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Tithonia diversifolia potential for forage production: selection of accessions occurring in Cerrado and Atlantic Forest Biomes in Brazil

[*Potencial de* Tithonia diversifolia *para produção de forragem: seleção de acessos ocorrendo nos biomas Cerrado e Mata Atlântica no Brasil*]

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ABSTRACT

Tithonia diversifolia (Mexican sunflower) is an invasive shrub of tropical and subtropical regions of the world, however, it has characteristics favorable to its use as an alternative forage source. The species' potential for use in animal feeding motivates us to evaluate your characteristics and productivity of *T. diversifolia* accessions from regions in the Cerrado and Atlantic Forest for cultivation as forage. *T. diversifolia* accessions were cultivated in the field and evaluated throughout the year in four seasons/cuts. The accessions showed high daily forage accumulation and crude protein content between 13.2 and 18.3%, with fiber content without limitation to animal consumption and digestibility. The photosynthetic rate and the efficiency of water use showed no variation between genotypes of *T. diversifolia*. The MC, DIA, PM, NL, and SRS accessions are indicated for use as forage, as they have higher production, protein, growth, and good chemical-bromatological characteristics.

Keywords: biomass accumulation, genotypes, shrub forage, mexican sunflower

RESUMO

Tithonia diversifolia *(girassol-mexicano) é um arbusto invasor de regiões tropicais e subtropicais do mundo, porém, com características favoráveis ao seu uso como fonte alternativa de forragem. O potencial da espécie para utilização na alimentação animal motiva a se avaliarem suas características e sua produtividade de acessos de* T. diversifolia *provenientes de regiões de Cerrado e Mata Atlântica para cultivo como forrageira. Os acessos de* T. diversifolia *foram cultivados em campo e avaliados ao longo do ano, em quatro épocas/cortes. Os acessos apresentaram elevado acúmulo diário de forragem e teor de proteína bruta entre 13,2 e 18,3%, com teor de fibra sem limitação ao consumo animal e à digestibilidade. A taxa fotossintética e a eficiência do uso da água não apresentaram variação entre os genótipos de* T. diversifolia*. Os acessos MC, DIA, PM, NL e SRS são indicados para uso como forrageira, pois apresentam maior produção, proteína, maior crescimento e boas características químico-bromatológicas.*

Palavras-chave: acumulação de biomassa, genótipos, forragem arbustiva, girassol-mexicano

INTRODUCTION

Tithonia diversifolia (Hemsl.) A. Gray is a perennial plant of shrubs native to Central America (Pérez *et al*., 2009). Its occurrence covers tropical and subtropical regions, with relative amplitude of precipitation conditions, including semiarid regions and high edaphoclimatic variation (GBIF…, 2023). The species was introduced as an ornamental plant in many countries and has become an invasive plant with intense changes in biodiversity (Dai *et al*., 2021), including the formation of monospecific populations (Kato-Noguchi, 2020). The success of *T. diversifolia* as an invasive plant in different regions of the globe (GBIF…, 2023) can be attributed to its adaptation to different soils,

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including low fertility, acidic soils, high aluminum content, and low iron content phosphorus (Ruíz *et al*., 2014), rapid growth (Silva *et al*., 2021) and different reproductive strategies (Santos-Gally *et al*., 2020). These characteristics are also desired in materials used for cultivation as forage plants. Despite being an invasive plant, it justifies its domestication and use by man, as has been done for other species (Nguyen *et al*., 2023; Macêdo *et al*., 2018).

The species has been studied as an alternative food source for ruminants, with results indicating greater availability to the animal of fermented organic matter for microbial protein synthesis (Galindo-Blanco *et al*., 2018). Diets based on *T. diversifolia* promoted greater body weight gain in pigs (Fasuyi and Ibitayo, 2011) and sheep (Cadena-Villegas *et al*., 2020) and improved meat and milk production in goats (Wambui *et al*., 2006) and the productive performance of birds (Rodrígues *et al*., 2018). In addition to its potential in animal feed, the species is also used as ornamental cover (Witt *et al*., 2019), green manure (Scrase *et al*., 2019), medicinal practices (Ajao and Moteetee, 2017; Dlamini *et al*., 2020) and antioxidant and antibacterial properties (Dlamini *et al*., 2020; Tagne *et al*., 2018). The adaptation of the species in coexistence with other plants and their adaptation (Witt *et al*., 2019) allied to the multipurpose accredit *T. diversifolia* as a component of interest for silvopastoral and agrosilvopastoral systems (Cardona *et al*., 2022).

Among *T. diversifolia* plants, high phenotypic and genetic diversity exists (Ruiz *et al*., 2018); however, there are no reports of its improvement or selection of superior materials used in animal feed. Due to its high protein content, high biomass productivity (Mauricio *et al*., 2017), and promising acceptance by cattle, sheep, and goats (García, 2017), the species has stood out as foraging of shrubs. Its use enables high weight gain, improved animal health, increased milk production, and greater profitability in the production system (Katongole *et al*., 2016). Such benefits could be improved using superior and selected genotypes of *T. diversifolia*. The characteristics of the species and its multiple uses lead to an interest in developing domesticated materials recommended for different cultivation situations. Information on variations in growth, chemical characteristics, physiological parameters, and forage accumulation of genetic materials of *T. diversifolia*, despite its relevant contribution to its use as a forage of shrubs, is still scarce in the literature, as well as the selection and improvement of the species.

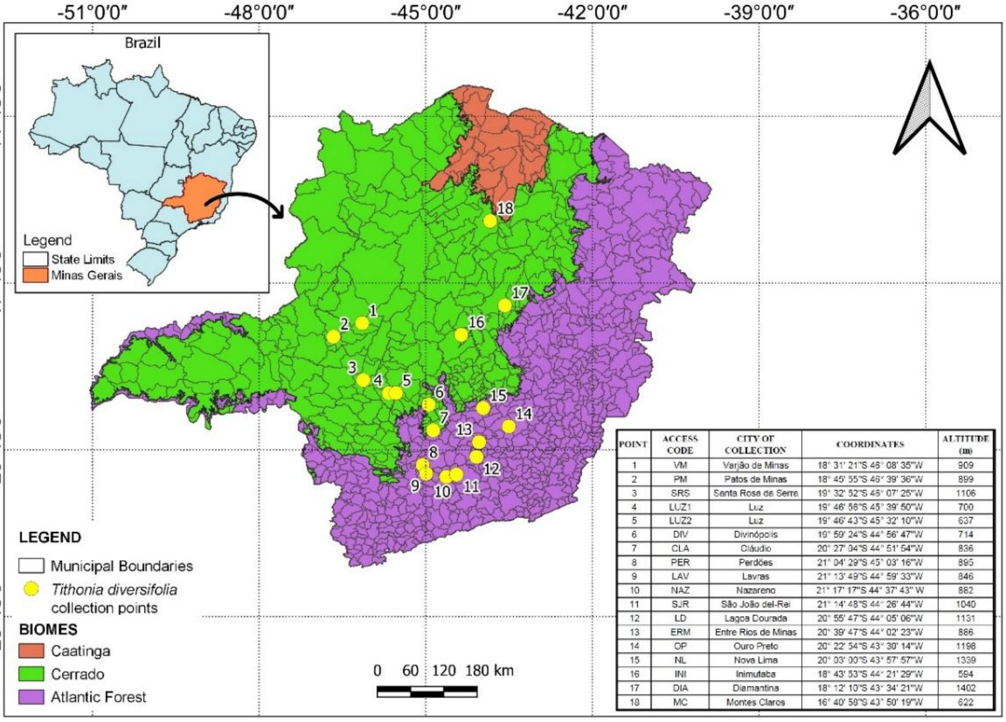
The objective of this work was to evaluate structural, physiological parameters, nutritive value and forage accumulation of *T. diversifolia* occurring in area Cerrado and Atlantic Forest Biomes in Brazil for cultivation as shrub forage plant to advance in the domestication of the species and in the selection of superior materials.

MATERIALS AND METHODS

T. diversifolia accessions were collected in different areas in the Cerrado and Atlantic Forest biomes of Minas Gerais, Brazil (Fig. 1). The minimum distance between each collection was 20 km (Machado *et al*., 1999) to distinguish the populations of the species. Each collection point was georeferenced using the Global Positioning System (GPSMAP® 60CSx), with accesses identified by code, alluding to the municipality (Fig. 1).

In the collection of *T. diversifolia* accessions, fragments of the stems of the plants, 40 cm in length, were obtained from the intermediate part of the stems, totaling 24 cuttings/accession. The stakes were wrapped in paper bags, previously moistened with water for preservation. The collected propagation material was taken to the experimental area for implantation in a germplasm bank.

The experimental area is located at a latitude of 16º40'59 South, a longitude of 43º50'19 West, and an average altitude of 620 m. The region’s climate is Aw, tropical, with dry winters and rainy summers (Climate, 2023), considered a transition region from Cerrado to Caatinga biomes in Brazil. The meteorological data for the trial period are shown in Fig. 2.



POINT	ACCESS CODE	CITY OF COLLECTION	COORDINATES	ALTITUDE (m)
1	VM	Varjão de Minas	18° 31' 21"S 46° 08' 35"W	909
2	PM	Patos de Minas	18° 45' 55"S 46° 39' 36"W	899
3	SRS	Santa Rosa da Serra	19° 32' 52"S 46° 07' 25"W	1106
4	LUZ1	Luz	19° 46' 56"S 45° 39' 50"W	700
5	LUZ2	Luz	19° 46' 43"S 45° 32' 10"W	637
6	DIV	Divinópolis	19° 59' 24"S 44° 56' 47"W	714
7	CLA	Cláudio	20° 27' 04"S 44° 51' 54"W	836
8	PER	Perdões	21° 04' 29"S 45° 03' 16"W	895
9	LAV	Lavras	21° 13' 49"S 44° 59' 33"W	846
10	NAZ	Nazareno	21° 17' 17"S 44° 37' 43" W	882
11	SJR	São João del-Rei	21° 14' 48"S 44° 26' 44"W	1040
12	LD	Lagoa Dourada	20° 55' 47"S 44° 05' 06"W	1131
13	ERM	Entre Rios de Minas	20° 39' 47"S 44° 02' 23"W	886
14	OP	Ouro Preto	20° 22' 54"S 43° 30' 14"W	1198
15	NL	Nova Lima	20° 03' 00"S 43° 57' 57"W	1339
16	INI	Inimutaba	18° 43' 53"S 44° 21' 29"W	594
17	DIA	Diamantina	18° 12' 10"S 43° 34' 21"W	1402
18	MC	Montes Claros	16° 40' 58"S 43° 50' 19"W	622

Figure 1. Map of the state of Minas Gerais – Brazil, with collection sites, their coordinates, and identification of the 18 accessions of *Tithonia diversifolia* in different areas in the Cerrado and Atlantic Forest biomes.

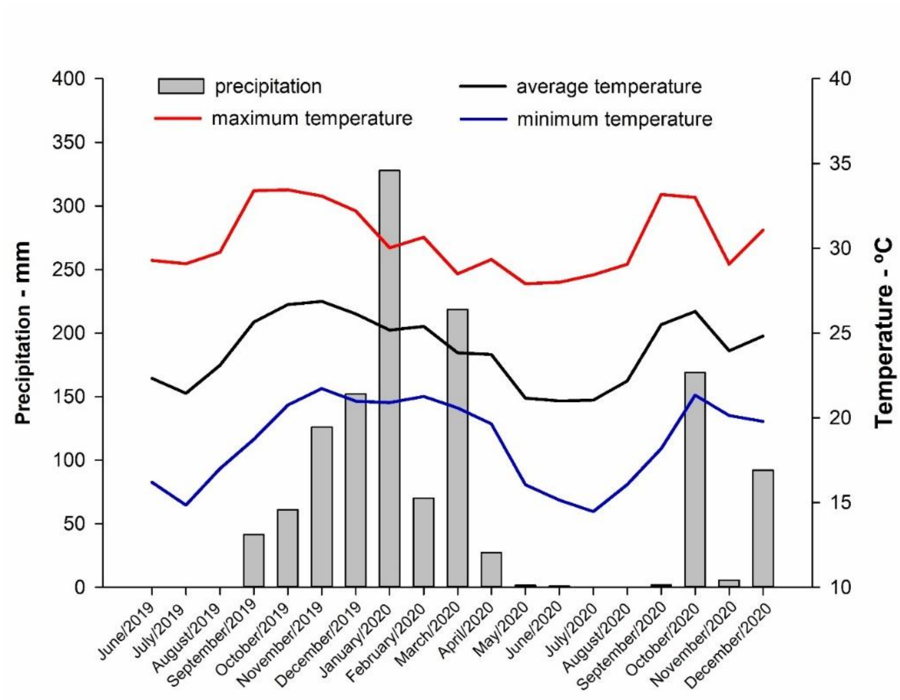


Figure 2. Monthly climatic data of the experimental area during the experiment in Montes Claros, Minas Gerais, Brazil.

The soil was classified as Cambisol Haplic Eutrophic, with clay-silty texture (Sistema…, 2013), whose physicochemical characteristics presented, in the layer from 0 to 20cm, were: 6.5 pH in water; 3.03 dag kg^{-1} MO; 6.6 and 122mg dm^{-3} of P and K, respectively; 7.5, 2.5, 1.33, 10.31 cmolc dm^{-3} of Ca, Mg, H+Al and CTC, respectively; 89% of base saturation, 28.0, 52.0 and 20.0% of sand, silt and clay, respectively.

Planting *T. diversifolia* accessions in soil tillage previously prepared with a disc harrow to a depth of 20cm was performed. The cuttings were planted in an upright position. The basal part of the cutting was deepened 10 cm in the soil, with a spacing of 2.0 x 0.4m. Due to the region's environmental conditions, complementary irrigation was used during cultivation. Dripper hoses mounted with emitters every 20cm, a flow rate of 1.5 L h^{-1}, and a pressure of 7 mca were used. The plants were irrigated with an average of 4.5 L day^{-1} $plant^{-1}$ at intervals of two days, in periods without precipitation.

The plants were cultivated in free growth and then cut 40 cm from the soil to stimulate shoots at 60, 120, and 170 days after implantation, before the evaluation period.

The experiment was carried out in randomized blocks with four replications, with the 18 accessions of *T. diversifolia* allocated in the plots (Fig. 1). Four evaluations/cuts were made over time in each plot. The cuts were made after 60 days of growth of *T. diversifolia* plants distributed in the first cut, referring to forage growth between November/2019 and January/2020, representing spring/summer; the second cut, between February/2020 and April/2020, in summer/autumn; the third, with growth occurring between May/2020 and July/2020, in autumn/winter; and the fourth cut, between September/2020 and November/2020, in spring. Therefore, the scheme of plots divided by the time for statistical analysis was considered.

The plants received 100 kg ha^{-1} nitrogen (urea with 45% of N) in cover seven days after each cut. Weed control was performed manually with a hoe whenever necessary.

Structural, physiological, and productive characteristics were evaluated in four cuts throughout the year. Plant height data were obtained with a graduated ruler to measure from soil level to the apex of the stems. The growth rate (cm day^{-1}) was calculated by dividing the height by the growth days, as described by Radford (1967). The diameter of the stems was measured with a caliper 10 cm from the growth base. Physiological evaluations were performed with an infrared gas exchange meter – IRGA (Analytical Development Co. Ltd, Hoddesdon, UK), on six fully expanded and healthy leaves, from the upper third of plants. The photosynthetic rate (A – μmol CO_2 m^{-2} s^{-1}) was evaluated, and the water use efficiency (WUE – μmol CO_2 mol^{-1} H_2O) was calculated by the ratio between the photosynthetic rate and the amount of water perspired.

The assessments were carried out in the morning from seven to eight o'clock, artificial light was used attached to the device's rod, with a radiation intensity of 1200 μmol m^{-2} s^{-1}. The CO_2 concentration was atmospheric.

The forage accumulation was determined by the harvest of the plants in the 2.4 m^2, cut at 40 cm above the ground. The plants were weighed on a scale to determine the weight of green matter. Three stems representing the plot were taken to determine the leaf/stem ratio, and the leaves and stems were separated and weighed. The different material was dried in an oven at 105º for 36h to determine the dry matter (DM), and then the leaf/stem ratio (L/S) was calculated.

The chemical-bromatological composition of the accessions was performed for the material of the first cut, produced in the spring/summer period, and for the third cut, carried out in the autumn/winter. Three stems containing leaves, pre-dried at 55°C for 36 hours, were ground in a Wiley-type knife mill with a 1 mm sieve and homogenized. From this material, a subsample was taken, which was directed to drying in an oven at 105º for 16h to determine the DM. Subsequently, the analysis of crude protein (CP), NDF, and ADF (Detmann *et al*., 2012) was performed. After determining the CP content of the samples, the crude protein productivity (kg ha^{-1}) was estimated by multiplying the average CP content of the forage by the dry forage mass of the corresponding cut.

The data on structural, chemical, physiological, and forage accumulation characteristics were submitted to variance analysis using the F test, and, when significant, the means of the variables isolated from the accessions were grouped, using the Scott-Knott test, at a significance level of 5%, using to the ExpDes.pt package of software R (R Core Team, 2021).

Multivariate analysis was also used for the best study of accessions, with the aid of the Multivariate Analysis package software R (R Core Team, 2021). The generalized Mahalanobis distance was estimated as a measure of dissimilarity, with which the dendrogram was obtained, using the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean). In addition, the canonical variables were analyzed. The forage accumulation data along the four cuts were presented descriptively.

RESULTS

T. diversifolia accessions showed structural and growth characteristics differences (Table 1). Access to MC showed the highest growth rate in the four annual cuts (Table 1). In the spring/summer cut, the MC and NL accessions formed the group with the highest daily growth. In the summer/autumn cut, the MC, NL, DIA, and PM accesses were grouped by the highest daily growth rate. In the third cut, performed in autumn/winter, the MC, PM, and DIA accessions stood out, while in the spring, only the MC accession presented a higher daily growth rate (Table 1). The daily growth rate of accessions ranged from 1.82 cm day^{-1} in autumn/winter up to 4.11 cm day^{-1} in summer/autumn, equivalent to 126%. In spring/summer, the MC, DIA, and PM accessions obtained the highest values for the stem diameter. In the cut carried out in summer/autumn, the PM, DIA, MC, NL, and SRS accessions have stood out. In autumn/winter, the DIA, MC, SRS, NL, and PM accessions had the most prominent stem diameter. In the fourth cut, in the spring, accessions were DIA, NL, SRS, and MC (Table 1).

Table 1. Growth rate (cm day^{-1}), diameter (mm), and leaf/stem ratio of *Tithonia diversifolia* accessions collected in different areas in the Cerrado and Atlantic Forest biomes in the state of Minas Gerais, Brazil

Accessions	Growth rate				Diameter				Leaf/Stem			
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 1	Cut 2	Cut 3	Cut 4	Cut 1^{ns}	Cut 2^{ns}	Cut 3	Cut 4^{ns}
VM	3.53c	3.79c	1.52d	2.82d	16.24d	13.08b	7.33b	12.19c	0.30	0.42	0.80c	0.46
PM	3.68c	4.37a	2.69a	3.35b	21.59a	16.59a	9.88a	13.68b	0.39	0.57	0.59d	0.45
SRS	3.92b	4.20b	2.13b	3.46b	20.31b	15.63a	10.32a	15.91a	0.52	0.56	0.57d	0.46
LUZ1	3.64c	4.13b	1.69c	3.19c	17.90c	13.93b	7.80b	11.97c	0.39	0.47	0.68d	0.58
LUZ2	3.81c	4.14b	1.37d	3.08c	16.99c	13.00b	6.77b	12.85c	0.46	0.49	1.57a	0.53
DIV	3.66c	3.92c	1.50d	3.20c	16.52d	12.21b	5.95b	11.58c	0.29	0.36	0.75c	0.38
CLA	3.61c	3.87c	1.31d	3.08c	16.40d	13.54b	6.52b	13.96b	0.46	0.38	0.90c	0.47
PER	3.63c	3.91c	2.06b	3.18c	16.87d	12.34b	7.99b	11.24c	0.32	0.32	0.63d	0.36
LAV	3.89b	4.24b	1.78c	3.30c	18.18c	13.79b	8.19b	14.24b	0.36	0.43	0.77c	0.47
NAZ	3.64c	3.91c	1.50d	3.03c	16.91c	13.76b	6.66b	11.96c	0.34	0.43	0.76c	0.46
SJR	3.37c	3.78c	1.34d	2.79d	15.14d	12.83b	6.42b	12.18c	0.47	0.42	1.13b	0.52
LD	3.78c	3.99c	1.72c	3.18c	16.10d	11.93b	7.46b	12.80c	0.33	0.39	0.76c	0.37
ERM	3.73c	4.00c	1.73c	3.02c	17.42c	12.65b	7.16b	11.56c	0.31	0.37	0.69d	0.42
OP	3.95b	4.25b	1.80c	3.38b	17.56c	13.27b	7.13b	14.10b	0.30	0.43	0.67d	0.50
NL	4.24a	4.51a	2.03b	3.68b	20.33b	16.01a	10.17a	16.30a	0.40	0.37	0.70d	0.42
INI	3.41c	3.85c	1.36d	2.77d	17.19c	13.16b	6.57b	11.73c	0.27	0.46	1.15b	0.56
DIA	4.00b	4.42a	2.42a	3.63b	22.20a	16.57a	11.22a	17.42a	0.45	0.46	0.67d	0.46
MC	4.42a	4.63a	2.73a	4.02a	23.10a	16.03a	11.19a	15.78a	0.41	0.35	0.65d	0.31
Means	3.78	4.11	1.82	3.23	18.11	13.91	8.04	13.42	0.38	0.43	0.80	0.45
CV 1 %		9.06				8.73				27.13		
CV 2 %		6.64				8.04				22.44		

The means followed by the same letter in the columns belong to the same group, according to the Scott Knott test, at the significance level of 5%. ns: It is not significant. CV1: Coefficient of variation associated with the plot (accessions). CV 2: Coefficient of interpretation related to subplot (cuts). Cut 1 – spring/summer; Cut 2 – summer/autumn; Cut 3 – autumn/winter; Cut 4 – spring, both with 60 days of forage growth between standardization cut and evaluation.

The leaf/stem ratio of *T. diversifolia* accessions showed no difference ($p>0.05$) among the accessions in the first, second, and fourth cut, with general averages of 0.38, 0.43, and 0.45, respectively. In the third cut, autumn/winter, LUZ2 accession showed the highest leaf/stem ratio, followed by INI and SJR (Table 1).

There were no differences (p>0.05) between *T. diversifolia* accessions for the physiological aspects evaluated. The average photosynthetic rate for the first, second, third, and fourth cut was 14.62; 5.55; 7.47, and 10.71 µmol of CO_2 m^{-2} s^{-1}, and for WUE it was 3.18; 3.78; 5.12 and 2.19µmol CO_2 mol^{-1} H_2O, respectively.

The *T. diversifolia* accessions showed a difference (p≤0.05) between the CP, NDF, and ADF contents, evaluated in the first cut (spring/summer) and in the third cut (autumn/winter) (Table 2). The accessions showed high CP levels, with the highest averages in the first cut ranging from 18.3% (LD) to 15.9% (PM). CLA access formed the lower group with the lowest CP value (Table 2). In the third cut, the group with the highest CP values ranged from 16.4% (NAZ) to 15.5% (PER), while group "b" presented the lowest values, between 14.8% (LUZ2) and 13.2% (LAV) (Table 2).

Table 2. Chemical-bromatological characteristics (%CP, %NDF, %ADF), Daily forage accumulation rate (kg ha^{-1} day^{-1} of DM), and CP productivity (kg ha^{-1}) of *Tithonia diversifolia* accessions collected in different areas in the Cerrado and Atlantic Forest biomes of the state of Minas Gerais, Brazil

Accessions	%CP		%NDF		%ADF		Daily forage accumulation rate				CP productivity	
	Cut1	Cut3	Cut1	Cut3	Cut1	Cut3	Cut1	Cut2	Cut3^{ns}	Cut4^{ns}	Cut1	Cut3^{ns}
VM	16.9a	15.9a	54.8c	43.4b	37.8b	26.6d	212.2b	152.5b	39.4	130.4	2485.1c	400.7
PM	15.9a	16.0a	54.7c	49.1a	38.2b	31.8a	218.0b	297.2a	140.1	238.6	2390.1c	1389.7
SRS	16.3a	16.1a	54.9c	46.0b	36.2b	29.0b	401.3a	253.5a	106.6	137.5	4536.5a	1077.3
LUZ1	17.0a	15.9a	55.5c	47.3a	37.5b	31.1a	236.5b	198.0b	69.0	139.1	2768.5c	698.8
LUZ2	15.0b	14.8b	55.5c	44.8b	38.7b	28.0c	164.4b	165.7b	30.4	116.2	1723.2c	283.8
DIV	14.6b	15.9a	58.0b	44.1b	39.8a	27.8c	185.2b	205.8b	43.2	171.3	1910.7c	437.4
CLA	13.3c	14.7b	59.5a	43.2b	39.2a	26.9d	193.6b	263.1a	53.4	150.3	1805.6c	472.4
PER	16.3a	15.5a	55.4c	45.6b	37.6b	27.2d	218.0b	233.3a	75.8	137.4	2390.2c	736.8
LAV	16.0a	13.2b	55.6c	44.9b	37.8b	29.4b	196.6b	189.3b	45.3	149.2	2194.6c	380.5
NAZ	16.1a	16.4a	59.9a	43.9b	39.6a	26.1d	164.2b	123.8b	44.3	144.3	1841.9c	457.8
SJR	16.1a	14.5b	55.9c	44.8b	38.6b	27.6c	152.9b	165.4b	42.6	115.1	1691.1c	392.2
LD	18.3a	15.6a	56.0c	44.7b	38.5b	28.2c	159.4b	142.0b	46.7	158.3	2059.8c	470.5
ERM	16.7a	16.2a	60.1a	44.9b	40.1a	27.8c	273.3b	255.0a	47.4	123.5	3203.8b	484.8
OP	15.6b	13.6b	58.1b	44.9b	40.4a	27.2d	250.4b	218.9a	76.7	209.6	2705.4c	656.0
NL	15.2b	14.3b	57.3b	43.2b	39.5a	28.6b	197.0b	236.5a	69.7	186.7	2054.3c	629.9
INI	15.4b	15.7a	59.7a	45.1b	40.9a	29.7b	150.1b	168.0b	46.4	184.2	1611.9c	477.6
DIA	16.8a	14.5b	53.8c	47.0a	37.3b	29.1b	185.8b	239.2a	96.9	188.6	2155.7c	892.6
MC	16.9a	13.9b	55.4c	47.5a	37.9b	29.0b	187.8b	240.5a	98.3	223.0	2196.3c	864.2
Means	16.0	15.1	54.7	43.4	38.6	28.4	208.1	208.2	65.1	161.3	2318.0	622.4
CV 1 %	7.58		2.37		3.12		45.57				42.97	
CV 2 %	7.02		2.82		2.89		28.08				40.45	

The means followed by the same letter in the columns belong to the same group, according to the Scott Knott test, at the significance level of 5%. ns: It is not significant. CV1: Coefficient of variation associated with the plot (accessions). CV 2: Coefficient of variation related to subplot (cuts). Cut 1 – spring/summer; Cut 2 – summer/autumn; Cut 3 – autumn/winter; Cut 4 – spring, both with 60 days of forage growth between standardization cut and evaluation.

The ERM, NAZ, INI, and CLA accessions were grouped with the highest values of NDF in the first cut, ranging from 60.1% to 59.5%, while the lowest values were from the third group formed, with a variation of 56.0% (LD) to 53.8% (DIA). In the third cut, the PM, MC, LUZ1, and DIA accessions obtained the highest levels of NDF, with a variation between 49.1% and 47.0%, while the group with the lowest levels of NDF varied between 45.9% (SRS) and 43.2% (CLA) (Table 2).

The accessions of *T. diversifolia* in the first cut were separated into two groups in terms of ADF levels: group "a", with higher levels of ADF, ranging from 40.9% (INI) to 39.2% (CLA); and group "b", with values between 38.7% (LUZ2) and 36, 2% (SRS). In the third cut, the highest levels were observed in the PM and LUZ1 accessions, while the lowest ADF values were in group "d", which ranged from 27.2% (PER) to 26.1% (NAZ) (Table 2).

The daily forage accumulation of *T. diversifolia* showed a difference ($p \leq 0.05$) between the accessions in the spring/summer and summer/autumn growth seasons (Table 2). In spring/summer, the highest productivity was observed in SRS accession (401.3 kg ha^{-1} day^{-1} of DM), a value about 204% higher than the average of group "b". In summer/autumn, the group "a", with higher yields, ranged from 297.2 (PM) to 218.9kg ha^{-1} day^{-1} (OP) of DM. Group "b" ranged from 205.8 (DIV) to 123.8kg ha^{-1} day^{-1} (NAZ) (Table 2). The daily forage accumulation of *T. diversifolia* DM from the third cut (autumn/winter) and the fourth cut (spring) presented averages of 65.1 and 161.3kg ha^{-1} day^{-1} of DM, respectively, for the accessions.

The crude protein productivity among the *T. diversifolia* accessions allowed the formation of three groups in the first cut, with the prominent group formed by the SRS accession (4536.5kg ha^{-1}), followed by the EMR access (3203.8kg ha^{-1}) in the second group. There was no difference ($p > 0.05$) between the accessions in the third cut, with a general average of 622.4kg ha^{-1} of CP (Table 2).

A cophenetic correlation coefficient of 0.84 was obtained, indicating a good representation of the dissimilarity matrix by the dendrogram for the study of the differences between the *T. diversifolia* accessions (Fig. 3). The cut-off point of the dendrogram was determined using the Mojena method (1977), at the height of 32, which demonstrates the formation of two groups: one composed of 13 accessions; and another includes the SRS, PM, MC, DIA, and NL accessions.

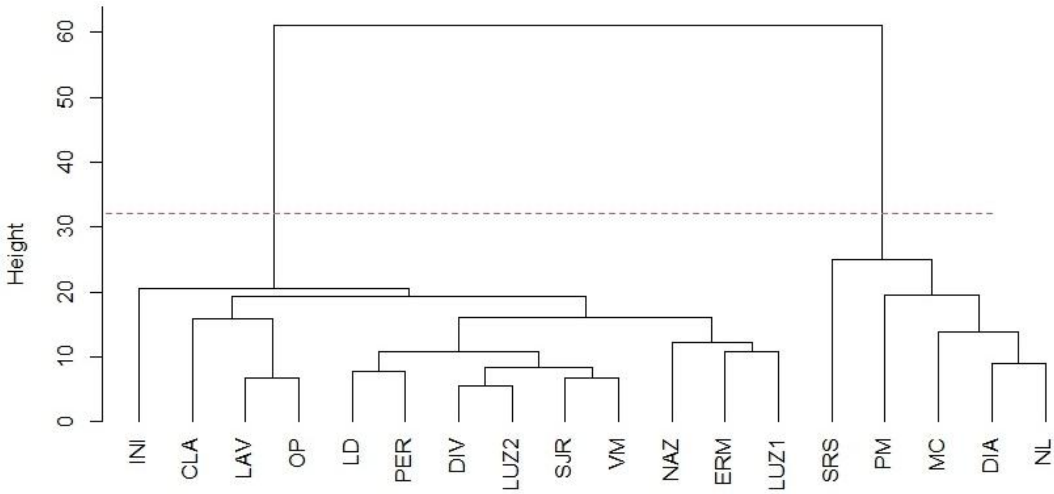


Figure 3. The dendrogram illustrates the pattern of dissimilarity obtained by the UPGMA method, based on Mahalanobis distance, in *Tithonia diversifolia* accessions collected in different areas in the Cerrado and Atlantic Forest biomes the state of Minas Gerais, Brazil.

From the first two canonical variables, it is possible to explain 73.34% of the variation in the data set (Fig. 4). A tendency was observed in the formation of two groups, following the same formation presented in the dendrogram (Fig. 3). The group composed of MC, DIA, PM, NL, and SRS tends to present higher values of daily forage accumulation (PROD), CP productivity (CPPROD), daily growth rate (GR), and stem diameter (DI). Accession LUZ2 presented a high leaf/stem ratio (LS). The ERM, PER, VM, LD, and NAZ accessions presented higher CP levels (CP). The LAV, OP, and CLA genotypes showed intermediate trait values.

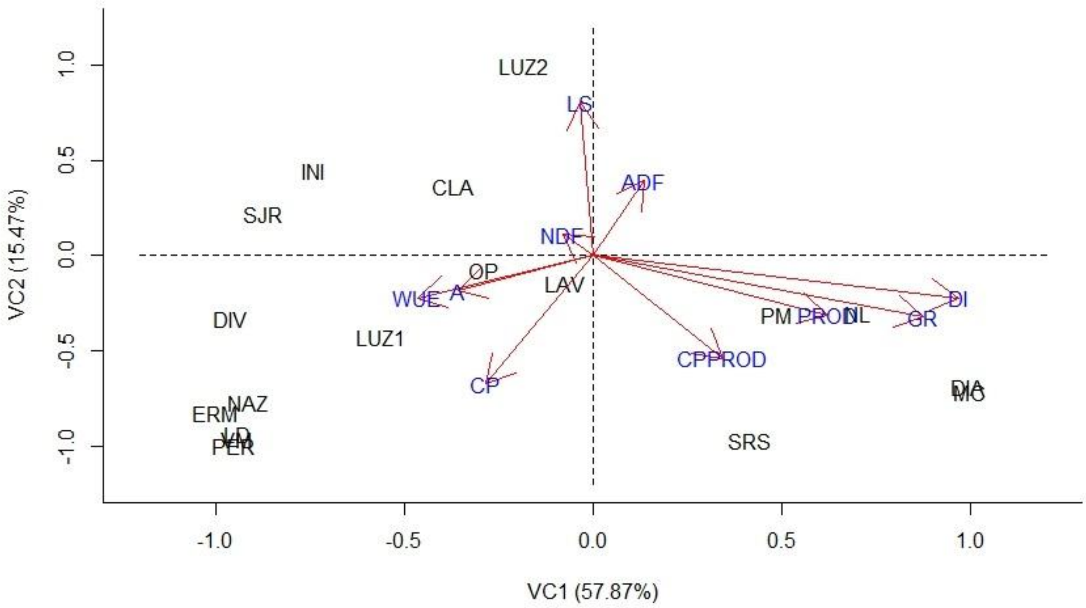


Figure 4. Graphic dispersion of scores on the two canonical variables (VC 1 and VC 2) in *Tithonia diversifolia* accessions collected in different areas in the Cerrado and Atlantic Forest biomes in the state of Minas Gerais, Brazil

DISCUSSION

The materials of *T. diversifolia* present differences in their structural (Table 1), bromatological and productive characteristics (Table 2 and Fig. 4), with relevant information to their selection and use as forage of shrubs. The phenotypic variations found among the accessions of *T. diversifolia* indicate possible genetic variability among the materials in the different regions of Minas Gerais, Brazil (Fig. 1). Despite the accessions being collected in different regions, the Cerrado and Atlantic Forest biomes of the state of Minas Gerais (Fig. 1), the excellent establishment and growth under the study conditions (Fig. 2), and the absence of differences ($p>0.05$) for the physiological aspects evaluated, reinforce the adaptation of *T. diversifolia* to different soil and climatic conditions, already reported in the literature (Silva *et al*., 2021; Tagne *et al*., 2018).

The average values for growth rate (Table 1) and daily forage accumulation (Table 2) of *T. diversifolia* accessions are considered high and very promising for using accessions as forage. The values found for daily forage accumulation (Table 2) are higher than those of tropical grasses in the rainy season, such as *Pennisetum purpureum*, with 125.6 kg ha^{-1} day^{-1} (Paciullo *et al*., 2008), and sugarcane varieties sugar, with 129kg ha^{-1} day^{-1} of DM (Cruz *et al*., 2014). In the literature, there is little information on daily forage accumulation rate of tropical shrubby forages and is limited to species with lower forage accumulation, such as *Leucaena leucocephala* (Tadros *et al*., 2012), *Morus alba* (Santillan *et al*., 2021) and *Gliricidia Sepitium* (Silva *et al*., 2022).

The *T. diversifolia* accessions showed high daily forage accumulation (Table 2) and good nutritional values, emphasizing the high levels and productivity of CP (Table 2). The forage availability of *T. diversifolia* accessions is characterized by its high accumulation in spring/summer and summer/autumn and low supply in autumn/winter, indicating seasonality in the production of *T. diversifolia* accessions in the most significant light restriction and low temperatures (Fig. 2), justified by being a species found in tropical and subtropical regions (Silva *et al*., 2021).

The daily forage accumulation (Table 2) obtained in this study was higher than those reported by other authors, and the planting densities, cultivation conditions, and genetic materials used must be considered. In Brazil, Silva *et al*. (2021) obtained a biomass of 17200kg ha^{-1} in irrigated areas and 8390kg ha^{-1} in rainfed areas in two successive cuts in the year. The SRS accession obtained high daily forage accumulation, with 401.3kg ha^{-1} day^{-1} of DM (27000kg ha^{-1}) in the first cut (spring/summer) (Table 2), indicating a superior genotype for this characteristic. The variations in biomass produced in the different studies reinforce the need to select superior genotypes of the species for use as forage. Regarding soil fertility and nutrients, *T. diversifolia* is an undemanding species (Ruíz *et al*., 2014) but responsive to nitrogen fertilization and irrigation (Silva *et al*., 2021), also used in this study.

Despite variations in the growth and daily forage accumulation of *T. diversifolia* accessions, photosynthetic rate and water use efficiency (WUE) did not show differences between materials. No difference was observed in characteristics related to photosynthesis between genotypes despite differences in material growth. The physiological assessment is punctual and carried out on healthy leaves in the middle third of the plants, which may not reflect the ability of the genotypes evaluated to produce new leaves and branches. The photosynthetic rate is essential in determining the genetic yield potential of genotypes of a crop, with the maximum photosynthetic potential related to the area of the plant leaf (Long *et al*., 2015). On the other hand, higher WUE values represent a plant's ability to survive in conditions of moderate to severe water deficit (Silva *et al*., 2021; Costa *et al*., 2020). Further studies should be conducted with the accessions to understand their adaptation under limited resources.

Tithonia diversifolia stands out for its high protein values (13.2 and 18.3% in the present study), reinforcing its importance as a forage and other uses in integrated systems of agricultural production. In the literature, the protein levels of the species vary from 14.1 to 16.5% for the entire plant (Gallego-Castro *et al*., 2017) and from 22.5 to 24.52% for leaves (Lezcano *et al*., 2012). One of the significant limitations of shrub and tree forages concerns low digestibility and intake. The fiber values of *T. diversifolia* accessions in the present study are higher than those reported in the literature for the species (Gallego-Castro *et al*., 2017). However, they are within limits for use as forage given that their NDF values (Tab. 2) are lower than those of *Stylosanthes guianensis*, 65.3% (Teixeira *et al*., 2010), *Cajanus cajan*, 70.40%, and *Leucaena leucocephala*, 75.59% (Nascimento and Silva, 2004), forage legumes important for the tropics. In the present study, all the accesses of *T. diversifolia* were within the range above the CP values; however, attention should be paid to the NDF values found (Table 2), which favors its use in the early stages of the plant.

The *T. diversifolia* accessions showed significant variation and morpho-agronomic potential for cultivation. The observed variations may be related to the different collection regions (Fig. 1) and adaptation of the materials to the cultivation conditions (Fig. 2). The different groups formed (Fig. 3 and Fig. 4) corroborate the high phenotypic and genetic diversity among plants of the species (Ruiz *et al*., 2018), reinforcing the need to select superior genotypes of *T. diversifolia* for use as forage. The materials MC, DIA, PM, NL, and SRS stood out for their high forage accumulation and nutritional composition throughout the four cuts of the year (Fig. 3 and Fig. 4). The results allow advances in the domestication of species and the selection of superior materials that occur in the Cerrado area and the Atlantic Forest Biomes of Brazil.

CONCLUSIONS

The accessions of *T. diversifolia* collected in areas of the Cerrado and Atlantic Forest of Minas Gerais present important variations in their growth, forage accumulation, and nutritional value, which must be observed for domestication of the species for use in animal feed.

The accessions MC, DIA, PM, NL, and SRS are recommended for use as forage because they present characteristics favorable for this purpose.

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