Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(3):280–291
doi: 10.17138/TGFT(9)280-291

Research Paper

Biomass production and nutritional properties of promising genotypes of *Tithonia diversifolia* (Hemsl.) A. Gray under different environments

Producción de biomasa y propiedades nutricionales de genotipos destacados de Tithonia diversifolia *(Hemsl.) A. Gray bajo diferentes condiciones ambientales*

JULIÁN ESTEBAN RIVERA[1*], TOMÁS E. RUÍZ[2], JULIAN CHARÁ[1], JUAN FLORENCIO GÓMEZ-LEYVA[3] AND ROLANDO BARAHONA[4]

[1]*Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria – CIPAV, Cali, Colombia.* cipav.org.co
[2]*Instituto de Ciencia Animal, San José de las Lajas, La Habana. Cuba.* ica.edu.cu
[3]*Laboratorio de Biología Molecular, TecNM-Instituto Tecnológico de Tlajomulco, México.* ittlajomulco.edu.mx
[4]*Universidad Nacional de Colombia, Sede Medellín, Colombia.* medellin.unal.edu.co

Abstract

Tithonia diversifolia is a shrub with excellent forage characteristics that has shown a wide genetic and phenotypic diversity. The objective of this study was to determine the biomass production and nutritional quality of seven genotypes of *T. diversifolia* with outstanding characteristics for ruminant nutrition, to analyze the Genotype x Environment (GxE) interaction of biomass production and to compare the performance of these genotypes with grasses offered normally in tropical conditions. For the GxE interaction the AMMI and SREG models were used, and evaluations were made in three environments. In the GxE analysis, the interaction was significant and effects of the environment on biomass productivity were observed with differences among genotypes. In the three environments, the high content of crude protein (28.89 g/100 g of DM), the low fiber content (30.95 g of neutral detergent fiber - NDF/100 g of DM) and the high percentages of *in vitro* degradation of DM for all the genotypes was adequate to be offered to ruminants. This study identified superior genotypes of *T. diversifolia* with good productive and adaptive performance for high-altitude and low-altitude zones with low fertility soils.

Keywords: Forage productivity, genetic diversity, GxE interaction, multivariate analysis, nutrient supply, SREG model.

Resumen

Tithonia diversifolia es un arbusto con excelentes características forrajeras que ha mostrado una amplia diversidad genética y fenotípica. El objetivo de este estudio fue determinar la producción de biomasa y la calidad nutricional de siete genotipos de *T. diversifolia* con características sobresalientes para la nutrición de rumiantes, analizar la interacción Genotipo x Ambiente (GxE) de la producción de biomasa y comparar el desempeño de estos genotipos con gramíneas ofrecidas normalmente en condiciones tropicales. Para la interacción GxE se utilizaron los modelos AMMI y SREG, y se realizaron evaluaciones en tres ambientes. En el análisis GxE, la interacción fue significativa y se observaron efectos del ambiente sobre la productividad de la biomasa con diferencias entre genotipos. En los tres ambientes, la composición química fue adecuada para ser ofrecida a los rumiantes. Cabe destacar el alto contenido de proteína bruta (28.89 g/100 g de MS), el bajo contenido de fibra (30.95 g de fibra detergente neutra - FDN/100 g de MS) y los altos porcentajes de degradación in vitro de la MS para todos los genotipos. Se puede concluir que existen genotipos superiores de *T.*

Correspondence: Julian Esteban Rivera, Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria – CIPAV.
Carrera 25 # 6 – 62 Cali, Colombia

diversifolia con capacidad de tener un buen rendimiento productivo y adaptativo para zonas de alta y baja altitud con suelos de baja fertilidad.

Palabras clave: Análisis multivariado, diversidad genética, interacción GxE, modelo SREG, oferta de nutrientes, productividad de forraje.

Introduction

Silvopastoral systems (SPS) have proven to be a suitable alternative to increase production efficiency and reduce the environmental impact of livestock systems (Jose et al. 2019). One of the shrub species used in Colombia and Mexico as a component of the SPS is *Tithonia diversifolia* (Hemsl.) A. Gray. *T. diversifolia* has excellent forage characteristics with high biomass productivity and nutritional value, and also wide phenotypic variation, which provides an opportunity to identify and select outstanding genotypes capable of achieving higher productivity (Ruiz et al. 2013). It has been grown under different edaphoclimatic conditions and exhibits a high degree of genetic diversity and variability in its agronomic properties, nutrient content, and adaptability (Holguín et al. 2015).

Although good productive responses are frequently reported in grazing ruminants receiving *T. diversifolia*-supplemented diets, greater benefits could be possible by carrying out evaluation and identification of different genotypes to select cultivars with desirable characteristics (Holguín et al. 2015; Rivera et al. 2018). One area of interest is to identify elite germplasm adapted to marginal conditions such as acid and low-fertility soils of the tropics and subtropics.

In successful forage selection programs, the influence of environmental factors on plant productivity and nutritional quality is the basis for identifying more efficient cultivars for animal nutrition and economic performance of farms (Schultze-Kraft et al. 2018). In recent years, the AMMI (*additive main effects and multiplicative interaction*) and SREG (*sites regression*) models have been used to determine the genotype-environment interaction (GxE) in agricultural crops, as well as their stability and adaptation to different environments (Bhartiya et al. 2017).

This study was carried out to measure biomass production and nutritional quality of different *T. diversifolia* provenances under different environment and management conditions and compare their chemical composition with the nutritional quality of two grasses usually offered in tropical conditions to identify stable genotypes with potential as feed. Variables measured included those associated with nutrient content and with agronomic performance.

Materials and Methods

Genotypes evaluated

Seven genotypes of *T. diversifolia* previously identified by Rivera et al. (2017) were included in this study (Table 1). These genotypes were previously selected based on the Dice dissimilarity index and the weighted forage potential index (WFPI) (Holguín et al. 2015) from a group of 30 populations collected in Colombia and Mexico. The selected genotypes presented outstanding performance in biomass production, number of stems and overall growth (Rivera et al. 2017).

Location of experiment

The study was carried out in three environments (environment 1: Tropical lowlands with fertilization; environment 2: Tropical lowlands without fertilization; environment 3: Tropical highlands without fertilization) during 2018 and 2019. Environments 1 and 2 were

Table 1. Location of the collection sites of the genotypes evaluated

Identification	Municipality	Department	masl (m)	Precipitation (mm/year)	Temperature (ºC)	Coordinates N	Coordinates W
Genotype 1	Granada	Meta	326	2410	27.2	3°53'42.06"	-74°11'48.72"
Genotype 2	Belén de los Andaquíes	Caquetá	232	2840	23.5	01°14'49.2"	-75°46'28.3"
Genotype 3	La Paz	Cesar	623	1220	27.2	10°14'18.66"	-73°6'21.539"
Genotype 4	Santa Rosa de Cabal	Risaralda	1870	2610	16.2	4°52'39.430"	-75°34'58.563"
Genotype 5	Encino	Santander	1608	870	22.3	6°11'26.52"	-73°8' 49.139"
Genotype 6	Charalá	Santander	1383	2130	23.4	6°16'46.8"	-73°9'49.499"
Genotype 7	Manizales	Caldas	2159	2545	16.3	5°0'51.538"	-75°33'58.302"

masl: meters above sea level

located in Meta, Colombia (3°47'21"N, 73°49'16"W) at 530 masl in a region classified as belonging to the tropical humid forest life zone (bh-T) (Holdridge 1978). Environment 3 was located in Caldas, Colombia (5°0'45"N, 75°25'47"W) at an altitude of 2300 masl, which corresponds to a lower montane moist forest (bh-MB) (Holdridge 1978).

Experimental design

An experimental area of 642 m^2 was established in each environment. In order to ensure genetic homogeneity, the planting material of all genotypes were produced in the laboratory using explant clonal reproduction. Each one of the 642 m^2 areas consisted of 21 plots (4 x 5.5 m) with three replicates of 36 plants for each genotype of *T. diversifolia* planted in a randomized complete block design. Neighboring pastures of *Urochloa brizantha* cv. Marandú in environment 1 and 2 and *Cenchrus clandestinus* in environment 3 were used as the reference for comparison with local feed supply. The level of fertilization used in environment 1 was in accordance with the extraction of nutrients of 40 days old *T. diversifolia* plants (Botero et al. 2019). These nutrients were applied by fertilizing with urea (46% N), ammonium phosphate (DAP) [$(NH_4)_2HPO_4$; 46% P_2O_5, 18% N] and potassium chloride (KCl, 60% K_2O) at a fertilizer rate of 16.22 g/plant (324 kg/ha), 2.15 g/plant (43 kg/ha) and 4.89 g/plant (98 kg/ha) respectively.

Soil analysis

Three soil samples were taken from 20–30 cm depth in each block at the beginning of the experiment. The following chemical and physical variables were measured: pH, electrical conductivity (E.C.) (dS/m), bulk density (g/cc), organic matter (%), texture, exchangeable acidity (mg/kg), exchangeable calcium (mg/kg), Iron (mg/kg), Manganese (mg/kg), Copper (mg/kg), Zinc (mg/kg), Boron (mg/kg), Phosphorus (mg/kg) and Cation exchange capacity (meq/100g). The different determinations were carried out at AGRILAB soil laboratory (Bogotá, Colombia).

Environmental conditions

During the experimental period, precipitation (mm), temperature (ºC), humidity (%), solar radiation (W/m2), dew point (ºC), wind speed (m/s) and THSW index (Thermal sensation due to wind), relative humidity and irradiance (instantaneous solar radiation) were recorded using a Vantage Pro 2TM (Davis ®) weather station.

Nutritional and agronomic variables

Morphological variables were measured during four harvests on five plants per plot, taking 2 measurements during the rainy and 2 during the dry season in each environment. A uniformity cut at 10 cm height was made 4 months after planting on the whole plot. For environment 3, harvests were made every 60 days by cutting 5 randomly selected plants per plot at 10 cm height when plants had reached an average plant height of 109.3 cm, and in environments 1 and 2, harvests were made every 40 days by cutting 5 randomly selected plants per plot at 10 cm height when plants had reached an average plant height of 95.5 and 69.1 cm, respectively. The cutting regimes were established based on the harvesting times usually used in each zone for the predominant forage species (*Urochloa brizantha* cv. Marandú and *Cenchrus clandestinus* respectively). Nutritional traits were determined at the Animal Nutrition Laboratory, the Colombian Corporation for Agricultural Research (AGROSAVIA) by near-infrared spectroscopy (NIRS) using two chemometric tools (GLOBAL and LOCAL) using a scanning VIS/NIR spectrometer (Foss NIRSystems model 6500) and the WinISI 4.7.0 software (Ariza-Nieto et al. 2018). The nutritional variables were determined using samples from one harvest in the dry season and one in the rainy season (Table 2).

Genotype-by-environment interaction and data analysis

For the GxE interaction analysis, AMMI (Mandel 1971) and SREG site regression analysis (Yan et al. 2000) models were used. Material stability was measured using the Shukla's Stability Variance. The analyses were performed in RStudio using the "*ggbiplot*", "*GGEBiplotGUI*" (GGEplot) and "*agricolae*" libraries (R Core Team 2019). Tukey's contrast test (0.05 significance level) was used when significant differences between means were detected, and when the data groups did not meet the conditions for a parametric analysis, the Kruskal-Wallis and Mann-Whitney tests were applied.

Table 2. Morphological and nutritional variables

Variables	Measurement method
Morphological variables	
Plant height (PlantH)	Measured using a tape measure from the base of the main stem to the flag leaf.
Stem diameter (StemDiam)	Measured using a vernier caliper at a height of 15 cm. The average of two randomly selected stems was used.
Leaf-stem ratio (Leaf:Stem)	Calculated from the fresh weight of stems and green leaves at harvest.
Number of branches (Bran, stems with leaves)	Measured by manual counting per plant at harvest.
Leaf area (LeafAre)	Mean of ten randomly collected leaves from each plant collected and analyzed in ImageJ® 1.47v software.
Green forage per plant (GreenF)	Fresh weight (g) of green leaves and small stems with diameters of less than 5 mm taken as a mean of 5 plants.
Dry forage per plant (DW)	Determined after drying the green forage for 72 hours in a forced-air oven at 65 °C (g/100 g).
Survival (Surv)	Calculated by the difference between the number of plants planted and the final number at harvest.
Presence of pests or diseases	Scored in each of the plots by observing for one minute the presence of pests causing evident damage to the plant material. If pests were present, damage was rated from 1 to 3, with 1 being low damage and 3 being severe damage.
Nutritional variables	
Dry Matter (DM)	Determined using NIRS FOSS 6500 LOCAL and GLOBAL models WinISI 4.7.0 software (Ariza-Nieto et al. 2018).
Crude protein (CP)	
Ether extract (EE)	
Neutral detergent fiber (NDF)	
Acid detergent fiber (ADF)	
Total digestible nutrients (TDN)	
in vitro DM degradability (IVDMD)	
Gross energy (GE)	
Net energy for lactation (NEL)	
Calcium (Ca)	Determined using AA and UV-VIS spectrophotometry. Based on methods NTC 5151 (ICONTEC 2003) and NTC 4981 (ICONTEC 2001) respectively.
Phosphorus (P)	

Results

Soil analysis

The soils in all sites were acidic with different levels of fertility (Table 3).

Environmental conditions

For environments 1 and 2, average temperature was 25.1 ± 1.3 ºC, relative humidity was 77.8 ± 9.4%, average dew point was 20.6 ± 1.17 ºC, wind speed was 0.68 ± 0.2 m/s, average THSW index was 27.7 ± 1.7 ºC, solar radiation was 478 ± 48.3 W/m^2, and accumulated precipitation was 1119 mm. During the rainy season the cumulative rainfall was 922.6 mm and during the dry season it was 195.4 mm. For environment 3, average temperature was 15.3 ± 0.85 ºC, relative humidity was 87.4 ± 5.4%, average dew point was 13.2 ± 0.77 ºC, wind speed was 0.42 ± 0.13 m/s, average THSW index was 15.5 ± 1.24 ºC, solar radiation was 249.9 ± 40.54 W/m^2, and accumulated precipitation was 905.9 mm. The cumulative rainfall during the rainy season was 673.6 mm and 232.3 mm during the dry season.

Nutritional and agronomic variables

Measurements of morphological and agronomic variables found in the three environments are presented in Table 4. During the evaluation period, the pest or disease damage was minimum (level 1) and occurred in environments 1 and 2 due to the presence of *Acromyrmex spp*. and *Atta spp*. The incidence of these ant attacks was not associated to a specific genotype of *T. diversifolia.*

The variables LeafAre, Leaf:Stem ratio, and Bran presented a positive significant correlation with the production of DW with Pearson coefficients of 0.86, 0.89 and 0.78, respectively. Significant differences between genotypes were found in each environment and there was also an effect of season in most variables. In environments 1 and 2, genotypes 7 and 5 had the highest growth. In environment 3, genotypes 4 and 7 had the highest growth

rates. In addition, there were significant differences in variables such as plant height, leaf size, stems per plant and leaf-stem ratio that were 1.5, 1.38, 1.67 and 1.63 times higher in fertilized plants, respectively.

Table 5 presents the results of the chemical analyses of *T. diversifolia* forage samples and evaluated grasses. In environments 1 and 2 some differences were observed among *T. diversifolia* genotypes (CP, IVDMD and P), but compared with the *U. brizantha* pasture, all genotypes show higher nutrient supply than this grass. In addition, despite not relevant finding a difference in the nutritional traits between environments 1 and 2, the greater growth observed in genotypes 5 and 7 (Table 4) allowed them to offer significantly more nutrients in terms of g per plant, compared to the other genotypes. The season had an effect on all parameters except NDF and GE content.

In environment 3 there were differences between *T. diversifolia* genotypes and with the C. clandestinus grass commonly used in the highland tropics of Colombia. CP, EE, NDF, ADF, TDN, IVDMD and NEL had differences between genotypes. The season also influenced the nutritional traits of the genotypes evaluated (Table 5).

Genotype-by-environment interaction

In environments 1 and 2, the genotypes with the highest DW yield were 7 (106.5 g per plant) and 5 (89.7 g per plant), and the genotypes with lowest DW were 1, 4 and 3 with an average of 65.8, 68.7 and 73.4 g/plant, respectively. Figure 1 shows the graphic representation of all genotypes in each environment according to its DW production. The genotypes at the most extreme points and close to the blue arrows are the best performing (Figure 1).

In environments 1 and 2, the production of DW in the rainy season was 1.4 times that of the low precipitation season, and the use of fertilizer increased DW production 1.9 times on average, and 2.3 times during the rainy season. The genotypes with best tolerance to the dry season represented by the smallest decrease in biomass production compared to the rainy season were 3, 1 and 2. In environment 3, the genotypes with the highest production were 4 and 7 with DW yield of 152.6 and 128.9 g/plant respectively, despite showing greater yield variability in this environment. The lowest performing genotypes were 1, 3 and 6. In this site, the genotypes decreased their yield on average by 13.5% as an effect of the dry season with genotypes 5 and 6 having the least reduction.

In the analysis of variance of the AMMI model (Table 6), genotypes, environments and GxE interaction presented significant differences for DW production per plant (Table 6).

In Figure 2 (left) the genotypes that were collected from similar environments are associated with better performance in that environment. In addition, the genotype ranking graph (right) shows that the genotypes closest to the center point are the best in all environments (7, 5 and 6).

Performance stability throughout environments

According to Shukla's stability index, the most stable genotypes were 7 and 5, due to their relatively high productive performance across environments. GxE interaction was also found in the survival of genotypes during the experimentation period (Figure 3). The average survival at the end of the experiment in environments 1 and 2 was 82.3% with the effect of fertilization (p=0.0068). In environment 3, the average survival rate was 65.8%.

Table 3. Chemical and physical characteristics of the soils

Characteristic	Environment 1	Environment 2	Environment 3
pH	4.72 (±0.03)	4.68 (±0.03)	5.45 (±0.08)
Electrical conductivity (dS/m)	0.06 (±0.01)	0.06 (±0.01)	0.17 (±0.02)
Bulk density (g/cc)	1.49 (±0.04)	1.47 (±0.05)	0.99 (±0.01)
Organic matter (%)	1.68 (±0.35)	1.45 (±0.30)	8.16 (±0.1)
Texture	Loam-Clay-Sandy	Loam-Clay-Sandy	Loam
Interchangeable acidity (mg/kg)	202 (±26.8)	203 (±18.2)	41.4 (±10.4)
Exchangeable calcium (mg/kg)	209 (±82.6)	178 (±65.4)	426 (±98.2)
Iron (mg/kg)	374 (±78.2)	374 (±75.1)	202 (±69.2)
Manganese (mg/kg)	6.97 (±3.16)	6.30 (±2.31)	18.2 (±8.55)
Copper (mg/kg)	0.86 (±0.24)	0.76 (±0.14)	2.80 (±0.70)
Zinc (mg/kg)	0.63 (±0.17)	0.50 (±0.15)	18.00 (±5.2)
Boron (mg/kg)	0.13 (±0.03)	0.14 (±0.01)	0.06 (±0.01)
Phosphorus (mg/kg)	6.03 (±1.95)	5.60 (±1.67)	13.7 (±2.08)
Cation exchange capacity (meq/100g)	3.40 (±0.42)	3.27 (±0.22)	3.46 (±0.59)

Table 4. Morphological and agronomic variables of the genotypes of *T. diversifolia*

Environment / Genotype	PlantH	StemDiam	Bran	Leaf:Stem	LeafAre
Environment 1					
Gen1	62.1bc	8.75bc	9.64ab	0.89	46.2c
Gen2	58.3c	8.04c	8.03b	0.98	44.9c
Gen3	63.8bc	8.93bc	8.72ab	0.96	54.2abc
Gen4	59.7c	8.43c	8.42ab	0.93	48.7bc
Gen5	75.1ab	10.1ab	9.75ab	0.96	59.4ab
Gen6	66.1bc	9.99ab	8.75ab	0.91	49.6bc
Gen7	84.5a	10.9a	11.1a	0.92	66.2a
p- value	<0.001*	<0.001*	0.013*	0.929	<0.001*
SEM	2.07	0.28	0.48	0.04	2.95
Season effect	<0.001*	0.004*	<0.001*	<0.001*	<0.001*
Dry season	60.6	9.71	6.55	0.71	36.73
Rainy season	72.6	8.72	11.6	1.14	67.92
Environment 2					
Gen1	98.6c	11.3b	11.4ab	0.78	74.01b
Gen2	97.9c	11.4b	11.1ab	0.83	71.3b
Gen3	90.4c	12.1ab	10.1b	0.86	75.3b
Gen4	92.3c	11.1b	12.6ab	0.83	74.9b
Gen5	117.1ab	13.2a	12.4ab	0.81	84.4b
Gen6	103.3bc	12.3ab	10.4ab	0.8	79.8b
Gen7	122.4a	12.5ab	13.9a	0.8	102.6a
p- value	<0.001*	0.002*	0.023*	0.578	0.001*
SEM	3.89	0.18	0.64	0.01	4.8
Season effect	<0.001*	0.029*	<0.001*	0.494	<0.001*
Dry season	82.3	11.7	8.23	0.808	52.8
Rainy season	123.5	12.3	15.2	0.824	107.4
Environment 3					
Gen1	75.5d	8.59c	13.84ab	0.75a	69.1c
Gen2	100abc	10.35ab	16.3ab	0.68ab	84.1a
Gen3	86.2c	9.85b	12.3b	0.74ab	72.3ab
Gen4	111a	10.98a	18.9a	0.65b	82.8ab
Gen5	101.5ab	9.97b	16.3ab	0.7ab	59.8c
Gen6	95bc	9.71b	15.2ab	0.72ab	71bc
Gen7	111.7a	10.23ab	18.3ab	0.66ab	77.9ab
p- value	0.003*	<0.001*	<0.001*	0.0105*	<0.001*
SEM	2.62	0.13	0.62	0.01	2.01
Season effect	<0.001*	0.014*	<0.001*	<0.001*	<0.001*
Dry season	89.1	9.74	12.7	0.65	65.9
Rainy season	104.2	10.2	18.9	0.74	78.9

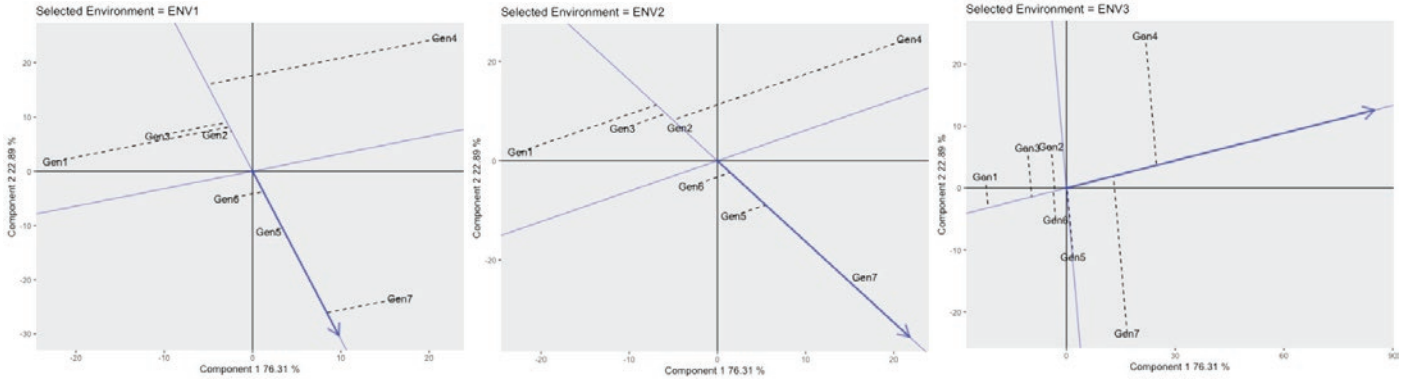

Figure 1. Dry biomass productivity GGEplot representation of the genotypes of *T. diversifolia* in the three environments.

Table 5. Nutritional traits (g/100 g of DM; Mcal/kg of DM) of the genotypes

Environment 1	Genotypes							Season		*U. brizantha*	*p- value*		SEM
Characteristic	Gen1	Gen2	Gen3	Gen4	Gen5	Gen6	Gen7	Rainy	Dry		Genotype	Season	
DM	15.0	16.0	15.5	15.9	15.5	15.6	15.3	14.3	16.8	22.7	0.252	<0.001*	0.223
CP	33.9ab	32.8abc	32.8abc	31.8bc	34.3a	31.4c	34.1ab	35.2	30.8	10.9	0.004*	<0.001*	0.488
Ash	15.0	15.0	15.4	14.9	15.0	15.0	14.7	15.4	14.6	7.9	0.657	0.001*	0.126
EE	1.98	2.00	2.10	2.19	2.10	2.20	1.93	1.49	2.65	1.59	0.662	<0.001*	0.102
NDF	31.2	31.7	30.8	32.2	31.3	31.8	31.3	30.6	32.3	65.3	0.951	0.016*	0.349
ADF	15.4	15.3	14.9	15.1	16.4	15.0	15.9	16.2	14.7	48.5	0.922	0.042*	0.371
Ca	1.60	1.41	1.56	1.47	1.33	1.68	1.49	1.27	1.74	0.41	0.503	<0.001*	0.061
P	0.44	0.46	0.45	0.44	0.45	0.42	0.44	0.51	0.38	0.20	0.933	<0.001*	0.012
TDN	76.0	75.1	75.2	74.4	76.0	74.1	75.9	76.4	74.1	51.7	0.265	<0.001*	0.349
IVDMD	82.8ab	81.9ab	82.1ab	81.2ab	82.9a	80.9b	82.7ab	83.6	80.5	57.7	0.013*	<0.001*	0.375
GE	4.29	4.28	4.28	4.27	4.30	4.26	4.29	4.29	4.26	4.09	0.849	0.053	0.008
NE_L	1.75	1.73	1.73	1.71	1.75	1.70	1.74	1.75	1.69	1.15	0.284	<0.001*	0.009
Environment 2	Genotypes							Season		*U. brizantha*	*p- value*		SEM
Characteristic	Gen1	Gen2	Gen3	Gen4	Gen5	Gen6	Gen7	Rainy	Dry		Genotype	Season	
DM	16.7	16.3	16.0	16.5	16.2	16.2	16.2	14.9	17.7	22.7	0.705	<0.001*	0.238
CP	29.6	27.8	30.3	27.3	28.9	30.5	29.8	31.1	27.2	10.9	0.118	<0.001*	0.470
Ash	14.8	14.2	14.9	14.3	15.2	15.3	14.8	15.3	14.3	7.94	0.222	0.002	0.165
EE	2.13	2.27	2.05	2.31	1.99	2.08	1.97	1.46	2.76	1.59	0.221	<0.001*	0.112
NDF	30.4	31.0	30.2	30.7	31.9	30.8	31.1	30.9	30.7	65.3	0.882	0.708	0.331
ADF	14.0	12.8	13.6	11.2	13.9	13.4	13.2	15.3	13.6	48.5	0.226	<0.001*	0.456
Ca	1.96	1.99	1.90	1.96	1.83	1.88	1.80	1.79	2.1	0.41	0.651	0.009*	0.039
P	0.37c	0.37bc	0.41abc	0.38bc	0.44a	0.44a	0.42ab	0.47	0.34	0.20	0.009*	<0.001*	0.012
TDN	73.6	72.0	73.6	72.0	72.5	73.9	73.4	73.9	71.9	51.7	0.181	<0.001*	0.298
IVDMD	80.3	78.5	80.3	78.5	79.1	80.6	80.0	80.7	78.5	57.7	0.181	<0.001*	0.320
GE	4.24	4.20	4.21	4.17	4.20	4.23	4.25	4.21	4.23	4.09	0.271	0.234	0.010
NE_L	1.69	1.65	1.69	1.65	1.66	1.70	1.68	1.7	1.64	1.15	0.138	<0.001*	0.007
Environment 3	Genotypes							Season		*C. clandestinus*	*p- value*		SEM
Characteristic	Gen1	Gen2	Gen3	Gen4	Gen5	Gen6	Gen7	Rainy	Dry		Genotype	Season	
DM	16.3	16.6	17.3	17.1	17.3	17.5	17	16.7	17.3	18.1	0.058	0.005*	0.127
CP	27.1ab	28.7a	27.1ab	29.5a	28.9a	25.4b	26.2b	27.5	27.1	20.9	0.005*	0.879	0.292
Ash	14.7	15.1	14.4	15.1	14.6	15.1	14.4	14.5	15	12.1	0.246	0.029*	0.106
EE	1.84c	2.34a	1.93bc	2.19ab	2.15abc	1.93bc	2.04abc	1.59	1.89	2.31	0.002*	0.095	0.029
NDF	32.1a	30.9ab	29.8bc	28.5c	29.7bc	31.6a	31.1ab	28.8	32.2	44.5	0.006*	0.008*	0.377
ADF	2.52	2.16	2.42	2.23	2.32	2.27	2.22	2.08	2.53	0.65	0.055	<0.001*	0.051
Ca	0.38	0.4	0.36	0.41	0.38	0.36	0.38	0.35	0.41	0.35	0.382	<0.001*	0.006
P	71.1bc	71.9abc	71.4abc	73.2a	72.5ab	70.1c	70.9bc	71.6	71.5	63.6	0.001*	0.802	0.202
TDN	77.6bc	78.5abc	77.9abc	79.8a	79.1ab	76.5c	77.4bc	78.2	78.1	67.6	0.001*	0.797	0.218
IVDMD	4.15	4.17	4.13	4.21	4.19	4.14	4.16	4.12	4.2	4.09	0.429	0.001*	0.012
GE	1.62bc	1.64abc	1.63abc	1.67a	1.65ab	1.60c	1.62bc	1.64	1.63	1.34	0.002*	0.801	0.005
NE_L	1.62bc	1.64abc	1.63abc	1.67a	1.65ab	1.60c	1.62bc	1.64	1.63	1.34	0.002*	0.801	0.005

DM: dry matter; CP: crude protein; Ash: ashes; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; Ca: calcium; P: phosphorus; TDN: total digestible nutrients; IVDMD: in vitro DM degradability; GE: gross energy; NE_L: net energy of lactation; SEM: standard error of the mean; * Different letters in the same row denotes statistical difference according to the Tukey test ($p < 0.05$).

Table 6. GxE interaction AMMI model. Analysis of variance for the dry matter production

	Df	Sum Sq	Mean Sq	F value	Variance (%)	Pr(>F)
Environment	2	72305	36152	98.5	53.3	0.0002 ***
Rep (Environment)	6	2201	367	1.23	1.62	0.294
Genotype	6	32820	5470	18.4	24.3	2.77E-14 ***
Environment x Genotype	12	28418	2368	7.98	20.9	2.97E-10 ***
Residuals	99	29360	297			

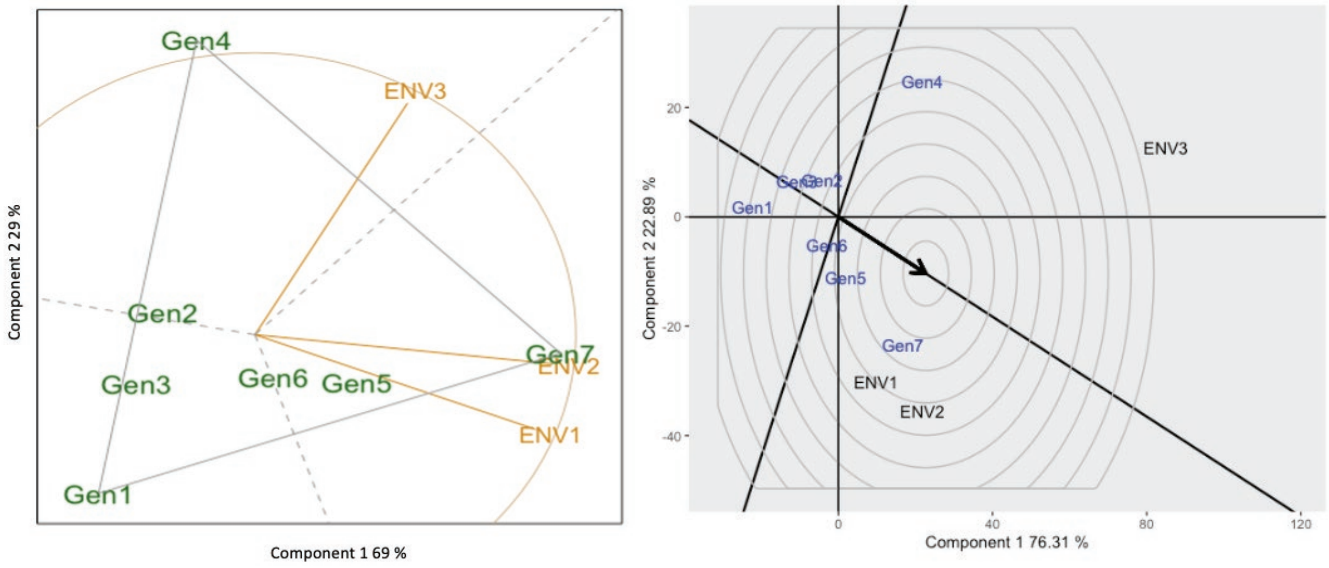

Figure 2. GGEplot dry matter yield of the *T. diversifolia* genotypes (left). Genotypes ranking with respect to the ideal genotype (right)

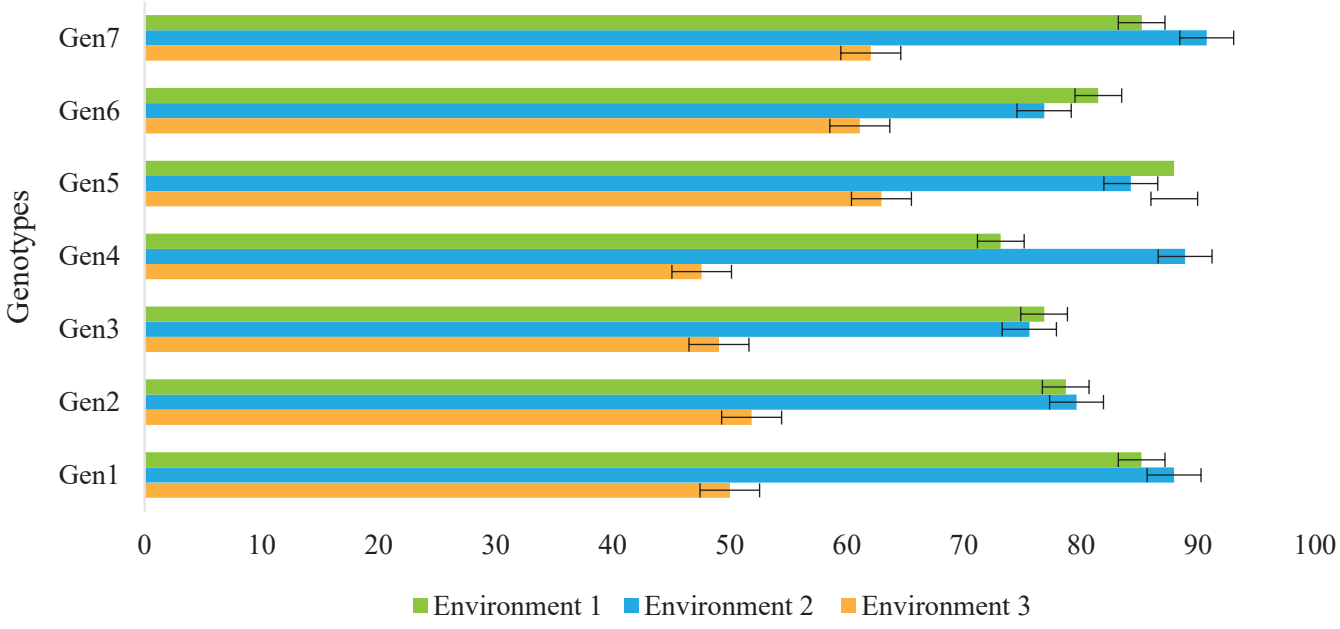

Figure 3. *Tithonia diversifolia* survival rate (%) in three environments in two experimental sites.

Discussion

According to Rivera et al. (2017), there is wide genetic diversity in the populations of *T. diversifolia* evaluated. A total of 105 fragments were amplified, of which 5% were monomorphic and 95% polymorphic. In addition, the analysis based on the genome proportion (genetic structure) of each population showed seven well-defined groups. In Mexico, Del Val et al. (2017) evaluated 20 materials for feed purposes from eight localities and obtained a total of 157 bands of which 33 were monomorphic and 124 polymorphic, indicating 79% polymorphism. Thus, in the dendrogram performed with the Dice coefficient and using the UPGMA classification method, no significant relationship was found among 10 samples and only two were similar (at a similarity level of 1.47, 2 groups were observed). Yang et al. (2012) found great genetic variability in collections of this species obtained from four regions in China and two in Laos. In this research, the mean values of Nei of genetic diversity (H) and the Shannon index of diversity (I) were 0.2937 and 0.432, respectively, and 84.62% of polymorphism was observed, demonstrating wide diversity of *T. diversifolia* materials and conferring it great adaptation to diverse environments.

T. diversifolia exhibited adaptation to the climatic and edaphic environments evaluated since outstanding genotypes were observed in each environment. The characteristics that could contribute to this adaptability are the large root volume, that improves the efficiency to obtain nutrients from the soil (Jama et al. 2000), the possibility to associate with different microorganisms, further favoring this property, especially in low fertility soils (Rivera et al. 2018) and its genetic diversity (Yang et al. 2012). The soils in the evaluation sites were diverse and could represent a large area of the tropics where soils are characterized by acidity and a range of fertility levels.

Morphological and agronomic characteristics showed great variability among genotypes and environments, and several of them (LeafAre, Leaf:Stem ratio and Bran) had a significant and direct correlation with DW production. Some of these traits related to leaf growth could be used to predict the growth of *T. diversifolia* and therefore employed for the selection of genotypes with greater productivity and adaptation (Ruiz et al. 2013). In addition, the variability found can be used strategically in selection programs and future varietal improvement. This could be carried out comprehensively with multi-criteria evaluations based on adaptability, productivity and nutritional quality (Holguín et al. 2015, Rivera et al. 2018). For example, genotypes 5 and 7 and genotypes 4 and 7 could be used in low-altitude and high-altitude, respectively in conditions similar to those evaluated in this study.

Ribeiro et al. (2016), reported high nutrient content in T. diversifolia, which can then be employed either as a supplement to diets based on tropical pastures, or as a forage source capable of partially replacing commercial concentrates in ruminant diets. The most outstanding chemical fractions in *T. diversifolia* are the high percentages of CP in leaves (>25%), the low fiber contents (NDF and ADF), the acceptable mineral contents (Ca and P) and the good degradability values of DM and energy. The results of this study are consistent with those reported by researchers such as Ribeiro et al. (2016), who identified its use in the feeding of high production dairy cattle, its nutritional value, as well as its fermentation dynamics.

The seven genotypes evaluated presented higher amounts of CP and lower percentages of fiber (NDF and ADF) than that reported by La O et al. (2012), who evaluated nine genotypes of *T. diversifolia* in Cuba and found protein and NDF values from 18.3 to 26.4 and from 14.8 to 25.7%, respectively. Likewise, in terms of CP, contents reported in this study are as high or even higher than those found in tropical legumes such as *Stylosanthes guianensis* (18.2%, Morgado et al. 2009) and *Arachis pintoi* (19.7%, Khan et al. 2013). Its degradability, energy, Ca and P content do not limit voluntary intake and nutrient availability at the ruminal level, despite the 60 days regrowth age used in environment 3. Although the genotypes at this site had significant differences, they all presented a high supply of nutrients. As a result of evaluations in the three environments, and two seasons, it was also possible to determine that the nutritional value of *T. diversifolia* is maintained under different environmental conditions, presenting a superior nutrient offer than that of tropical pastures. According to Rivera et al. (2015) the inclusion of *T. diversifolia* in Brachiaria-based systems can support an increased number of animals per hectare and increase milk production as well as milk quality.

Identifying stable genotypes adapted to different conditions and with the ability to achieve high performance in variable environments has been an ongoing challenge in the study of forage species (Liang et al. 2015). The characterization of stable genotypes across different environments is an important task, although difficult to achieve due to the frequent influence of GxE interactions (Senger et al. 2016). Yan (2002) indicated

that typically, the environment explains most of the total yield variation (up to 70% or more), while genotypes and GxE interaction are generally small. This is specifically true for traits like plant yield, as for example found in this study.

The SREG chart (Figure 2) identifies the ideal genotype as the one with a high score on the first axis of the principal component (CP1) that is associated with high yields and scores close to zero on the second axis of the principal component (CP2). This is related to good stability (Figure 1 and Figure 2) as shown by genotypes 5 and 7. Furthermore, in the GGE Biplot, the genotypes located towards the center of the figure are less representative than those located at the corners or vertices of the polygon, which are considered more responsive (positively or negatively, genotypes 6, 3 and 2) to the environmental conditions. Genotypes located in sectors of the SREG chart where there are no sites, are considered to have poor performance behavior in most of the sites evaluated (Yan et al. 2001) (Genotypes 1, 2 and 3).

In the genotype classification, the graph of the so-called "ideal" genotype is shown (Figure 2). An "ideal" genotype is one with the highest performance in test environments and stable performance (Yan and Kang 2002). Although such an "ideal" genotype may not exist in reality, it can be used as a reference for the evaluation of different genotypes. A genotype is more desirable if it is closer to the "ideal" genotype (Yan and Kang 2002) as was the case of genotypes 7 and 5.

The production of biomass found in this study was lower than those reported by Alonso Lazo et al. (2015), who evaluated four grazing frequencies and different planting distances in Cuba and found weights between 1,400 and 2,300 g of green weight/plant and from 200 to 600 g of dry weight of the entire plant. These weights were similar to those reported by Gallego et al. (2015) in Colombia under conditions similar to those given in environment 3, and where Ruiz et al. (2013) recorded weights of 100 green leaves between 110 and 190 g at 42 days, and between 150 and 240 g at 60 days. Botero et al. (2019) found a positive response of *T. diversifolia* when it was fertilized. The response found by these authors was greater than that reported in this study (2.5 times more DW when *T. diversifolia* was fertilized).

Significant differences were found in the survival rate of the genotypes (Figure 3) for both montane rain forest and tropical rain forest conditions, evidencing that high rainfall in clay loam soils negatively affects the survival of T. diversifolia. For this parameter, Gallego et al. (2015), found survival rates above 90% for plants from three establishment methods under highland conditions after one month of sowing. The differences between the two studies are probably due to the adverse environmental conditions during seedling establishment in this research.

Conclusions

T. diversifolia has the ability to adapt to different edaphoclimatic conditions and offer a high amount of nutrients for ruminants. The high percentage of CP, the low fiber values and the high percentages of energy and degradability of DM are outlined as the most remarkable characteristics in this species. Despite its wide plasticity, environmental conditions modify the yield of *T. diversifolia* genotypes showing GxE interaction and favoring the possibility of identifying and selecting genotypes with greater productive potential that are better adapted to specific sites. In this research, genotypes 5 and 7 were the most outstanding in site 1 and genotype 4 was the most outstanding material in site 2, while genotypes 5 and 6 showed stability across sites.

Acknowledgements

The authors wish to thank the Colombian Sustainable Cattle Ranching project, funded by the Government of the United Kingdom and the Global Environment Facility, for supporting the field and laboratory work, and to El Porvenir and Los Alpes farms for their help during the experiments and for providing access to their facilities. Thanks also to Minciencias for supporting J. Rivera's Ph.D. studies (National Doctorates 727-2015) and to CIPAV (contract 80740-006-2020) and the Colombian Autonomous Fund for Science, Technology and Innovation Francisco José de Caldas.

References

(Note of the editors: All hyperlinks were verified 27 August 2021).

Alonso Lazo J; Achang Fagra G; Tuffi Santos LD; Arruda Sampaio R. 2015. Productivity performance of *Tithonia diversifolia* in grazing with different rest times during both seasons of year. Livestock Research for Rural Development Volume 27, Article#115. (In Spanish). lrrd.org/lrrd27/6/alon27115.html

Ariza-Nieto C; Mayorga OL; Mojica B; Parra D; Afanador-Tellez G. 2018. Use of LOCAL algorithm with near infrared spectroscopy in forage resources for grazing systems in

Colombia. Journal of Near Infrared Spectroscopy 26(1): 44–52. doi: 10.1177/0967033517746900

Bhartiya A; Aditya JP; Singh K; Pushpendra; Purwar JP; PJ; Agarwal A. 2017. AMMI & GGE biplot analysis of multi environment yield trial of soybean in North Western Himalayan state Uttarakhand of India. Legume Research 40(2):306–312. doi: 10.18805/lr.v0iOF.3548

Botero J; Gómez A; Botero M. 2019. Yield, agronomic parameters and nutritional quality of *Tithonia diversifolia* in response to different fertilization levels. Revista Mexicana de Ciencias Pecuarias 10(3):789–800. doi: 10.22319/rmcp.v10i3.4667

Del Val R; Miranda JM; Flores MX; Solorio B; Solorio FJ; Gómez-Leyva JF; González-Palomares S. 2017. Diversidad genética de *Tithonia diversifolia* (Hemsl) Gray de Michoacán: Análisis con marcadores de ADN-SSR. Reaxion Ciencia y Tecnología Universitaria 4(3):10–14. bit.ly/3zpWhBs

Gallego L; Mahecha-Ledesma L; Angulo-Arizala J. 2015. Crecimiento y desarrollo de *Tithonia diversifolia* Hemsl. A Gray en condiciones de trópico alto. Actas 3° Congreso Nacional de Sistemas Silvopastoriles - VIII Congreso Internacional de Sistemas Agroforestales, Puerto Iguazú, Argentina, 7–9 Mayo 2015. p. 53–58.

Holdridge LR, eds. 1978. Ecología basada en zonas de vida. Instituto Interamericano de Cooperación para la Agricultura (IICA), San José, Costa Rica. repositorio.iica.int/handle/11324/7936

Holguín V; Ortíz S; Velasco A; Mora JR. 2015. Evaluación multicriterio de 44 introducciones de *Tithonia diversifolia* (Hemsl.) A. Gray en Candelaria, Valle del Cauca. Revista de la Facultad de Medicina Veterinaria y de Zootecnia 62(2):57–72. doi: 10.15446/rfmvz.v62n2.51995

ICONTEC (Instituto Colombiano de Normas Técnicas y Certificación). 2001. Alimentos para animales. Determinación del contenido de fosforo. Método espectrofotométrico. NTC 4981. ICONTEC, Bogotá, Colombia. bit.ly/2WxHOoo

ICONTEC (Instituto Colombiano de Normas Técnicas y Certificación). 2003. Alimento para animales. Determinación de los contenidos de calcio, cobre, hierro, magnesio, manganeso, potasio, sodio y zinc. Método de espectrometría de absorción atómica. NTC 5151. ICONTEC, Bogotá, Colombia. bit.ly/2V30RXo

Jama B; Palm CA; Buresh RJ; Niang A; Gachengo C; Nziguehbh G; Amadalo B. 2000. *Tithonia diversifolia* as a green manure for soil fertility improvement in western Kenya. A review. Agroforestry Systems 49(2):201–221. doi: 10.1023/A:1006339025728

Jose S; Walter D; Mohan Kumar B. 2019. Ecological considerations in sustainable silvopasture design and management. Agroforestry Systems 93:317–331. doi: 10.1007/s10457-016-0065-2

Khan MT; Khan NA; Bezabih M; Qureshi MS; Rahman A. 2013. The nutritional value of peanut hay (*Arachis hypogaea* L.) as an alternate forage source for sheep. Tropical Animal Health and Production 45:849–853. doi: 10.1007/s11250-012-0297-8

La O O; González H; Orozco A; Castillo Y; Ruiz O; Estrada A; Ríos F; Gutiérrez E; Bernal H; Valenciaga D; Castro BI; Hernánez Y. 2012. Composición química, degradabilidad ruminal *in situ* y digestibilidad *in vitro* de ecotipos de *Tithonia diversifolia* de interés para la alimentación de rumiantes. Revista Cubana de Ciencia Agrícola 46(1):47–53. redalyc.org/articulo.oa?id=193024313008

Liang S; Ren G; Liu, J; Zhao X; Zhou M; McNeil D; Ye G. 2015. Genotype-by-environment interaction is important for grain yield in irrigated lowland rice. Field Crops Research 180:90–99. doi: 10.1016/j.fcr.2015.05.014

Mandel J. 1971. A new analysis of variance model for non-additive data. Technometrics 13(1):1–18. doi: 10.1080/00401706.1971.10488751

Morgado ES; Almeida FQ de; Silva VP; Gomes AVC; Galzerano L; Torres H; Rodrigues LM. 2009. Digestion of carbohydrates of forages in horses. Revista Brasileira de Zootecnia 38(1):75–81. (In Portuguese). doi: 10.1590/S1516-35982009000100010

R Core Team. 2019. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA. rstudio.com

Ribeiro RS; Terry SA; Sacramento JP; Silveira SR; Bento CBP; Silva EF da; Montovani HC; Gama MAS da; Pereira LGR; Tomich TR; Mauricio RM; Chaves AV. 2016. *Tithonia diversifolia* as a supplementary feed for dairy cows. Plos One 11:e0165751. doi: 10.1371/journal.pone.0165751

Rivera JE; Chará J; Gómez-Leyva JF; Ruíz T; Barahona R. 2018. Phenotypic variability and phytochemical composition of *Tithonia diversifolia* A. Gray for sustainable animal production. Livestock Research for Rural Development 30, Article#200. (In Spanish). lrrd.org/lrrd30/12/rive30200.html

Rivera JE; Cuartas CA; Naranjo JF; Tafur O; Hurtado EA; Arenas FA; Chará J; Murgueitio E. 2015. Effect of an intensive silvopastoral system (iSPS) with *Tithonia diversifolia* on the production and quality of milk in the Amazon foothills, Colombia. Livestock Research for Rural Development. 27, Article#189. (In Spanish). lrrd.org/lrrd27/10/rive27189.html

Rivera JE; Gómez-Leyva JF; Castaño K; Morales JG; Barahona R; Del Val R. 2017. Molecular, chemical and morphological diversity in *Tithonia diversifolia* (hemsl.) Gray for animal feed in Colombia and Mexico. Libro de Actas IX Congreso Internacional Sobre Sistemas Silvopastoriles. Manizales, Colombia, 6–8 September 2017. p. 249–255. (In Spanish).

Ruiz TE; Torres V; Febles G; Díaz H; González J. 2013. Growth performance of ecotypes of *Tithonia diversifolia* according to morphological components. Livestock Research for Rural Development 25, Article#154. (In Spanish). lrrd.org/lrrd25/9/ruiz25154.html

Schultze-Kraft R; Rao IM; Peters M; Clements RJ; Bai C;

Liu G. 2018. Tropical forage legumes for environmental benefits: An overview. Tropical Grasslands-Forrajes Tropicales 6(1):1–14. doi: 10.17138/tgft(6)1-14

Senger E; Martin M; Dongmeza E; Montes JM. 2016. Genetic variation and genotype by environment interaction in *Jatropha curcas* L. germplasm evaluated in different environments of Cameroon. Biomass and Bioenergy 91:10–16. doi: 10.1016/j.biombioe.2016.04.017

Yan W. 2002. Singular value partitioning in bi-plot analysis of multi-environment trial data. Agronomy Journal 94(5):990–996. doi: 10.2134/agronj2002.0990

Yan W; Cornelius PL; Crossa J; Hunt LA. 2001. Two types of GGE biplots for analyzing multi-environment trial data. Crop Science 41(3):656–663. doi: 10.2135/cropsci2001.413656x

Yan W; Hunt LA; Sheng Q; Szlavnics Z. 2000. Cultivar evaluation and mega environment investigation based on the GGE biplot. Crop Science 40(3):597–605. doi: 10.2135/cropsci2000.403597x

Yan W; Kang MS. 2002. GGE Bi-plot Analysis: A graphical tool for breeders, geneticists, and agronomists. CRC Press. Boca Raton, FL, USA. doi: 10.1201/9781420040371

Yang J; Tang L; Guan Y; Sun W. 2012. Genetic diversity of an alien invasive plant Mexican Sunflower (*Tithonia diversifolia*) in China. Weed Science, 60(4):552–557. doi: 10.1614/WS-D-11-00175.1

(Received for publication 11 February 2021; accepted 18 August 2021; published 30 September 2021)