration of the variability among the first variables, usually above 80% [16].

However, other authors recommend as viable a cumulative variance above 70% in the first canonical variables [31,32]. Note also that cluster analysis can be complemented by other visualisation techniques, such as canonical variables, as a way of facilitating geometric interpretation [16]. That facilitation justifies the use of canonical variables analysis in the present study, since the first two variables explain 72.59% of the total variation (Table 3), and this analysis additionally complements the cluster analysis and provides a structural simplification of the data.

Fig. 3 shows the scatterplot of the 70 *C. procera* accessions based on the first two canonical variables, with the formation of six distinct groups, taking as reference the agreement with the groups formed by the Tocher and UPGMA methods, indicating the characters' potential to represent the genetic diversity among the genotypes studied.

Table 3. Canonical variables and their relative (root (%)) and cumulative (cumulative root (%)) importance for the 23 agronomic characters evaluated in the 70 *C. procera* accessions

Canonical variable	Root	Root (%)	Cumulative %
1	68.864273	64.762112	64.762112
2	8.327747	7.831673	72.593785
3	5.369828	5.049954	77.643739
4	4.69763	4.417798	82.061537
5	3.136434	2.949601	85.011138
6	2.542641	2.391179	87.402317
7	2.23268	2.099682	89.501998
8	1.931751	1.816679	91.318678
9	1.468157	1.3807	92.699378
10	1.325425	1.246471	93.945849
11	1.149742	1.081254	95.027103
12	0.877134	0.824884	95.851987
13	0.805589	0.757601	96.609588
14	0.692235	0.650999	97.260587
15	0.665455	0.625815	97.886402
16	0.566546	0.532797	98.419199
17	0.490764	0.46153	98.880729
18	0.394032	0.37056	99.251289
19	0.245188	0.230583	99.481871
20	0.213711	0.20098	99.682852
21	0.151786	0.142745	99.825597
22	0.098641	0.092765	99.918362
23	0.086809	0.081638	100.00

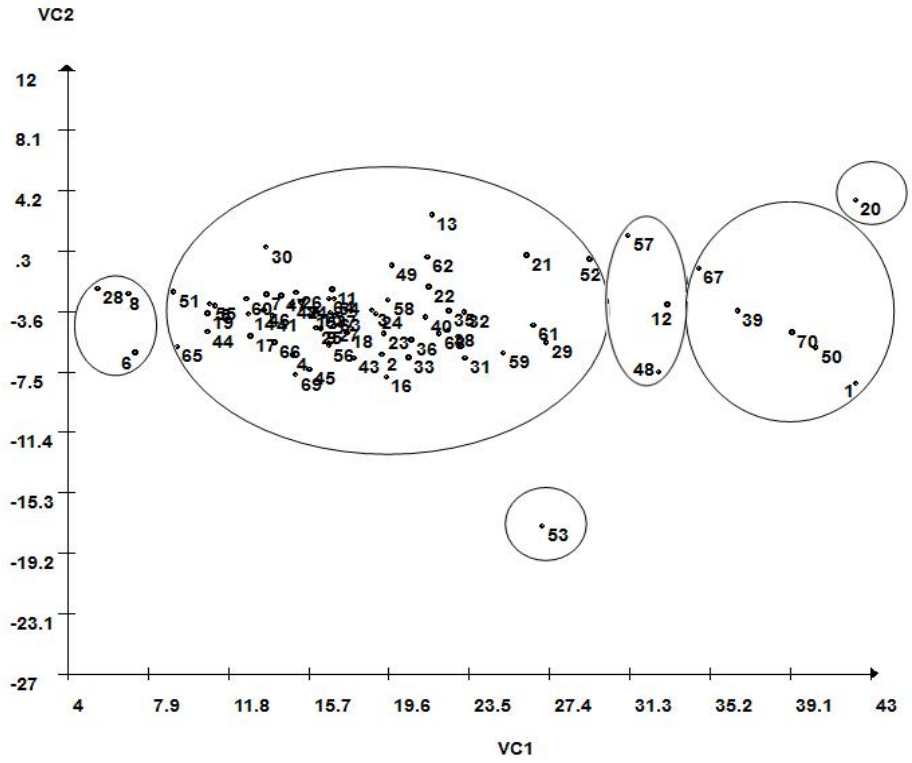

Fig. 3. Scatter plot of scores of the 70 *C. procera* accessions for the first two canonical variables, established by the 23 agronomic characters. Circles delimit the groups formed

The group compositions obtained by the different analyses, mainly supported by the estimates of genetic parameters, provide support for the identification of promising accessions. These accessions are indicated for use in breeding programs focused on forage production, considering the importance of the agronomic characteristics in the evaluation of productive potential.

Table 4 shows the means of the 23 agronomic characters of accessions 1, 12, 20, 39, 48, 50, 57, 67 and 70, selected based on the study of genetic diversity.

For quantifying the relative contribution of the agronomic characters to the genetic diversity, the method proposed by Singh [33] was used, when possible, to obtain the values of S.j (where S is the measure of relative importance for variable j) for the 23 characters from the dissimilarity measure D^2. The variables TFM, LFM and SFM were observed to contribute the most to the differentiation of the 70 accessions, with 85.23% of the total variance; and the SDM, LDM and TDM characters contributed with 14.62%. These results identify the agronomic characters to be used in future studies aimed at forage production. Note that plant phytomass is one of the main characteristics of forage plants because this character represents the productive potential of the plant. Therefore, plants with the potential to reach relatively high phytomass, and therefore relatively high percentages of dry matter, are of great importance for future *C. procera* breeding programs.

The other characters, despite their importance, yielded the lowest estimates of explain S.j. This result highlights the need to perform future research with the accessions of the present study, aiming to investigate other characteristics, such as secondary metabolites, as a means to optimise the species breeding program. Mohamed et al. [34] reported that *C. procera* latex has a high content of active compounds, including cardiotonic glycosides, alkaloids, terpenes, resins, lipids, flavonoids, tannins and steroids. Among these secondary metabolites, cardiotonic glycosides are the most important, toxic action in humans and animals. Thus, research that investigates these glycosides in the accessions of the present study is an important future priority.

Table 4. Means of the 23 agronomic characters of accessions 1, 12, 20, 39, 48, 50, 57, 67 and 70, selected based on the study of genetic diversity

Accessions	SEI	FEP	SVI	CAA	CBA	CAB	CBB	SD
1	15.11	96.67	0.34	38.85	11.35	34.05	6.7	26.06
12	15.11	96.67	0.37	34.3	7.5	32.6	6.25	25.16
20	2.94	26.67	1.01	36.15	8.6	32.3	6.3	31.35
39	17.17	98.33	0.32	38.35	10.35	35.15	8.2	27.58
48	16.56	100	0.4	33.7	7.8	29.95	5.45	25.63
50	13.94	95	0.39	41.2	12.65	34.45	7.2	33.13
57	13.36	80	0.56	35.5	7.85	31.1	6.4	26.24
67	16.17	98.33	0.31	41.65	12.35	34.6	7	28.34
70	17	100	0.32	39.85	11.4	31	5.5	27.15
Accessions	**HEI**	**NL**	**ALW**	**ALL**	**LA**	**TLA**	**BF**	**NFI**
1	125	14	8.79	17.44	118.76	1682.54	154	19
12	108.5	13.67	9.58	18.61	138.42	1898.94	198	17
20	130	15	9.97	19.14	154.53	2358.78	176	14
39	117	15.33	8.96	17.72	123.3	1886.88	180	16
48	123	15.67	8.42	16.86	106.99	1679.08	163	24
50	113	14.67	8.55	17.53	113.09	1672.22	170	16
57	110	16	8.16	16.96	107.00	1712.05	179	17
67	123	15	8.32	17.41	112.81	1693.92	204	15
70	128	15	9.58	19.15	140.60	2114.50	163	17
Accessions	**NFrI**	**SFM**	**LFM**	**TFM**	**SDM**	**LDM**	**TDM**	
1	2	62.96	112.79	175.75	16.81	12.90	29.71	
12	2	49.68	86.6	136.28	13.70	11.05	24.75	
20	2	59.45	92.72	152.17	12.07	15.81	27.88	
39	4	54.05	89.99	144.04	16.10	10.38	26.48	
48	4	46.98	85.2	132.18	14.14	10.33	24.47	
50	4	56.81	112.22	169.03	17.13	14.85	31.98	
57	2	55.57	47.71	103.28	8.52	8.22	16.75	
67	2	52.23	85.88	138.11	14.30	10.05	24.34	
70	3	56.12	92.56	148.68	16.36	10.24	26.60	

4. CONCLUSION

Genetic variability was expressed by all agronomic characters among the 70 *C. procera* accessions used in the study.

The phytomass characters were the ones that contributed to about 85.23% of the total variance among the *C. procera* genotypes. Accessions 1, 12, 20, 39, 48, 50, 57, 67 and 70 showed good agronomic performances and good forage potential as well. They were selected for inclusion in the *C. procera* breeding program for use as parents in hybridisation programs and for conservation for future studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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