

Morphological and productive characterization of *Macroptilium lathyroides* (L.) Urb. native to the semi-arid region of Brazil

Gabriella Pinheiro de Albuquerque¹, Mércia Virginia Ferreira dos Santos¹, Ana María Herrera Ângulo², Márcio Vieira da Cunha¹, Mario de Andrade Lira Junior¹, José Carlos Batista Dubeux Júnior³, Natália Viana da Silva¹, Alexandre Carneiro Leão de Mello¹

¹ Universidade Federal Rural de Pernambuco, Recife, PE, Brazil. E-mail: gabriella.zootec@gmail.com; mercia.vfsantos@ufrpe.br; marcio.cunha@ufrpe.br; mariolirajunior@gmail.com; natalia.viana@ufrpe.br; alexandre.lmello@ufrpe.br

² Universidad Nacional Experimental del Táchira, San Cristóbal, Táchira, Venezuela. E-mail: <u>aherrera@unet.edu.ve</u>

³ University of Florida, North Florida Research and Education Center, Marianna, FL, United States of America. E-mail: <u>dubeux@ufl.edu</u>

ABSTRACT: Fifteen accessions of *Macroptilium lathyroides* (L.) Urb. were collected in three semi-arid Brazilian municipalities and evaluated to characterize their morphology and yield in a greenhouse for three 60-day growth cycles. In the first two cycles, chlorophyll a and b, carotenoids, and soil plant analysis development index (SPAD) were estimated, and root dry mass was determined in the third cycle. Data were analyzed by analysis of variance and Kruskall-Wallis test when appropriate. Principal component analysis and clustering were performed for all accessions. Leaf length, leaf number, leaf mass and total aerial mass production differed between accessions and growth cycles. Root dry mass differed between accessions and SPAD index and stem dry mass between growth cycles. The first two principal components (representing 67% of the variation) generated three clusters based on leaf and leaflet length, plant height, and total aerial mass. The variation in plant height, leaf number, and leaf and total aerial mass in all accessions of *M. lathyroides* indicates that there is merit in collecting further germplasm of this species to support future breeding programs. Access 62F (Bom Jardim) stands out considering most of the morphological and productive.

Key words: genetic potential; native legumes; photosynthetic pigments; selection; water stress

Caracterização morfológica e produtiva de *Macroptilium lathyroides* (L.) Urb., nativa do semiárido brasileiro

RESUMO: Quinze acessos de *Macroptilium lathyroides* (L.) Urb. foram coletados, caracterizando sua morfologia e produtividade em casa de vegetação durante três ciclos de crescimento (60 dias). Nos dois primeiros ciclos foram estimadas clorofila a e b, carotenoides e índice de desenvolvimento de plantas do solo (SPAD) e a massa seca de raízes foi determinada no terceiro ciclo. Utilizou-se análise de variância e teste de Kruskall-Wallis quando adequado. Análise de componentes principais e agrupamento foram realizados para todos os acessos. O comprimento foliar, número de folhas, massa foliar e a produção de massa aérea total foram diferentes entre os acessos e os ciclos de crescimento. A massa seca das raízes apresentou diferença entre os acessos e o índice SPAD e a massa seca do caule entre os ciclos de crescimento. Os dois primeiros componentes principais (representando 67% da variação) geraram três grupos a partir do comprimento da folha e do folíolo, altura da planta e massa aérea total. A variabilidade na altura das plantas, número de folhas e massa aérea total e foliar em todos os acessos de *M. lathyroides* indica que há mérito em coletar mais germoplasma desta espécie para apoiar futuros programas de melhoramento. O acesso 62F (Bom Jardim) destaca-se pela maioria das características morfológicas e produtivas avaliadas.

Palavras-chave: potencial genético; leguminosas nativas; pigmentos fotossintéticos; seleção; estresse hídrico



* Mércia Virginia Ferreira dos Santos - E-mail: mercia.vfsantos@ufrpe.br (Corresponding author) Associate Editor: Ricardo Gallo

Introduction

Legumes are one of the most ecologically important plant families due to symbiosis with rhizobia and biological nitrogen fixation (<u>Sprent et al., 2017</u>). The use of native legumes in tropical pastures, besides being a forage resource, improves and conserves degraded or potentially degradable soils (<u>Santos et al., 2023</u>). This is particularly important where high temperatures, low rainfall and high evapotranspiration prevail, which over time may require restoration of pastures (Muir et al., 2011; Pérez-Fernández et al., 2016).

To succeed in the introduction of native legumes, the germplasm must be previously explored (Menezes et al., 2015; Muir et al., 2019). Biomass production, morphology, nitrogen fixation capacity and seed availability under water stress are some of the characteristics which should be considered in the selection of native legumes for soil conservation and ruminant feed. These characteristics, and the lower input required by native germplasm increase their economic viability for introduction into the system (Toniutti et al., 2015; Muir et al., 2016), which could minimize fertilizer use, restore soil function and biodiversity, increase environmental services and system sustainability (Muir et al., 2011; Toniutti et al., 2015; Bhatt et al., 2016).

Macroptilium species are considered a potential forage native resource under water stress (Santos et al., 2014), although little productive and chemical composition information is known (Silva et al., 2015). This legume genus, native to tropical America, is represented by 21 species distributed mainly in Northern Argentina, Venezuela's savannas, Southern Uruguay and several regions of Brazil and Mexico (Torres-Colín et al., 2010; Ciotti et al., 2014; <u>Prabhukumar et al., 2016; Alatorre-Hernández et al., 2018</u>). According to <u>Sousa et al. (2013</u>), it was introduced to tropical and subtropical India, Australia, Africa, and southeastern North America.

Macroptilium lathyroides stands out among the forage species of the genus (Santos et al., 2013; Bortolini et al., 2015), although it was previously known as *Phaseolus lathyroides* L. (Cook & Schultze-Kraft, 2015). This is an annual with erect growth, up to 1.5 m height, used for conservation of degraded areas and animal feed (Fuentes et al., 2010; Castell-Puchades et al., 2016). However, due to its wide adaptability (found in areas with low and erratic rainfall and flooding conditions), short reproductive cycle, and nonlimiting seed availability, it has been reported as a weed, mainly in soybeans, rice, and guava (Concenco et al., 2012; Ciotti et al., 2014; Hoyos et al., 2015; Silva et al., 2017).

Although the species is not well understood, it fixes between 10-30 kg N ha⁻¹ (Freitas et al., 2011) and <u>Alatorre-Hernández et al. (2018)</u> observed high dry matter yield, protein, and digestibility, reporting 4000 kg DM ha⁻¹, 152.8 g kg⁻¹ DM and 595.6 g kg⁻¹ DM, respectively.

This agroecological versatility coupled with the lack of morphological and productive information indicate the need for more data to minimize its introduction risk (Flores &

<u>Bautista, 2012</u>). <u>Santos et al. (2013)</u> indicates the potential of collection and characterization of *M. lathyroides* accessions due to the variability presented at plant height, leaf number and potentially nitrogen fixation (Figure 1). This trial, thus, aimed to characterize the morphology and production of some native *M. lathyroides* accessions collected in three locations in the Brazilian semi-arid region.

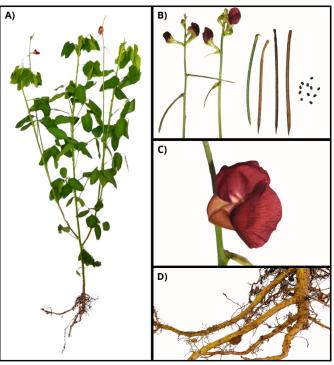


Figure 1. *Macroptilium lathyroides* (L.) Urb. A) General appearance of the plant; B) Inflorescence, pods, and seeds; C) Flower; D) Nodules on the roots.

Materials and Methods

Site and experimental design

The experiment was carried out in a greenhouse between August 2012 and April 2013 in Recife, Pernambuco, Brazil, located at 08°01'13.4"S and 34°57'14.9"W , with climate classified according to Köppen-Geiger (Peel et al., 2007) as tropical dry-summer (As'). The treatments consisted of 15 accessions of *Macroptilium lathyroides* (L.) Urb., on a completely randomized design, with three replications. The accessions were collected in three municipalities in Pernambuco (Figure 2), Brazil characterized according to conditions of average temperature and average annual rainfall (Table 1), and later identified in the Dárdano de Andrade Herbarium, Agronomic Institute of Pernambuco, Brazil.

Establishment and maintenance of forage legume

Seeds were immersed in sodium hypochlorite (1.5%) followed by sulfuric acid to break dormancy. In August 2012, they were planted in 20 L plastic pots (four seeds/pot) with drainage and filled with 1.5 kg of gravel in the bottom + 10 kg of Cohesive Yellow Argisol soil collected at 0 to 20 cm of depth.

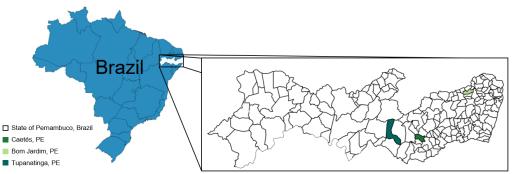


Figure 2. Location of three municipalities within the state of Pernambuco, Brazil.

Accoss	Geographic coordinates		Altitude	Municipality
Access	Longitude	Latitude	(m)	Climatic zone - Köppen-Geiger
19D	36°33′05″	8°49′66″	786	Caetés
26D	36°35′76″	8°47′77″	827	Aw: Tropical wet-dry climate
31D	36°36′93″	8°46′95″	858	Annual Pp > 750 mm; Tm > 18°C
				Tupanatinga
36E	37°15′97″	8°46'00"	628	Aw: Tropical wet-dry climate
				Annual Pp > 750 mm; Tm > 18°C
06F	35°33'78"	7°48′06″	323	
08F	35°33'79"	7°48'06"	323	
27F	35°32'68″	7°47′46″	297	
37F	35°33′14″	7°47′52″	351	
54F	35°31′82″	7°48′39″	177	Bom Jardím
62F	35°31′27″	7°48′18″	163	As': Tropical dry summer
72F	35°31′64″	7°45'67"	208	Annual Pp > 1200 mm; Tm > 18°C
81F	35°28′87″	7°44'39"	157	
109F	35°37′71″	7°49'02"	425	
110F	35°37′71″	7°49'02″	425	
111F	35°37'71″	7°49'02″	425	

Table 1. Seed collection sites for Macroptilium lathyroides	es (L.) Urb. accessions, in the state of Pernambuco, Braz
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Pp: Precipitation; Tm: Average temperature.

Soil characteristics were: pH (water, 1: 2.5) = 6.6, Mehlich-I, P = 57 mg dm⁻³, Na⁺ = 0.25 cmolc dm⁻³, K⁺ = 0.41 cmolc dm⁻³, Ca⁺²+Mg⁺² = 3.65 cmolc dm⁻³, Ca⁺² = 2.9 cmolc dm⁻³, Al⁺³ = 0.0 cmolc dm⁻³, H⁺ + Al⁺³ = 2.28 cmolc dm⁻³, and C= 6.11 g kg⁻¹. The plants were irrigated daily (200 ml of water) until 30 days after planting and subsequently, on alternate days with 400 ml of water. Plants were thinned 60 days after planting, keeping four plants in each pot.

Data collection and sampling

Evaluations were done every 60 days for three cycles, measuring leaf and leaflets length and width, considering five representative expanded leaves of each plant with a manual caliper (0.1 cm precision); leaf number per plant through manual counting; and plant height from the soil surface until the insertion of the last leaf of the last vegetative node visible in the plant with a ruler (0.1 cm precision).

Photosynthetic pigments and SPAD (soil plant analysis development) index were evaluated in the first two cycles of growth. The SPAD index was determined on five expanded leaves per plant using a SPAD 502 chlorophyll meter (Konica Minolta, Japan). After reading, samples of

leaf tissue were collected to determine the chlorophyll a and b and carotenoids content following <u>Lichtenthaler</u> (1987).

Yield was obtained by cutting at 20 cm height, followed by separation of leaves and stems. After the third evaluation, roots were extracted to determine root dry mass per plant. Leaves, stems, and roots were dried to constant weight in a forced circulation oven, at 55 ± 2 °C.

Statistical analysis

Analysis of variance was performed for the variables described above (except for leaf number and chlorophyll *b*), with Scott-Knott's grouping test (p < 0.05). Due to the absence of normal distribution and homogeneity of variance for leaf number and chlorophyll *b*, the Kruskall-Wallis analysis was used, considering access and growth cycle as sources of variation. Analyzes were performed using SAS University Edition software and InfoStat (version 2018).

A principal components analysis performed access variability, considering the variables evaluated in the three growth cycles. Subsequently, a cluster analysis was performed, considering the morphological and productivity variables best described in the principal components.

Results

Morphological characteristic

M. lathyroides accessions differed for morphological characteristics such as leaf length and leaf number (<u>Table 2</u>). Accessions 62F and 19D (p < 0.05) had longer leaves, and accessions 36E and 111F had the most leaves per plant (p < 0.01). There were also differences between the growth cycles (p < 0.01) for leaf length and leaf number, with higher values in the first cycle (<u>Table 3</u>).

The other morphological characteristics had significant interactions access × growth cycle factors. Both for leaf width and leaflet length (Table 4) most accessions had higher measurements in the first cycle, decreasing throughout the assessments, except for accessions 62F and 81F which did not differ for the first two cycles; and 37F, which had lower values for all three cycles.

For leaflet width (p < 0.05), lower values were also observed at the third cycle for all accessions (<u>Table 4</u>) but differing from the first and second cycles only for accessions 111F, 81F, 06F, 72F and 62F.

In general, plant height presented lower values (p < 0.01) for the third cycle in relation to the first (<u>Table 5</u>). For the first and second cycles accessions 111F, 72F, 36E, 31D, 19D and 26D were taller, decreasing in the third cycle, while accessions 110F, 109F, 81F, 08F and 54F were consistently lower.

Photosynthetic pigments and SPAD index

Differences were not found for photosynthetic pigments, due to *M. lathyroides* accessions, cycles or their interaction (p > 0.05), with values consistently higher for chlorophyll *a* $(0.90 - 1.25 \text{ mg g}^{-1})$ than chlorophyll *b* $(0.43 - 0.54 \text{ mg g}^{-1})$. The SPAD index was higher in the first than the second cycle at 45.44 and 35.86, respectively (p < 0.01). There was a positive and significant correlation between SPAD index and photosynthetic pigments (p < 0.01), but with low coefficients, r = 0.27, 0.31 and 0.33 when associated with chlorophyll *a*, *b* and carotenoids, respectively.

Productive characteristic

M. lathyroides accessions differed for productive characteristics such as leaf mass, total aerial mass, and root

Table 2. *Macroptilium lathyroides* (L.) Urb. native accessions of Brazilian Semi-arid morphological and productive characteristics.

A	Leaf length	Leaf	Aerial m	ass production (g	DM pot ⁻¹)	Root production
Access	(cm)	number	Leaf	Stem	Total mass	(g DM plant⁻¹)
110F	6.01 B	11 AB	2.38 B	3.69 A	6.07 B	1.50 AB
111F	6.10 B	20 A	1.86 C	3.77 A	5.63 B	1.47 AB
81F	5.82 B	9 B	1.29 C	3.05 A	4.34 C	1.36 AB
109F	5.68 B	12 AB	1.87 C	3.36 A	5.23 C	1.62 AB
06F	4.93 C	10 AB	1.59 C	3.71 A	5.30 C	1.49 AB
72F	5.05 C	12 AB	1.29 C	3.25 A	4.55 C	1.48 AB
37F	4.37 C	10 AB	1.87 C	4.06 A	5.93 B	1.28 AB
62F	6.11 A	10 AB	3.77 A	4.12 A	7.89 A	2.68 A
27F	5.80 B	9 AB	0.98 C	3.55 A	4.54 C	0.42 B
08F	5.41 B	12 AB	0.89 C	2.41 A	3.30 C	1.07 AB
54F	5.66 B	12 AB	2.78 B	3.68 A	6.47 B	2.11 A
36E	5.87 B	20.58 A	0.91 C	3.85 A	4.75 C	1.82 AB
31D	4.19 C	16 AB	1.33 C	3.26 A	4.58 C	1.07 AB
19D	5.63 A	17 AB	1.65 C	3.67 A	5.32 C	1.12 AB
26D	4.87 C	17 AB	1.39 C	2.90 A	4.29 C	1.53 AB
CV %)	10.89	29.04	43.35	12.66	20.40	33.45
F-Test						
Access	0.0269	0.0074	<0.0001	0.0820	<0.0001	0.0319

CV: Coefficient of variation.

Means followed by common uppercase letter in the column, within each variable, are not different by Scott-Knott test (p > 0.05).

Table 3. *Macroptilium lathyroides* (L.) Urb. native accessions of Brazilian Semi-arid with morphological and productive characteristics by evaluation cycle.

Characteristic		Cycle	CV	F-Test	
Characteristic	1	2	3	(%)	F-lest
Morphological					
Leaf length	7.67 A	5.52 B	3.08 C	33.69	<0.0001
Leaf number	15 A	14 A	9 B	20.72	<0.0001
Aereal mass production					
Leaf	2.44 A	1.41 B	1.29 B	30.03	<0.0001
Steam	3.95 A	3.34 B	3.20 B	9.36	0.0058
Total	6.39 A	4.74 B	4.49 B	16.15	<0.0001

CV: Coefficient of variation.

Means followed by common uppercase letter in the column, within each variable, are not different by Scott-Knott test (p > 0.05).

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	Growth cycle of 60 days								
Access	1	2	3	1	2	3	1	2	3
	L	Leaf width (cm)		Le	Leaflet length (cm)		Leaflet width (cm)		
110F	7.73 Aa	5.28 Ba	2.80 Ca	5.02 Aa	3.05 Ba	2.05 Ba	2.97 Aab	2.18 ABa	1.60 Ba
111F	8.00 Aa	5.79 Ba	3.72 Ca	4.53 Aab	3.45 ABa	2.42 Ba	2.53 Aab	2.39 Aa	1.99 Aa
81F	6.90 Aa	5.34 Aa	2.90 Ba	4.28 Aab	4.3 Aa	1.23 Ba	3.14 Aab	2.64 Aa	1.13 Ba
109F	5.64 Aab	5.15 Aa	4.04 Aa	3.57 Aab	3.76 Aa	2.69 Aa	1.77 Aab	2.88 Aa	1.81 Aa
06F	6.01 Aab	3.79 Ba	2.68 Ba	3.7 Aab	3.54 Aa	1.78 Ba	2.72 Aab	2.70 Aa	1.32 Ba
72F	5.55 Aab	6.14 Aa	3.07 Ba	4.18 Aab	3.2 ABa	2.13 Ba	2.24 Aab	2.41 ABa	1.29 Ba
37F	4.38 Ab	5.72 ABa	3.23 Ba	2.77 Ab	3.05 Aa	2.28 Aa	1.67 Ab	2.60 Aa	1.68 Aa
62F	6.70 Aab	6.50Aa	2.83 Ba	4.55 Aab	3.74 Aa	1.98 Ba	2.91 Aab	2.58 ABa	1.70 Ba
27F	6.22 Aab	4.89 Aa	2.67 Ba	4.27 Aab	3.27 ABa	1.78 Ba	2.94 Aab	2.07 ABa	1.34 Ba
08F	7.03 Aa	5.53 Aa	2.70 Ba	4.37 Aab	2.86 Ba	1.71 Ba	3.30 Aa	2.02 Ba	1.39 Ba
54F	7.05 Aa	5.91 Aa	2.52 Ba	4.4 Aab	2.71 Ba	1.51 Ba	2.88 Aab	2.14 Aa	0.98 Ba
36E	5.50 Aab	5.14 ABa	3.43 Ba	4.02 Aab	3.45 Aa	3.29 Aa	1.95 Aab	2.00 Aa	1.82 Aa
31D	5.68 Aab	3.65ABa	2.70 Ba	3.88 Aab	2.33 ABa	1.68 Ba	2.15 Aab	1.50 Aa	1.88 Aa
19D	7.75 Aa	5.05 Ba	3.00 Ca	4.25 Aab	3.15 ABa	2.25 Ba	2.60 Aab	2.00 Aa	1.75 Aa
26D	5.06 Ab	5.48 Aa	4.53 Aa	3.05 Aab	3.69 Aa	2.14 Aa	1.85 Aab	3.03 Aa	2.13 Aa
CV (%)		17.21			21.71			24.98	
F-Test									
Access × growth		0.0012			0.0208			0.0100	

CV: Coefficient of variation.

Means followed by common lowercase letter in the column for access, and uppercase in the row for growth cycle, are not different by Scott-Knott test (p > 0.05).

Table 5. Macroptilium lathyroides (L.) Urb. access × growth
cycle interaction for plant height (cm).

A = = = = =		Growth cycle	
Access	1	2	3
110F	68.39 Ab	77.30 Aa	71.00 Aa
111F	127.83 Aa	107.92 Aba	73.08 Ba
81F	80.83 Aab	90.75 Aa	93.92 Aa
109F	85.50 Aab	76.00 Aa	73.67 Aa
06F	87.08 Aab	102.00Aa	71.33 Aa
72F	111.33 Aa	109.44 Aa	61.08 Ba
37F	86.25 Aab	82.14 Aa	61.78 Aa
62F	44.43 Ab	89.53 Aba	68.17 Ba
27F	88.00 Aab	94.50 Aa	66.88 Aa
08F	63.75 Ab	78.67 Aa	74.33 Aa
54F	54.78 Ab	78.69 Aa	60.64 Aa
36E	122.63 Aa	99.50 Aba	72.88 Ba
31D	110.00 Aab	91.50 Aa	70.75 Aa
19D	106.50 Aab	125.00 Aba	62.50 Ba
26D	130.34 Aa	93.75 Aba	62.50 Ba
CV (%)		22.40	
F-Test			
Access × growth		0.0143	

CV: Coefficient of variation.

Means followed by common lowercase letter in the column for access, and uppercase in the row for growth cycle, are not different by Scott-Knott test (p > 0.05).

production (Table 2). Access 62F stand out with highest leaf, total aerial (p < 0.01) and root mass (p < 0.05). The accession 54F presented the highest values for root mass (Table 2). No differences were observed for stem mass.

The same pattern was observed for leaf, steam, and total aerial mass yield for cycle assessment, with the highest values at the first cycle (p < 0.01, Table 3), as the morphological variables. No differences were observed for root production.

Principal components and cluster analysis

The first two principal components which explain 67% of the between-access variability (Table 6), are composed of six of the nine variables. Within each principal component, variables were selected based on the correlation matrix, selecting those with the highest coefficient, which were not significantly correlated. Thus, leaf mass production and leaf number, which are highly associated (p < 0.01) with the total aerial mass production (0.94) and plant height (0.70), were not included in the components.

Cluster analysis formed three groups of *M. lathyroides* accessions, which could indicate low variability among them. Nevertheless, the characteristics used for the cluster showed differential behaviors for each access group (<u>Table 7</u>).

Table 6. Principal component vectors for morphological andproductive characteristics of *Macroptilium lathyroides* (L.)Urb. accessions.

Variables	PC-1	PC-2	PC-3
Plant height	-0.19	0.49*	0.33
Leaf length	0.40*	0.29	-0.17
Leaf width	0.38	0.31	-0.13
Leaflet length	0.33	0.43*	0.00
Leaflet width	0.27	0.17	-0.48
Leaf number	-0.07	0.42*	0.50
Total aerial mass	0.43*	-0.27	0.30
Leaf mass	0.43*	-0.29	0.12
Steam mass	0.33	-0.18	0.50
Variance (%)	42	25	18
Cumulative variance (%)	42	67	84

PC: principal component; (*) Weight variables for each component.

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Variables		CV		
variables	C-1	C-2	C-3	(%)
Plant height (cm)	89.60	68.10	82.33	28.16
Leaf length (cm)	5.51	5.93	4.30	40.96
Leaflet length (cm)	3.20	3.22	2.67	35.51
Total aerial mass (g DM/pot)	4.72	6.78	5.26	32.37
Number of accessions	10	3	2	
Accessions	19D, 36E, 111F, 26D, 08F, 81F, 27F, 109F, 72F, 06F	54F, 62F, 110F	37F, 31D	

		1.1.
Table 7 Productive and morphological	characteristics of the generated clusters	and their corresponding accessions
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Discussion

Morphological characteristics

The longer leaf length of accessions 62F and 19D (Table 2) might require higher nutrient or photoassimilate availability. These plants, under inadequate supply of any required nutrients can severely restrict normal plant growth and development (Brouder & Volenec, 2020), Still, they may also provide greater leaf area for increased light capture. According to Mitchell et al. (2020), the increase in leaf length is accompanied by reduced growth in leaf width and thickness. However, this shift in growth to produce thinner leaves allows faster establishment of functional leaf area per unit of the substrate to quickly capture sunlight and reestablish a positive C balance for the plant.

The greater leaf number of accessions 36E and 111F, could lead to greater leaf biomass, due to the relationship between leaf number and biomass accumulation potential of plants (Silva et al., 2010). However, a greater number of leaves per plant (92 - 188) have been observed in accessions of M. lathyroides collected in Pernambuco, Brazil (Borges et al., 2019). Furthermore, higher actual values for leaf mass production were found for access 62F, the accessions 36E and 111F on the lower leaf yield group and the second highest in total aerial mass group. According to Calado et al. (2016), those plants capable of producing more leaves could additionally provide better quality fodder for ruminants. This could also mean an advantage on litter quality and nutrient cycling, due to their higher nutrient content. Furthermore, these legumes transfer biologically fixed nitrogen to the herbs and grasses grow alongside them in mixed swards (Howieson et al., 2008), with economic and ecological benefits in terms of lesser fertilizer application and soil sustainability, through the provision of ecosystem services that include reduced greenhouse gas emissions compared to a N fertilizer-based cropping system (Jhariya et al., 2018). Highlighting the relevance of biological fixation for soil fertility preservation in semiarid environments, along with the need for research on the forage potential of native species (Santos et al., 2010).

Regarding the results per cycle, both leaf length and number decreased through the cuts, with the first cycle to the second reduction of 28 and 44%, and from the second to the third of 8 and 33%, for leaf length and leaf number, respectively (Table 3). Although irrigation was constant

irrigation was not adjusted through cycles considering temperature or evapotranspiration. It is possible, thus, that these climatic changes in the last cycle have limited soil water availability and consequently foliar development. Morphological changes and reduction of productive characteristics were observed by <u>Medeiros et al. (2023)</u> working with native legume *Desmanthus pernambucanus* [L.] Thellung plants, associated with restricted leaf expansion (<u>Liu et al., 2021</u>) and reduced photosynthetic capacity (<u>Queiroz et al., 2021</u>) under water deficit conditions. The accessions that stood out in the first two cycles in the leaf width (62F) and leaflet length (81F), had stronger declines between the second and third cycles of 56 and 71%, respectively (<u>Table</u> <u>3</u>). This indicate they might be most sensitive to the more stressful conditions of the third growth cycle.

Average heights of different accesses of *M. lathyroides* have been reported between 32-68 cm (Borges et al., 2019). Higher values were observed in the present study in the first cutting cycle. Decreased plant height may be associated with morphological alterations resulting from cutting in successive cycles. According to Jennings & Foster (2020) in upright species, the terminal bud is at or near the top of the plant, upward growth of the main shoot is halted when the terminal bud is removed by cutting or grazing. A decrease in plant height up to 55% in *M. lathyroides* accessions was observed at greenhouse experiment conditions when irrigation frequency decreased from 2 to 10 days without irrigation (Silva et al., 2017). However, irrigation conditions were constant in the present experiment.

There is considerable variability within each legume species there is considerable plant-to-plant variation. Plant breeders have narrowed the species variation within a cultivar, making the plants more consistent and predictable in their response to management (Jennings & Foster, 2020).

Photosynthetic pigments and SPAD index

The content of the photosynthetic pigment's chlorophyll *a*, *b*, and carotenoids vary between and within species as a function of plant development (<u>Costache et al., 2012</u>). *M. lathyroides* accessions of three different origins, had similar pigment contents, even with the morphological differences, mainly in the leaves, observed between accessions. This statement allows to infer about the similarity of the accessions in light capture potential. However, the period of greatest stress as reflected in the morphological variables

was not evaluated for these variables. Factors such as salinity, high temperatures (<u>Shareef et al., 2020</u>), and nutrient levels are directly associated with the content of photosynthetic pigments, mainly chlorophyll (<u>Siqueira et al., 2023</u>).

Chlorophyll *a*, the main photosynthetic pigment in higher plants, cyanobacteria, and algae (<u>Ghosh et al., 2018</u>), had higher values than chlorophyll *b* in all accessions, a difference associated with higher nitrogen demand in the chemical structure of this pigment, which is observed mainly in plants Fabaceae by the nitrogen fixation capacity (<u>Uvalle et al., 2008</u>). Carotenoids, which are plant pigments not visible in plants and fruits, may prevent damage to chlorophyll molecules in the presence of high light intensities (<u>Butnariu, 2016</u>).

Regarding cycles, there was a decrease in SPAD index of 15% in the first cycle to the second, reaching indexes below 40. According to <u>Lin et al. (2015)</u> SPAD index readings can achieve acceptable chlorophyll estimates in case of fresh leaves, but not under water-stressed conditions. These values could represent the threshold for the beginning of the decrease of the photosynthetic potential of the plant associated with the relative amount of chlorophyll (<u>Silva et al., 2014</u>). Some water or thermal stress conditions could affect the integrity of the cells in the organs of C₃ plants, with reflected photosynthetic potential consequences (<u>Killi et al., 2017</u>).

Productive characteristics

Accession 62F, from Bom Jardim, Pernambuco-Brazil, had the highest leaf, total aerial, and root masses (Table 2), maintaining the balance between shoot development (morphological and productive) and root development. However, considering all the characteristics, accessions 111F, 54F and 81F, also showed potential. At present, little has been referenced on the morphological development of M. lathyroides accessions, in semi-arid zones. Average total aerial mass for *M. lathyroides* accessions was between 3.30 to 7.89 g DM pot⁻¹. Both thermal and water stress have been referred to as limiting plant growth in field conditions (Lipiec et al., 2013; Karatassiou et al., 2014; Imadi et al., 2016). Similar values were observed by Araújo et al. (2011). However, variability was related to soil type, with averages of 2.00 and 9.75 g DM pot⁻¹ in Fluvic Neosol and Regolithic Neosol soil, respectively.

Principal components and cluster analysis

Due to the importance of leaf, leaflet length, plant height, and total aerial mass production in the principal component analysis of *M. lathyroides* accessions (<u>Table 6</u>), henceforward, special attention should be given to these characteristics.

The first group had accessions from the three collection sites, characterized by the tallest plants with lower total aerial plant yield and included, accessions 19D and 26D from Caetés; 36D from Tupanatinga; and 111F, 08F, 81F, 27F, 109F, 72F and 06F from Bom Jardim. The second group had the higher leaf and leaflet length, and total aerial mass and lower plant height and was formed by Bom Jardim accessions 54F, 62F and 110F. The third group included accessions 37F (Bom Jardim) and 31D (Caetés), with leaf and leaflet length inferior to the previous groups, but intermediate total aerial mass production and plant height (Table 7).

No relationship was found between the morphological and productive diversities and accession origin. However, this analysis might guide future research in relation to the development of varieties, considering that the lowest plant height and the longest leaves also characterized the highest highest-yielding access. This will allow more specific assessments in relation to better adaptation to certain conditions (Schultze-Kraft et al., 2018), selection criteria and genetic improvement (Acuña et al., 2012) and specifically in the case of *M. lathyroides* accessions, which are unexplored, to evaluate as many morphological, agronomic, phenological and productive characters as possible. According to <u>Borges et al. (2019)</u> these assessments will support future breeding programs using a minimum descriptors quantity to protect cultivars.

Conclusion

The variation in plant height, leaf number, and leaf and total aerial mass in all accessions of *Macroptilium lathyroides* (L.) Urb. indicates that further germplasm of this species is merited to support future breeding programs.

Access 62F of *M. lathyroides*, from Bom Jardim-Pernambuco, Brazil, stands out considering most of the morphological and productive characteristics evaluated. However, additional information, such as genetic divergence assessment, is necessary to evaluate the potential of *M. lathyroides* accessions fully.

Compliance with Ethical Standards

Authors contributions: Conceptualization: MVFS, MVC, MALJR, JCBDJ, ACLM; Data curation: GPA, MVFS; Formal analysis: GPA, AMHA, MVC, MALJR, NVS; Funding acquisition: MVFS; Investigation: GPA, MVFS, MVC, NVS; Methodology: MVFS, MVC, MALJR, JCBDJ, ACLM; Project administration: MVFS, MVC, JCBDJ, ACLM; Resources: GPA, MVFS; Supervision: MVFS, MVC; Validation: GPA, MVFS, AMHA, MVC, NVS; Visualization: GPA, MVFS, AMHA; Writing – original draft: GPA, MVFS, AMHA, MVC, NVS; Writing – review & editing: MVFS, MVC, MALJR, JCBDJ, NVS, ACLM.

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