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Genetic and phenotypic variability of *Tithonia diversifolia* (Hemsl.) A. Gray. in Colombia

Variabilidad genética y fenotípica de *Tithonia diversifolia* (Hemsl.) A. Gray. en Colombia

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The inclusion of *T. diversifolia* in diets based on tropical grasses has proven potential to improve nutrient availability in these diets, as well as enhance animal production in different productive conditions. However, recent studies have evidenced that there are better genotypes that could be selected to enhance the use of this species. This paper shows the principal results obtained in the determination of the genetic and phenotypic variability of *T. diversifolia* in materials collected in Colombia. Initially, a genetic assessment is presented permitting to identify seven outstanding genotypes as animal feed and with great genetic diversity. These materials have been spread to conduct studies for determining genotype-environment interaction in biomass yield, fermentative performance, chemical composition, and sexual propagation. The results evidence that *T. diversifolia* materials can be identified and selected for having better adaptation to specific conditions than others, better growth, higher offer of nutrients, and capacity of modifying the dynamic of fermentation in the rumen.

Key words: *adaptation, Mexican sunflower, chemical composition, genetic diversity, genotype-environment interaction, biomass yield, silvopastoral systems*

La inclusión de *T. diversifolia* en dietas basadas en gramíneas tropicales ha demostrado su potencial para mejorar la disponibilidad de nutrientes en estas dietas y aumentar la producción animal en diferentes condiciones de producción. Sin embargo, investigaciones recientes han evidenciado que existen genotipos superiores que se podrían seleccionar para mejorar el aprovechamiento de dicha especie. Este trabajo muestra los principales resultados obtenidos en la determinación de la variabilidad genética y fenotípica de *T. diversifolia* en materiales colectados en Colombia. Inicialmente, se presenta una evaluación genética que permitió identificar siete genotipos destacados para la alimentación animal y con gran diversidad genética. Estos materiales se han propagado para desarrollar estudios orientados a determinar la interacción genotipo ambiente de la producción de biomasa, comportamiento fermentativo, composición química y propagación sexual. Los resultados evidencian que se pueden identificar y seleccionar materiales de *T. diversifolia* con mejor adaptación a condiciones específicas que otros, mejor crecimiento, mayor oferta de nutrientes y capacidad de modificar la dinámica de fermentación en el rumen.

Palabras clave: *adaptación, botón de oro, composición química, diversidad genética, interacción genotipo ambiente, producción de biomasa, sistemas silvopastoriles*

Introduction

At population as at species level, genetic and phenotypic variability provides plants and animals with the capacity of responding to challenges and threats (Govindaraj *et al.* 2015). The assessment and identification of genotypes of plant species for animal production is of great importance to select those having desirable traits such as browsing resistance, fast growth, good nutritional offer, adaptation to specific environmental conditions, among others (Ruiz *et al.* 2013, Holguín *et al.* 2015 and Rivera *et al.* 2021a).

It is known that *Tithonia diversifolia* (Hemsl.) A. Gray has been found for several years in Africa, Asia, and South America. This condition has favored its growth in uncountable environments, providing great diversity in agronomic, chemical, and adaptation properties (Ruiz *et al.* 2013, Miranda *et al.* 2015, Holguín *et al.*2015 and Luo *et al.* 2016). Colombia is no stranger to this condition; so, there is

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possibility for identifying and selecting better genotypes to reach greater use of this species in bovine feeding.

T. diversifolia, mostly known as Mexican sunflower, is a shrub that, due to faculties of adapting to multiple environmental, soil, and management conditions, regrowth capacity, fast growth, and great nutritional value and nutrient supply, has demonstrated its potential for animal feeding (Olabode *et al.* 2007, Ribeiro *et al.* 2016 and Mauricio *et al.*2017). However, Holguín *et al.* (2015) and Rivera *et al*. (2021a) mention that not all the populations are appropriate to all environments; and, therefore, identifying those of greatest forage potential is fundamental for a better use.

This research has as main purpose to present the most outstanding results in the determination of the genetic and phenotypic variability of *T. diversifolia* in plant materials collected in Colombia and Mexico. From this viewpoint, the search has been addressed to identify outstanding genotypes or plant origins that get adapted to specific conditions of livestock production. A genetic assessment was performed to identify seven genotypes, spread and analyzed as to their genotype-environment (G x E) interaction, fermentative performance, and propagation through trials that were briefly described in the four sections of this paper.

I. Genetic diversity of plant materials of *T. diversifolia* in Colombia and Mexico

At present, in Colombia and in other countries where *T. diversifolia* is used for animal feeding, its genetic diversity analysis is scarce and limited, hampering the planning and designing of preservation and management strategies, as well as the selection of germplasm lines and the genetic improvement of this species (Ruiz *et al.* 2013 and Luo *et al.* 2016). Herein, some analyses are included to describe genetic variability of plant materials collected in Colombia and Mexico for ruminant feeding to later move forward to agronomic performance studies in experimental plots that are also described in the following sections.

Materials and Methods

Some samples of *T. diversifolia* were collected in 31 places distributed in Colombia and Mexico. In Colombia, the collections were conducted in six eco-regions: Eje Cafetero (EEC), Valle del Río Cesar (EVRC), Piedemonte orinocense (EPO), Bajo Magdalena (EBM), Caquetá (CAQ), and Santander y Boyacá (ESB); and, in Mexico, in the states of Michoacán (MICH) and Jalisco (JAL).

For the DNA extraction, the DNeasyPlant mini Kit (Qiagen ®) commercial kit was used. The PCR amplifications were performed according to the protocols developed in the Laboratory of Molecular Biology at the Technological Institute of Tlajomulco (Mexico). For the amplification of the fragments, forty-five primers were assessed; out of which, eleven were selected (seven oligos ISSR or Inter Simple Sequence Repeats and four for the cytochrome gene P450) (Yamanaka *et al.* 2003).

The generated data were analyzed by means of a grouping, using the UPGMA method (Unweighted Pair-Group Method), and a dendrogram was generated by means of the NTSYS statistical software (Numerical Taxonomy System for Personal Computer, version 2.02 PC), which served to establish genetic distances. Each oligo was calculated the number of observed and effective alleles, (Na, Ne), number of polymorphic bands (P), index of genetic diversity of Nei (H) and index of Shannon (I) with the utilization of the POPGENE software, version 3.2 (Yeh *et al.* 1999).

Results and Discussion

Out of a total of 105 amplified fragments, 5 % were monomorphic and 95 % polymorphic. The size of the DNA fragments from the PCR varied from 300 up to 2,500 pb. The measures of genetic variation are shown in table 1. The amount of effective alleles in the ISSR under study varied from 1.0 to 1.9. The diversity values (H) brought about a total of 6.0 % to 48.8 % of heterozygosis. The Shannon data indices (I) ranged from 0.13 a 0.67, representing a measure of genetic diversity with an average of 0.432 ± 0.227 (table 1) and showing high polymorphism in *T. diversifolia.*

The cluster had a high cophenetic correlation (0.874) and a clustering coefficient of 0.8, which meant high correspondence among groups and differences among them (figure 1). The dendrogram served to separate the genotypes and, thus, generate data for the selection in each group.

Figure 2 shows the genetic structure of the 31 collections of *T. diversifolia* under study. The analysis based on the genome ratio of each population showed five well-defined groups, which agreed with the grouping obtained in the clustering analysis (figure 1).

The genetic structure clearly showed the existence of homogenous groups that can be used as selection criteria for materials to be improved and adapted to each soil and climate condition. Previous studies have proven that *T. diversifolia* has great phenotype diversity, variability that has favored its productivity, nutritional composition, and adaptability to different productive conditions (Yang *et al.* 2012 and Luo *et al.* 2016). These aspects have been assessed by Ruiz *et al.* (2010) and Holguín *et al.* (2015), who identified that this species may vary considerably as to regrowth capacity, agronomic performance, and chemical composition.

Table 1. Parameters of genetic diversity used in the analysis

Oligos	Number of samples	Na*	Ne*	H*	I*	P
(GA)8YT	32	2	1.8824	0.4688	0.6616	10
(AG)8C	32	2	1.7534	0.4297	0.6211	9
(GA)8C	32	2	1.2047	0.1699	0.3111	9
(AG)8YC	32	2	1.9321	0.4824	0.6755	8
(CT)8AGA	32	2	1.3581	0.2637	0.4334	9
T(CT)7CC	32	2	1.8824	0.4688	0.6616	6
(CT)8RG	32	2	1.0644	0.0605	0.1391	10
CYP1A1F/CYP2B6R	32	2	1.5193	0.3418	0.5253	8
CYP1A1F/heme2B6	32	2	1.0644	0.0605	0.1391	10
CY2C19F/CYP21A1R	32	2	1.1327	0.1172	0.2338	13
CY2C19F/heme2B6	32	2	1.8221	0.4512	0.6435	13
Average	32	2	1.4734	0.2812	0.432	
SD		0	0.3631	0.1787	0.2267	

* Na: number of observed alleles, Ne: number of effective alleles (Kimura and Crow 1964), H: genetic diversity of Nei (1973); I: index of Shannon (Lewontin 1972); P: number of polymorphic loci

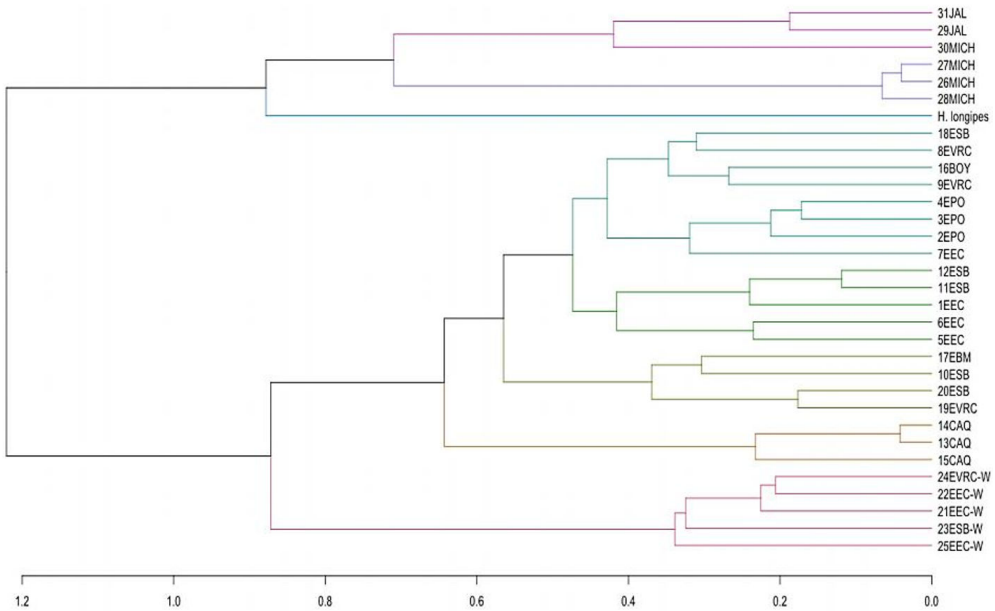
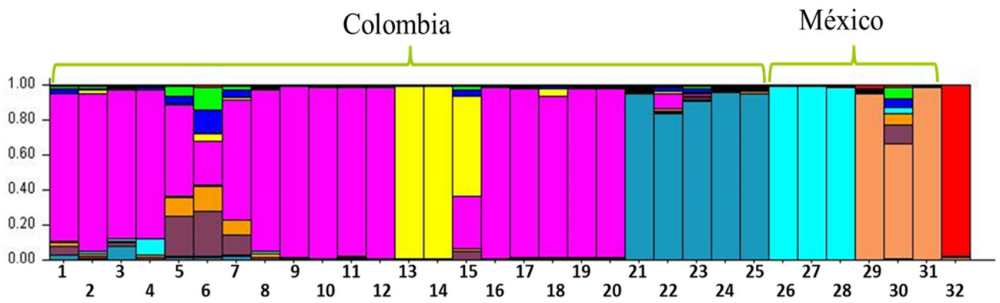


Figure 1. Dendrogram generated by the method of UPGMA for the 31 collections of T. diversifolia from Mexico and Colombia, using the index of dissimilitude of Dice

II. Genotype-environment interaction and agronomic performance of outstanding materials of *T. diversifolia* in Colombia

For the selection of forage plants, the determination of the influence of environmental factors on the quality and possible interactions with the genotypes of interest should be the basis for identifying more efficient and economical nutritional sources that strengthen the agricultural systems (Schultze-Kraft *et al.* 2018). Recently, the AMMI (Additive Main Effects and Multiplicative Interaction) and SREG (Biplot Method Using Sites Regression) models have been useful tools to determine genotype-environment interaction in agricultural crops (Bhartiya *et al.* 2017 and Carter *et al.*2018). These models include analysis of variance and principal component analysis (PCA). The analysis of variance permits studying the principal effects of the genotypes and the environment and the PCA the GxE interaction, which is addressed in a multivariate form for its graphic interpretation (Alejos *et al.* 2006). Next, it is shown herein the results of the GxE interaction from seven outstanding materials that were identified in Colombia,



K=9 **1**:1EEC; **2**: 268EPO; **3**: 1416EPO; **4**: 1EPO, **5**: 2583EEC, **6**: 2388EEC, **7**: 953EEC; **8**: 1250EVRC; **9**: 1716EVRC; **10**: 225ESB; **11**: 2785ESB; **12**: 2529ESB; **13**: 1CAQ; **14**: 2CAQ; **15**: 3CAQ, **16**: 1BOY; **17**: 3700EBM; **18**: 1ESB; **19**: 1EVRC, **20**; 2ESB, **21**: 1-1EEC; **22**: 1EEC-UNAL, **23**: 1ESB-Carr, **24**: 1250EVRC, **25**: 1EEC-SENA, **26**: 1MICH; **27**: 2MICH; **28**: 3MICH; **29**: 1JAL; **30**: 4MICH; **31**: 2JAL; **32**: *H. longipes*.

Figure 2. Genetic structure of the collections conducted in Colombia and Mexico

previously described in the analysis of genetic diversity. The amplification of the analysis may be consulted in Rivera *et al.* (2021a).

Materials and Methods

The assessment of the genetic diversity was complemented with some growth measures in the field to choose the genotypes to be studied in experimental plots. The weighted forage potential index (WFPI) was used in each cluster (Holguín *et al.* 2015) in the genetic analysis to compare and identify the plant materials of highest agronomic potential, which were those of greatest valuation. The identified materials (7) were spread as clones in the laboratory, and were sown in three plots arranged in a completely randomized block design using all the materials in two localities: Environment (1) low tropics without fertilization - REG1, Environment (2) low tropics with fertilization - REG1Fert, and Environment (3) high tropics without fertilization - REG2. For the selection of the localities, representative criteria for soil and climate were used in the sites, as well as growth potential of *T. diversifolia* (rainfall, temperature, sun radiation, soil and cattle breeding potential).

The experimental plots corresponded to environments 1 and 2. They were sown in the municipality of San Luis de Cubarral (Meta, Colombia) at 530 m a.s.l., with average annual rainfall of 4100 mm, and average temperature of 24.8 ºC, classified as tropical humid rainforest (bh-T) (Holdridge 1986) (3°47'21.43"N, 73°49'15.93"O). The plots in the environment 3 were sown in the municipality of Villamaría (Caldas, Colombia) (5°0'44.92"N, 75°25'47.28"O), at an altitude of 2,300 m a.s.l., with average temperature of 15 ºC, and annual rainfall of 2850 mm, corresponding to low mountainous humid rainforest (bmh-MB) (Holdridge 1986).

For analyzing GxE interaction for biomass yield, two non-parametric methods were used: AMMI analysis (Additive Main Effects and Multiplicative Interaction) (Mandel 1971) and SREG analysis (Site Regression Analysis) (Yan *et al.* 2000). Two samplings were carried out in rainy season, and two, in dry season, every 40 and 60 d, respectively, in each locality.

Results and Discussion

In REG1 and REG1Fert environments, the plant materials of greatest yield were Gen7 (106.51 g) and Gen5 (85.92 g). Those of lowest DM yield were Gen1, Gen4 and Gen3, with averages of 65.85, 68.67, and 73.54 g of DM every 40 d. Figure 3 shows the representation of each of the plant materials in each environment according to DM yield.

As average, DM yield in rainy season was 1.4 times higher than in dry season. The use of fertilizers increased DM/plant in 98.6 % as average in both seasons, with greater influence in rainy season (2.3 times). The materials of best response to fertilization were genotypes 2, 4, and 6; and those of worst response were 7 and 3. The genotypes with best response in dry season, represented by lower decline in biomass yield in respect to rainy season, were genotypes 3, 1, and 2; although none were in the group of genotypes with best performance in these conditions. Genotypes 4 and 1 were those contributing the most to variability for being further away from the axes represented by each of the environments in REG1.

For REG2 (figure 3), the materials with greatest yield were Gen4 and Gen7, with DM yields per plant of 152.63 and 128.87 g, respectively, despite having greater variability. The materials of lowest yield were Gen1, Gen3 and Gen6, with 63.58, 82.73, and 94.42 g of DM/plant every 60 d, respectively. In this area, the materials decreased their yield in 13.5 % as average, compared to the rainy and dry seasons. The materials in which season influenced the least were Gen5 and Gen6.

In the analysis of variance of the AMMI model (table 2), the genotypes, as well as the environments and the GxE

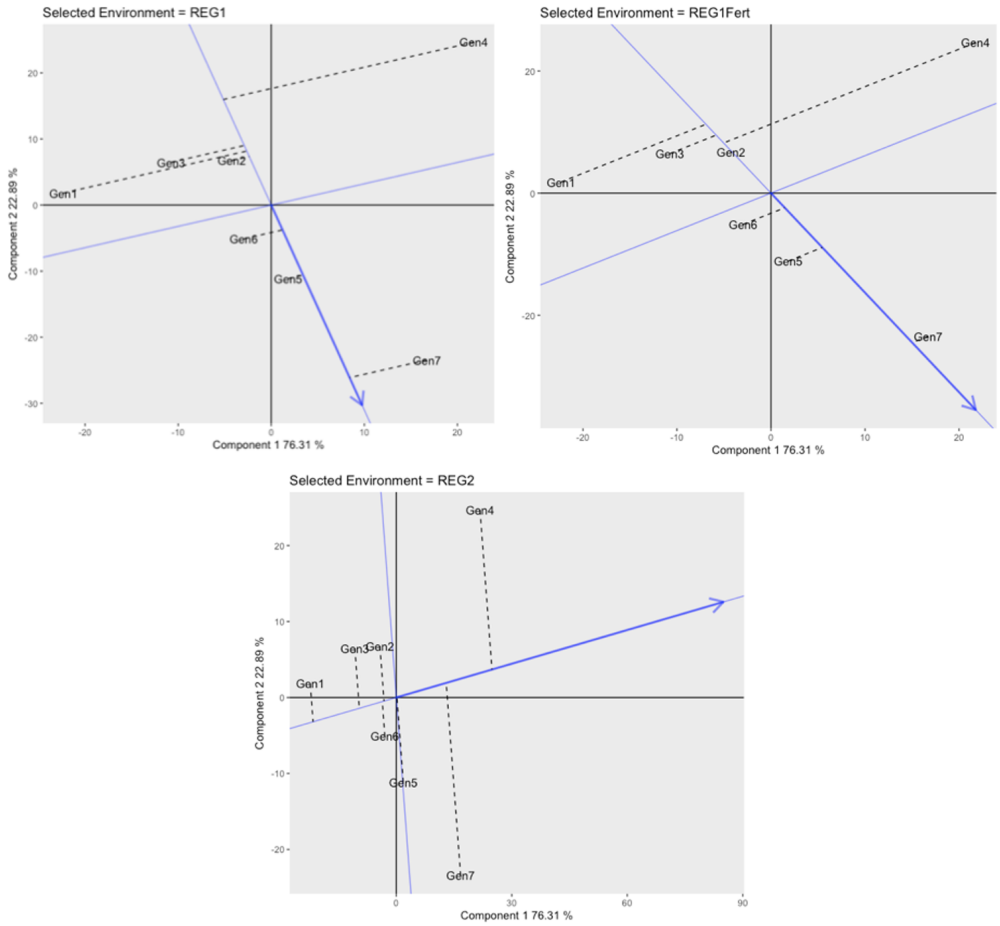


Figure 3. Representation GGEplot of DM yield in *T. diversifolia* genotypes under study in each locality

Table 2. Analysis of variance of the AMMI model for GxE interaction in DM yield

	Df	Sum Sq	Mean Sq	F value	Variation, %	Pr(>F)	
Environment	2	72305	36152	98.55	53.26	0.000026	***
REP (environment)	6	2201	367	1.237	1.621	0.294	
Genotype	6	32820	5470	18.44	24.17	2.77E-14	***
Environment:genotype	12	28418	2368	7.98	20.93	2.97E-10	***
Residues	99	29360	297				

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1Coefficient of var: 19.45; DM average: 88.50

interaction, presented significant differences for DM yield per plant ($p<0.05$). This condition is shown graphically in figure 4. The plant materials Gen7 and Gen5 were associated with the best yields in the areas REG1 and REG1Fert, and Gen4, in REG2. Gen7 and Gen5 were identified as ideal genotypes since they were closer to the origin of the circumferences shown in figure 5.

Finally, according to the index of Shukla, Gen7 and Gen5 were the most stable. This is due to their relative high productive yield, which was determined in the three environments under study, and due to the low variability.

III. Fermentative performance of different *T. diversifolia* materials

It has been proven that some phytochemical compounds such as tannins, saponins, and essential oils contribute to diminish enteric methane production and to modify the rates of gas production at rumen level due to their inhibiting effects on certain groups of microorganisms in the rumen (Delgado *et al.* 2012, Barahona *et al.* 2013, Banik*et al.*2013 and Bhatta *et al.*2013). *T. diversifolia*, because of its chemical traits, may modify the fermentative performance in

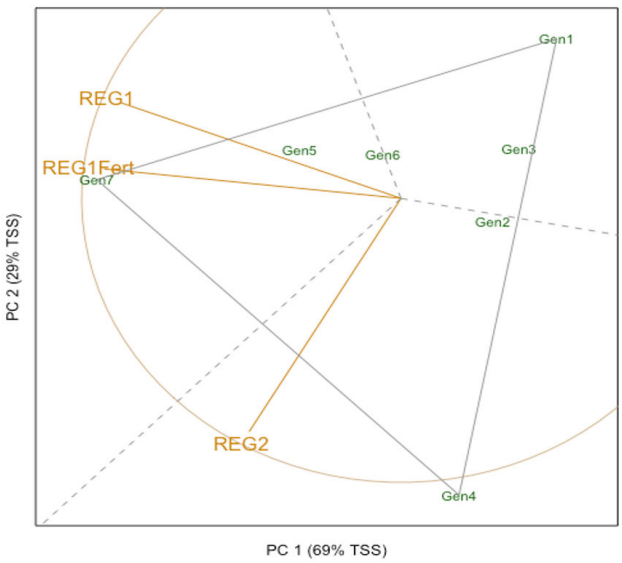


Figure 4. GGEplot of DM yield of *T. diversifolia* genotypes in three environments

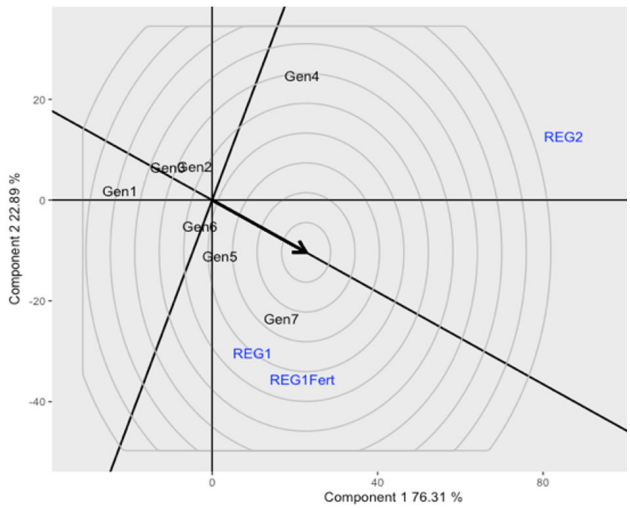


Figure 5. Ranking of genotypes in respect to the ideal genotype

diets based on tropical pastures, and may present differences among genotypes (Rivera *et al.* 2021a). The main outcomes as to the fermentative performance of seven outstanding genotypes from Colombia are presented in the following paragraphs. For further information, Rivera *et al.* (2021b) can be consulted.

Materials and Methods

Seven outstanding *T. diversifolia* genotypes were assessed and identified in experimental plots in high tropical (5°0'44.92"N, 75°25'47.28"O - 2,300 m a.s.l.) and low tropical conditions (3°47'21.43"N, 73°49'15.93"O - 530 m a.s.l.), according to descriptions previously presented in papers on GxE interaction. The genotypes were mixed with pasture traditionally used in each locality, according to Donneys *et al.* (2015) and Molina *et al.* (2015). As stated by these authors, the intake in *T. diversifolia* systems at high density associated with *Cenchrus clandestinus* (Hochst. ex Chiov.) is 80 % pasture and 20 % shrub (High tropics), out of the total DM in the diet. For the mixture with pastures of the *Urochloa* genus, such as *Urochloa brizantha* cv Marandú, the intake is 75 % for pasture and 25 % for *T. diversifolia* (low tropics) (DM of the diet).

Fermentative performance, methane production (CH_4), generation of short-chain volatile fatty acids (VFA), and DM degradability of different plant materials were studied by means of the *in vitro* gas production technique (Theodorou *et al.*1994), with use of a completely randomized design (Rivera *et al.* 2021b).

Results and Discussion

For low tropics conditions, *T. diversifolia* inclusion provoked significant differences in all the variables measured, except ether extract and crude energy ($p<0.05$), for the diet based on *U. brizantha,* but there were no differences among diets including *T. diversifolia*. In general, the inclusion of *T. diversifolia* decreased DM and fiber content in the diet, but augmented minerals and nitrogen-free extract supply, besides improving DM degradability ($p<0.05$). Also, it is noteworthy that the use of fertilization in *T. diversifolia* increased CP, Ca, P, and Enl, and degradability in respect to diets of *T. diversifolia* without fertilization.

The inclusion of *T. diversifolia* in diets based on *U. brizantha* modified some parameters of *in vitro* rumen fermentation. Its use had significant effects on the variables of maximum rate of gas production (MRGP) and lag phase (LP, time taken for colonization of rumen feed, and fermentation start) ($p<0.05$), but not on the inflection point time (IPT) and gas at the inflection point (GIP). The fertilization of *T. diversifolia* represented significant rise in the maximum rate of gas production, as compared to the diets without fertilization ($p<0.05$). Among the mixtures, the genotypes 1, 3, and 7 presented lower values of MRGP. The mixture of the genotypes 2 and 3 showed higher values of LP ($p=0.007$). According to the chemical characteristics of the genotypes of *T. diversifoliay* and *C. clandestinus* (high tropics) and according to the percentage of inclusion, the inclusion of the shrub had significant ($p>0.05$) effects on none of the parameters under study for this grass.

As to DM degradability, *T. diversifolia* and *U. brizantha* mixtures had differences at 24 h time, but diet fermentation at 72 h including *T. diversifolia* presented, as average, 4.72 % more DM degradation than the diet with 100 % grass ($p<0.05$), but without differences with those including *T. diversifolia*. The fertilized materials had significant differences in regards to the diets with *T. diversifolia* genotypes without fertilization ($p<0.05$). In respect to high

tropics diets, the inclusion of different *T. diversifolia* genotypes in diets based only on *C. clandestinus,* disminished in 4.6 %, significantly, the degradation at both times. However, there were not differences in the *T. diversifolia* mixtures.

As to VFA production at 24 h of incubation, *U. brizantha* had lower production of propionic acid (mol/100 mol) with differences among mixtures including genotypes 5, 6, and 7 of *T. diversifolia* ($p=0.0321$). Genotypes 2 and 4 were those of largest amount of acetic acid, and the Gen6 diet produced the lowest values of total VFA for *T. diversifolia* mixtures (mol/100 mol). The A:P ratio had also significant differences, with the highest values in the diet of 100 % *U. brizantha* ($p=0.004$), condition also found for acetic acid ($p=0.012$). At 72 h, the production of acetic, propionic, and butyric acid, total production of total VFA, and the A:P ratio had significant ($p<0.05$) differences for the values in mol/100 mol, as for Mmol/L. The inclusion of *T. diversifolia* produced lower amounts of acetic acid and higher production of propionic acid; besides, the A:P ratio was lower in all *T. diversifolia* diets. The mixture of Gen4, Gen7and 100 % *U. brizantha* were the trials with lower production of butyric acid. Meanwhile, the diets Gen4 and 100 % *U. brizantha* were those of highest production of total VFA, with significant differences in respect to the mixtures Gen1, Gen5 and Gen6. The use of fertilizer had significant differences in the acetic, propionic and butyric acids variables, as well as in the A:P ratio.

As to methane production in low tropics diets, *T. diversifolia* inclusion diminished significantly the CH_4production in respect to the diet with 100 % *U. brizantha,* especially in the emissions per mg of CH_4/g of DMD at 72 h. As average, at 72 h, the use of the shrub declined in 1.2, 2.42, and 5.79 units of Ym, mg de CH_4/g of IDM and mg of CH_4/g of DDM, respectively. Moreover, in *T. diversifolia* mixtures*,* Gen2, Gen4, and Gen7 had the lowest emissions in terms of mg de CH_4/g of IDM ($p=0.006$), and the highest Ym was that of 100 % *U. brizantha* and the diet with Gen6. In the diets under study, fertilization had neither significant effect nor differences in the mixtures including mg of CH_4/g of IDM ($p=0.006$), and the highest Ym was that of 100 % *U. brizantha* and the diet with Gen6. For the evaluated diets, fertilization had no significant effect or differences among the mixtures that included *T. diversifolia* (Rivera *et al.* 2021), and in the diets of the high tropics the *T. diversifolia* inclusion did not have significant differences for the units of measure and the times under study ($p>0.05$).

IV. Propagation and growth of different outstanding *T. diversifolia* materials in Colombia

T. diversifolia can reproduce asexually and gamic seeds, which grant great capacity of reproduction and colonization of new habitats to this plant (Ruiz *et al.* 2009 and Obukohwo and Umar 2014). This species blooms and produces seeds throughout the year, especially in October and November; although, due to environmental conditions, it can be of annual flowering (Pérez *et al.* 2009 and Chagas-Paula *et al.* 2012). The typically mature plants produce between 80,000 and 160,000 seeds per square meter annually, out of which 70 % fully development. Nevertheless, De Guerra (1996) and Obukohwo and Umar (2014) reported germination percentages below 30 % in natural conditions.

Although field observations indicate that *T. diversifolia* has a great capacity for growing through clones (Ruiz *et al.*2009); currently, it is known that the plant material from sexual seeds may favor the growth of larger roots, more vigorous plants, higher persistence of crops, and faster recovery after cutting and grazing. However, it is still hard to produce good quality seed material (Romero *et al.* 2014), and, on top of that, there are genotypes in this species with different germination capacity (Ruiz *et al.* 2018). On the following section, the main results of the study of seven outstanding genotypes in Colombia are shown in terms of propagation and reproduction. For further information, Rivera *et al.* (2021c) could be consulted.

Materials and Methods

Seven outstanding genotypes of *T. diversifolia* were assessed and identified as to genetic diversity, and they were examined in the aforementioned trials.

The variables vegetative stage length (d), reproductive stage length (d), length of the drying of the achenes (d), flowering stage (d), flowerheads per plant (#), seeds per flowerhead (#), seeds per plant (#), full seeds (%), empty seeds (%), and rudimentary seeds (%) were measured. Besides, two trials were evaluated prior to germination, and one trial, without previous processing for sexual seed germination. The trials prior to germination were water at 80ºC during 10 min, according to Akinola *et al.* (2000), Agboola *et al.* (2005) and Nasreen *et al.* (2015), and sulfuric acid (H_2SO_4) at 50 % during 5 min (Muoghalu and Chuba 2005). The seeds were stored after collection for four months with the goal of reducing the physiological latency (Agboola *et al.* 2005 and Santos-Gally *et al.* 2020). Germination was evaluated in experimental pots in the laboratory for 20 days.

Results and Discussion

The genotypes assessed had significant differences in all growth variables, except for the time of the drying of the achenes, and the percentage of rudimentary seeds ($p<0.05$). Genotype 1 (140.1 d) was the material that demanded the longest time for growing and flowering combined (flowering stage). Genotype 4 (127.2 d) was the one having the shortest time with significant ($p<0.05$) differences.

Table 3. Germination percentage in sexual seeds for different *Tithonia diversifolia* genotypes

Trial	Genotypes							Fertilization		Genotype	Fertilization	SEM
	Gen1	Gen2	Gen3	Gen4	Gen5	Gen6	Gen7	No	Si	*p- value*		
Trial 1	32.62^{b}	45.36^{a}	54.02^{a}	45.82^{a}	47.69^{a}	53.99^{a}	48.62^{a}	42.65	51.11	<0.001	0.002	1.53
Trial 2	39.54^{b}	51.64^{a}	58.18^{a}	54.01^{a}	52.89^{a}	62.23^{a}	56.88^{a}	46.94	59.46	0.001	<0.001	1.89
Trial 3	17.53^{c}	22.31^{abc}	31.97^{a}	30.34^{ab}	19.68^{a}	31.83^{a}	26.59^{abc}	23.36	30.15	0.001	0.025	1.31

Trial 1: without previous treatment; Trial 2: water at 80 ºC for 10 min; Trial 3: Sulfuric acid immersion

*Different letters in the same row denote statistical difference according to the test of Tukey ($p<0.05$).

In regards to sexual seed production, fertilization had significant effect by generating 2.14 times more seeds ($p<0.05$); and, in general, it increased the time in the stages under study in around five days. Genotypes 5 and 7 were the plant materials with the highest production of flowerheads per plant, the highest percentage of full seeds, and the largest number of seeds per plant, which was associated with the highest number of branches. Likewise, the percentage of empty seeds was noteworthy in all the genotypes, as well as that of rudimentary seeds, which accounted for the fact that more than 30 % of the seeds had no physical viability.

In regards to germination (table 3), there were significant differences among genotypes and trials ($p = 0.000118$). As average, germination percentage was 46.87, 53.53, and 25.97 % for the control, the use of water at 80 ºC, and the sulfuric acid, respectively; and this process started at three days after sowing. The germination of seeds without treatment was significantly lower in the genotype 1, as compared with the other six genotypes ($p<0.0001$). Moreover, this same genotype had the lowest values when water and sulfuric acid treatments were used, but it did not show significant differences in respect to genotypes 2 and 7. Fertilization increased, as average, 9.2 % the germination of the different genotypes. The trial that reached the best germination was that of the use of water at 80 ºC; and the worst germination was that of sulfuric acid. On the whole, genotypes 3 and 6 were the ones having the best germination percentages, but they had significant differences, only with genotype 1.

Conclusions

According to the trials performed, it was concluded that *T. diversifolia* is a species that can be used as forage shrub in different soil and climate conditions, thanks to its high genetic diversity. In spite of identifying high nutritional quality in all the genotypes under trial, based on the high content of CP (>25 %), energy, and minerals such as Ca and P, and of providing low values of NDF and ADF (49 and 46 %, respectively) and high *in vitro* degradability, there is the possibility of choosing better materials to increase growth and biomass yield in specific productive conditions.

Genotypes 5 and 7 were identified as those of best agronomic performance in conditions of acid and low quality soils in warm areas, and genotype 4 proved to be the one possessing better growth in high tropics conditions due to its GxE interaction.

Due to its chemical traits, this shrub modifies fermentation parameters by increasing propionic acid generation and the general efficiency of the fermentative process, and by decreasing the A:P ratio. Moreover, *T. diversifolia* in low quality diets has the potential of diminishing the CH_4 releases in *in vitro* conditions. However, differences were found among some of the genotypes under study as to their potential to modify CH_4 fermentation and production dynamics.

At last, *T. diversifolia* has genotypes able of showing growth and flowering stages significantly different among them by modifying the reproduction and sexual seed time. Furthermore, thanks to the differentiated growth, there are genotypes with greater production of viable sexual seed, associated with the number of stems and flowers per plant as it was the case of genotypes 5 and 7. The data provided in these trials could be the basis for establishing programs of selection, characterization, improvement, and preservation of better genotypes of this species devoted to animal production.

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