



# Phenomic characterization of *Crotalaria* germplasm in Embrapa's genebank, Brazil

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**Abstract:** This study aimed to evaluate the morphological diversity of 22 *Crotalaria* accessions conserved in the Embrapa Cerrados germplasm bank, Brazil, with emphasis on their potential use in breeding and genetic resource management. Sixteen morphological descriptors were analyzed, revealing substantial phenotypic variation among accessions. Plant height ranged from 45 to 146cm, and leaflet and seed traits also showed broad variation, reflecting a wide adaptive spectrum. The Shannon–Weaver (H) and Simpson (D) diversity indices indicated consistently high diversity across traits (mean H = 0.964; mean D = 0.940), confirming the existence of a rich genetic base within the collection. Principal component analysis showed that the first two components explained 63.72% of total variance, with reproductive traits such as seed length, seed width, and 100-seed weight contributing most to variation among accessions. Cluster analysis grouped the accessions into five distinct morphological clusters, identifying genotypes with superior plant vigour, leaf area, or seed characteristics as potential parents for future breeding programmes. The observed morphological variability highlights the significant genetic diversity within *Crotalaria* germplasm, supporting its value for selection, hybridization and conservation. The combination of diversity indices and multivariate analyses provides a robust framework for identifying promising accessions for breeding programmes targeting biomass production, seed yield and environmental adaptation. These findings reinforce the importance of maintaining and characterizing germplasm collections as strategic reservoirs for sustainable crop improvement and the long-term conservation of genetic resources.

**Keywords:** Tropical legume, genetic resources, genetic diversity, morphology, cluster analysis

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## Introduction

The use of legumes in sustainable agricultural production systems has been widely recommended due to their ability to fix atmospheric nitrogen, produce high biomass, and promote physical, chemical and biological soil improvements. Among these legumes, the genus *Crotalaria* (Fabaceae) has stood out due to its hardiness, adaptation to tropical regions, and high potential for use as a cover crop and green manure (Daimon, 2006).

Studies by Koudahe *et al* (2022), Silva *et al* (2019) and Arone *et al* (2024) highlight the strategic role of *Crotalaria* in agroecological systems focused on the sustainability

of tropical soils, especially due to its efficiency in nutrient cycling, restoration of degraded areas, and ecological multifunctionality.

The genus *Crotalaria* comprises over 300 species, some of which are popularly known as ‘rattlepod’ due to the characteristic rattling sound their mature seeds make inside their dry pods (Polhill, 1968). These fast-growing plants are widely used in agricultural systems, performing various ecological functions, such as green manure through biological nitrogen fixation (BNF), carbon fixation, nutrient cycling, nematode control, and attracting pollinating insects (Muller-Salman and Kotschi, 1994). Despite this agricultural potential, which can be utilized in a variety of ways, there is still little information available on the genetic diversity stored in biological collections of the species. These genetic resources are extremely important for the selection and development of new cultivars for agricultural use.

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*Crotalaria* species are mostly annual herbaceous plants, with some perennial or shrubby species. They have erect stems, which can reach 0.5 to 3 metres in height, with variable branching and the presence of trichomes, giving the plant a pubescent appearance (National Research Council, 1979). The leaves are alternate, simple (as in *C. juncea*) or trifoliate, and vary in shape from lanceolate to elliptical (Devecchi et al, 2014).

The root system is taproot-like, with a deep, well-developed taproot, accompanied by a vigorous secondary root system (Bolleddu et al, 2023). This morphological characteristic favours nutrient absorption at depth, improves soil porosity, and contributes to soil decompaction (Lanna et al, 2021). The flowers are papilionate, usually yellow, and arranged on terminal branches. The fruits are legume-like, with several seeds that produce a characteristic sound when dry, giving rise to the common name ‘rattlepods’ (Muli et al, 2025).

The conservation and characterization of genetic resources, both *in situ* and *ex situ*, is strategic to ensure the functional resilience of the genus in the face of climate change and agricultural intensification. National and international germplasm banks, such as those of Embrapa Cerrados, the International Livestock Research Institute (ILRI), the International Center for Tropical Agriculture (CIAT) and the US Department of Agriculture (USDA), have played a relevant role in the collection, characterization and provision of *Crotalaria* accessions for use in research and development (Muli et al, 2022).

Genetic and morphological studies reveal significant intraspecific and interspecific variability between cultivated and native *Crotalaria* species, offering significant selection potential for breeding programmes aimed at developing new cultivars with pest resistance, greater biomass production, adaptation to low-fertility soils, and greater efficiency in biological nitrogen fixation (Muli et al, 2022; Odhoch et al, 2025).

*Crotalaria* species exhibit a high adaptability to the Brazilian Cerrado, particularly *C. ochroleuca*, *C. juncea*, *C. breviflora* and *C. spectabilis*, which have been extensively evaluated for biomass production, BNF, and their effects on soil fertility and biology (Silva et al, 2025). Dry matter production can range from 5 to 18t/ha, depending on soil and climate conditions and management practices (Abranches et al 2021; Lima Filho et al, 2023).

Studies conducted in the Cerrado region indicate that species such as *C. ochroleuca* and *C. juncea* are particularly efficient at accumulating nutrients: average N, P and K contents in the shoots can reach 25g/kg, 3.5g/kg, and 15g/kg, respectively (Silva et al, 2025). Furthermore, these legumes accumulate high amounts of fibres such as cellulose and lignin, contributing to the formation of stable organic matter in the soil (Guerra et al, 2007; Arone et al, 2024).

BNF is one of the most valued attributes of the *Crotalaria* genus, with reports of fixation exceeding 300kg N/ha in crops of *C. juncea* and *C. spectabilis* in association with efficient strains of *Bradyrhizobium* (Ferreira et al, 2021). This symbiosis significantly reduces the need for nitrogen fertilizers and improves nutrient use efficiency in integrated systems (Ferreira et al, 2021).

Furthermore, *Crotalaria* possesses bioactive compounds with allelopathic and nematicidal properties. Species such as *C. spectabilis* and *C. juncea* are used in the management of plant-parasitic nematodes, such as *Meloidogyne incognita* and

*Pratylenchus brachyurus*, due to the production of alkaloids that are toxic to these organisms (Wang et al, 2002; Oka, 2010).

Despite the traditional focus on its use as a cover crop, there is growing interest in improving *Crotalaria* seed productivity, especially given its expanding use in sustainable agricultural systems. Average seed production ranges from 500 to 1,200kg/ha, depending on the species, planting density, and environmental conditions (da Silva et al, 2022). Research focused on genetic improvement and harvest management has sought to optimize the viability and vigour of the seeds produced (da Silva et al, 2022).

Characterizing and evaluating the genetic diversity of these materials is essential to promote the use of genetic resources stored in germplasm banks, in addition to guiding actions to expand the available genetic base. Breeding programmes and the development of new cultivars can utilize the information resulting from efforts to characterize genetic variability and identify superior genotypes, helping to meet the demands of current and future agriculture. In this context, the study aimed to evaluate the genetic variability among the *Crotalaria* accessions stored in Embrapa Cerrados Germplasm Bank using morphological descriptors.

## Materials and methods

Twenty-two accessions of *Crotalaria* spp. in the Embrapa Cerrados Germplasm Bank, located at Planaltina/DF, Brazil, were evaluated to determine their phenomic diversity (Table 1).

**Table 1.** List of *Crotalaria* spp. accessions in the Germplasm Bank of Embrapa Cerrados. \*, Brazilian germplasm system number.

Id Number	BRA Number*	Species
2	00158911-8	<i>Crotalaria mitosa</i>
3	00158912-6	<i>Crotalaria spectabilis</i> Roth
4	00158913-4	<i>Crotalaria</i> sp.
6	00158921-7	<i>Crotalaria anagyroides</i> Kunth
7	00158916-7	<i>Crotalaria</i> sp.
8	00158917-5	<i>Crotalaria</i> sp.
10	00158919-1	<i>Crotalaria</i> sp.
11	00158920-9	<i>Crotalaria</i> sp.
12	00158914-2	<i>Crotalaria</i> sp.
13	00158915-9	<i>Crotalaria retusa</i> L.
14	00158922-5	<i>Crotalaria spectabilis</i> Roth
16	00158925-8	<i>Crotalaria</i> sp.
19	00158928-2	<i>Crotalaria striata</i> DC.
20	00158929-0	<i>Crotalaria ochroleuca</i> G. Don
21	00158939-9	<i>Crotalaria incana</i> L.
22	00158930-8	<i>Crotalaria mucronata</i> Desv.
24	00158932-4	<i>Crotalaria spectabilis</i> Roth
25	00158933-2	<i>Crotalaria retusa</i> L.
26	00158934-0	<i>Crotalaria retusa</i> L.
28	00158936-5	<i>Crotalaria grantiana</i> Harv.
29	00158937-3	<i>Crotalaria paulina</i> Schrank
30	00158938-1	<i>Crotalaria pallida</i> Aiton

Seeds of each accession were sown in 60-cell plastic trays filled with a suitable substrate, remaining there for 90 days after germination. After this period, they were transplanted to the field at Embrapa Cerrados Research Center, in Planaltina/DF/Brazil (15°35'34,42"S e 47°43'53,41"W), in a clayey red latosol, in single-row plots containing seven plants per plot, spaced with 0,5m among plants and 1,5m between lines. After 90 days, the vegetative measures were performed and the seeds characteristics were collected according to the maturation of the seeds, from each accession, using 16 qualitative and quantitative descriptors as presented in Table 2.

A graduated ruler and precision callipers were used to accurately measure morphological data. Seed weight was measured using samples of 100 seeds, with five replicates per accession. Seed colour was classified according to the Royal Horticultural Society (RHS, 2015) colour chart, and seed shape was defined based on pre-existing morphological classes (heart and kidney).

Genetic variability among the accessions with respect to the morphological descriptors was examined by calculating Simpson's (1949) and Shannon and Weaver's (1949) diversity indices. These indices give a measure of phenotypic diversity and range from zero to one, where one represents great genetic diversity and zero the opposite or no genetic diversity. The indices correspond to the probability that two individuals randomly selected from a group of populations will have the same morphological feature. The formulas for calculating both indices are presented below.

- Shannon–Weaver (H):

$$H = - \sum_{i=1}^n p_i \ln(p_i)$$

- Simpson (D):

$$D = 1 - \sum_{i=1}^n p_i^2$$

where  $i = 1$  to  $n$ , and  $p$  is the proportion of the total morphotypes made up of the  $i^{\text{th}}$  morphotype.

The data were organized in digital spreadsheets and subjected to descriptive and multivariate analyses using R software (R Core Team, 2024). Principal component analysis (PCA) and cluster analysis were performed using the Ward method (Ward, 1963), with the aim of observing the genetic variability among the accessions and separating them into similarity groups. Qualitative descriptors were not used for PCA due to their categorical nature, making them unsuitable for PCA analysis.

## Results

A wide range of variation was observed among the evaluated morphological descriptors (Table 3), indicating substantial phenotypic diversity within the studied accessions. Plant height exhibited the largest variation, with a mean of 81.4cm and a broad range from 45 to 146cm (SD = 26.8), reflecting differences in overall plant vigour and architecture. Similarly, plant diameter varied considerably (20.5–99cm, mean = 63.6cm), suggesting diverse growth habits and canopy structures among genotypes.

Traits related to branching showed moderate variability. The main branch diameter ranged from 4.6 to 17cm, while main branch length varied between 9 and 61.5cm. The number of primary branches exhibited a relatively high dispersion, highlighting morphological heterogeneity in plant architecture.

Comparing the individual accessions, accession #25 (BGF 6494) had plants with the tallest average height (145cm) and largest average diameter (92cm), exhibiting an upright growth habit. The smallest accession #13 (BGF 6476) had an average height of 45cm and a diameter of 54cm, exhibiting a semi-upright growth habit. Both accessions are *Crotalaria retusa* L. (Table 4).

**Table 2.** List of evaluated morphological descriptors for *Crotalaria* sp.

Morphological descriptor	Code	Replications	Unit	Equipment used
Plant height	AP	7	cm	Graduated ruler
Plant diameter	DP	7	cm	Graduated ruler
Growth habit	HC	7	Categories	Visual evaluation
Main branch diameter	CM	7	mm	Digital callipers
Main branch length	CRP	7	mm	Digital callipers
Number of primary branches	NRP	7	Count	Manual counting
Central leaflet length	CFC	7	mm	Digital callipers
Central leaflet width	LFC	7	mm	Digital callipers
Central leaflet L/W ratio	RC_LFC	7	mm	Calculated
Petiole length	CP	7	mm	Digital callipers
Leaflet shape	LS	7	Categories	Visual evaluation
Seed length	CS	7	mm	Digital callipers
Seed width	WS	7	mm	Digital callipers
Seed shape	FDS	7	Categories	Visual evaluation
Seed colour	Srgb	7	Colour chart	Visual evaluation
100-seed weight	PCS	5	g	Precision scale

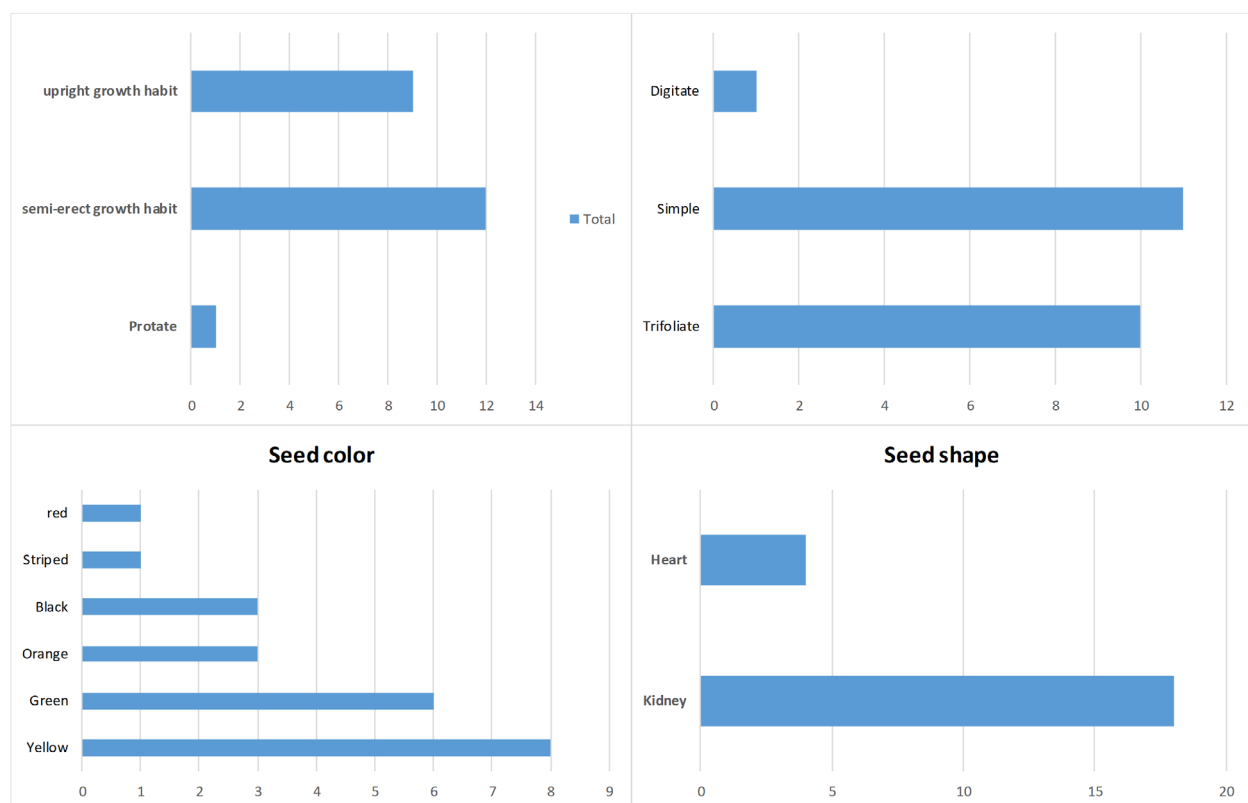
**Table 3.** Descriptive statistics of quantitative descriptors

Morphological descriptor	Mean	Standard deviation	Variance	Range
Plant height (cm)	81.4	26.8	718.3	45–146
Plant diameter (cm)	63.6	18.7	350.2	20.5–99
Main branch diameter (cm)	9.7	3.2	10.2	4.6–17
Main branch length (cm)	31.6	13.5	182.3	9–61.5
Number of primary branches	10.7	4.7	22.3	6–25
Central leaflet length (mm)	60.1	26.3	692.7	10.7–105
Central leaflet width (mm)	27.0	11.2	125.4	4.6–51
Central leaflet L/W ratio	2.6	1.5	2.3	1.47–8.4
Petiole length (mm)	20.9	15.3	233.9	2–44
Seed length (mm)	2.5	0.6	0.36	2–3.57
Seed width (mm)	3.3	0.9	0.87	2.16–4.68
100-seed weight (g)	1.2	0.7	0.52	0.40–2.19

Leaflet traits also displayed marked variation. Central leaflet length ranged from 10.7 to 105mm, and leaflet width from 4.6 to 51mm. The length-to-width ratio (L/W) varied from 1.47 to 8.4, suggesting the coexistence of both broad and elongated leaflet morphotypes. Petiole length ranged from 2 to 44mm, showing substantial variability in leaf structure.

Seed-related traits were comparatively less variable. Seed length and width ranged from 2 to 3.57mm and 2.16 to 4.68mm, respectively, with low standard deviations. The 100-seed weight exhibited moderate variation, consistent with the general trend of smaller seed-size diversity compared with vegetative descriptors.

Regarding leaflet shape, it was observed that ten accessions had trifoliate leaves, 11 had simple leaves, and only one had a digitate leaf, demonstrating significant morphological variation in this characteristic (Figure 1). In addition, significant differences in shape and colour of the seeds were observed. The majority of the accessions (18) presented a kidney shape, and the remaining accessions (4) presented a heart shape (Figure 1). In relation to the colour of the seeds, six different types were observed: yellow (6), green (6), orange (2), black (4), red (1), and striped (1) were observed (Figure 1).

**Figure 1.** Frequency distribution of growth habit, leaflet shape, seed colour and shape observed on the *Crotalaria* germplasm.

**Table 4.** Plant height (AP); plant diameter (DP); growth habit (HC); main branch diameter (DHP); main branch length (CHP); number of primary branches (NRP); central leaflet length (CFC); central leaflet width (LFC); petiole length (CP); leaflet shape (LS); seed length (CS); seed width (WS); Seed colour (Srgb) and 100-seed weight (PCS) of *Crotalaria* accessions.

ID #	AP (cm)	DP (cm)	HC	CM (mm)	CRP (cm)	NRP	CFC (mm)	LFC (mm)	RC_LFC (cm)	CP (mm)	LS	CS (mm)	WS (mm)	FDS	Srgb	PCS (g)
2	78	62	Upright	11	30	6	60.75	34	1.81	31	trifoliate	2.3	2.18	heart	yellow	0.532
3	83	63	Upright	9	26	7	43.4	24.9	1.76	33.2	trifoliate	2.15	2.8	kidney	green	0.693
4	69	60	Upright	6	25	7	65	36	1.8	35	trifoliate	2.18	2.88	kidney	green	0.664
6	93	64	Upright	12	22.5	10	39	20.5	1.92	31	trifoliate	2.65	2.47	heart	yellow	0.661
7	59	68	Semi-erect	7	42.2	9	47.5	18.7	2.52	2.7	simple	3.19	4.2	kidney	yellow	1.88
8	53	51	Semi-erect	6.5	42.5	10	50.3	16.4	3.1	2.3	simple	3.28	4	kidney	yellow	1.77
10	102	75	Semi-erect	6.3	61.5	9	85	3.5	26.8	2	simple	2.5	2.79	kidney	black	0.760
11	54	49	Semi-erect	6	41	8	48.8	17.5	2.81	2.1	simple	3.28	4.13	kidney	yellow	1.83
12	58	99	Prostrate	10	38	15	42.5	17.5	2.5	44	trifoliate	2.37	3	kidney	orange	0.777
13	45	54	Semi-erect	7	48	8	58.2	20.7	2.88	2.4	simple	3.36	4.35	kidney	yellow	2.09
14	99	51	Semi-erect	13	28	19	105	42.5	2.5	4.5	simple	3.57	4.61	kidney	black	2.19
16	67	53	Upright	12	32	11	98.9	40.2	2.48	4.8	simple	3.01	3.68	kidney	red	1.08
19	115	83	Upright	16	16	8	48.1	32.8	1.47	28.5	trifoliate	3.08	2.7	heart	green	0.878
20	146	92	Upright	17	9	25	57.5	7.75	8.4	31.2	trifoliate	2	3.31	kidney	orange	1.13
21	115	76	Upright	7.6	14	12	82	29	2.8	8	simple	2.4	2.87	kidney	green	0.719
22	67	20.5	Semi-erect	4.6	18	10	46	21.7	2.22	36	trifoliate	2.48	2.85	kidney	striped	0.766
24	63	36	Semi-erect	8.8	22	6	103.7	51	2	5.6	simple	3.57	4.68	kidney	black	2.04
25	52	44	Semi-erect	7	26.5	9	48	16.8	2.9	2.9	simple	3.27	4.42	kidney	yellow	1.96
26	54	47	Semi-erect	6	35	7	43.2	14.8	2.9	2.5	simple	3.41	4.31	kidney	yellow	2.1
28	70	46	Semi-erect	7.8	19.5	9	10.7	4.6	2.5	5.4	digitate	2	2.16	kidney	orange	0.4
29	82	84	Upright	11	28	6	41.6	21	1.97	39.4	trifoliate	2.34	2.54	kidney	green	0.583
30	90	80	Semi-erect	10.5	24.6	8	53.5	24.7	2.1	38.6	trifoliate	2.62	2.43	heart	green	0.634

**Table 5.** Shannon–Weaver (H) and Simpson (D) diversity indices for quantitative descriptors

Descriptor	Shannon–Weaver (H)	Simpson (D)
Plant height (cm)	0.984	0.950
Plant diameter (cm)	0.985	0.950
Main branch diameter (cm)	0.981	0.949
Main branch length (cm)	0.974	0.947
Number of primary branches	0.974	0.946
Central leaflet length (mm)	0.975	0.948
Central leaflet width (mm)	0.966	0.945
Central leaflet L/W ratio	0.971	0.943
Petiole length (mm)	0.865	0.920
Seed length (mm)	0.990	0.952
Seed width (mm)	0.942	0.939
100-seed weight (g)	0.956	0.942
Total	0.964	0.940

Shannon–Weaver (H) and Simpson (D) indices were calculated using the continuous values of quantitative morphological descriptors, treating each accession as a unique morphotype (Table 5).

Diversity values were consistently high across all evaluated traits. Shannon accurately represents class richness and the presence of rare classes, showing values closer to 1 when morphologically unique accessions are present, even if at low frequency. Simpson, on the other hand, is guided by the concentration in a few classes (dominance). The value is high when many accessions are very closely related/duplicated.

The Shannon–Weaver index (H) ranged from 0.865 for petiole length to 0.990 for seed length, with a total mean of 0.964. Similarly, Simpson's index (D) varied from 0.920 for petiole length to 0.952 for seed length, with a total mean of 0.940.

Among vegetative traits, plant height (H = 0.984; D = 0.950), plant diameter (H = 0.985; D = 0.950), and main branch diameter (H = 0.981; D = 0.949) showed the highest diversity values. Leaflet traits displayed slightly lower but still high values, with central leaflet length (H = 0.975; D = 0.948) and width (H = 0.966; D = 0.945) presenting similar diversity patterns.

Seed traits exhibited high and uniform diversity levels. Seed length had the highest indices among all descriptors (H = 0.990; D = 0.952), followed by seed width (H = 0.942; D = 0.939) and 100-seed weight (H = 0.956; D = 0.942). Overall, both indices indicated substantial phenotypic variability among the evaluated quantitative traits.

PCA was performed to discriminate among accessions and group them into different clusters/groups. The goal of PCA is to provide a reduced dimension model that would indicate measured differences among groups. It can also contribute to a better understanding of the set of variables by describing how much of the total variance is explained by each one. With this objective, PCA was performed only on quantitative variables. Thus, only 11 variables were used: plant height (AP); plant diameter (DP); main branch diameter (DHP); main branch length (CHP); number of primary branches (NRP); central leaflet length (CFC); central leaflet width (LFC); petiole length (CP); seed length (CS); seed width (WS); and 100-seed weight (PCS).

The PCA revealed that the first two components explain 63.72% of the total data variation, which indicates a good representation of the genetic variability among the evaluated accessions (Table 6).

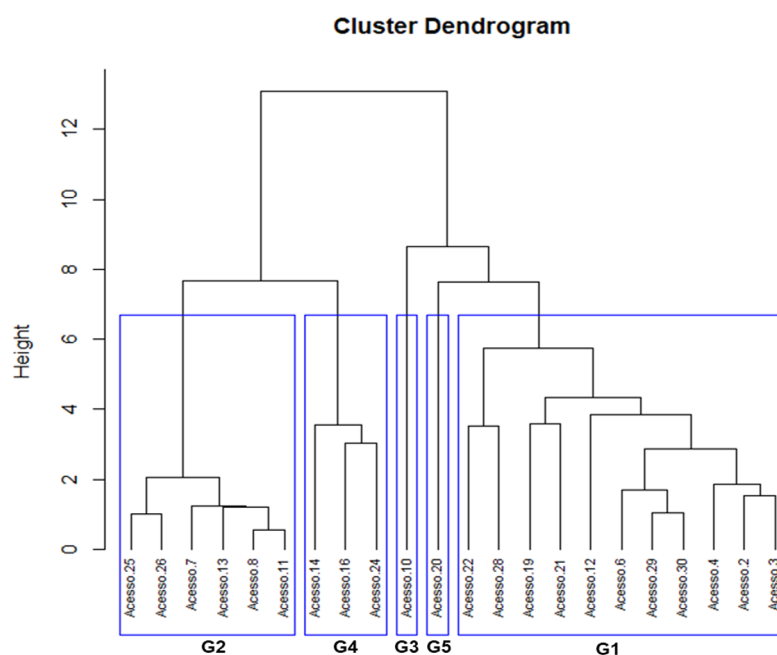
**Table 6.** Vector loadings and percentage of variation explained by the first three principal components for morphological characteristics of *Crotalaria sp.*

Variable	PC1	PC2	PC3
Eigen Value	4.56	2.45	1.47
% Explained	41.48	22.24	13.37

The loadings of morphological descriptors on each component indicated that PC1 was primarily influenced by seed length, seed width, and 100-seed weight (Table 7). PC2 was formed based on main branch diameter (CM), central leaflet length (CFC) and number of primary branches (NRP).

**Table 7.** Loadings of morphological descriptors on principal components. AP (plant height); DP (plant diameter); CM (main branch diameter); CRP (main branch length); NRP (number of primary branches); CFC (central leaflet length); LFC (central leaflet width); CP (petiole length); CS (seed length); WS (seed width); PCS (100-seed weight).

Descriptor	PC1	PC2
AP	-0.686	0.555
DP	-0.651	0.194
CM	-0.538	0.692
CRP	0.517	-0.357
NRP	-0.269	0.632
CFC	0.329	0.699
LFC	0.199	0.541
CP	-0.788	-0.079
CS	0.868	0.295
WS	0.871	0.356
PCS	0.872	0.306



**Figure 2.** Dendrogram of the 22 *Crotalaria* accessions based on morphological descriptors using the Ward method

The first two principal components were used to execute a cluster analysis using the complete linkage clusters method (Sokal and Michener, 1958). The dendrogram resulting from this analysis is presented in Figure 2.

A cluster analysis using the Ward method allowed the 22 accessions to be divided into five groups based on similarity. Group 1 was the largest, with 11 accessions, followed by Group 2 with six accessions, and Group 4 with three accessions. Groups 3 and 5 each had only one accession; these accessions correspond to the tallest and largest plants (Table 8).

Group 1 was represented by nine different species, demonstrating that it included the largest diversity. Group 2 was mostly represented by *C. retusa* accessions. Group 3 was formed by a single *Crotalaria* sp. accession. Group 4 gathered mostly accessions of *C. spectabilis* and Group 5 was formed by a single accession of *C. ochroleuca*.

## Discussion

### Phenotypic variation

The morphological evaluation of the accessions revealed substantial variability across all traits, indicating a high degree of phenotypic diversity. Most of the accessions presented an upright (11 accessions) or a semi-erect growth

habit (10). This variation is critical for selection depending on the intended agronomic purpose, such as forage production or adaptation to specific environments (Sayed et al, 2022). Similarly, plant diameter (DP) showed moderate variation, reflecting differences in plant architecture that may influence light interception and biomass accumulation (Solbrig, 1994). Accessions with greater height and diameter may be prioritized in breeding programmes aiming to maximize biomass, while more compact forms could be selected for environments requiring denser planting or easier management.

Branch traits, including main branch diameter (DHP) and main branch length (CHP), exhibited considerable diversity. Such variation affects structural stability and biomass yield, particularly in accessions with higher numbers of branches (NRP), which ranged from 6 to 25. These branching traits are important for breeding strategies targeting canopy architecture optimization, light distribution and overall productivity (Nelson and Moser, 1994).

Leaf size influences photosynthetic capacity, transpiration and overall plant growth, contributing to adaptation under different environmental conditions. Accessions with larger leaf areas could be prioritized for breeding programmes focused on maximizing biomass accumulation, while smaller leaves may be advantageous for water-limited environments (Weraduwege et al, 2015). Accessions #2, 4, 14, 16, 19, 21

**Table 8.** Averages of morphological descriptors by group AP (plant height); DP (plant diameter); CM (main branch diameter); CRP (main branch length); NRP (number of primary branches); CFC (central leaflet length); LFC (central leaflet width); CP (petiole length); CS (seed length); WS (seed width); PCS (100-seed weight).

Cluster	Nº	AP	DP	CM	CRP	NRP	CFC	LFC	CP	WS	CS	PCS
G1	11	83.6	67.0	9.6	23.8	8.9	48.4	24.2	30.0	2.4	2.6	0.7
G2	6	52.8	52.6	8.7	16.0	5.7	42.5	17.5	25.0	3.3	3.4	2.1
G3	1	102.0	75.0	6.1	18.0	9.0	55.0	34.5	20.0	2.5	2.8	0.8
G4	3	80.0	72.0	17.0	40.0	5.0	65.0	30.0	14.0	2.0	2.0	1.0
G5	1	146.0	92.0	17.0	90.0	25.0	57.5	7.8	31.2	2.0	3.3	1.1

and 24 presented the larger leaf area among the germplasm.

Seed weight (PCS) ranged from 0.40 to 2.19g, indicating potential differences in seedling vigour and establishment. Twelve of the 24 accessions (half) had a seed weight below 1g, while the other half exceeded 1g. The presence of approximately seven accessions classified only as *Crotalaria* sp. within these two groups complicates the determination of whether this difference is genuinely species-related. Nevertheless, there are three accessions classified as *C. spectabilis*. The PCS for these accessions presented large variability: 0.7g; 2g and 2.2g. Variation in seed weight is a critical trait for germplasm evaluation and breeding, as it often correlates with seedling performance, establishment success, and yield potential (Gnan *et al*, 2014). Accessions with higher seed weights may be selected to enhance early growth and establishment in breeding programmes targeting improved crop performance.

The observed diversity in seed shape and colour, as well as in leaf morphology (trifoliolate, simple, and digitated), confirms significant genetic variability and underscores the importance of *ex situ* conservation in the Embrapa Cerrados germplasm bank. The presence of accessions with unique characteristics, such as heart- or kidney-shaped seeds and distinct growth habits, highlights the availability of genetic resources for breeding programmes targeting seed production, biomass yield and nutrient-use efficiency (Arone *et al*, 2024; Muli *et al*, 2021).

Overall, the observed morphological variation suggests a rich genetic base within the evaluated accessions. Vegetative traits, particularly plant height, leaflet size and branching characteristics, exhibited higher coefficients of variation than reproductive traits, emphasizing their contribution to morphological differentiation among accessions. Integrating these phenotypic evaluations into germplasm selection strategies can facilitate the identification of superior accessions, providing a foundation for efficient breeding programmes. This broad genetic diversity could also be exploited in breeding programmes targeting plant architecture and leaf morphology, resulting in new cultivars with improved adaptability, biomass productivity, or specific agronomic characteristics. Future studies combining molecular marker analysis with these morphological traits would provide a more comprehensive understanding of the genetic potential and heritability of key traits.

## Diversity index

The analysis of morphological traits in this study provides valuable insights into the genetic diversity of the evaluated accessions. The Shannon–Weaver (H) and Simpson (D) diversity indices offer a quantitative assessment of this diversity, which is crucial for effective breeding and germplasm selection strategies.

The quantitative morphological descriptors showed an average Shannon–Weaver diversity index (H) of 0.964 and an average Simpson diversity index (D) of 0.940. When H and D values are high and close to 1 in a germplasm collection calculated from morphological descriptors, this generally indicates high phenotypic diversity and low dominance of a morphological state. Traits such as AP (H = 0.984), DP (H = 0.985), and CS (H = 0.990) exhibited high diversity, indicating a broad genetic base. Conversely, traits like NRP (H = 0.65) showed lower diversity, suggesting potential

areas for improvement through breeding.

A high H value indicates many states present (class richness) and/or a relatively balanced distribution among these states (evenness). In practice, this means that the collection covers a wide range of phenotypes and is not concentrated in a few morphological types.

A high D value (approaching 1) indicates the absence of a clearly dominant morphological state among the analyzed descriptors. This suggests that, based on these descriptors, the germplasm bank contains few ‘identical’ accessions, resulting in lower redundancy (fewer evident phenotypic duplicates).

These results indicate that the collection exhibits good stratification and represents a wide variation, which is valuable for *ex situ* conservation. The existing diversity, already well distributed, increases the potential for assembling core collections with minimal loss of variability, while the presence of extreme or rare phenotypes provides additional options for pre-breeding.

Accessions with unique trait profiles, especially those with lower diversity, can serve as valuable sources of novel alleles. Incorporating these into breeding programmes can broaden the genetic base and introduce beneficial traits (Fu *et al*, 2015).

The diversity indices can inform the development of selection indices that prioritize traits with optimal diversity levels, balancing the need for improvement with the preservation of genetic variability.

Diversity assessments in other legume species have demonstrated that combining diversity indices such as Shannon–Weaver and Simpson with multivariate analyses is an effective approach for characterizing germplasm collections and guiding breeding and conservation strategies (Upadhyaya *et al*, 2002; Carvalho and Quesenberry, 2009).

## Principal component analysis

PC1 was primarily influenced by reproductive characteristics, specifically seed length, seed width, and 100-seed weight (Table 6). These positive loadings suggest that seed size and weight were the main sources of variation among the accessions. Conversely, a morphological trade-off was indicated by the negative correlations of PC1 with vegetative traits, such as petiole length (-0.788), plant height (-0.686), and plant diameter (-0.651). This pattern – a trade-off between vegetative growth and seed size – is consistent with findings reported by Devecchi *et al* (2014), Lanna *et al* (2021) and Carvalho and Quesenberry (2009).

PC2 was predominantly associated with plant height (AP), main branch diameter (CM), number of primary branches (NRP), central leaflet length (CFC) and central leaflet width (LFC).

## Cluster analysis

Group 1 (11 accessions) represents the ‘mainstream’ ideotype: medium plant height and canopy diameter, moderate stem and primary-branch development, intermediate leaflet and petiole dimensions and the smallest seeds with the lowest 100-seed weight. Agronomically, this profile fits a general-purpose cover/green-manure pool with reasonably balanced architecture. Group 2 (6 accessions) is a compact pool but clearly stands out for larger, heavier seeds, which is typically associated with better field emergence

and seed-lot robustness – useful for seed production and for service-crop cultivars where establishment reliability is a priority, including intercropping scenarios where lower stature reduces competition. Group 3 (1 accession) and Group 4 (3 accessions) concentrate ‘leaf-and-structure’ extremes: Group 3 has tall and wide plants with large central leaflets and moderate branching, suggesting strong shading potential for weed suppression and fast canopy closure (Abranches *et al*, 2021). Group 4 combines medium height with thick stems and very large leaflets but fewer primary branches, pointing to structural robustness and a broad-leaf canopy that can be valuable for biomass/canopy design depending on lodging behaviour. Group 5 (1 accession) is the most extreme architecture ideotype – very tall, very wide, exceptionally branched (#25) with very long main-branch length – indicating a high-biomass, high-competitiveness pool for aggressive cover, soil protection, and rehabilitation, while its comparatively heavier seeds support establishment.

These findings are consistent with previous studies on *Crotalaria* species, where PCA and cluster analyses have been employed to assess morphological diversity and accessions grouping based on phenotypic traits (Muli *et al*, 2021; Yaradua *et al*, 2018). The combination of PCA and cluster analysis in this study provides a comprehensive understanding of the morphological variation among *Crotalaria* accessions, highlighting the potential for selection and breeding programmes aimed at improving agronomic performance.

The comprehensive morphological evaluation of the *Crotalaria* accessions revealed a high degree of phenotypic and genetic diversity across vegetative and reproductive traits. The wide variation observed in plant height, diameter, branching pattern, leaflet morphology and seed characteristics underscores the existence of a rich and heterogeneous genetic base within the studied germplasm. The consistently high Shannon–Weaver and Simpson diversity indices confirm that the collection maintains a broad spectrum of variability, which is essential for effective germplasm conservation and future breeding initiatives.

The principal component and cluster analyses provided a clear separation of accessions into distinct morphological groups, indicating that the evaluated traits successfully captured meaningful genetic differentiation. Accessions exhibiting superior plant vigour, leaf area, or seed size were identified as potential parents for breeding programmes targeting biomass accumulation, seed production, or adaptability to diverse environments. Meanwhile, genotypes with contrasting phenotypes represent valuable resources for introgressing novel alleles and maintaining broad genetic variability within the species.

## Conclusion

The results of this study have practical implications for both breeding and germplasm conservation. The identification of distinct phenotypic groups and accessions with superior agronomic traits provides a solid foundation for developing improved cultivars with enhanced biomass production, seed yield and environmental adaptability. Accessions representing divergent morphological clusters should be prioritized in hybridization programmes to maximize heterosis and maintain broad genetic variability. Furthermore, the high within-collection diversity observed reinforces the importance of continuous phenotypic and molecular characterization in

germplasm banks to ensure the long-term preservation of genetic resources and their effective utilization in sustainable agricultural systems.

In summary, the study demonstrates that morphological characterization, combined with diversity indices and multivariate analyses, is a powerful approach for assessing genetic variability in *Crotalaria*. The identified diversity provides a solid foundation for future breeding efforts aimed at optimizing plant architecture, seed yield, and adaptation, while supporting the conservation and strategic utilization of genetic resources essential for crop improvement and ecosystem sustainability.

## Author contributions

JMMS conducted data collection and manuscript writing. JVM performed the data analysis. AKBR, CTK, MRF and GJB contributed to manuscript review. MAC served as germplasm curator and contributed to experimental planning, data analysis and manuscript writing.

## Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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