ISSN: 2346-3775

Tropical Grasslands -Forrajes Tropicales

Online Journal

Vol. 9 No. 3

September 2021

Published by:

International Center for Tropical Agriculture
Since 1967 Science to cultivate change

Centro Internacional de Agricultura Tropical (CIAT),
Cali, Colombia

In association with:

Chinese Academy of Tropical Agricultural Sciences (CATAS),
Haikou, Hainan, P.R. China

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50 years publishing on tropical forages - from *Tropical Grasslands* and *Pasturas Tropicales* to *Tropical Grasslands-Forrajes Tropicales*

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Creation of Tropical Grasslands-Forrajes Tropicales

The online journal *Tropical Grasslands-Forrajes Tropicales* (ISSN official abbreviation *Trop. Grassl.-Forrajes Trop.*) was created in 2012 as a successor to the former journals, *Tropical Grasslands*, published during 1967–2010 by the Tropical Grassland Society of Australia Inc., and *Pasturas Tropicales*, published during 1979–2007 by the International Center for Tropical Agriculture (CIAT). *Tropical Grasslands* was an important vehicle for disseminating the work of authors from all countries on research and development in the assessment, management and utilization of pastures and fodder crops in tropical agriculture, as well as reviews in these topics (Pulsford 2010). During the 44 years of production, 160 issues were produced, 18 of which were proceedings of conferences on tropical pastures (Tropical Grasslands 2010). On the other hand, *Pasturas Tropicales*, directed at Spanish speakers and focusing initially for Latin American audiences was first published as Pastos Tropicales, being essentially a newsletter. Scientific publication became increasingly important for the journal and from 1986, its 8th volume, the name was changed to *Pasturas Tropicales*, which was published as an imprint journal until 2007.

The name of the new journal *Tropical Grasslands-Forrajes Tropicales* indicates both its bilingualism (English or Spanish, with abstracts in both languages) and the desire to continue the tradition of both former journals (Schultze-Kraft et al. 2013).

The initial steps to establish the journal were made possible through a seed money grant received from an anonymous donor in memory of Dr. José M. Toledo, leader of the former CIAT Tropical Pastures Program in the 1980s. During the 5-year period 2013–2017, the journal was sponsored by grants from the Chinese Academy of Tropical Agricultural Sciences (CATAS) and the Australian Centre for International Agricultural Research (ACIAR). Since 2019, CATAS has been the sole sponsor and co-publisher. An official foundation ceremony was held at CATAS on 13 December 2012 in Danzhou, Hainan, PR China. CATAS has continuously afforded grant support since 2013 and the journal is currently published in association with CATAS. This allows publication without any article submission and processing charges. Dr. Liu Guodao, CATAS Vice president serves as co-chair of the Management Committee, and and Dr. Huan Hengfu, CATAS Senior researcher, serve as member of the Journal Editorial Board.

The inaugural issue of the journal was presented at the 22nd International Grassland Congress, 15–19 September 2013 in Sydney, Australia (Schultze-Kraft et al. 2013), and was the result of a co-publication agreement with the Organizing Committee. It contained those papers relevant to tropical pastures and forages that were presented at the Congress, including keynote papers, presented papers and poster papers.

Characteristics of the journal

The main features of the journal are that it is international, published online only, open access (no charges for subscription or publication fees), bilingual (English and Spanish), peer reviewed and guided by an Editorial Board composed of the world's leading tropical forage scientists. Further information on the journal is available at its website (www.tropicalgrasslands.info). All issues of the former journals *Tropical Grasslands* and *Pasturas Tropicales* can also be accessed there. *Tropical Grasslands-Forrajes Tropicales* follows the publication series of the Australian CSIRO *Tropical Agriculture Genetic Resources Communication* (ISSN 0159-6071) published during 1980–2000. By kind permission of CSIRO, the issues that deal with (sub)tropical forages can be accessed on the website as well.

The journal is published every four months by CIAT in Cali, Colombia, with three issues: the first published on January 30th, the second published on May 31st and the third published on September 30th.

The editors Lyle Winks and Rainer Schultze-Kraft

Since its inception *Tropical Grasslands-Forrajes Tropicales* has counted on two dedicated editors, Rainer Schultze-Kraft and Lyle Winks. Without their dedication, ideas and work ethics for almost 10 years the creation and continuity of the journal, with increasing standards and distribution would not have been possible.

Prof. Dr. Rainer Schultze-Kraft is a forage scientist with 50 years of experience. Receiving his PhD on forages from the University of Giessen, Germany in the 70s, he moved on to CIAT working on forages until the late 80s. After that he received a call from the University of Hohenheim, Germany, to continue his academic work on tropical forages there until his retirement in the early 2000s, when he became a CIAT Emeritus Scientist. His work on tropical forages - with a focus on tropical forage legumes - has received worldwide scientific recognition across the tropics in Asia, Africa and the Americas. He acted as Managing and Spanish Editor of the journal.

Lyle Winks is an agricultural scientist, who specialised in ruminant nutrition and worked as a researcher in the Queensland Department of Primary Industries (QDPI) for almost 30 years. He published numerous papers on pasture-based beef production in north Queensland, and became Director of the Beef Cattle Husbandry Branch of QDPI (1984-92), supervising its research throughout the State. From 1992-2010 he was the Editor of *Tropical Grasslands*, publishing 18 volumes (74 issues) during that time. He currently runs a beef production enterprise in SE Queensland. He acted as English Editor of the journal.

Current status of the journal

By June 2021, *Tropical Grasslands-Forrajes Tropicales* had published 334 papers. These included 177 in special issues (115 contributions to the International Grassland Congress 2013 and 62 contributions to the International Leucaena Conference 2018) and 157 in regular issues.

The journal is indexed in all major abstract and citation databases of peer-reviewed literature. The Journal is indexed in the core collection of Science Citation Index Expanded and two additional indexes of Clarivate Web of Science, which provides the best-known impact factor indicator (formerly: ISI Journal Impact Factor). The current Journal Impact Factor is 0.611 (2020) and 0.897 (5-yr IF). The journal is also indexed in Scopus, and the CiteScore for 2020 was 1.50, making considerable progress since 2017 (0.90), 2018 (1.00) and 2019 (1.30). CiteScore is the way Scopus measures the impact of its indexed journals. The SCImago Journal & Country Rank is a portal that includes the journals and country scientific indicators developed from the information contained in the Scopus® database (Elsevier B.V.). These indicators can be used to assess and analyze scientific domains. For 2020, the Journal had a score of 0.26, and in 2019 was ranked in the second highest value amongst the set of agronomy journals ranked by Scimago Journal Rank (Q2).

Since 2018, the Journal is indexed in SciELO, a bibliographic database, digital library, and cooperative electronic publishing model of open access journals. SciELO was created to meet the scientific communication needs of developing countries (with a focus on Latin America) and provides an efficient way to increase visibility and access to scientific literature. In 2019, the Journal was included in the National Bibliographic Index (Publindex) of the Colombian Ministry of Science Technology and Innovation, based on indicators and standards of scientific quality at the international level. In the 2020 classification the journal was ranked as “A2”, in accordance with the fulfillment of the internationally recognized evaluation criteria for scientific publications related to the processes of editorial management, evaluation, visibility and impact. In 2019, the Journal was also included in the Regional Online Information System for Scientific Journals of Latin America, the Caribbean, Spain and Portugal (Latindex), a network of institutions that work jointly to gather and disseminate information of serial scientific publications produced in Latin America.

SHERPA RoMEO (S/R) is an online resource that aggregates and analyzes publisher open access policies from around the world, and uses different colors to help highlight publisher's archiving policies. In 2018, the journal reached the classification as a RoMEO Green Journal, the highest open access category. Updated annually, the Information Matrix for Journal Analysis (MIAR in Spanish) database gathers key information for the identification and analysis of journals. The system creates a correspondence matrix between the journals and the databases and repositories that index or include them. The ICDS (Composite Index of Secondary Dissemination in Spanish) is an indicator that shows the visibility of the journal in different scientific databases of international scope. A high ICDS means that the journal is present in different information sources of international relevance. The ICDS 2020 was 10.3. The Journal is also accepted/registered in several major databases/networks (EBSCO, ROAD, AGRIS, CABI, DOAJ and Google Scholar), by which international visibility is increased. The Journal´s current Google Scholar h-index is 20; the i10-index is 50.

Authors

With the mission of providing high-quality tropical forage knowledge to a wide group of stakeholders in the tropics and making it accessible to the less developed countries (Urrea-Benítez et al. 2020), 78% of the 1,837 authors who have published in the journal so far are from the global tropics (Africa, Asia, Latin America). The authors came from 52 countries (Figure 1).

Reception Web metrics

The number of total visits and the number of unique visitors to its website have been increasing steadily. In 2020, the back end (the part that connects to the database and the server that uses the website) of the website was upgraded, to a newer and safer server. This also included the purchase of HTTPS certificates to establish secure connections. However, this also made the former statistics provided by AWStats/CIAT IT become obsolete and from this year the website is keeping track of the user statistics via Google Analytics. Recent statistics indicate 69,552 pageviews for the year 2020, while for the first six months of 2021 (i.e. until June 2021) the number of visits increased to 56,186 pageviews.

Users of the journal are worldwide, with currently half from the Americas and 25% from Asia (Figure 2). The number of users from Africa is steadily increasing as internet access is becoming more widespread, critical for an online journal. Open Access allows unrestricted use of the journal. Of interest is also the analysis of the operating system from where people are browsing the website, as an indicator of the main language of the users. While English, Spanish, Portuguese, Spanish and Chinese speakers are well represented, francophone users are so far under-represented (Figure 3).

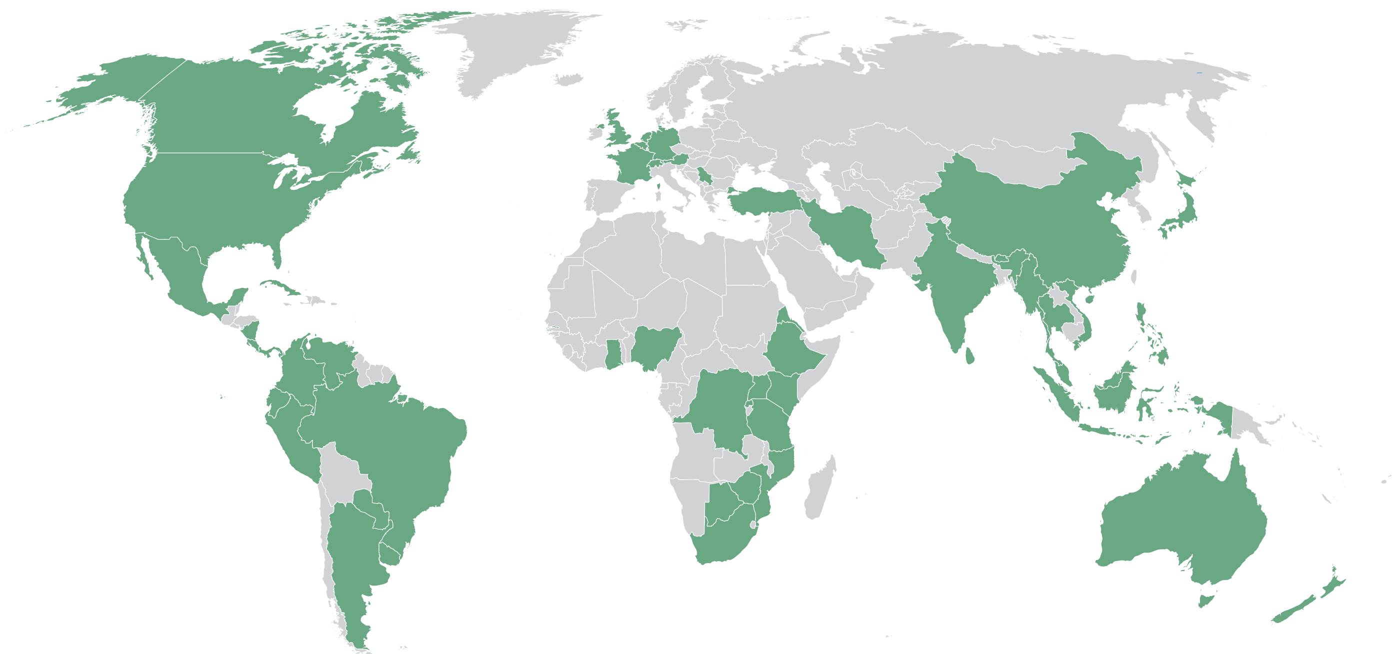

Figure 1. Countries of the authors published in the journal by June 2021. Source: Google Analytics.

Disclaimer: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Tropical Grasslands-Forrajes Tropicales concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

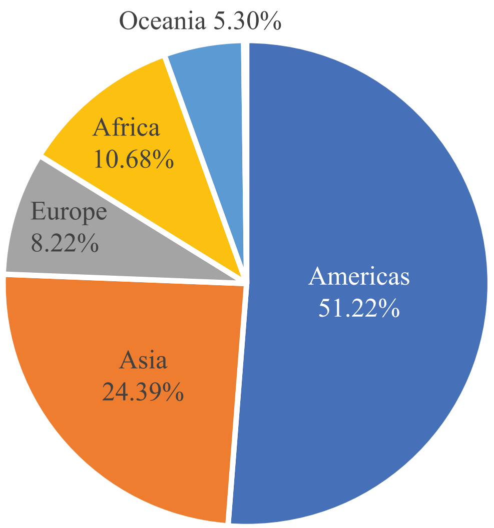

Figure 2. Location of users of the journal by continent. Source: Google Analytics.

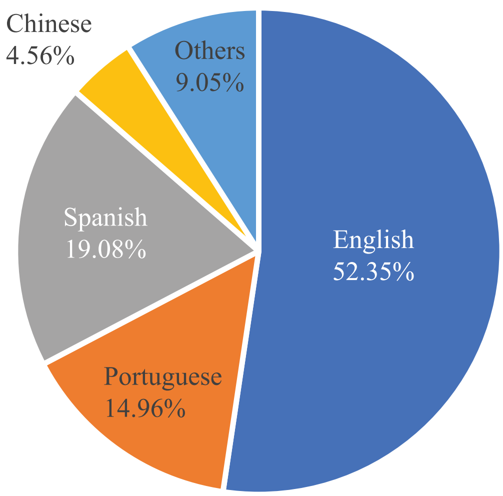

Figure 3. Main language of the users of the journal based on the browser/operating system. Source: Google Analytics.

Outlook

The journal is in a transition period to the new editors, Dr. Jean Hanson, English and Managing Editor and Dr. Danilo Pezo, Spanish Editor. Both have worked with the International Livestock Research Institute (ILRI) for many years and have long and broad experience in tropical forages. Jean Hanson is an Emeritus fellow with ILRI in Ethiopia and Danilo Pezo is currently working with CATIE in Costa Rica. The new editorial team will continue to be well supported by Jose Luis Urrea, Communications Specialist at The Alliance of Bioversity International and CIAT, Colombia.

In the coming year the new editorial team will work on further modernizing the journal including a fully automatized submission and review process. Further effort will be placed on increasing the quality and number of contributions and the reach of the journal to the global south, in particular Asia and Africa. In addition, an assessment of how to increase utilization from Francophone countries will be initiated aiming to maintain *Tropical Grasslands-Forrajes Tropicales* as the foremost journal on tropical forages at the global level.

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(Note of the editors: All hyperlinks were verified 20 September 2021).

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doi: 10.17138/TGFT(9)249-255

Research Paper

Canopy characteristics of 'Mavuno' hybrid brachiariagrass and 'Marandu' palisadegrass harvested at different harvest intensities

Características del dosel de la pastura brachiaria *híbrida 'Mavuno' y la pastura 'Marandu' recolectadas a diferentes intensidades de cosecha*

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Abstract

'Mavuno' is a newly released brachiariagrass (*Urochloa* hybrid) cultivar with limited information available in the literature. The objective of this study was to compare forage characteristics of this cultivar and 'Marandu' palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster cv. Marandu] harvested at 2 different stubble heights during 2 growing seasons (January–April). The study was conducted in Araguaína, TO, Brazil in 2017 and 2018. Treatments were the factorial arrangement of 2 brachiariagrass cultivars, Mavuno and Marandu, harvested at 2 harvest intensities, 5 and 15 cm stubble height, distributed in a randomized complete block design with 4 replicates. Response variables were canopy height, forage accumulation, proportion of leaf, stem and dead material, and concentration of crude protein (CP) and in vitro digestible organic matter (IVDOM). Mavuno and Marandu did not differ (P>0.05) in forage accumulation (mean = 3,800 kg DM/ha/harvest) and IVDOM concentration (mean = 637 g/kg); however, Mavuno had lower CP concentration (101 vs. 110 g/kg), greater proportion of stems (16 vs. 13%) and less dead material (4 vs. 6%) than Marandu (P<0.05). Harvesting at 5 cm stubble height rather than 15 cm increased herbage accumulation per harvest (4,100 vs. 3,500 kg DM/ha) with decreased proportion of leaves (77 vs. 84%) and CP concentration (101 vs. 115 g/kg) (P<0.05). Our data suggest that Mavuno is a useful addition to the range of brachiariagrass cultivars for sowing in tropical regions and further studies are needed to evaluate the long-term persistence of Mavuno under different management practices in a range of environmental situations. While harvesting at 5 cm stubble height rather than 15 cm increased forage accumulation but reduced CP concentration, regardless of cultivar, longer-term effects on the stability of these pastures with these harvest frequencies and heights are open to question and studies should be continued for longer periods to assess longevity of stands under the 2 management strategies. Applying maintenance fertilizer during the growing season might have prevented the marked decline in dry matter accumulation as the season advanced and this hypothesis should be tested.

Keywords: Harvest severity, nutritive value, tropical pastures, *Urochloa* spp.

Resumen

"Mavuno" es un cultivar de pasto brachiaria (híbrido de *Urochloa*) recientemente liberado con información limitada disponible en la literatura. El objetivo de este estudio fue comparar las características del forraje de este cultivar y el pasto 'Marandu' [*Urochloa brizantha* (Hochst. Ex A. Rich.) R.D. Webster cv. Marandu] cosechado a 2 alturas diferentes de rastrojo durante 2 temporadas de crecimiento (enero–abril). El estudio se realizó en Araguaína, TO, Brasil en 2017 y 2018. Los tratamientos fueron el arreglo factorial de 2 cultivares de *Brachiaria*, Mavuno y Marandu, cosechados a

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2 intensidades de cosecha, 5 y 15 cm de altura de rastrojo, distribuidos en un diseño de bloques completos al azar con 4 repeticiones. Las variables de respuesta fueron altura del dosel, acumulación de forraje, proporción de hoja, tallo y material muerto, y concentración de proteína cruda (PC) y materia orgánica digestible in vitro (IVDOM). Mavuno y Marandu no difirieron (P> 0.05) en la acumulación de forraje (media = 3,800 kg MS / ha / cosecha) y la concentración de IVDOM (media = 637 g / kg); sin embargo, Mavuno tuvo menor concentración de PC (101 vs 110 g / kg), mayor proporción de tallos (16 vs 13%) y menos material muerto (4 vs 6%) que Marandu (P <0.05). La cosecha a 5 cm de altura de rastrojo en lugar de 15 cm aumentó la acumulación de forraje por cosecha (4.100 vs 3.500 kg MS / ha) con una proporción menor de hojas (77 vs 84%) y concentración de PC (101 vs 115 g / kg) (P <0,05). Nuestros datos sugieren que Mavuno es una adición útil a la gama de cultivares de *Brachiaria* para la siembra en regiones tropicales y se necesitan más estudios para evaluar la persistencia a largo plazo de Mavuno bajo diferentes prácticas de manejo en una variedad de situaciones ambientales. Si bien la cosecha a 5 cm de altura de rastrojo en lugar de 15 cm aumentó la acumulación de forraje pero redujo la concentración de PC, independientemente del cultivar, los efectos a largo plazo sobre la estabilidad de estos pastos con estas frecuencias y alturas de cosecha están abiertos a cuestionamientos y los estudios deben continuar por más tiempo para evaluar la longevidad de las plantaciones bajo las 2 estrategias de manejo. La aplicación de fertilizantes de mantenimiento durante la temporada de crecimiento podría haber evitado la marcada disminución en la acumulación de materia seca a medida que avanzaba la temporada y esta hipótesis debería ser probada.

Palabras clave: Pastos tropicales, severidad de la cosecha, *Urochloa* spp., valor nutritivo.

Introduction

Brachiariagrasses (*Urochloa* spp.) are among the most commonly planted forage species in tropical regions, and Marandu palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster cv. Marandu] has been one of the most frequently used cultivars in Brazil, representing approximately 35% of the total forage seed production in the country (Jank et al. 2011). Palisadegrass is primarily used in extensive grazing systems that are subjected to relatively low levels of inputs such as commercial fertilizer and liming (Miles et al. 2004). Despite the widespread use of palisadegrass, areas of Marandu have declined for unknown reasons in some regions of Brazil (Barbosa 2006). Therefore, new brachiariagrass cultivars need to be tested to potentially replace Marandu in those areas.

Mavuno is a hybrid brachiariagrass registered in Brazil (MAPA nº 30488) and was released as a commercial cultivar in April 2013. It originated from a cross between ruzigrass [*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins] and U. *brizantha*, that has been used for forage systems in tropical regions; however, there is limited scientific information available about Mavuno. Da Silva et al. (2020) observed that Mavuno had greater herbage accumulation and higher nutritive value than 'Tifton 85' bermudagrass (*Cynodon* spp.) and Jiggs bermudagrass [*Cynodon dactylon* (L.) Pers.] in Florida, USA, but had similar forage characteristics to 'Mulato II' brachiariagrass, which is also a *Urochloa* hybrid cultivar.

Harvest frequency and intensity are the most influential factors in terms of warm-season perennial grass herbage accumulation, nutritive value and persistence (Sollenberger and Burns 2001). In general, forage harvested at shorter stubble height could have decreased residual leaf area and root growth, which may limit the regrowth rate of the pasture (Inyang et al. 2010).

Depending on only limited numbers of brachiariagrass species and cultivars makes the livestock industry in tropical regions vulnerable to infestations by pests and diseases. Therefore, it is important to diversify the genetic sources of brachiariagrass to create more resilient grazing systems in different regions of the world. The objective of this study was to compare Marandu with the new Mavuno brachiariagrass under different harvest intensities in a tropical region. We hypothesized that Mavuno and Marandu would have similar forage accumulation and nutritive value.

Material and Methods

The study was conducted at the Federal University of Tocantins, Araguaína, Brazil (07º5' S, 48º12' W; 277 masl), from January to April 2017 (Year 1) and December 2017 to April 2018 (Year 2). The experimental period chosen covered the growing season at the experimental location, where only infrequent and scarce rainfall occurs in the spring months (September–December). The soil type was Entisol (psamments, quartzipsamments). Initial soil characterization (0–20 cm horizon) indicated that mean pH was 5.3 and Mehlich-1 extractable P, K, Mg and Ca concentrations were 5, 20, 340 and 145 mg/kg, respectively. According to the Köppen climatic classification, the region has a tropical humid summer with well-defined rainy and dry seasons, with average annual rainfall of 1,828 mm. The rainfall, minimum and maximum temperatures during the experimental period are presented in Table 1.

Treatments were the factorial arrangement of 2 brachiariagrass cultivars, Mavuno and Marandu, and 2 harvest intensities, 5 and 15 cm stubble height, distributed in a randomized complete block design with 4 replicates. The harvest intensity treatments provided a comparison between a moderate harvest stubble height (15 cm) and a short stubble height, which may modify production of the pasture due to limited residual leaf area (Giacomini et al. 2009).

Plot size was 3 × 3 m with 0.5 m alleys between plots and 1.0 m between blocks. On 16 January 2016, the existing vegetation in the experimental area was sprayed with glyphosate [N- (phosphonomethyl) glycine; Roundup Ultra 2, Monsanto Company, St Louis, MO, USA] at a level of 0.8 kg/ha, following which the seedbed was disked with a tandem disk until there was no remaining vegetation on the soil surface. Approximately 14 d after the soil preparation, seed was sown into the plots manually in rows 30 cm apart at a depth of 2 cm. The seeding rate for both grasses was 10 kg/ha, following the recommendation of the seed company (Wolf Seeds) for those cultivars and seed lots. Plots received 30 kg N, 13 kg P and 25 kg K/ha approximately 14 d after germination. Plots were clipped in January 2017 (Year 1) and December 2017 (Year 2) at the respective treatment stubble height, fertilized with 60 kg N, 6 kg P and 50 kg K/ha and evaluated every 28 d thereafter until April each year. The fertilizer sources were urea, simple superphosphate and potassium chloride and rates chosen were used to represent the limited fertilizer strategies used by producers in tropical and subtropical regions.

Measurements

Before each harvest, canopy height was measured using a calibrated stick at 5 random points per plot from ground level to the highest point reached by leaves or stems with no disturbance of the sward. An area of 0.75 m^2 was harvested manually and subsamples were dried at 55 ºC for 72 h and used to assess herbage accumulation, morphological composition and nutritive value. For determining morphological composition, a subsample was taken and manually separated into leaf, stem and dead material. The remaining forage on each plot was clipped at the same stubble height and removed from the plots after each harvest.

Tiller density and tiller mass were evaluated before the forage was harvested. The tillers in one 0.25 m^2 metal ring per experimental unit were counted and the data used to estimate tiller density/m^2. Tillers were harvested, dried at 55 ºC for 72 h and tiller dry mass was calculated.

A further subsample for nutritive value determination was taken, dried in the same way and ground to pass a 1 mm stainless steel screen in a Wiley mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA). The nutritive value analyses were conducted on whole-plant samples (leaf + stem). In vitro digestibility of organic matter (IVDOM) was determined using the two-stage technique described by Tilley and Terry (1963) and modified by Moore and Mott (1974). The micro-Kjeldahl technique was used with a modification of the aluminum block digestion described by Gallaher et al. (1975) for N determination. Crude protein was estimated by multiplying N concentration by 6.25.

Statistical analysis

The data were analyzed using the PROC MIXED technique of SAS (SAS Institute Inc. 1996). Response

Table 1. Monthly rainfall, minimum and maximum temperatures during the experimental period in Year 1 and Year 2 in Araguaína, TO, Brazil (INMET 2018) and long-term average rainfall (1984–2018).

Month, year	Rainfall (mm)	Average rainfall (mm)	Max temperature (°C)	Min temperature (°C)
Year 1				
January 2017	292	257	30.7	21.4
February 2017	345	265	30.6	21.7
March 2017	252	286	31.0	22.0
April 2017	208	221	31.4	22.2
Year 2				
December 2017	256	227	30.5	22.4
January 2018	256	257	30.7	21.7
February 2018	345	265	30.3	22.3
March 2018	315	286	30.7	22.5
April 2018	124	221	32.0	22.0

variables were canopy height, forage accumulation, CP, IVDOM and leaf, stem and dead material proportions. Cultivar, harvest intensity, months and their interactions were considered fixed effects. Blocks and year were considered random effects. Month was analyzed as a repeated measurement and the covariance structure selected based on the least Akaike information criterion value. Normality of residues and homogeneity of variances were tested using conditional studentized residual plots. Treatments were considered different when P≤0.05 by LSD test. Main effects and interactions not discussed in the Results and Discussion sections were not significant (P>0.05). Main effects were not discussed if there was a significant (P<0.05) interaction with the respective independent variable.

Results

Canopy height differed between cultivars and harvest intensities, with no cultivar × harvest intensity interaction (Table 2). Mavuno was taller than Marandu at harvest and forage harvested at 15 cm was taller than that harvested at 5 cm. In addition, month of harvest had an effect on canopy height (Table 3), which declined progressively from January to April (P<0.05).

There were no differences in forage accumulation between cultivars (P>0.05), but pasture harvested at 5 cm accumulated more forage than that at 15 cm (P<0.05; Table 2). As reflected by differences in canopy height, forage accumulation declined progressively from January to April (P<0.05; Table 3).

While leaf proportion in harvested forage was similar for the 2 cultivars (mean = 80.5%), Marandu had less stem (13 vs. 16%) and more dead material (6 vs. 4%) than Mavuno (P<0.05) (Table 2).

There was a cultivar × harvest intensity interaction for tiller density (Table 4; P<0.05). Tiller density of both cultivars did not differ (370 tillers/m^2) when harvested at 5 cm, but Mavuno had greater (P<0.05) tiller density than Marandu when harvested at 15 cm (475 vs. 375 tillers/m^2). Tiller density declined as the season progressed for both harvest intensities but the differences were significant (P<0.05) only when harvested at 5 cm. Tiller mass was greater when harvested at 5 cm than when harvested at 15 cm (Table 2) and tiller mass declined progressively from January to April (P<0.05) (Table 3).

There were cultivar, harvest intensity (Table 2) and month effects on CP concentrations (Table 3) but no significant interactions among the variables. Mavuno had lower CP concentration than Marandu and forage

Table 2. Effects of brachiariagrass cultivars, Mavuno (*Urochloa* hybrid) and Marandu [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster], and harvest intensity (5 and 15 cm stubble height) on canopy height at harvest, forage accumulation/harvest, proportion of leaf, stem and dead material, tiller mass and crude protein (CP) and in vitro digestible organic matter (IVDOM) concentrations in 2017 and 2018 (means for two years).

Parameter	Cultivar		Harvest consistency		s.e	P value		
	Mavuno	Marandu	5 cm	15 cm		cv.	Harvest height	cv. × Harvest height
Canopy height (cm)	31A[1]	28B	27b[2]	33a	0.71	<0.01	<0.01	0.34
Forage accumulation (kg DM/ha)	3,874	3,744	4,100a	3,500b	277	0.62	0.04	0.30
Leaf (%)	80	81	77b	84a	1.41	0.36	<0.01	0.83
Stem (%)	16A	13B	17a	12b	1.06	<0.01	<0.01	0.56
Dead material (%)	4B	6A	6a	4b	0.54	<0.01	<0.01	0.22
Tiller mass (g DM/m^2)	1.0	1.0	1.2a	0.9b	0.07	0.98	<0.01	0.96
CP (g/kg)	101B	110A	101b	115a	3.1	0.03	0.05	0.16
IVDOM (g/kg)	634	640	640	634	7.0	0.19	0.27	0.09

[1]Cultivar means followed by the same upper-case letters are not different (P>0.05).
[2]Harvest consistency means followed by the same lower-case letters are not different (P>0.05).

Table 3. Effects of month of harvest on canopy height and forage accumulation, plus crude protein (CP) and in vitro digestible organic matter concentrations (IVDOM) of brachiariagrass cultivars, Mavuno (*Urochloa* hybrid) and Marandu [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster], harvested at 2 harvest intensities (5 and 15 cm stubble height) in 2017 and 2018 (means for two years).

Parameter	January	February	March	April	s.e
Canopy height (cm)	43a[1]	31b	24c	22c	1.05
Herbage accumulation (kg DM/ha)	5,660a	4,330b	2,980c	2,260c	438
Tiller mass (g/m2)	1.4a	1.1b	0.8bc	0.6c	0.12
CP (g/kg)	110ab	101b	119a	82c	0.53
IVDOM (g/kg)	620b	650a	638a	640a	0.83

[1]Means within rows followed by the same lower-case letter are not different (P>0.05).

Table 4. Harvest intensity × month effects on proportion of leaf, stem and dead material and tiller density in brachiariagrass cultivars, Mavuno (*Urochloa* hybrid) and Marandu [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster], harvested in 2017 and 2018 (means for two years).

Harvest intensity (stubble height)	January	February	March	April	s.e
Leaf (%)					
5 cm	76bA	75bA	74bB	85aB	1.4
15 cm	76cA	79cA	87bA	94aA	
s.e.		1.8			
Stem (%)					
5 cm	22aA	21aA	15bA	12bA	1.0
15 cm	22aA	18aA	6bB	4bB	
s.e.		1.7			
Dead material (%)					
5 cm	3bA	4bA	11aA	5bA	0.5
15 cm	2bA	3bA	6aB	3bA	
s.e.		0.8			
No. of tillers/m^2					
5 cm	392aA	383aA	349bB	330cB	9
15 cm	401aA	411aA	406aA	383aA	
s.e.		9			

Within parameters, means within columns followed by the same upper-case letters and means within rows followed by the same lower-case letters are not different (P>0.05).

harvested at 15 cm had higher CP concentration than that harvested at 5 cm. Crude protein concentration was highest in March and lowest in April (P<0.05). Conversely, IVDOM was not affected by either cultivar or harvest height (Table 2) but was lower in January than in the remaining months (P<0.05) (Table 3).

Discussion

The greater canopy height for Mavuno than for Marandu was likely due to its greater proportion of stems and stem elongation resulted in forage with greater height. While Da Silva et al. (2020) observed that Mavuno had greater canopy height than Mulato II, Jiggs and Tifton 85 bermudagrass, the correlation coefficients of canopy height with forage accumulation and light interception were only r = 0.60 and r = 0.56, respectively, indicating that canopy height may not be an accurate indicator of forage accumulation. This finding was supported by results from our study because the 2 cultivars did not differ in forage accumulation despite Mavuno being taller than Marandu.

The decrease in canopy height and forage accumulation from January to April may be related to the fact that fertilizer was applied only at the beginning of the experimental period, considering that rainfall and temperature during the experimental period were relatively uniform (Table 1), except for April 2017, when rainfall was lower than in other months. In addition, there is a decrease in daylength from 12 h and 31 min to 11 h and 51 min from December to May, which has potential to decrease forage accumulation as well.

Harvesting at 5 cm resulted in greater forage accumulation than harvesting at 15 cm despite displaying lesser height at all stages. This reinforces the findings of Rodrigues et al. (2014), who harvested 'Xaraes' palisadegrass at different stubble heights (10, 20, 30, 40 and 50 cm) and observed an increase in herbage accumulation with decreasing stubble height.

The similar proportion of leaves in Mavuno and Marandu canopies (mean 80.5%) was likely the main factor leading to similar IVDOM for forage from the 2 grasses. While Mavuno had a greater proportion of stems than Marandu, it also had less dead material and the effects of these traits on IVDOM may have tended to cancel each other out. It is well reported in the literature that stems have greater concentrations of structural carbohydrates than leaves, and structural carbohydrates are generally less digestible than non-structural carbohydrates, which are commonly present at higher proportions in leaves (Chapman et al. 2014). It is also well documented that senescent material may have lost a significant proportion of cell contents, leading to decreased digestibility (Dubeux Jr et al. 2006).

The greater proportion of stems in forage harvested at 5 than at 15 cm was expected because there is a greater proportion of stems in the bottom layers of warm-season forage canopies as reported by Vendramini et al. (2019) and Pontes et al. (2017).

Mavuno harvested at 15 cm had greater tiller density than Marandu, but there was no difference in tiller mass, indicating that Mavuno had less weight per tiller. The same trend was observed with harvest intensity because forage harvested at 5 cm had lower tiller density but greater tiller mass than forage harvested at 15 cm. Euclides et al. (2019) observed that increasing grazing intensity, i.e. grazing to a shorter stubble height, decreased persistence of palisadegrass. The greater decline in tiller density during the growth period when forage was cut at 5 cm rather than 15 cm in our study suggests that the more severe cutting height could result in reduced persistence of these pastures over time.

Mavuno has lower CP concentration than Mulato II under similar management systems and Da Silva et al. (2020) suggested that Mavuno may have an intrinsically lower CP concentration than other selected brachiariagrasses. The marked reduction in CP concentration observed in April was likely due to the run-down in soil N levels since no fertilizer was applied after commencement (~90 d) plus limited rainfall during April (Table 1).

The slight increase in stem proportion in the forage harvested at 15 cm failed to decrease IVDOM of forage as the stems produced were relatively immature (28 days). While we expected Mavuno to have greater IVDOM than Marandu, there was no difference in IVDOM between the cultivars. Both Mavuno and Mulato II are hybrids of ruzigrass and palisadegrass, and among the brachiariagrass species ruzigrass is known to have consistently highest digestibility (Barnard 1969; Rosa et al. 1983). The harvest intervals employed and fertilizer management used in this study may have negated the potential differences in IVDOM between Mavuno and Marandu.

In summary, the absence of marked differences in performance between Mavuno and Marandu during the study suggests that Mavuno is a suitable option for broadening the range of brachiariagrass genetic resources in tropical regions. However, Mavuno had greater canopy height and proportion of stem in the canopy, and lower CP concentration than Marandu, when harvested at a fixed harvest frequency of 28 d. While harvesting at 5 cm stubble height rather than 15 cm increased herbage accumulation but reduced CP concentration, regardless of cultivar, longer-term effects on the stability of these pastures with these harvest frequencies and heights are open to question and studies should be continued for longer periods to assess longevity of stands under the 2 management strategies. Applying maintenance fertilizer during the growing season might have prevented the marked decline in dry matter accumulation as the season advanced and this hypothesis should be tested.

Further studies are warranted to evaluate the effects of additional abiotic and biotic factors on production and survival of Mavuno in a range of environmental situations.

Acknowledgments

We thank the CAPES foundation for sponsoring the assistantship of the first author's Ph.D. program.

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(Note of the editors: All hyperlinks were verified 28 May 2021).

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(Received for publication 30 April 2020; accepted 8 May 2021; published 30 September 2021)

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(3):256–267
doi: 10.17138/TGFT(9)256-267

Research Paper

Effects of stubble height and season of the year on morphogenetic, structural and quantitative traits of Tanzania grass

Efectos de la altura residual y de la estación del año en las características morfogénicas, estructurales y cuantitativas del pasto Tanzania

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Abstract

The objective of this study was to evaluate regrowth period (RP), morphogenetic, structural and productive characteristics of the guinea grass cultivar Tanzania [*Megathyrsus maximus* (syn. *Panicum maximum*)] under different stubble heights (SH) during dry (DS) and rainy (RS) seasons in the eastern Amazon region. The treatments were: 5, 15, 25, 35, 45 and 55 cm SH, distributed in a randomized complete block design with 6 replicates. In the 2 seasons, RP decreased linearly with increase in SH, and was considerably shorter in the RS (47 d). Leaf appearance rate decreased linearly from 0.071 to 0.051 leaves/tiller/d with increasing SH, and it was higher during the RS. Increase in SH increased leaf elongation rate, stem elongation rate and leaf area index. In the RS, climatic conditions favored the morphogenesis, resulting in higher herbage accumulation (8,693 kg DM/ha) than in the DS (2,597 kg DM/ha). In associating seasons with SH, we recommend that Tanzania grass be managed at SH between 35 and 45 cm in the DS, resulting in RP from 61 to 64 days, and at SH of 35 cm in the RS, resulting in RP of 41 days. Studies to test this management strategy seem warranted.

Keywords: Amazon biome, dry season, herbage accumulation, *Megathyrsus maximus*, rainy season.

Resumen

En el estudio se evaluaron el período de rebrote (RP), las características morfogénicas, estructurales y productivas de pasturas de Tanzania [*Megathyrsus maximus* (syn. *Panicum maximum*)] en diferentes alturas de rastrojo (SH) y estaciones del año, estación seca (DS) y estación lluviosa (RS) en la región Amazónica oriental. Los tratamientos incluyeron cinco SH: 15; 25; 35; 45 y 55 cm en un diseño de bloques completamente al azar con seis repeticiones. En las dos estaciones el RP disminuyó linealmente con el aumento de SH, y fue considerablemente menor en la RS (47 d). La tasa de aparición de hojas disminuyó linealmente de 0.071 a 0.051 hojas/macolla/d con el aumento de SH, y fue mayor durante la RS. El aumento en SH proporcionó aumento en la tasa de alargamiento de hoja, en la tasa de alargamiento del tallo, y en el índice de área foliar. En la RS, las condiciones climáticas favorecieron la morfogénesis del cultivar Tanzania, lo que resultó en mayor acumulación de forraje (8,693 kg DM/ha) que DS (2,597 kg DM/ha). En la asociación de estaciones con SH, recomendamos que pasturas de Tanzania se maneje en SH entre 35 y 45 cm en DS, correspondiente a RP de 61 a 64 días, y en el SH de 35 cm en la RS, correspondiente a RP de 41 días. Los estudios para probar esta estrategia de gestión parecen justificados.

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Palabras clave: Acumulación de biomasa, Amazonia oriental, época lluviosa, época seca, *Megathyrsus maximus.*

Introduction

In aiming to intensify pasture management, producers in Brazil largely use cultivars of guinea grass [*Megathyrsus maximus* (syn. *Panicum maximum*)], a species of African origin well adapted to tropical conditions. A cultivar widely used is Tanzania, due to its favorable traits such as high production potential and nutritional value (Paciullo et al. 2016). However, this cultivar is also very demanding in terms of soil fertility (Pezzopane et al. 2016) and management owing to its high stem elongation rates, notably near flowering time.

Tanzania grass pastures have gained prominence in the Amazon biome thanks to the expansion of Brazilian livestock to this region, despite very strict environmental laws (Nascimento et al. 2019). Thus, many concerns arise regarding the management of this forage plant due to the different soil-climatic conditions found in the region, such as highly acidic, phosphorus-poor and high-water-table soils, annual precipitation above 2,000 mm, plus minimum temperature above 20 °C and availability of light during most of the year.

In addition to the soil-climatic peculiarities of the region that influence herbage production, it is important to understand how grasses respond to the intensity/severity (Silva et al. 2016, 2019; Gomide et al. 2019) and frequency of defoliation (Moura et al. 2017; Pedreira et al. 2017) under the specific conditions mentioned above. Defoliation frequency can be determined based on light interception (LI) by the canopy, which, according to recent research (Pedreira et al. 2017; Tesk et al. 2018; Silva et al. 2019), has shown potential for use when the level of 95% is adopted. At this stage pastures are considered to have high proportions of leaves and low proportions of stem and dead material in the herbage mass, in addition to better quality (Brougham 1956; Korte et al. 1982; Parsons et al. 1988).

Defoliation intensity/severity can be predefined based on stubble height (SH) and is widely used in research on forage plants in tropical (Silva et al. 2016; Pereira et al. 2018; Gomide et al. 2019; Tesk et al. 2020) and temperate regions (Kohmann et al. 2017; Insua et al. 2020). As such, it has become a management tool on many farms. However, studies on effects of SH on morphology and forage production in pastures in Brazil have been located in regions where soil-climatic characteristics are very different from those encountered in the Amazon biome. This reinforces the need for studies like the present one, which may assist in decision-making in the field with a view to optimizing pasture use and, consequently, reducing environmental impacts in the region.

The hypothesis tested in this experiment was that, when associated with differing SH following harvesting, climatic conditions typical of the Amazon region (precipitation regime, mainly) affect both the structure and production of Tanzania grass. Therefore, the objective of this study was to examine the morphogenetic and structural traits and herbage production of *Megathyrsus maximus* cv. Tanzania managed under 5 different SHs throughout an experimental year in the eastern Amazon region.

Material and Methods

Experimental site

The experiment was conducted on the experimental farm at the Federal Rural University of the Amazon (UFRA), located in Igarapé-Açu, Pará, Brazil (01º07'21" S, 47º36'27" W; 50 masl), from August 2017 to August 2018, in plots of Tanzania grass established in 2014. The soil is classified as a Yellow Latosol (Oxisol) with a sandy-loam texture and a low slope gradient. Soil analysis performed in the 0 20 cm layer revealed the following chemical characteristics: pH ($CaCl_2$) = 4.7; organic matter = 7.98 g/kg; P (ion-exchange resin extraction method) = 1.54 mg/dm^3; K = 3.0 mmolc/dm^3; Ca = 28.0 mmolc/dm^3; Mg = 28.0 mmolc/dm^3; H + Al = 47.2 mmolc/dm^3; cation-exchange capacity = 106.2 mmolc/dm^3; and base saturation = 55.7%.

The local climate is classified as a tropical monsoon (Am) type according to the Köppen classification, with a short dry season and heavy rains during the rest of the year. Total precipitation during the experimental period was 2,270 mm, consisting of 130 mm from September to December 2017 and negative soil water balance (96 to 175 mm monthly deficit) (characterized as the dry season, DS), and 2,140 mm from January to August 2018 (rainy season, RS) with positive soil water balance from February to July (15–380 mm monthly surplus) (Table 1). Mean temperature during the experimental period was 27.5 °C.

Experimental design and management

Treatments consisted of 5 Tanzania grass stubble heights (SH: 15, 25, 35, 45 and 55 cm) evaluated for a full year with data separated into dry (DS) and rainy (RS) seasons.

The experiment was laid out as a randomized complete block design with 6 replicates, in a total of thirty 3 × 4 m experimental units spaced 1 m apart.

Table 1. Monthly rainfall and water balance in Igarapé-Açu, PA, Brazil.

Month	Precipitation (mm)		Soil water balance (mm)
	Climate mean (1994–2017)	Experimental period (2017–2018)	
Sep	44.7	34.2	-98
Oct	22.0	17.1	-152
Nov	18.5	0.0	-175
Dec	96.6	78.9	-96
Jan	236.1	153.3	0
Feb	308.0	514.4	380
Mar	456.7	303.4	125
Apr	389.4	408.8	255
May	258.5	381.8	245
Jun	199.6	146.3	20
Jul	155.0	134.8	15
Aug	62.4	97.2	1

In June 2017, still in the RS, the various plots were harvested at the appropriate stubble heights for implementation of the study. Subsequently, plots received the equivalent K dose of 100 kg/ha in the form of KCl plus 200 kg N/ha in the form of urea divided into 3 equal applications (January, March and May 2018). After this harvest, light interception (LI) by the canopy in each plot was monitored using the AccuPAR LP-80 canopy analyzer (Decagon®) throughout the regrowth period of the grass, with readings taken daily at 3 points per plot, until the canopy reached 95% LI. Upon reaching 95% LI, the plots were harvested at the appropriate SH (treatments) using a mower.

The harvest intervals in the plots and the number of harvest events, according to the SH and the evaluation periods, are shown in Table 2. The total number of harvest events was determined by the length of the regrowth period (RP) in each treatment, i.e. time to reach 95% LI. Regrowth period was calculated as the time (days) between one harvest and the subsequent one.

Morphogenetic and structural traits

Leaf blades and stems were measured on 5 tillers per plot, once weekly, during the regrowth period. After each harvest, 5 new tillers were selected.

All leaves of each tiller were numbered and classified as expanded (with the ligule visible), expanding (no visible ligule) or senescent (when the end of the leaf blade showed some sign of senescence). Leaves with more than 50% of the blade length compromised by senescence were considered dead. In the expanded leaves, length was measured from the tip of the blade to its ligule. For expanding leaves, length was measured from the tip of the blade to the ligule of the youngest fully expanded leaf. In the case of senescent leaves, length of the green leaf blade was measured from the ligule to the point where senescent tissue was visible. The length of stem plus sheath was measured from ground level to the ligule of the last fully expanded leaf.

The data were used to estimate the rates of leaf appearance (LAR, leaves/tiller/d), leaf elongation (LER, cm/tiller/d), stem elongation (SER, cm/tiller/d) and leaf senescence (LSR, cm/tiller/d) for each tiller. The number of live leaves per tiller (NLL) was also determined by direct counting. Leaf lifespan (LLS, days) was determined using the values of LAR and NLL per tiller (Lemaire and Chapman 1996). Phyllochron (PHY, days/leaf) was calculated as the inverse of LAR. Final leaf size (FLS, cm) was determined as the length of expanded leaf blades. Leaf and stem elongation rates and LSR were obtained by dividing the difference between the final and initial lengths of the green or senescent leaf blades or stems, by the number of days in the regrowth period. Leaf appearance rate was calculated by dividing the number of expanded leaves per tiller by the number of days in the regrowth period.

Height of forage in plots in pre-harvest condition was measured whenever the plot reached 95% LI, at 5 points per plot, using centimeter-graded rules. The indirect leaf area index (LAI) was also determined in the pre-harvest condition using the AccuPAR PAR/LAI LP-80 canopy analyzer (Decagon®). Readings were taken at 3 points per plot along with the measurement of LI.

Table 2. Duration of dry and rainy seasons and number of harvest events (in parentheses) of Tanzania grass for different stubble height (SH) treatments in Igarapé-Açu, PA, Brazil.

SH (cm)	Season of the year		Total harvest events
	Dry	Rainy	
15	10 Sep – 31 Dec 2017 (1)	01 Jan – 04 Aug 2018 (3)	4
25	20 Sep – 31 Dec 2017 (1)	01 Jan – 03 Aug 2018 (4)	5
35	01 Sep – 31 Dec 2017 (2)	01 Jan – 10 Aug 2018 (5)	7
45	01 Sep – 31 Dec 2017 (2)	01 Jan – 05 Aug 2018 (5)	7
55	01 Sep – 31 Dec 2017 (3)	01 Jan – 06 Aug 2018 (7)	10

Tiller population density (TPD) was evaluated whenever the plots intercepted 95% of light. For this assessment, the total number of live tillers within a 1 m × 0.5 m frame per plot was counted and the result expressed as number of tillers per square meter.

Tiller demography was evaluated in a single tussock per plot. Immediately after the grass was cut to implement the SH, the tillers present in the tussock were counted. These tillers were marked and termed 'zero-generation tillers' (G0). At the subsequent harvest, live tillers from G0 were counted, and new emerged tillers, termed 'generation-one tillers' (G1), were marked. The total number of dead tillers was always counted in each evaluation cycle until the end of the experimental period. Tiller counts were made successively, totaling 5, 6, 8, 8 and 10 generations for SHs of 15, 25, 35, 45 and 55 cm, respectively. From the obtained data, the following variables were calculated and expressed as tillers/100 tillers per regrowth period, following Bahmani et al. (2003): tiller appearance rate (TAR) = number of emerged tillers divided by the number of existing tillers at the previous marking event; tiller mortality rate (TMR) = number of dead tillers divided by the number of existing tillers at the previous marking event; and tiller survival rate (TSR) = number of remaining tillers divided by the number of tillers existing at the previous marking event.

Herbage accumulation and morphological composition

Herbage accumulation above the SH was measured by collecting the herbage within a 1 m × 0.5 m quadrat per plot whenever the canopy reached 95% LI. The collected samples were halved: one half being used to determine DM concentration, and the other for a manual separation of the morphological components, namely: leaf blade (LB), stem + sheath (ST) and dead material (DeM). Samples were then weighed and dried in a forced-air oven at 60 °C for 72 h, and the proportions of each morphological component were calculated based on the weight of the components. Herbage accumulation was calculated for each harvest cycle and, at the end of each period, all accumulations were summed, generating the total herbage in each period (THA).

Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS software (SAS Institute Inc. 2008). The model used for all studied variables contained the effects of SH, season of the year and their interactions, which were considered fixed effects, whereas the blocks (replicates) and their interactions were considered random effects. The procedure of repeated measures over time was used with the variance components as a covariance structure. Treatment means were considered different and interactions significant when P≤0.05. Polynomial orthogonal contrasts were used when SH effects were observed. Means as a function of seasons were compared by the F-test.

Results

There was no significant interaction effect (P>0.05) between SH and season for the length of the regrowth period (RP) (Table 3). The RP decreased linearly with increase in SH and was considerably shorter in the RS than in the DS (P≤0.05).

Morphogenetic and structural traits

There was a significant interaction effect between SH and season (P≤0.05) for the morphogenetic variables LLS and SER. LAR, PHY and LER variables were influenced by both SH and season (P≤0.05), whereas LSR was influenced by SH only (Table 4).

Table 3. Effects of stubble height and season of the year on the duration of the regrowth period (RP) of Tanzania grass (*Megathyrsus maximus*) in Igarapé-Açu, PA, Brazil.

Season	Stubble height (cm)					Effect	Mean	s.e.
	15	25	35	45	55			
	Regrowth period (d)							
Dry	115.6	96.3	64.1	61.4	59.6		79.3a	1.15
Rainy	71.1	53.7	41.2	40.7	29.4		47.3b	0.68
Mean	93.1	75.0	52.6	51.2	44.6	L (<0.0001)		
s.e.	1.32	1.21	1.21	1.21	1.28			

Within stubble heights seasonal means followed by different letters are different (P≤0.05).
L: observed significance level for linear effects of SH.

Table 4. Effects of stubble height and season of the year on leaf appearance rate (LAR), leaf elongation rate (LER), phyllochron (PHY), leaf senescence rate (LSR), leaf lifespan (LLS) and stem elongation rate (SER) of Tanzania grass (*Megathyrsus maximus*) in Igarapé-Açu, PA, Brazil.

Season	Stubble height (cm)					Effect	Mean	s.e.
	15	25	35	45	55			
				LAR (leaves/tiller/d)				
Dry	0.063	0.051	0.048	0.058	0.053		0.050b	0.003
Rainy	0.080	0.068	0.060	0.058	0.050		0.064a	0.002
Mean	0.071	0.059	0.054	0.058	0.051	L (<0.0001)		
s.e.	0.003	0.003	0.004	0.003	0.004			
				LER (cm/tiller/d)				
Dry	1.45	1.60	1.67	2.22	2.38		1.86b	0.239
Rainy	2.92	3.29	3.60	3.37	3.97		3.43a	0.239
Mean	2.18	2.44	2.64	2.79	3.18	L (<0.0001)		
s.e.	0.234	0.234	0.247	0.234	0.247			
				PHY (d/leaf)				
Dry	15.87	19.61	20.83	17.24	18.87		18.48a	1.60
Rainy	12.50	14.71	16.67	17.24	18.87		15.99b	1.50
Mean	14.09	16.95	18.52	17.24	18.87	L (<0.0001)		
s.e.	1.58	1.58	1.50	1.58	1.50			
				LSR (cm/tiller/d)				
Dry	0.138	0.152	0.217	0.269	0.353		0.226	0.025
Rainy	0.114	0.193	0. 239	0.307	0.363		0.243	0.026
Mean	0.126	0.172	0.228	0.288	0.358	Q (<0.0001)		
s.e.	0.060	0.060	0.036	0.036	0.025			
				LLS (d)				
Dry	69.49a	58.72a	45.01a	42.78a	41.85a	Q (0.0201)	51.56	1.44
Rainy	44.44b	37.93b	35.69b	35.18b	24.73b	L (<0.0001)	35.59	0.74
Mean	56.94	48.32	40.35	38.98	33.29			
s.e.	2.062	1.618	1.618	1.618	1.618			
				SER (cm/tiller/d)				
Dry	0.060a	0.069a	0.074b	0.075b	0.052b	NS	0.065	0.007
Rainy	0.071a	0.091a	0.104a	0.117a	0.138a	L (0.0079)	0.100	0.006
Mean	0.065	0.080	0.089	0.096	0.095			
s.e.	0.011	0.011	0.011	0.011	0.011			

Within stubble heights means for seasons followed by different letters are different (P≤0.05).
L, Q: observed significance level for linear and quadratic effects of SH, respectively.

Leaf appearance rate decreased linearly with increasing SH (P≤0.05) and overall was greater during the RS than during the DS (0.064 vs. 0.050 leaves/tiller/d) (Table 4). In contrast, average LER increased linearly as SH increased but was greater during the RS than during the DS (3.43 vs. 1.86 cm/leaf/d). Phyllochron increased linearly with increase in SH (P≤0.05), since LAR declined linearly, and was longer during the DS than in the RS (18.48 vs. 15.99 d/leaf).

Leaf senescence rate increased quadratically (P≤0.0001) with increase in SH, with no significant effect of season. Leaf lifespan decreased with increasing SH, the response being quadratic during the DS (P≤0.05) and linear during the RS (P≤0.0001). On average, LLS was greater during the DS of the year at all SHs (P≤0.05). Stem elongation rate was not significantly affected by SH during the DS (P>0.05) but increased linearly (P≤0.05) with increase in SH during the RS. For SH of 35, 45 and 55 cm, SER was higher during the RS than during the DS (P≤0.05) (Table 4).

In the analysis of structural traits (Table 5), a significant interaction effect between SH and season (P≤0.05) was observed for FLS and NLL. Final leaf size increased linearly with increasing SH during the DS (P≤0.05) with no effect of SH during the RS. Leaves were larger (P≤0.05) during the RS at all SHs except 55 cm. Number of live leaves decreased with increasing SH in both seasons, linearly (P≤0.05) during the DS and quadratically (P≤0.05) during the RS. At SHs of 35, 45 and 55 cm, NLL was higher in the RS than in the DS

(Table 5). Tiller population density was affected only by SH (P≤0.05), decreasing quadratically as SH increased (P≤0.05). In the pre-harvest condition, i.e. upon reaching 95% LI, canopy height was not altered (P>0.05) by SH or season. Average height of Tanzania grass at 95% LI was 75.0 ± 0.40 cm in the DS and 76.5 ± 0.46 cm in the RS. Leaf area index rose linearly with increasing SH (P≤0.05), ranging from 2.78 in the plots managed at 15 cm to 3.97 in those managed at SH of 55 cm (Table 5).

As regards tiller demographic variables, a significant interaction effect between SH and season (P≤0.05) was detected for tiller mortality rate (TMR) (Table 6). A linear reduction in TMR was observed as SH increased in both seasons (P≤0.05). For most SHs TMR was higher in the RS but differences were significant (P≤0.05) only for 15 and 25 cm SH. Tiller appearance rate also decreased linearly with increasing SH (P≤0.05) and was higher during the RS than in the DS. TSR also increased linearly with increase in SH (P≤0.05) in both seasons and was higher during the DS than in the RS (67.5 vs. 60.3%) (Table 6).

Herbage accumulation and morphological composition

There was a significant interaction effect between SH and season for proportions of leaf blade and dead material (P≤0.05). Isolated effects of SH (P≤0.05) and season of the year (P≤0.05) were detected for THA and stem + sheath (Table 7).

Total herbage accumulation decreased linearly with increase in SH in both seasons (P≤0.05). Only about 22% of THA occurred in the DS (P≤0.05) (Table 7).

Leaf blade proportion increased linearly (P≤0.05) with increase in SH in both seasons (Table 7). While leaf proportion was greater (P≤0.05) in the DS than the RS at 15 cm SH, at 55 cm SH the reverse was the case. During the RS, DeM proportion decreased linearly with increasing SH but showed a quadratic response during the DS (P≤0.05). ST proportion responded quadratically to increasing SH (P≤0.05), and at the height of 55 cm this component was not found in the samples of herbage accumulated above the residual stubble. Stem + sheath percentage was higher in the RS (6.21%) than in the DS (5.38%).

Table 5. Effects of stubble height and season of the year on final leaf size (FLS), number of live leaves (NLL), tiller population density (TPD) and leaf area index (LAI) of Tanzania grass (*Megathyrsus maximus)* in Igarapé-Açu, PA, Brazil.

Season	Stubble height (cm)					Effect	Mean	s.e.
	15	25	35	45	55			
				FLS (cm)				
Dry	24.1b	25.6b	28.3b	28.3b	31.7a	L (0.0003)	27.6	1.16
Rainy	35.0a	30.9a	33.4a	34.6a	33.9a	NS	33.6	1.18
Mean	29.6	28.2	30.8	31.4	32.8			
s.e.	1.22	1.15	1.15	1.15	1.15			
				NLL				
Dry	3.82a	3.64a	3.33b	3.02b	2.67b	L (<0.0001)	3.29	0.08
Rainy	3.81a	3.85a	3.97a	3.78a	3.19a	Q (0.0235)	3.72	0.08
Mean	3.81	3.74	3.65	3.40	2.93			
s.e.	0.11	0.10	0.09	0.09	0.09			
				TPD (tillers/m²)				
Dry	264	258	260	234	224		228	3.60
Rainy	272	274	276	220	222		232	3.22
Mean	268	266	268	228	224	Q (0.0295)		
s.e.	6.34	5.36	5.08	5.08	5.08			
				LAI				
Dry	2.63	3.27	3.64	3.96	4.02		3.50	0.08
Rainy	2.93	3.27	3.65	3.67	3.91		3.55	0.03
Mean	2.78	3.43	3.64	3.86	3.97	L (<0.0001)		
s.e.	0.09	0.09	0.09	0.09	0.09			

Within stubble heights means followed by different letters are different (P≤0.05).
L, Q: observed significance level for linear and quadratic effects of SH, respectively.

Table 6. Effects of stubble height and season of the year on tiller mortality rate (TMR), tiller appearance rate (TAR) and tiller survival rate (TSR) of Tanzania grass (*Megathyrsus maximus*) in Igarapé-Açu, PA, Brazil.

Season	Stubble height (cm)					Effect	Mean	s.e.
	15	25	35	45	55			
				TMR (tillers/100 tillers)				
Dry	42.4b	31.0b	27.2a	26.0a	23.8a	L (0.0005)	30.3	1.14
Rainy	58.9a	44.5a	36.4a	35.3a	23.4a	L (<0.0001)	39.7	1.03
Mean	50.7	37.7	31.8	30.6	23.6			
s.e.	2.04	1.63	1.63	1.63	1.63			
				TAR (tillers/100 tillers)				
Dry	37.9	31.0	30.9	31.9	22.1		30.5b	1.73
Rainy	58.7	49.0	39.1	39.1	28.8		42.9a	1.70
Mean	48.3	40.0	35.0	35.5	25.4	L (<0.0001)		
s.e.	2.69	2.83	2.69	2.69	2.69			
				TSR (tillers/100 tillers)				
Dry	46.6	68.1	72.8	74.0	76.2		67.5a	1.69
Rainy	41.1	55.5	63.6	64.7	76.6		60.3b	0.87
Mean	43.8	61.8	68.2	69.4	76.4	L (<0.0001)		
s.e.	2.41	2.22	2.22	2.22	2.22			

Means followed by different letters comparing the effect of seasons are different (P≤0.05).
L: observed significance level for linear effects of SH

Table 7. Effects of stubble height and season of the year on total herbage accumulation (THA) and leaf blade (LB), dead material (DeM) and stem (ST) proportions of Tanzania grass (*Megathyrsus maximus*) in Igarapé-Açu, PA, Brazil.

Season	Stubble height (cm)					Effect	Mean	s.e.
	15	25	35	45	55			
				THA (kg DM/ha)				
Dry	3,503	2,671	2,510	2,304	1,647		2,527b	125
Rainy	10,142	9,262	8,664	7,913	7,482		8,693a	187
Mean	6,822	5,966	5,587	5,109	4,565	L (<0.0001)		
s.e.	197	179	197	197	197			
				LB (g/kg DM)				
Dry	611a	762a	780a	777a	793b	L (0.0004)	745	16.0
Rainy	561b	756a	798a	833a	857a	L (<0.0001)	761	12.0
Mean	587	759	789	805	825			
s.e.	17.5	16.2	16.2	16.2	16.2			
				DeM (g/kg DM)				
Dry	201a	198a	163a	163a	209a	Q (<0.0001)	187	10.0
Rainy	228a	167a	166a	154a	142b	L (<0.0001)	172	10.0
Mean	215	183	165	159	176			
s.e.	15.8	15.8	15.8	15.8	15.8			
				ST (g/kg DM)				
Dry	182	45.9	32.5	9.8	0.0		53.8b	3.1
Rainy	198	59.5	39.7	12.8	0.0		62.1a	3.1
Mean	190	52.7	36.1	11.3	0.0	Q (<0.0001)		
s.e.	8.2	8.2	8.2	8.2	0.0			

Means followed by different letters comparing the effect of seasons are different (P≤0.05).
L: observed significance level for linear effects of SH

Discussion

Shorter regrowth periods in pastures managed during the RS, as compared with the DS, are mainly due to contrasting climatic conditions between the seasons. Tillering is directly affected by the light intercepted by the canopy (Paciullo et al. 2016), as well as by changes in temperature and water availability (Tilley et al. 2019), which was highly contrasting between the two seasons. Environmental conditions adverse to tiller development lead to less herbage accumulation after harvest (Table 7), causing plants to expend more time and larger amounts of reserves to re-intercept 95% LI (Silva et al. 2019). In this study, we observed that differences in regrowth period between the two seasons declined as SH was increased up to 45 cm. For instance, at SH of 15 cm the difference between seasons in regrowth period was 44.5 days, whereas at 45 cm this difference was only 20.7 days.

In grass species, the regrowth period can also be influenced by harvesting or grazing, especially as a function of SH, which is directly related to the remaining LAR. Pastures managed under lower SH need longer intervals to recover leaf area to be able to achieve 95% LI. As a consequence, they show a longer regrowth period, which, in practice, is not recommended. The management of Tanzania grass in an intermittent grazing system has been recommended in Brazil with a pre-grazing height of 70 cm (LI = 95%) (Euclides et al. 2014; Zanine et al. 2018) and a post-grazing SH close to 50% of the pre-grazing height, i.e. 35 cm. However, what differs in the studies are the varying regrowth periods required by the grass to start from SH of 35 cm and reach 95% LI (70 cm). These differences in regrowth period are usually related to factors such as time of year, soil management and fertility and the climate of the region, warranting the development of studies in different parts of the country. On average, the regrowth period of Tanzania grass in the RS in central Brazil has ranged between 21 and 32 days, at pre- and post-grazing heights of 70 and 35 cm, respectively. In our study, this regrowth period was 41.2 days, as the grass reached 95% LI at the greatest average height (76.5 cm). This was probably due to the typical excess rainfall that occurs in the region during the RS (2,086 mm in 2018), leaving part of the months with a cloudy sky.

Morphogenetic and structural traits

Decreasing LAR in tropical grasses in response to increases in SH (Table 4) are usually associated with the longer pseudostems that occur at greater stubble heights, which, in turn, result in a greater distance to be traveled by the leaf until its exposure above the tube (Lemaire and Chapman 1996). This is better understood when we analyze the increase in PHY, i.e. the time taken for two consecutive leaves in the tiller to appear, following an increase in SH. Phyllochron follows an inverse response pattern to that of LAR; at lower SH, PHY tends to decrease and LAR tends to increase, possibly related to the plant's need to restore the photosynthetic apparatus shortly after harvest or a more intense grazing event. Similar results showing a decrease in LAR and an increase in PHY following increases in SH were described by Silva et al. (2016) in Tifton-85 pastures, reinforcing the inverse relationship between these variables.

Higher LER occurs at higher SH (Table 4), due to the elongation of pseudostems. Shorter pseudostems favor a rapid leaf emergence, resulting in lower LER. The opposite is also true, as leaves take longer to emerge from longer pseudostems, resulting in higher LER. In this respect, Zanine et al. (2018) observed an increase in LER from 11.54 to 15.21 cm/tiller/d as SH was increased from 30 to 50 cm in Tanzania grass pastures managed at 95% LI in the pre-grazing condition during the RS. The higher LER (3.43 cm/tiller/d) seen in the RS was likely due to the greater water availability during this season than in the DS (2,087 vs. 169 mm). Barbosa et al. (2011) studied the effects of defoliation intensities and frequencies in Tanzania grass and observed LER of 4.16 and 1.16 cm/tiller/d in the rainy and dry seasons, respectively, and associated this difference with climatic differences between the two seasons.

The increase in LSR in response to increasing SH observed in both seasons (Table 4) is related to intraspecific competition for light, which reduces the quantity and quality of the light that penetrates the pasture as the grass becomes taller. As an adaptation response to this competition, the plant starts to invest in elongating its internodes to elevate leaves to the top of the pasture, where light is more abundant. Simultaneously, the leaves located at the base of the tussocks become more shaded, which accelerates leaf senescence (Duchini et al. 2013). For this reason, during the regrowth period, both SER and LSR exhibit the same response pattern. These results corroborate those described by Silva et al. (2016) in Tifton-85 grass.

Leaf lifespan expresses the tissue flow occurring in the plant, which is normally higher during the DS (Table 4). The longer LLS in the DS may be the result of low precipitation as well as lower day-length, which are typical of this season in tropical conditions. In this scenario, grasses extend the lifespan of green leaves and reduce leaf tissue turnover, which results in lower LAR and LER as well as a longer PHY, as previously discussed. It is thus clear that these traits are influenced by seasons of the year, which are directly related to environmental conditions.

The shortened LLS in response to increasing SH in both seasons (Table 4) can be better understood when considered together with the leaf senescence process, which increased along with increasing SH. Once senescence is established, nutrients are redirected to younger leaves, which reduces the photosynthetic activity of older leaves and leads to a reduction in LLS (Oliveira et al. 2007), as observed in our study.

From SH of 35 cm upwards, higher SER was observed during the RS (Table 4), which may be associated with a larger amount of leaves remaining after harvest at these greater SHs. These remaining leaves usually cause shading on the tillers, prompting them to elongate their stem to capture light in the upper layers of the canopy, which also explains the linear increase in SER along with increasing SH. Another explanation for the difference in SER between the seasons may be related to the reproductive stage of the cultivar, which produces inflorescences during the summer (RS) under tropical conditions. As inflorescence-emergence approaches, tropical grasses elongate their stems so that the inflorescences reach and remain in the upper strata of the pasture (Pedreira et al. 2017), in an attempt to facilitate seed dispersal. Zanine et al. (2018), working with the same guinea grass (cv. Tanzania) also observed a higher mean SER in summer than in winter (13.38 vs. 4.87 mm/tiller/d) and in pastures under greater SH (50 vs. 30 cm) during summer (15.21 vs. 11.54 mm/tiller/d).

Leaf blade length, represented by FLS, is a structural variable that responds to the intensity of defoliation. Higher values for this variable are associated with greater SH, agreeing with the greater leaf sheath length (Volaire et al. 2014). Thus, the distance to be traveled by the leaf blade inside the pseudostem is greater, which results in an extended elongation time and, consequently, a longer new leaf (Duru and Ducrocq 2000). However, this response pattern was observed only during the DS, which still provided the lowest FLS as compared with the RS for stubble heights up to 45 cm (Table 5). This reduction in FLS during the DS may be associated with the lower average LER observed in this season (Table 4), as these two traits are known to be correlated (Lemaire and Chapman 1996).

Since it is inversely related to LSR, the number of live leaves per tiller decreased in both seasons with increase in SH (Table 5), whereas average LSR increased (Table 4). This is likely because, as they contained larger proportions of senescent leaves at the base of the canopy, pastures with greater SH had a higher percentage of dead leaves, which resulted in a lower NLL per tiller, as well as the greater SH displaying lower LAR (Table 4). These effects were also described by De Carvalho et al. (2016) and Silva et al. (2016) in Tifton-85 grass managed under different grazing intensities.

Stubble height is a factor that affects tiller density, with higher densities being commonly observed in shorter pastures (lower SH) and vice-versa (Lima et al. 2017; Santana et al. 2017). This fact can be explained as a response to the tiller size/density compensation mechanism existing in higher plant communities (Matthew et al. 1995). By using this mechanism, grasses regulate pasture leaf area and, consequently, the ability to intercept incoming light. This can cause greater shading at the base of the canopy under higher LAI, which can reduce the stimulation of the basal and axillary buds for the production of new tillers. Indeed, LAI rose linearly with increasing SH, which reinforces the premise described. This increase in LAI may be associated with the increase in LER as SH was raised.

When managed under lower SH, Tanzania grass may exhibit a higher TMR (Table 6), which is likely due to the removal of the apical meristem at harvest, as it is considered a tall grass, reaching 1.2 m in height in free growth. On the other hand, lower SH also promotes higher TAR, which indicates that there was a balance between deaths and the appearance of tillers at the lower SH, which contributed to the perenniality of the cultivar. The inverse relationship between SH and TAR observed in the study can be explained by the greater light intensity that reaches the base of the canopies managed under lower SH, in addition to the higher LAR (Sbrissia et al. 2010) (Table 4). In theory, the appearance of a new leaf allows the development of a new tiller (Skinner and Nelson 1992).

Knowledge about seasonal variations in the rates of tiller appearance, mortality and, consequently, survival, is important for understanding the mechanisms involved in perenniality and tiller turnover in pastures. During the RS, although the pastures had a higher TAR (42.9 tillers/100 tillers), it was not high enough to compensate for lower tiller survival (60.29 tillers/100 tillers) than in the DS (67.53 tillers/100 tillers), and this condition could negatively affect plant persistence and pasture productivity. On the other hand, during the DS, despite the low rate of tiller appearance, their survival was high, in an effort to maintain pasture persistence under these climatic conditions.

Herbage accumulation and morphological composition

With increase in SH the plants needed a shorter time interval and, probably, smaller amounts of reserves to reach 95% LI, i.e. a shorter regrowth period (Silva et al. 2019). This led to a decrease in THA as compared with the pastures with lower SH, which required a longer interval to intercept 95% of incident light again and thus accumulated more herbage. Other evaluated factors that also help to explain the decreasing THA in response to the increase in SH were the reductions in leaf and tiller appearance rates and tiller density as well as increasing leaf senescence, as SH was increased. Similarly, Hamilton et al. (2013) reported a reduction in the accumulation of ryegrass and tall-fescue herbage when SH was increased from 2 to 15 cm.

Only about 22% of THA occurred in the DS, which would be due to climatic conditions being favorable for regrowth of the grass in the RS (higher precipitation rates, mainly), favoring greater number of harvests in that period, which is consistent with the premise that tropical pastures in Brazil produce 60–80% of the total herbage mass in the RS and the remaining 20–40% in the DS (Pedreira et al. 2005; Fernandes et al. 2014; Oliveira et al. 2020). This more favorable environment reduced the average regrowth period from 80.1 days in the DS to 51.2 days in the RS, resulting in more harvests being possible during the RS. In addition, variables directly related to herbage accumulation, such as LAR, LER and SER, were higher in the RS. Our findings confirm the existence of production seasonality in grasses of the genus *Megathyrsus*, corroborating the results reported by Luna et al. (2016) and Santos et al. (2016). One must also remember that the RS was twice as long as the DS.

The proportion of leaf blade (LB) in herbage accumulated above the stubble increased with increasing SH in both seasons, whereas the average proportion of ST decreased (Table 7). When we increased SH, we harvested the herbage in the upper strata of the canopy, where there is a higher proportion of leaves and a lower percentage of stems. In practice, to maximize herbage accumulation, Tanzania grass pastures must be managed at lower SH. However, this accumulated herbage contains a lower proportion of green leaves and a higher proportion of stems than forage from a greater SH, where there is less THA with a higher proportion of green leaves and a lower proportion of stems. Therefore, in ideal terms, a compromise should be made in choosing the SH to balance the amount of accumulated herbage with its nutritional value.

The higher proportion of LB observed at the SH of 15 cm during the DS (Table 7) may be due to the greater removal of morphological components, which put the plants in an even more adverse condition than is ‘normal’ in the DS. Thus, the plants needed to rebuild their leaf area and maintain more leaves alive (NLL) to ensure the perenniality of the cultivar. The higher proportion of LB in the herbage accumulated at SH of 55 cm during the RS may be related mainly to the effect of herbage harvest in the upper stratum, as highlighted above, as well as the greater precipitation occurring during this season.

Long regrowth periods, resulting from low SH, culminated in undesirable changes in the structure of the forage canopy, characterized by an increased proportion of ST and DeM in the herbage accumulated above the stubble. Under grazing conditions this can result in herbage losses due to the amount of material largely rejected by grazing animals, thereby negatively influencing harvest efficiency and the nutritional value of the produced material.

The observed decrease in the proportion of ST in the accumulated herbage with increasing SH was due to the harvest intensities themselves. In tropical pastures, it is known that, from a certain point (strata closer to the ground level), the stem component starts to represent a much more significant percentage of the stratum. This can affect herbage intake, since intake can be affected by components associated with the architecture and the morphological and botanical composition of the pasture, which define its structure. Stem is the component that most restricts intake due to the physical barrier it imposes on the grazing process (Laca and Lemaire 2000). Fontes et al. (2014) examined the effect of defoliation intensities in *Brachiaria* grasses and also observed that higher defoliation intensities resulted in a higher proportion of stems in the samples.

This study made it possible to confirm the hypothesis that interactions between seasons of the year in the eastern Amazon region and SH affect the structure and production of Tanzania grass. In the DS, Tanzania grass changed its morphogenetic and structural traits to result in only 22.6% of annual total herbage accumulation occurring during the DS. The remaining 77% of herbage accumulation was due to the beneficial changes in the morphogenetic and structural traits of the grass provided by the favorable climatic conditions occurring in the RS. Results from this study lead us to conclude that Tanzania grass should be managed at SH between 35 and 45 cm in the DS, corresponding to RP from 61 to 64 days, and at SH of 35 cm in the RS, corresponding to RP of 41 days. This should result in a compromise between yield and quality of forage produced. Studies to assess the outcome of such a strategy seem warranted.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoa de Nível Superior (CAPES) for the first author's scholarship.

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(Note of the editors: All hyperlinks were verified 9 July 2021).

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(Received for publication 31 July 2020; accepted 24 June 2021; published 30 September 2021)

doi: 10.17138/TGFT(9)268-279

Research Paper

Effect of sowing rate and date on establishment and growth of *Trichloris crinita*, a native American pasture grass from arid environments, in the Arid Chaco of Argentina

Efecto de la densidad y fecha de siembra en el establecimiento y crecimiento de Trichloris crinita*, una gramínea forrajera nativa de ambientes áridos de las Américas, en el Chaco Árido de Argentina*

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Abstract

In arid regions, revegetation with locally adapted native species can improve forage production and help ameliorate soil degradation. We investigated the effects of 3 sowing dates and 3 sowing rates of *Trichloris crinita* cv. Chamical-INTA, a perennial forage grass native to arid and semi-arid regions, on pasture establishment parameters in the Argentinian Arid Chaco phytogeographical region. Sowing date significantly influenced plant density and soil coverage at the end of the growing season, with the latest sowing date increasing mean plant density and soil coverage by 42–66% and 16–38%, respectively, relative to the 1st and 2nd dates. Conversely, the later sowing dates (2nd and 3rd dates) exhibited significantly lower mean values for all plant growth-related traits, i.e. tillers per plant, plant height and percentage of flowering plants. Sowing rate had a strong effect on plant density at the end of the growing season but not on plant growth parameters. Under the conditions of this study, using intermediate sowing densities (7.5 kg seed/ha) and sowing early in the season, when temperatures were still mild, delivered the best results in terms of pasture density and establishment efficacy. Early sowing resulted in a greater percentage of flowering plants and seed set prior to the first winter frosts, which should ensure ongoing establishment of plants in the next wet season. Longer-term studies to examine the survival of plants and possible increase in plant density over time are necessary to determine if this procedure has sustainable benefits for pastures in the area.

Keywords: Degraded areas, forage productivity, pasture management, plant density, vegetation recovery.

Resumen

En regiones áridas y semiáridas, la siembra de especies nativas adaptadas localmente puede contribuir a mejorar la productividad forrajera y atenuar la degradación del suelo. Este trabajo investigó los efectos de tres densidades y tres fechas de siembra en la implantación y el establecimiento de *Trichloris crinita* cv. Chamical-INTA, una gramínea forrajera nativa de zonas áridas, en la región fitogeográfica del Chaco Árido, Argentina. La fecha de siembra influyó significativamente en la densidad de plantas establecidas y cobertura del suelo al final de la época de crecimiento, con siembras en la tercera fecha mostrando incrementos para estas variables de 42–66% y 16–38%, respectivamente,

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comparado con las siembras en la primera y segunda fechas. Contrariamente, las fechas de siembra tardías (segunda y tercera fechas) mostraron valores significativamente menores para todos los parámetros de crecimiento evaluados (p.ej. brotes por planta, altura de planta y porcentaje de floración). La densidad de siembra tuvo un fuerte efecto sobre la densidad final de plantas establecidas, pero no en los parámetros de crecimiento. Las siembras tempranas con densidad intermedia (7.5 kg/ha) tuvieron los mejores resultados en términos de eficacia del establecimiento de la pastura. La siembra temprana resultó en un mayor porcentaje de plantas florecidas al final de la temporada, y las semillas resultantes deberían favorecer el establecimiento de la pastura en la temporada siguiente. A futuro, serán necesarios estudios de largo plazo que monitoreen la supervivencia y densidad de plantas en el tiempo, a fin de determinar si estas prácticas poseen beneficios sostenibles para las pasturas de *T. crinita* en esta región.

Palabras clave: Áreas degradadas, densidad de plantas, manejo de pasturas, productividad forrajera, recuperación de vegetación.

Introduction

Drylands, i.e. arid, semi-arid and dry subhumid regions combined, cover nearly 41% of the Earth's land surface and are home to more than 38% of the total global population (Global Land Project 2005; Millennium Ecosystem Assessment 2005). Severe land degradation is present on 10–20% of these lands, affecting ~250 million people, mainly in developing countries (Millennium Ecosystem Assessment 2005), and current expectations are that these estimates will increase over time due to climate change and population growth.

In arid and semi-arid regions, land desertification, characterized by low fertility and organic matter concentration in the soil, is widespread, and this situation is often aggravated by overgrazing by domesticated animals (Papanastasis 2009). Different studies have estimated that 20–73% (with a mean of 60%, considering estimates from all studies) of the world's grazing areas are moderately to severely degraded (Lund 2007). Moreover, loss of perennial grasses in rangelands, often accompanied by severe soil erosion and salinity, is a frequent component of desertification processes in arid regions (Waters and Shaw 2003).

The traditional strategy to address these degradation issues has been to reseed the degraded areas with introduced perennial species. However, in recent decades the trend has moved towards the use of native species, with a clear recognition of their intrinsic adaptive and ecological value (Waters and Shaw 2003). Selection of drought-tolerant species, with adequate seed available, and the utilization of appropriate and sustainable management practices, especially with regard to pasture establishment, are critical for a successful revegetation program (Quiroga et al. 2013).

Trichloris crinita (Lag.) Parodi [syn. *Leptochloa crinita* (Lag.) P.M. Peterson & N. Snow (Peterson et al. 2012; 2015)] (Chloridoideae, Poaceae) is a perennial grass native to arid and semi-arid regions of North and South America (Peterson et al. 2007). Under natural conditions, it behaves as a typical aestival species, growing whenever soil water is available and the temperature is above 10 °C (Seligman et al. 1992). In these dry lands, the species is widely recognized for its good forage quality, drought tolerance, resistance to trampling and grazing, rapid growth and aggressive competition with other native species (Kozub et al. 2017). In environments with low water availability, it is used as forage for range grazing and for restoration of degraded rangelands (Passera et al. 1992; Cavagnaro and Trione 2007; Guevara et al. 2009).

These arid environments are typical in the north- and central-west part of Argentina. The 'Arid Chaco' region, located in the 'Chaco' phytogeographical province, is one of these drylands, presenting an east-to-west annual rainfall gradient of 250–550 mm, with 80% of the rainfall occurring in mid-summer (November–January) (Morello et al. 1985). In this region, extensive rearing of beef cattle and, to a lesser extent, goats, is the main productive activity (Rueda et al. 2013), with pasture grasses being the main feed source for livestock. However, in recent decades, forage production has decreased steadily as a consequence of land degradation due to overgrazing, thereby altering the landscape to shrublands with extensive areas of bare soil (Blanco et al. 2005; Karlin 2013). Under this scenario, applying revegetation strategies, for example by sowing seeds of locally-adapted native grasses, such as *T. crinita*, may be effective in increasing plant numbers and therefore forage production, as reported in previous studies with other grass species (Passera et al. 1992; Blanco et al. 2005; Quiroga et al. 2009; Mora et al. 2013).

Initial seedling growth and establishment are stages of extreme susceptibility to a wide range of stresses, due to the small seedling size, and these stages are critical for achieving productive pastures (Praat 1995; Skinner

2005; Bertram 2008). To date, we are unaware of any studies to evaluate the effects of sowing density and date on seedling survival and pasture establishment using *T. crinita*. We hypothesized that early sowing of *T. crinita* using high sowing densities would improve the implantation and establishment of the pasture in an arid environment, such as the Argentinian 'Arid Chaco' phytogeographical region. The objective of this work was to investigate how different sowing rates and dates affect the establishment and growth of *T. crinita* pasture in the year of implantation.

Material and Methods

A field trial was carried out in the Agricultural Experimental Field of the 'Universidad Nacional de La Rioja Sede Chepes' (31°20' S, 66°38' W). The experiment began with the sowing of the grass in the spring of 2014 and finished with the first frost of autumn of 2015.

Three sowing rates were used: 0.25 g/m^2, now referred to as low sowing rate (LR); 0.75 g/m^2, intermediate sowing rate (IR); and 1.25 g/m^2, high sowing rate (HR); they are equivalent to 2.5, 7.5 and 12.5 kg seed/ha, respectively, or 300, 900 and 1,500 caryopses/m^2. An unseeded plot was used as a Control, with the objective to obtain complementary information on the dynamics of the natural vegetation, as determined by the seed bank already on site. Three sowing dates were used: 22 November 2014 (sowing date 1; after the first rains); 22 December 2014 (sowing date 2); and 21 January 2015 (sowing date 3). Spikelets of *Trichloris crinita* cv. Chamical-INTA with a mean germination rate of 85% were sown into a soil with superficial tillage (5 cm-deep soil movement with a hand rake). After depositing seeds on the soil surface, the soil was raked to incorporate the seeds and to avoid random dispersal by the wind. The soil was classified as typic torriorthent, with the following characteristics: silt loam texture, low organic matter content (1.5% of soil mass), pH of 8.2, conductivity of 2.12 mS/cm, 0.1% total nitrogen and a C:N ratio of 9.7. A complete randomized block design was used. The total trial area was 144 m^2 and consisted of 12 treatments of sowing rate × sowing date combinations, with 3 replicates, totaling 36 experimental units (plots) of 4 m^2 (2 × 2 m) each.

Every 15 days for the following 180 days (until 21 May 2015), the following parameters were measured: density of *T. crinita* plants and other narrow-leaf (grasses) and broad-leaf plants; number of tillers per plant; and plant height of *T. crinita*. The percentage of flowering plants was estimated on 21 May at the end of the growing season for all treatments, i.e. 180, 150 and 120 days after 1st, 2nd and 3rd sowing dates, respectively. The percentage of the land surface covered with plants was estimated using photo images taken from above on 6 April 2015, i.e. 135, 105 and 75 days after the 1st, 2nd and 3rd sowing dates, respectively. This date was arbitrarily selected as a point in time, when the pasture appeared to have reached its maximum vegetative growth and the leaves of some plants began to senesce. All measurements were performed in selected and standardized areas by using a quadrat of 1 m2 in the center of each experimental unit.

Mean daytime temperature (T) and the distri-bution of rainfall were monitored during the experiment. Temperature data were obtained from the agrometeorological station of the National Institute of Agricultural Technology (INTA), located at El Portezuelo (EEA INTA La Rioja AER El Portezuelo), La Rioja, Argentina, located ~50 km from the experimental site. The amount and distribution of precipitation were recorded with a rain gauge located on the site of the experiment.

The data were analyzed using mixed linear models with a factorial structure, treating sowing date, sowing rate and their interactions as fixed effects, while treating the 3 blocks as random effects. Different structures of residual variance were considered, and the best models were selected using the Akaike (AIC) and Schwarz (BIC) information criteria (Di Rienzo et al. 2017). All statistical and graphic analyses were performed with InfoStat version 2018 software (Di Rienzo et al. 2018). The data were expressed as mean ± standard error, and P values <0.05 were considered significant, using the DGC test (Di Rienzo et al. 2002).

The photographs taken to determine the percentage of the soil surface covered with plants, using canopy area as the criterion, were analyzed with CobCal® 2.0 software (Ferrari et al. 2009), which estimates the area or percentage of vegetation coverage based on colorimetric analysis.

Results

Weather conditions during the experiment

Mean day temperature and rainfall throughout the growing season are shown in Figure 1A.

Mean daily temperature for each vegetative period, i.e. from the relevant sowing date to 21 May 2015, was 23.6 °C ± 4.4; 23.5 °C ± 4.6; and 22.6 °C ± 4.3, for the 1st, 2nd and 3rd sowing dates, respectively. In general,

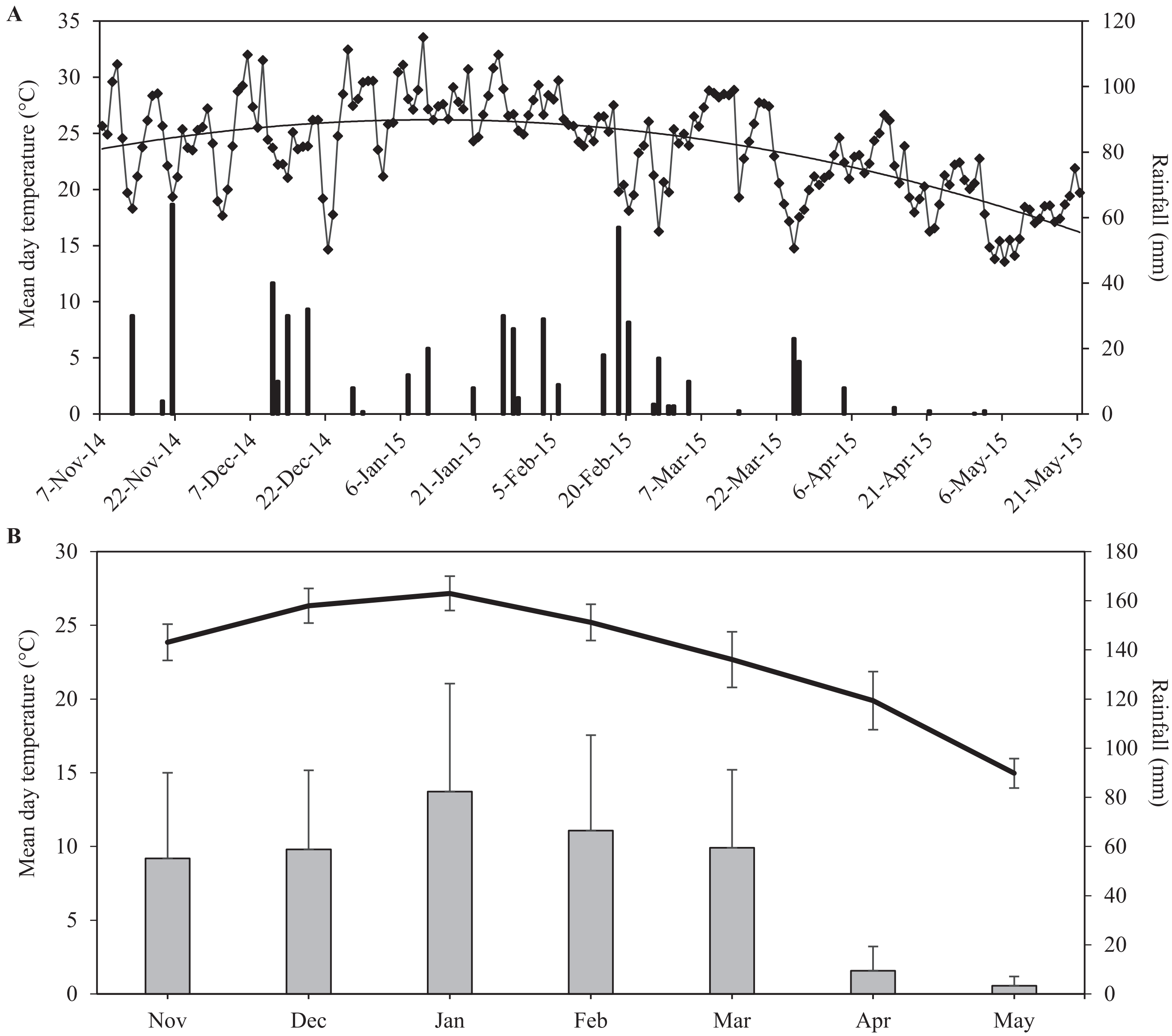

Figure 1. A. Time-course variation of mean day temperature (upper graph part with adjusted trend line) and rainfall (histogram in the lower graph part) in the growing season at the site of the experiment. B. Time-course variation of mean day temperature (black line) and mean precipitation (histogram) during the growing season (November–May) at the site of the experiment for the decade 2011–2021. Dispersion bars indicate standard deviations.

mean temperature increased steadily from the 1st sowing date (22 November 2014) until early February (6 February 2015), reaching a maximum of 33.6 °C on 10 January 2015. Additionally, Figure 1B presents weather conditions at the site of the experiment for the last decade.

Total precipitation during the experiment was 548 mm, with amounts for the 3 growing periods being 450 mm for the 1st sowing date, 338 mm for the 2nd sowing date and 289 mm for the 3rd date. In November 2014, 98 mm of rainfall were registered, with 64 mm received before the 1st sowing date (21 November 2014).

Plant density of T. crinita and other species

Both sowing date ($P=0.0051$) and sowing rate ($P<0.0001$) of *T. crinita* seed had significant impacts on final plant density (FPD) at the end of the experiment, i.e. 135, 105 and 75 days after the 1st, 2nd and 3rd sowing dates, respectively, but there was a significant ($P=0.0142$) interaction between sowing date and sowing rate (Table 1; Figure 2). The highest values for FPD occurred for the intermediate sowing rate (IR; 0.75 g/m^2) treatment at the 1st and 3rd sowing dates ($P<0.05$), with means of 135 and

131 plants/m², respectively. The lowest FPD (22 plants/m²) was for the low sowing rate (LR; 0.25 g/m²) at the 1st sowing date (Figure 2). Among the Control plots, only those for the 2nd sowing date presented *T. crinita* plants, with a mean FPD of 2 plants/m². Presumably, these plants developed from *T. crinita* seeds that were part of the natural seed bank in the soil, as this species is native to arid and semi-arid regions of Argentina, including the Arid Chaco, where the experiment was conducted.

Figure 3 depicts the time-course variation of *T. crinita* plant density throughout the study. At all sowing rates for the 1st sowing date, minimal emergence of *T. crinita* seedlings was observed at 15 days after sowing, but emergence increased significantly in the following 15 days, reaching a maximum plant density (MPD) 45–60 days after sowing (DAS), with mean values of 48, 195 and 199 plants/m² for LR, IR and HR, respectively ($P<0.05$). Plant density then declined steadily until April with final populations of 22, 135 and 81 plants/m² for LR, IR and HR, respectively ($P<0.05$).

For the 2nd sowing date, there was little emergence at observation dates before 5 February (45 DAS), when peak plant numbers were recorded (58, 145 and 164 plants/m² for LR, IR and HR, respectively; $P<0.05$). A rapid decline in plant numbers occurred in the following 2 weeks, after which plant density remained relatively stable until the end of the experiment, with FPDs of 43, 99 and 60 plants/m² for LR, IR and HR, respectively ($P<0.05$).

Table 1. Effects of sowing date and sowing rate, and their interaction, on final plant density of *Trichloris crinita* and other narrow- and broad-leaf species, mean number of tillers per plant, plant height and percentage of flowering plants of *T.crinita*, plus percentage of soil covered with vegetation at the end of the study (last measurement).

	Plant density at end of study			Tillers per plant	Plant height	% soil coverage	% flowering plants
	Trichloris crinita	Narrow-leaf species	Broad-leaf species				
Sowing date	6.8**	ns	ns	6.1**	55.5***	4.6*	16.8***
Sowing rate	37.7***	6.1**	5.1**	ns	ns	19.5***	ns
Date × rate	3.5*	ns	ns	ns	ns	3.8**	ns

Numbers are the F value from ANOVA.
Vegetation cover was determined with the software CobCal 2.0 (Ferrari et al. 2009) using digital photographs taken from above.

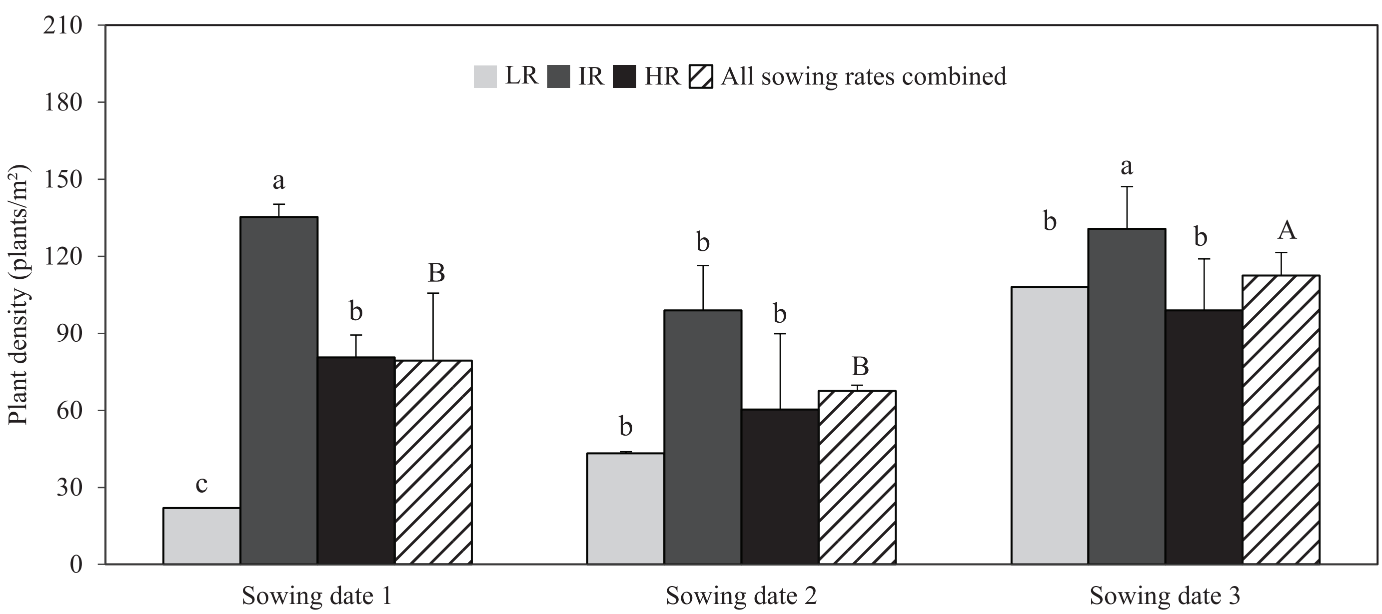

Figure 2. Plant density at the end of the study (FPD) for *Trichloris crinita* for each sowing date and sowing rate, i.e. at 180, 150 and 120 days after the 1st, 2nd and 3rd sowing dates, respectively. Low (LR), intermediate (IR) and high sowing rates (HR) correspond to sowing densities of 0.25, 0.75 and 1.25 g seed/m2, respectively. Columns represent means and bars are the standard errors. Lower-case letters indicate significant differences among all different combinations of sowing dates and rates and upper-case letters indicate comparisons among sowing dates considering all sowing rates for a given sowing date combined. Columns with the same letter are not significantly different at $P<0.05$.

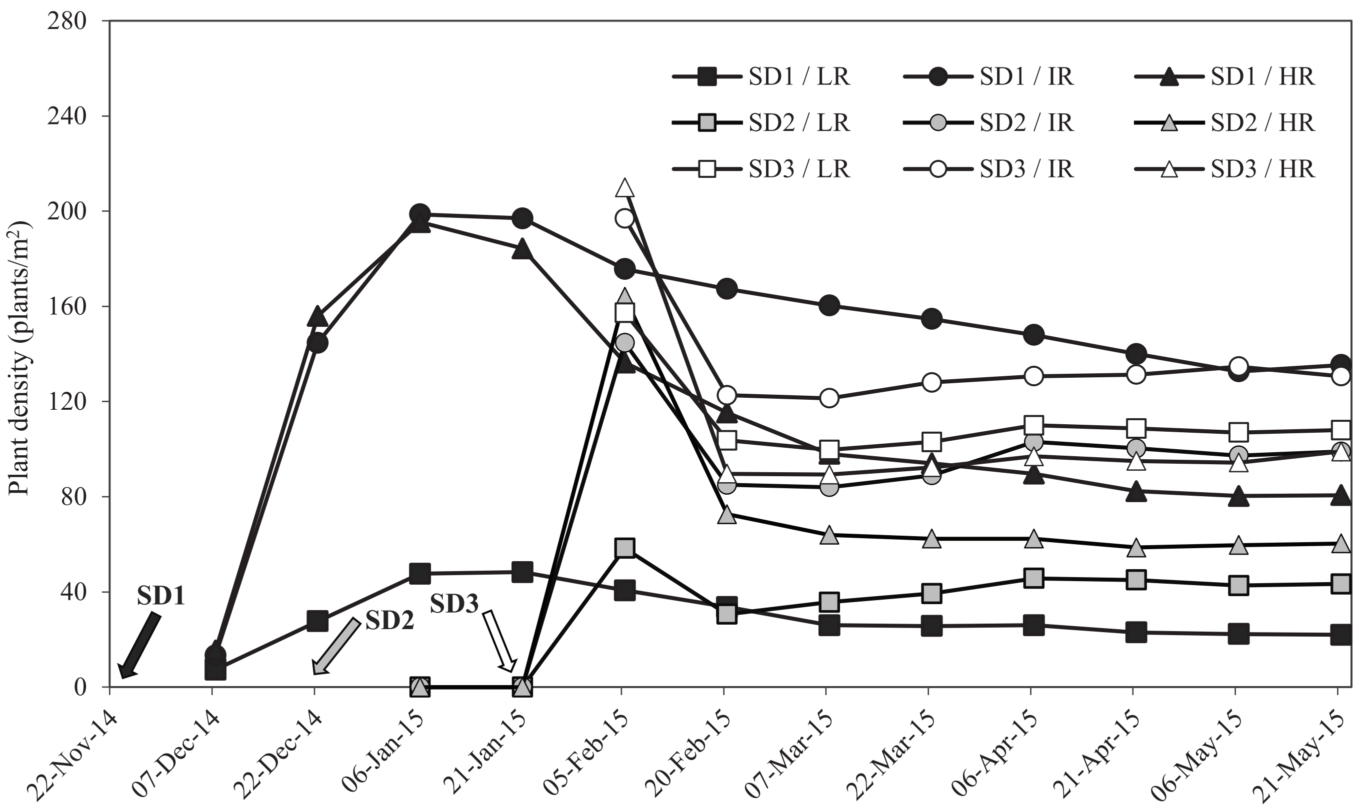

Figure 3. Time-course variation of mean density (plants/m^2) of *Trichloris crinita* during the growing season. Arrows for SD1, SD2 and SD3 indicate the 1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates, respectively. For each sowing date, 3 sowing rates were used: low (LR; 0.25 g seed/m^2), intermediate (IR; 0.75 g seed/m^2) and high (HR; 1.25 g seed/m^2).

For the 3rd sowing date, peak plant emergence was detected 15 DAS, with plant densities of 157, 197 and 210 plants/m^2 for LR, IR and HR, respectively ($P<0.05$). During the next 15 days, plant density decreased rapidly and then remained relatively stable until the end of the experiment, when plant numbers were 108, 131 and 99 plants/m^2 for LR, IR and HR, respectively.

While all plots contained some non-*T. crinita* plants, with monocots more abundant than dicots (Figure 4), Control plots had higher frequency of these plants ($P<0.05$) than plots where *T. crinita* was sown (Table 1; Figure 4). The most frequent grasses were: *Aristida adscensionis, Digitaria californica, Cenchrus ciliaris, Pappophorum caespitosum, Chloris virgata, Sporobolus pyramidatus and Setaria leucopila*, whereas the main broad-leaf species were: *Flaveria bidentis, Gomphrena tomentosa, Allionia incarnata* and *Solanum elaeagnifolium*.

Production of tillers

For *T. crinita*, while tiller density at the end of the study was not significantly affected by sowing rate on any sowing date ($P>0.05$), when data for different sowing rates were combined within sowing dates, tiller numbers were greater for the first sowing date than for the subsequent sowing dates (9.8 vs. 7.3 and 7.1 tillers/plant for progressive sowings) (Table 1; Figure 5; $P=0.105$). Production of tillers throughout the growing

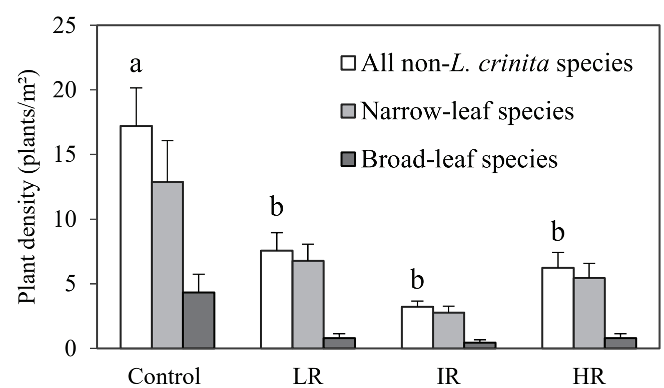

Figure 4. Plant density (plants/m^2) for non-*Trichloris crinita* species at the end of the growing season for Control (0 g seed/m^2) and the sowing rates low (LR; 0.25 g seed/m^2), intermediate (IR; 0.75 g seed/m^2) and high (HR; 1.25 g seed/m^2). Columns represent mean value ± standard error. Columns for all non-*T. crinita* species with the same letter are not significantly different at $P<0.05$.

season revealed that, despite the lack of significant variation among sowing rate treatments at the end of the experiment, there was a tendency for higher tillers per plant (TPP) in the IR treatment than in LR and HR treatments for the 3 sowing dates (Supplementary Figure S1). For the 1st sowing date, mean TPP remained at 1 during the first 60 DAS, then increased steadily to reach an overall mean of 9.8 TPP at the end of the experiment. TPP for the IR treatment was higher than for LR and HR treatments during most of the growing season (Supplementary Figure S1 A). In general, similar TPP variation patterns were observed for all sowing dates, with different sowing rate treatments not varying much from each other, except for a tendency in favor of IR for the early part of the growing season (Supplementary Figures S1 B and C).

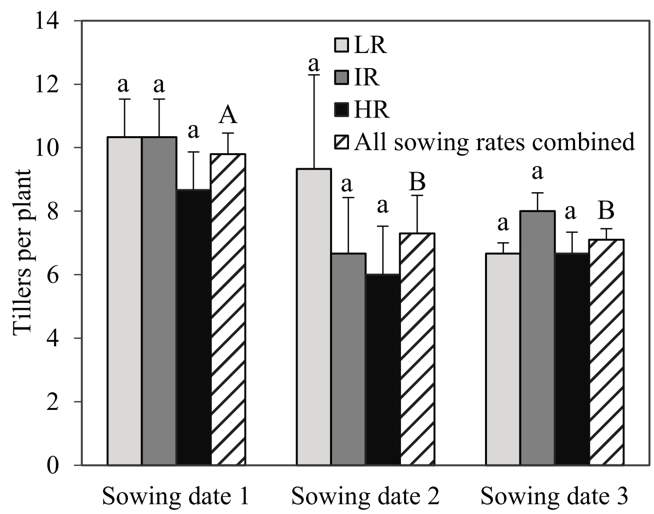

Figure 5. Tillers per plant in *T. crinita* at the end of the growing season, as influenced by sowing date and sowing rate. Low (LR; 0.25 g seed/m^2), intermediate (IR; 0.75 g seed/m^2) and high sowing rates (HR; 1.25 g seed/m^2) were used on the 1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates. Columns represent mean value ± standard error. Lower-case letters indicate differences among the different combinations of sowing dates and rates, while upper-case letters indicate differences among sowing dates combining all sowing rates. Columns with the same letter are not significantly different (P>0.05).

Plant height

Plant height was significantly influenced by sowing date (P<0.0001) but not by sowing rate (Table 1). Overall, *T. crinita* plants sown earliest (1st date) were 62–75% taller than those sown on the 2nd and 3rd dates (Figure 6). The time-course variation for this trait, as influenced by sowing date and rate, is presented in Supplementary Figure S2. Variation among the sowing rate treatments was evident for the 1st sowing date, with IR presenting the tallest plants, followed by HR and LR (Supplementary Figure S2 A), whereas no clear differences among the sowing rate treatments were observed for the 2nd and 3rd sowing dates (Supplementary Figures S2 B and C).

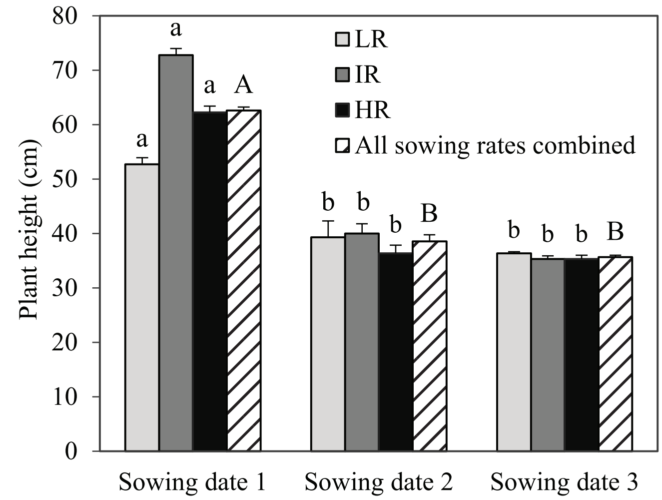

Figure 6. Height of *Trichloris crinita* plants at the end of the growing season for each sowing date and rate. Low (LR; 0.25 g seed/m^2), intermediate (IR; 0.75 g seed/m^2) and high sowing rates (HR; 1.25 g seed/m^2) were used on the1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates. Columns represent mean value ± standard error. Lower-case letters indicate differences among the different combinations of sowing dates and rates, while upper-case letters indicate overall differences between sowing dates. Columns with the same letter are not significantly different (P>0.05).

Vegetation cover

The percentage of soil coverage was significantly affected by sowing rate (P<0.0001), sowing date (P=0.0214) and their interaction (P=0.0094) (Table 1; Figure 7). Soil coverage was highest in the IR treatment at all sowing dates but differences were significant for only the 1st (84.6% coverage) and 3rd (77.3% coverage) sowing dates. The lowest soil coverage was observed in the LR plots sown early in the season.

Percent of flowering plants

The percentage of flowering plants of *T. crinita* at the end of the growing season was affected by sowing date (P=0.0001) but not sowing rate (Table 1). Early-sown plots (1st date) had significantly higher percentage of flowering plants (75–88%) than plots sown on the 2nd (32–37%) and 3rd (40–48%) dates, regardless of the sowing rates used (Figure 8).

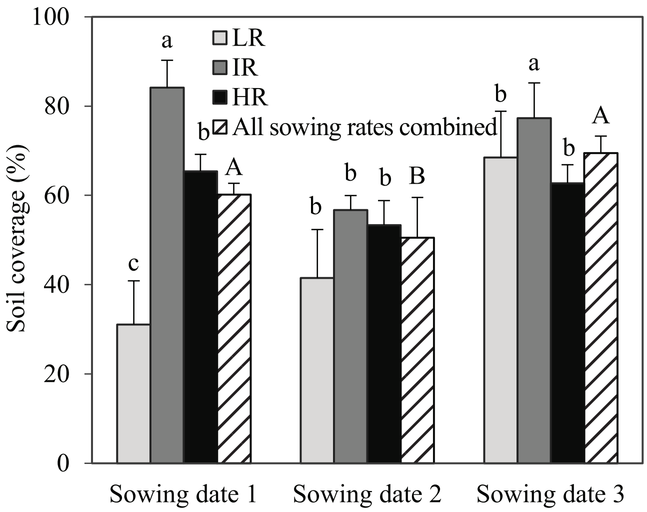

Figure 7. **S**oil coverage (%) of established *Trichloris crinita* plants at the end of the growing season for each sowing date and rate. Low (LR; 0.25 g seed/m^2), intermediate (IR; 0.75 g seed/m^2) and high sowing rates (HR; 1.25 g seed/m^2) were used on the 1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates. Columns represent mean value ± standard error. Lower-case letters indicate differences among the different combinations of sowing dates and rates, and upper-case letters indicate overall differences between sowing dates. Columns with the same letter are not significantly different (P>0.05).

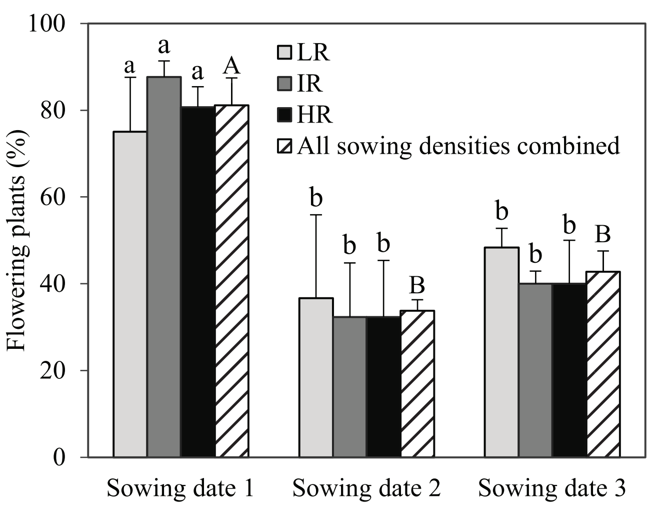

Figure 8. Percentage of *Trichloris crinita* plants flowering at the end of the growing season for each sowing date and rate. Low (LR; 0.25 g seed/m^2), intermediate (IR; 0.75 g seed/m^2) and high sowing rates (HR; 1.25 g seed/m^2) were used on 1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates. Columns represent mean value ± standard error. Lower-case letters indicate differences among the different combinations of sowing dates and rates, and upper-case letters indicate overall differences between sowing dates. Columns with the same letter are not significantly different (P>0.05).

Discussion

To the best of our knowledge the present study examined, for the first time, the effects of sowing rate and date on establishment of *T. crinita* pastures. Although *T. crinita* has been used for decades, as forage and for revegetation purposes, it was not until recently that cultivars of this species were developed and seed was made available in sufficient quantities for widespread use. To date, 6 *T. crinita* cultivars can be found at the Argentinian Cultivar Registry of the National Institute of Seeds (INASE). Thus, the use of a reliable and genetically-certified seed source of *T. crinita* cv. Chamical-INTA, the cultivar used in this study, provides confidence in the genetic homogeneity of the plant material used in this study.

Effects of sowing rate

The plots in which intermediate sowing rates (IR) were used had higher plant density and soil coverage at the end of the growing season than plots of low (LR) and high sowing rates (HR) and this was consistent for all sowing dates (Figures 2 and 7). Competition among individuals may explain the effects of sowing rate on final plant density in the experimental plots. According to Harper (1977), Burton et al. (2006) and Brooker et al. (2008), the density of established plants increases with sowing rate up to a maximum, which depends on the characteristics of the crop and its interaction with the environment, and then decreases due to excessive competition for resources among the plants. This behavior was clearly observed throughout the growing season for all sowing rates and dates analyzed (Figure 3). In other words, all treatments revealed an initial peak in plant density followed by a decrease, presumably due to the high competition among the plantlets, but the IR treatment showed a much higher initial peak than the LR treatment, and a more gradual decline after the peak than the HR treatment, resulting in the IR treatment having the highest density of established plants and highest soil coverage at the end of the experiment. Thus, in terms of density of a *T. crinita* pasture under agroecological conditions similar to those used in the present study, our results suggest an optimum sowing rate of 0.75 g seed/m^2, which translates to 7.5 kg seed/ha.

The present study was conducted during the growing season (from spring to autumn), in which implantation and establishment of the pasture takes place. After the non-vegetative winter period, and re-sprouting of the plants in spring, we evaluated plant density, considering only those plants that were visually sprouted and had resumed their vegetative growth; these densities were similar to those recorded at the end of the previous season (data not presented). This suggests that at the end of the first growing season the pasture was fully established.

A significant negative relationship was found between the density of established *T. crinita* plants and the density of all other (non-*T. crinita*) plants [the Pearson's correlation coefficient (r) value was -0.66; P<0.0001], which probably resulted from the natural seed bank in the soil and included other grasses and broad-leaf species. IR and Control plots had the lowest and highest density of non-*T. crinita* plants, respectively (Figure 4). The number of non-*T. crinita* plants in Control plots increased slowly and gradually during the growing season, whereas in plots sown with *T. crinita* the number of plants of other species did not vary throughout the experiment (data not presented). Altogether, these data clearly reflect the competitive pressure of *T. crinita* plants on other native grasses and dicots in determining the final pasture composition of plant species. This is in agreement with Quiroga et al. (2009) and Blanco et al. (2013), who proposed that the sowing of seeds in semi-arid environments can effectively increase the plant density of desirable species in the short term. In the particular case of *T. crinita*, sowing of this species in dry regions of South America, where much of the land is extensively degraded, may contribute to the recovery of the ground cover and increase forage grass availability.

Effects of sowing date

Sowing date had significant effects, although smaller than those of sowing rate (Table 1), on plant density of *T. crinita* at the end of the growing season, with the latest sowing date presenting higher overall mean plant numbers (for all densities combined) than the earlier sowing dates (Figure 2). These results are likely due to the more favorable (lower) temperatures and higher rainfall received during the establishment phase of the plantlets after the 3rd sowing date, as compared with the warmer and drier conditions affecting plantlets from the 1st and 2nd dates (Figure 1).

As was to be expected, vegetative growth parameters of individual plants, such as plant height and number of tillers per plant (TPP), as well as the percentage of flowering plants at the end of the growing season, were strongly influenced by sowing date. The greater plant heights in plots from the earliest sowing date, regardless of sowing rate, were likely due to the longer vegetative growth period of these early-sown plants, as compared with those sown later in the season, as evidenced from comparisons of the time-course variation for this trait among different sowing dates (Supplementary Figure S2). Similarly, overall production of tillers per plant was significantly higher for plots sown on the 1st date than for plots sown on the 2nd and 3rd dates (Figure 5), and monitoring of this trait revealed similar variation in tillering as for plant height (Supplementary Figure S1). Thus, a significant positive correlation (r=0.66; P=0.0002) was observed between *T. crinita* plant height and TPP. This positive association is not surprising, as both traits are considered sub-components of the same overall vegetative growth.

The strong effect of sowing date on percentage of plants of *T. crinita* flowering at the end of the growing season, with the early planting presenting, on average, a 0.9–1.4-fold increase compared with later sowings, was likely a function of plants from the earlier sowing being more mature following a longer growth period, thereby allowing a larger number of plants to reach the reproductive stage, as compared with plants sown later. Flowering is affected by a combination of physiological age and day length, and older plants experienced a longer growth period and more hours of day-length than younger plants.

Influence of climatic factors

Mean annual rainfall in the Arid Chaco region varies from 450 mm in the east to 200 mm in the west, with 80% occurring between November and March (Morello et al. 1985). Thus, the climatic conditions in the present study were rather atypical for this region, given that 548 mm of rainfall occurred during the experiment (Figure 1). For this reason, the results of the present study are suitable for seasons and/or regions with this amount of rainfall. Therefore, there is a need to verify the results of this experiment in other seasons with different water conditions.

From the beginning of the experiment until 6 January 2015, when the highest plant density for the 1st sowing date was reached, a total of 219 mm of rainfall was received, representing 40% of the total rainfall received. This provided plants from the 1st sowing date with adequate soil moisture in the early stages and throughout the growing season.

January was the warmest month, presenting the highest monthly mean temperature (27.4 °C) (Figure 1). This may explain why seedlings from the 2nd sowing date (22 December 2014) did not emerge until the first week of February, i.e. 45 DAS, which coincided with the emergence of seedlings from the 3rd sowing date (Figure 3). January also coincided with the beginning of the decline in plant density for the 1st sowing date. This suggests that the abundant rainfall received before January favored the initial establishment of seedlings from the first sowing, whereas the subsequent high temperatures in January were less favorable for seedling establishment and general plant growth, i.e. plant height and TPP, in plots from the 2nd and 3rd sowing dates. Nonetheless, comparably higher plant density and soil coverage were observed in plots from the 3rd sowing date (Figures 2 and 7), presumably due to the lower temperatures and rainfall received in this period (Figure 1).

Conclusions

To the best of our knowledge, this study is the first to document the effects of sowing date and sowing rate on the establishment and plant growth parameters of *Trichloris crinita*, a good-quality pasture grass native to arid regions. Results showed that, under the environmental and management conditions of the present work, sowing date significantly influenced plant density and soil coverage at the end of the growing season, with the latest (3rd) sowing date increasing both parameters. On the other hand, sowing date had the opposite effect on individual plant growth-related parameters evaluated, with the earliest (1st) sowing resulting in greater production of tillers, plant height and percentage of flowering plants at the end of the season. Since the study terminated with the first frost, earlier flowering from the first sowing should ensure more seed was produced to support plant populations over the subsequent year. Sowing rate had a strong significant effect on the final plant density of the pasture (and therefore on soil coverage) but not on individual plant growth parameters, with intermediate sowing densities yielding the densest plant stands at the end of the growing season. Thus, using ~0.75 g seed per m^2 (7.5 kg/ha) and sowing early in the season, when temperatures were still mild, delivered the best results in terms of pasture density and establishment efficacy, which is likely due to the more favorable initial growing conditions in which the plants were able to take advantage of available water, nutrients and light resources and develop sufficiently to combat later periods of high temperatures. We used aerial photos to assess ground cover, i.e. area of canopy was the criterion. A more effective assessment of amount of soil coverage is the measurement of basal area of the stand and this method should be utilized as well in future studies. In addition, longer-term studies are warranted to determine the survival of the species over time and its contribution to the pasture in terms of plant numbers and dry matter yields. Since the rainfall received during the study was at the top end of the range of annual precipitation expected in the region, further studies are needed to determine the outcome of sowings where more typical rainfall patterns and amounts were received.

Acknowledgments

The authors acknowledge the National University of La Rioja (UNLaR) ‘Sede Chepes’ for providing the necessary infrastructure for the development of the experimental work, and the National Institute of Agricultural Technology (INTA) E.E.A. La Rioja, for providing seed of *Trichloris crinita* cv. Chamical-INTA.

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(Note of the editors: All hyperlinks were verified 14 July 2021).

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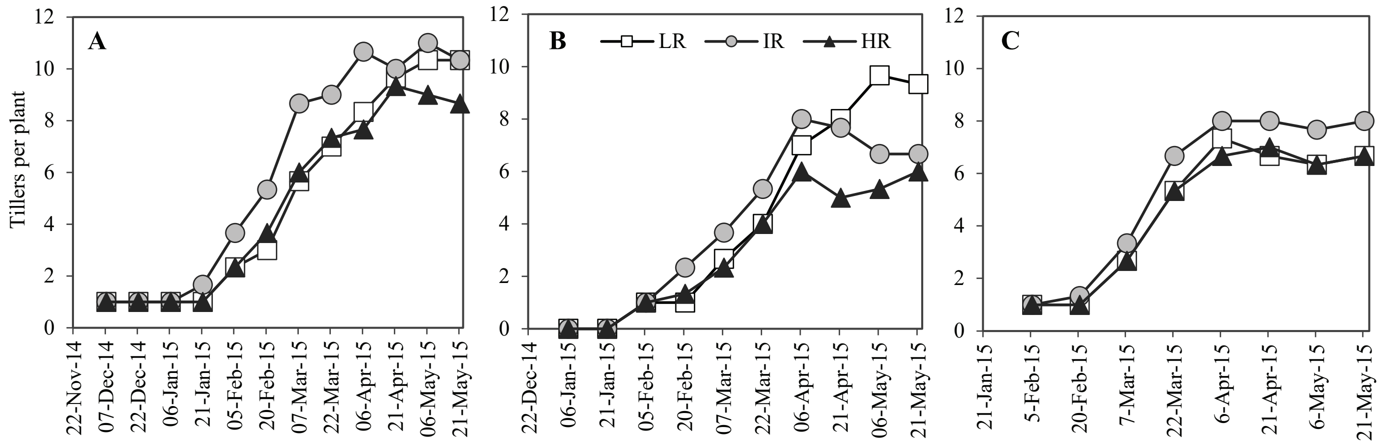
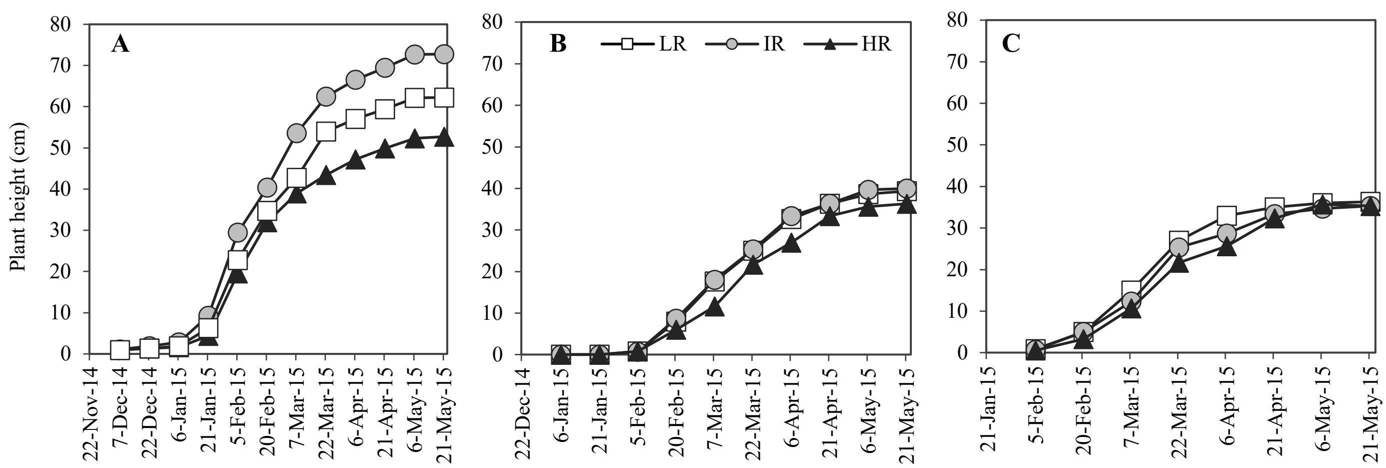
Supplementary Data



Supplementary Figure S1. Time-course variation of the mean number of tillers per plant produced by Trichloris crinita plants during the growing season, for the 1st (22 November 2014) (A), 2nd (22 December 2014) (B), and 3rd (21 January 2015) (C) sowing dates. Data points for low sowing rate (LR, 0. 25 g seed/m^2) are indicated by clear squares, intermediate sowing rate (IR, 0.75 g seed/m^2) by gray circles and high sowing rate (HR, 1.25 g seed/m^2) by black triangles.



Supplementary Figure S2. Time-course variation for mean plant height of *Trichloris crinita* during the growing season for the 1st (22 November 2014) (A), 2nd (22 December 2014) (B), and 3rd (21 January 2015) (C) sowing dates. Data points for low sowing rate (LR, 0. 25 g seed/m^2) are indicated by clear squares, intermediate sowing rate (IR, 0.75 g seed/m^2) by gray circles and high sowing rate (HR, 1.25 g seed/m^2) by black triangles.

(Received for publication 19 October 2020; accepted 6 July 2021; published 30 September 2021)

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*

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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.*

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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

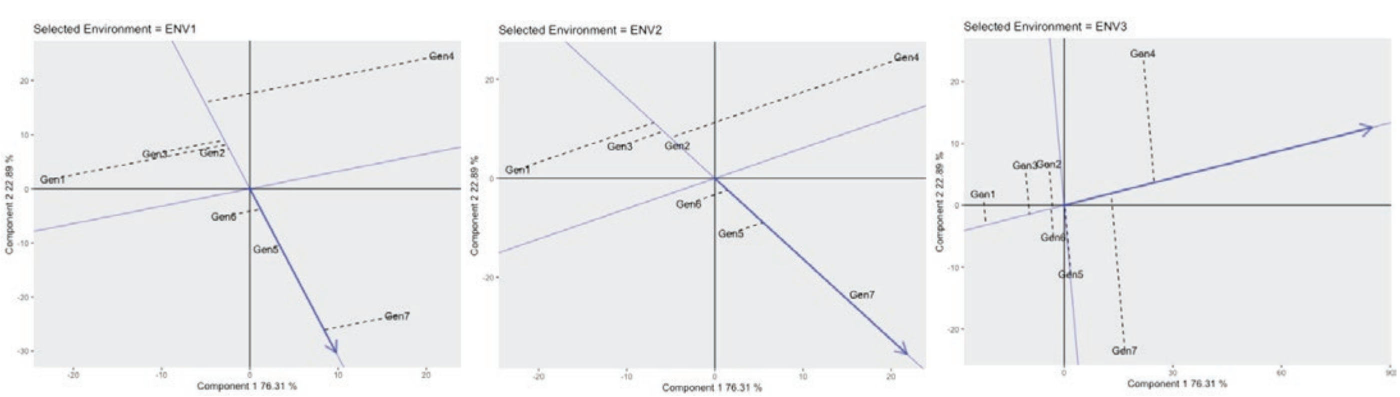

Figure 1. Dry biomass productivity GGEplot representation of the genotypes of *T. diversifolia* in the three environments.

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			

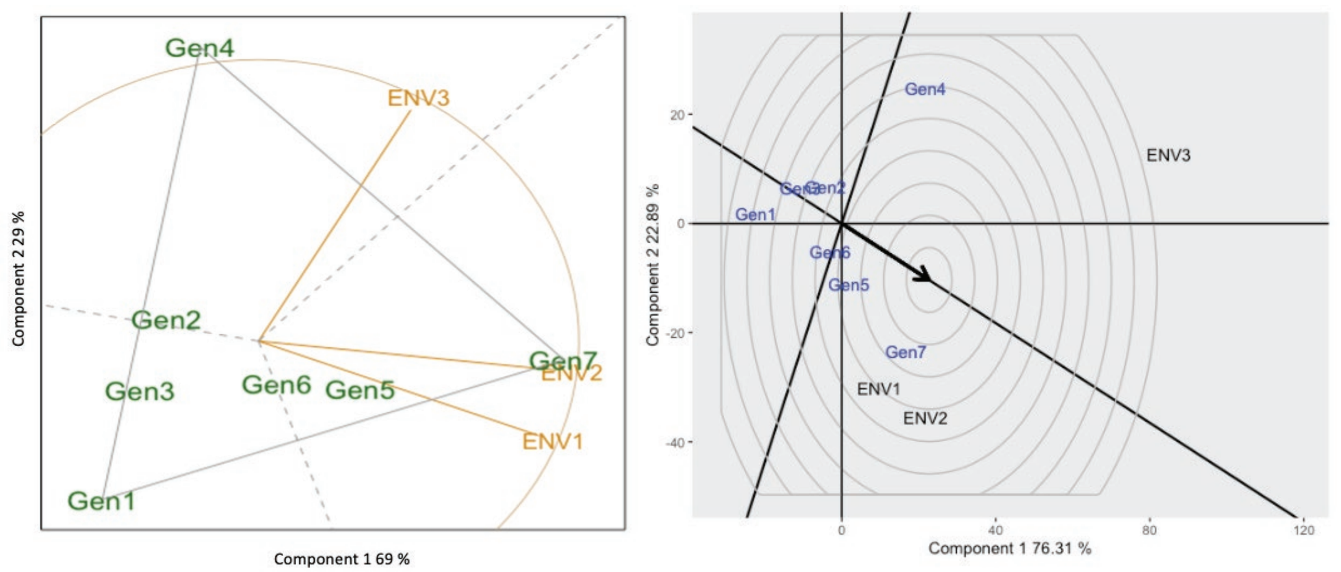

Figure 2. GGEplot dry matter yield of the *T. diversifolia* genotypes (left). Genotypes ranking with respect to the ideal genotype (right)

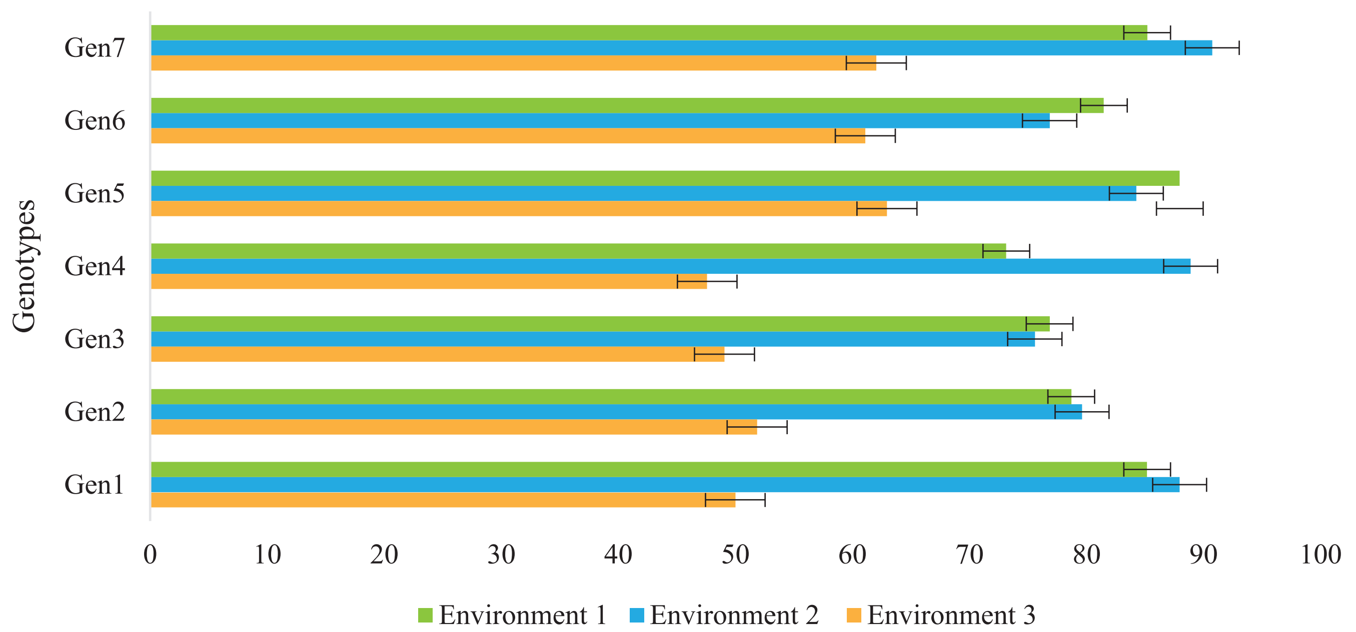

Figure 3. *Tithonia diversifolia* survival rate (%) in three environments in two experimental sites.

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.

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

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

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(3):292–299
doi: 10.17138/TGFT(9)292-299

Research Paper

Evaluation of ten perennial forage grasses for biomass and nutritional quality

Evaluación de biomasa y calidad nutricional en diez gramíneas forrajeras perennes

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Abstract

A study was carried out to evaluate 10 perennial forage grass accessions from 4 species for herbage dry matter yield and nutritional quality at Holetta Agricultural Research Center. The evaluated grass species and varieties were one Desho grass (*Pennisetum*) variety Kulumsa, four *Urochloa decumbens* (ILRI-14721, ILRI-14720, ILRI-13205 and ILRI-10871), three *Urochloa ruziziensis* (ILRI-14813, ILRI-14774 and ILRI-13332) and two *Setaria sphacelata* (ILRI-143 and ILRI-6543) accessions. Plant height and forage dry matter yield were significantly affected by accession over years, during the establishment and production phases. Combined analysis indicated that the tested accessions varied significantly for plant height with the *Setaria* accessions taller than the other tested species. Combined data analysis revealed that forage dry matter yield significantly varied according to species and Desho grass (variety Kulumsa) was higher in dry matter yield than the other grasses tested. Fiber contents (NDF, ADF and ADL) were significantly influenced by accession. Crude protein yield differed among the accessions and Desho grass had higher crude protein, followed by *U. decumbens* 13205, *U. decumbens* 14721 and *S. sphacelata* 6543. Based on dry matter yield and crude protein *U. decumbens* 13205, *U. ruziziensis* 13332, *S. sphacelata* 6543 and Desho grass (var. Kulumsa) are recommended as alternative forage grasses for the study area and similar agro-ecologies.

Keywords: Desho grass, forage yield, *Urochloa*, *Setaria*, crude protein.

Resumen

Se llevó a cabo un estudio para evaluar 10 accesiones de gramíneas forrajeras perennes de 4 especies para determinar el rendimiento de materia seca y la calidad nutricional del forraje en el Centro de Investigación Agrícola de Holetta. Las especies y variedades de gramíneas evaluadas fueron una pasto Desho (*Pennisetum*) variedad Kulumsa, cuatro accesiones de *Urochloa decumbens* (ILRI-14721, ILRI-14720, ILRI-13205 e ILRI-10871), tres de *Urochloa ruziziensis* (ILRI-14813, ILRI-14774 e ILRI -13332) y dos de *Setaria sphacelata* (ILRI-143 e ILRI-6543). La altura de la planta y el rendimiento de materia seca del forraje se vieron afectados significativamente por la accesión a lo largo de los años, durante las fases de establecimiento y producción. El análisis combinado indicó que las accesiones probadas variaron significativamente la altura de la planta en las accesiones de *Setaria*, siendo más altas que las otras especies probadas. El análisis de datos combinados reveló que el rendimiento de materia seca del forraje varió significativamente según la especie y el pasto Desho (variedad Kulumsa) fue mayor en rendimiento de materia seca que los otros pastos evaluados. El contenido de fibra (NDF, ADF y ADL) se vio significativamente influenciado en cada accesión. En cuanto a rendimiento de proteína cruda el pasto Desho fue el mayor, seguido por *U. decumbens* 13205, *U. decumbens* 14721 y *S. sphacelata* 6543. Basado en el rendimiento de materia seca y proteína cruda *U. decumbens* 13205, *U. ruziziensis*

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13332, *S. sphacelata* 6543) y pasto Desho (var. Kulumsa) se recomiendan como pastos forrajeros alternativos para el área de estudio y condiciones agroecológicas similares.

Palabras clave: proteína cruda, *Pennisetum,* rendimiento forrajero, *Setaria*, *Urochloa*.

Introduction

The central highland of Ethiopia is characterized by a crop-livestock mixed farming system. Livestock is an integral component of most of the agricultural activities in the country. The share of the livestock subsector in the national economy is estimated to be 12–16% of the total Gross Domestic Product (GDP), 30–35% of the agricultural GDP (LMA 1999), 19% of the export earnings (FAO 2004) and 31% of the total employment (Feleke 2003). Although Ethiopia has a large livestock population (CSA 2016), the productivity of livestock is low with the major hindrances being shortage of feed resources in terms of quantity and quality (Demeke et al. 2017). To combat these nutritional constraints, the use of locally available and introduced forage species adapted to the local agro-ecological conditions is recommended. The cultivation of high quality forages with high herbage yield and adaptability to biotic and abiotic environmental stresses is one of the options to increase livestock production under smallholder farmer conditions (Tessema 2005). The introduction of promising improved forage crops like *Urochloa, Setaria* and Desho grass is an advocated strategy to alleviate the prevailing feed crisis in the country.

Most of the *Urochloa* (previously named as *Brachiaria*) species and varieties that have been developed are resistant to Napier grass stunt and smut disease affecting Napier grass varieties in Eastern Africa (Ghimire et al. 2015; Maass et al.2015). *Urochloa* is well adapted to low-fertility soils and diseases. It withstands heavy grazing and sequesters carbon through its large root system with enhanced nitrogen use efficiency and minimized greenhouse gas emissions (Arango et al. 2014; Moreta et al. 2014). *Urochloa decumbens* (Stapf) R. D. Webster (previously named as *Brachiaria decumbens* Stapf) is reported to be drought resistant and resilient when grown on infertile soils, producing high herbage yields with relatively low levels of fertilizer inputs. *U. ruziziensis* (R. Germ. & C. M. Evrard) Crins [previously named as *Brachiaria ruziziensis* (R. Germ. & C. M. Evrard)] plays an important role in soil erosion control and ecological restoration. The grass has high dry matter yield potential (Rodrigues et al. 2014). *U. ruziziensis* also produces abundant roots which contribute to the collection of water, soil aggregation and aeration (Kluthcouski et al. 2004). Recent studies indicate that adoption of *U. brizantha* (Hochst. ex A. Rich.) R. D. Webster [previously named as *Brachiaria brizantha* (Hochst. ex A. Rich.)] cultivars as cut-and-carry fodder for dairy cattle have increased milk production on participating farms in Kenya by 15–40% (Schiek et al. 2018). Similarly, use of the *Urochloa* hybrid Mulato II fodder in dairy and beef enterprises in Rwanda enabled a 30% increase in milk production and a 20% increase in meat production (CSB 2016).

Setaria sphacelata (Schumach.) Stapf & C. E. Hubb. is a perennial C_4 grass, which can produce more than 20 t DM/ha annually (Taylor et al. 1976; Sithamparanathan 1979). It has been recommended for use in tropical and subtropical countries with a minimum yearly rainfall of 750 mm or 580 mm on very fertile soils (Botha 1948). However, it grows better in wetter areas with no prolonged dry season (Rattray 1960). *S. sphacelata* has the desirable forage attributes of high yield (Singh et al. 1995), high crude protein concentration (de Almeida and Flaresso 1991) and good animal performance (Jones and Evans 1989).

Desho [*Cenchrus glaucifolius* (Hochst. ex A. Rich.) Rudov & Akhani] formally known as *Pennisteum glaucifolium* Hochst. ex A. Rich. is a perennial grass and is palatable to cattle, sheep and other herbivores (FAO 2010). Desho grass serves as a business opportunity for farmers in Ethiopia (Shiferaw et al. 2011; Tilahun et al. 2017). According to Lukuyu et al. (2011), it is very important to have chemical composition and utilization information of locally available feeds for their inclusion into livestock feeding programs. Despite their significant potential for forage production, there is little research on the comparative advantage of producing Desho, *Urochloa* and Setaria species in the central highlands of Ethiopia. The present study aimed to evaluate the performance of *Urochloa, Setaria* and Desho grass species and varieties and recommend the best ones with combined attributes of high herbage yield and quality for wider distribution among livestock producer communities in the Ethiopian highlands.

Materials and Methods

Description of the study area

The experiment was conducted at the Holetta Agricultural Research Center (HARC), Ethiopia, during the main cropping seasons of 2014 to 2019 under rain-fed conditions. HARC is located at 9°00'N latitude and 38°30'E longitude at an altitude of 2,400 m.a.s.l. It is characterized by a long term (30 years) average annual rainfall of 1,055 mm, average relative humidity of 60.6%, and average maximum and minimum air temperature of 22.2°C and 6.1°C, respectively. Rainfall is bimodal and about 70% of the precipitation falls in the period from June to September while the remaining thirty percent falls in the period from March to May (EIAR 2005). The soil type of the area (Table 1) is predominantly red Nitosol (Keneni 2007).

Experimental treatments and design

The study involved ten perennial forage grass species and varieties (Table 2). Seeds of the *Urochloa* and *Setaria* species were obtained from the International Livestock Research Institute (ILRI), while Desho grass was obtained from the Debre Zeit Research Center (DZARC). The experiment was conducted as a Randomized Complete Block Design (RCBD) with three replications. The experimental fields were ploughed and harrowed to a fine seedbed. The seeds were grown in a nursery and vegetative parts in the form of root splits from mature plants were used for planting which was accomplished at the beginning of the main rainy season (in mid-June). Plot size was 7.2 m^2 (3x 2.4m). The root splits were planted with the intra and inter row spacing of 0.25 m and 0.5 m respectively. The spacing between plots and blocks was 1.5 m. Phosphorus fertilizer was uniformly applied to all plots at planting in the form of diammonium phosphate (DAP, 18% N, 20% P, 1.5% S) at the rate of 100 kg/ha. After every harvest, the plots were top dressed with 100 kg urea (46% N)/ha of which one-third was applied at the first shower of rain (in May) and the remaining two-thirds applied during the active growth stage of the plant, during the mid-growing season (July–August).

Table 1. Properties of soils in the study area

Parameter	Values	Method of Analysis
pH (1:2.5 H_2O)	4.94	Potentiometric method
Organic carbon (%)	1.79	Dichromate oxidation method (Walkley and Black 1934)
Total nitrogen (%)	0.20	Kjeldhal method (Jackson 1958)
Available P (ppm)	5.60	Olsen method (Olsen et al. 1954)
CEC (meq/100 g)	18.24	NH_4OAc method (pH=7)
Na^+ (meq/100 g)	0.16	NH_4OAc method (Okalebo et al. 1993)
K^+ (meq/100 g)	5.03	NH_4OAc method (Okalebo et al. 1993)
$Ca2^+$ (meq/100 g)	29.50	NH_4OAc method (Okalebo et al. 1993)
$Mg2^+$ (meq/100 g)	13.75	NH_4OAc method (Okalebo et al. 1993)
P(mg kg^{-1})	5.6	NH_4OAc method (Okalebo et al. 1993)
Texture		
Sand (%)	18	Bouyoucos hydrometric method
Silt (%)	15	Bouyoucos hydrometric method
Clay (%)	67	Bouyoucos hydrometric method

Source: Holetta Agricultural Research Center meteorological data report

Table 2. Evaluated grass species

Species	Common name	Accession	Country of origin
Cenchrus glaucifolius (Hochst. ex A. Rich.) Rudov & Akhani)	Desho grass	Kulumsa	Ethiopia
Setaria sphacelata (Schumach.) Stapf & C. E. Hubb.	common setaria	ILRI-143= cv. Kazungula	Zambia
Setaria sphacelata (Schumach.) Stapf & C. E. Hubb.	common setaria	ILRI-6543= cv. Narok	Kenya
Urochloa decumbens (Stapf) R. D. Webster	signal grass	ILRI-10871 = cv. Basilisk	Uganda
Urochloa decumbens (Stapf) R. D. Webster	signal grass	ILRI-13205	Kenya
Urochloa decumbens (Stapf) R. D. Webster	signal grass	ILRI-14720	Rwanda
Urochloa decumbens (Stapf) R. D. Webster	signal grass	ILRI-14721	Rwanda
Urochloa ruziziensis (R. Germ. & C. M. Evrard) Crins	ruzi grass	ILRI-13332	unknown
Urochloa ruziziensis (R. Germ. & C. M. Evrard) Crins	ruzi grass	ILRI-14774	Burundi
Urochloa ruziziensis (R. Germ. & C. M. Evrard) Crins	ruzi grass	ILRI-14813	Burundi

Data collection

This experiment involved two phases, namely establishment (in mid June 2014) and productive phases (2015–2019). Data were collected on vigor, plant height at harvesting and forage dry matter yield. Plant vigor was recorded during the establishment phase (mid June 2014–June 2015) on a scale from 1–5 and converted to a percentage. Plant height was measured from the ground to the highest leaf at the time of forage harvesting stage. Plant height and number of tillers per plant were recorded from 6 randomly selected plants from the whole plot. For the determination of biomass yield, Setaria accessions were harvested at 10% flowering stage using a quadrat measuring 3 * 2.4 m^2 (7.2 m^2) areas. Desho and *Urochloa* were harvested at >40cm before flowering, the height of cutting determined by previous studies. The plot was cut twice per year in May–June and October–November. Weight of the total fresh biomass yield was recorded from each plot in the field and a 500 g sub-sample was taken from each plot to the laboratory to determine dry matter yield. Sub-samples were oven dried at 65°C for 72 hours. The oven dried samples were ground to pass through a 1 mm sieve for laboratory analysis. Before scanning, the samples were dried at 60 °C overnight in an oven to standardize the moisture and then 3 g of each sample was scanned by Near Infra-Red Spectroscopy (NIRS). Percentage dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and *in-vitro* dry matter digestibility (IVDMD) were predicted using a calibrated NIRS (Foss 5000 apparatus and WinISI II software).

Statistical analysis

The analysis of variance (ANOVA) procedure of the SAS general linear model (GLM) (ersion 9.4 was used to analyse the quantitative data (SAS 2002). The LSD test at 5% significance was used for comparison of means.

Results

Plant vigor and height

The result of the analysis indicated that vigor was significantly (P<0.001) affected by species (Table 3) with a rating of more than 50% plant vigor except for *U. decumbens* 14720. The plant vigor percentage performance of the species was positively associated with the forage dry matter yield during the establishment year showing plant vigor can be a good indicator of the forage dry matter yield potential.

The result of a combined analysis during the production phase (2015–2019) showed that plant height at harvesting was significantly (P<0.001) influenced by accession (Table 3). *S. sphacelata* accessions were significantly (P<0.001) taller than the other evaluated perennial forage grass species in 2014, 2015, 2018 and 2019 experimental years but in 2018 the plant height recorded for *S. sphacelata* accessions was non-significant (P>0.05) with Desho grass (variety Kulumsa).

Dry matter yield

Forage dry matter yield was significantly (P<0.001) different for accessions over the production years (Table 4). Desho grass had significantly (P<0.001) higher forage dry matter yield than other evaluated grasses in 2014, 2015, 2016 and 2017, excluding *U. decumbens* 13205 that was not significantly (P>0.05) different from Desho grass in the 2016 production phase. In 2018 *U. decumbens* 10871, *U. decumbens* 13205 and *U. decumbens* 14721 had higher (P<0.001) forage dry matter yield than the other grasses.

The forage dry matter yield increased with production years for the first three consecutive years (2014–2016) for each evaluated grass species, exclusive of *S. sphacelata* 143 which showed a slight decrease from the first (2015) year of production to fourth year (2018) of production. However, in the third and fourth years of production all accessions showed a decrease in forage dry matter yield except *U. ruziziensis* 13332 and *U. decumbens* 10871. During the fifth (2018) year of production to the end of this experiment, all evaluated grasses showed biomass yield increase. Desho grass had higher forage dry matter yield during the establishment phase (2014) than the other grasses.

Forage chemical composition

Nutritional qualities of the perennial forage grass species evaluated at Holetta are presented in Table 5. NDF and ADF content were significantly (P<0.001) different among the accessions and *U. ruziziensis* had lower ADF and NDF content than the other grasses.

ADL was significantly (P<0.05) different among the accessions. IVDMD and crude protein percentage were not significantly (P>0.05) influenced either by species or accession.

Crude protein yield (CPY) was significantly (P<0.001) different among the accessions. Despite having the lowest CP percentage, Desho grass had higher (P<0.001) CPY than *U. ruziziensis*, *S. sphacelata* and *U. decumbens* accessions, except *U. decumbens* 14721 and *S. sphacelata* 6543.

Table 3. Vigor and plant height (cm) of perennial forage grass species at harvest.

S. No	Species	Plant height (cm) in year						Productive phase	Combined analysis	Vigor (%)
		2014	2015	2016	2017	2018	2019			
1	*U. ruziziensis* ILRI-14813	44.13^{cde}	42.23^{d}	76.20^{d}	38.87^{e}	54.19^{bc}	39.20^{d}	50.14^{d}	49.14^{e}	60.00^{cde}
2	*U. ruziziensis* ILRI-14774	34.67^{de}	49.45^{cd}	50.87^{e}	50.00^{e}	45.00^{c}	37.80^{d}	46.62^{d}	44.63^{e}	56.20^{def}
3	*U. ruziziensis* ILRI-13332	52.20^{c}	61.67^{c}	81.40^{d}	42.77^{e}	65.00^{bc}	39.73^{d}	58.11^{d}	57.13^{de}	70.00^{bc}
4	*U. decumbens* ILRI-14721	40.67^{cde}	59.93^{cd}	103.03^{bc}	112.77^{abc}	67.88^{b}	56.13^{bc}	79.95^{bc}	73.40^{c}	53.40^{def}
5	*U. decumbens* ILRI-10871	45.23^{cd}	66.67^{c}	92.37^{cd}	102.77^{abc}	68.09^{b}	60.30^{bc}	78.04^{c}	72.57^{c}	80.00^{ab}
6	*U. decumbens* ILRI-14720	29.23^{e}	54.72^{cd}	94.80^{cd}	101.13^{bcd}	59.58^{bc}	61.97^{bc}	74.44^{c}	66.90^{cd}	46.60^{f}
7	*U. decumbens* ILRI-13205	41.33^{cde}	60.55^{c}	91.77^{cd}	92.77^{d}	64.55^{bc}	54.73^{c}	72.87^{c}	67.62^{cd}	63.40^{cd}
8	*S. sphacelata* ILRI-143	119.47^{a}	130.83^{a}	119.37^{ab}	114.47^{ab}	113.22^{a}	82.53^{a}	112.08^{a}	113.31^{a}	76.60^{b}
9	*S. sphacelata* ILRI-6543	121.67^{a}	130.00^{a}	127.00^{a}	117.74^{a}	116.22^{a}	85.00^{a}	115.19^{a}	116.27^{a}	50.00^{ef}
10	Desho grass (var. Kulumsa)	89.43^{b}	91.67^{b}	105.30^{bc}	100.00^{cd}	95.47^{a}	65.57^{b}	91.60^{b}	91.24^{b}	90.00^{a}
	Mean	61.80	74.77	94.21	88.33	74.93	58.30	77.91	75.22	64.62
	CV	15.18	14.13	12.54	8.97	16.99	10.87	23.20	25.48	9.27
	Significance level	***	***	***	***	***	***	***	***	***

***=P<0.001; Means with the same letter are not significantly different.

Table 4. Dry matter yield (t/ha) per year of perennial forage grass species tested per year at Holetta from 2014 to 2019.

S. No	Species	Dry matter yield (t/ha) in each year						Productive phase	Combined analysis
		2014	2015	2016	2017	2018	2019		
1	*U. ruziziensis* ILRI-14813	2.43^{cd}	13.04^{bc}	19.11^{cde}	8.37^{d}	7.64^{de}	9.75^{cde}	11.58^{e}f	10.06^{de}
2	*U. ruziziensis* ILRI-14774	1.30^{de}	5.07^{e}	12.23^{e}	8.16^{d}	4.33f	8.08^{de}	7.56f	6.52^{e}
3	*U. ruziziensis* ILRI-13332	3.45^{c}	17.37^{bc}	24.12^{bcd}	8.70^{d}	7.62^{de}	11.53^{cde}	13.87^{de}	12.13^{cd}
4	*U. decumbens* ILRI-14721	0.96^{e}	10.92^{cde}	30.07^{ab}	23.24^{b}	17.04^{a}	19.78^{ab}	20.21^{bc}	17.00^{bc}
5	*U. decumbens* ILRI-10871	0.76^{e}	7.39^{de}	25.03^{bc}	14.93^{cd}	18.62^{a}	20.92^{a}	17.38^{bcd}	14.61^{bcd}
6	*U. decumbens* ILRI-14720	0.64^{e}	10.03^{de}	23.32^{bcd}	16.70^{bc}	12.40^{bc}	13.57^{bcd}	15.20^{cde}	12.78^{cd}
7	*U. decumbens* ILRI-13205	1.19^{de}	14.23^{bcd}	36.53^{a}	22.77^{b}	18.36^{a}	18.30^{ab}	22.04^{ab}	18.56^{b}
8	*S. sphacelata* ILRI-143	5.27^{b}	18.62^{b}	23.26^{bcd}	14.70^{cd}	9.86^{cd}	8.41^{de}	14.97^{de}	13.35^{bcd}
9	*S. sphacelata* ILRI-6543	5.74^{b}	18.41^{b}	17.19^{de}	12.20^{cd}	5.88^{e}f	7.22^{e}	12.18^{e}f	11.11^{de}
10	Desho grass (var. Kulumsa)	13.14^{a}	33.41^{a}	36.55^{a}	34.48^{a}	13.54^{b}	15.69^{abc}	26.75^{a}	24.27^{a}
	Mean	3.49	14.84	24.74	16.43	11.53	13.33	44.15	14.06
	CV	24.39	27.75	18.45	27.41	16.54	27.72	16.17	59.18
	Significance level	***	***	***	***	***	***	***	***

***=P < 0.001; Means with the same letter are not significantly different.

Table 5. Nutrient content of perennial forage grasses

S. No	Species	DM%	Ash%	NDF%	ADF%	ADL%	CP%	CPY (t/ha)	IVDMD%
1	*U. ruziziensis* ILRI-14813	91.35^{de}	17.72^{a}	61.95^{b}	29.92^{b}	4.44^{bc}	6.33	0.64^{cd}	59.81
2	*U. ruziziensis* ILRI-14774	91.37^{de}	16.72^{abc}	63.55^{b}	31.27^{b}	4.74^{abc}	6.72	0.44^{d}	59.56
3	*U. ruziziensis* ILRI-13332	91.16^{e}	17.14^{ab}	62.68^{b}	30.68^{b}	4.27^{c}	6.22	0.75^{bcd}	60.92
4	*U. decumbens* ILRI-14721	91.60^{bcd}	16.52^{abcd}	67.14^{a}	34.72^{a}	4.86^{ab}	5.57	0.95^{abc}	55.35
5	*U. decumbens* ILRI-10871	91.83^{bc}	15.25^{cd}	69.48^{a}	37.50^{a}	5.25^{a}	5.58	0.81^{bc}	55.68
6	*U. decumbens* ILRI-14720	91.47^{cde}	15.56^{bcd}	67.55^{a}	35.32^{a}	5.19^{a}	6.87	0.88^{bc}	57.16
7	*U. decumbens* ILRI-13205	91.72^{bcd}	16.26^{abcd}	68.03^{a}	35.34^{a}	4.84^{ab}	5.57	1.04^{ab}	56.26
8	*S. sphacelata* ILRI-6543	91.70^{bcd}	14.94^{d}	67.55^{a}	35.74^{a}	4.80^{abc}	6.98	0.92^{abc}	54.14
9	*S. sphacelata* ILRI-143	92.26^{a}	15.69^{bcd}	66.99^{a}	36.32^{a}	4.59^{bc}	6.96	0.77^{bc}	54.51
10	Desho grass (variety Kulumsa)	91.92^{ab}	12.92^{e}	69.29^{a}	37.64^{a}	4.48^{bc}	5.04	1.23^{a}	56.44
	Mean	91.64	15.87	66.42	34.45	4.75	6.18	0.84	56.98
	CV	0.27	5.83	2.27	4.97	7.06	13.22	59.77	4.37
	Significance level	**	***	***	***	*	ns	***	ns

DM% = Dry matter percentage; Ash% = Ash percentage; NDF% = Neutral detergent fiber percentage; ADF% = Acid detergent fiber percentage; ADL% = Acid detergent lignin percentage; CP% = Crude protein percentage; CPY = Crude protein yield; IVDMD = In-vitro dry matter digestibility; ** = P<0.01; * = P<0.05; *** = P<0.001; ns = non-significant; Means with the same letter are not significantly different.

Discussion

Desho and *Setaria* showed better vigor than *Urochloa*, suggesting that Desho and *Setaria* were faster to establish and had superior competition against the weeds than *Urochloa* species especially in the establishment phase. This can be an important characteristic to establish these forages on soil bunds for soil conservation in the livestock-crop mixed area. Soil bunds are available for free grazing during the non-cropping season and these grasses can tolerate the grazing due to their fast establishment characteristics. *S. sphacelata* accessions and Desho grass were taller during the establishment year, possibly due to the morphological vertical growth characteristics of the species and plant vigor. Plant height differences can be attributed to the morphological and physiological differences among the cultivars (Nguku 2015), Setaria having different morphology to the *Urochloa* accessions.

U. ruziziensis accessions provided the highest forage dry matter yield in the establishment phase, suggesting that this species is fast growing and more easily established than *U. decumbens* accessions. During the production phase, Desho grass produced significantly more forage dry matter yield than other evaluated grass species. This implies Desho grass is more adaptable to Nitosol and cold air conditions than *U. ruziziensis*, *U. decumbens* and *S. sphacelata* grasses. The *Urochloa* accessions are true tropical plants and their growth almost stops when temperatures drop below about 20 °C. *Setaria* is more subtropical and will continue to grow at much lower temperatures than the *Urochloa* accessions. Forage dry matter yield increased with production years for the first three consecutive years due to climatic variation (rainfall pattern, temperature, frost). Desho grass had the highest forage dry matter yield and more vigorous growth that resulted in the well-established root system that enabled the grass to extract better growth resources from the soil.

Although differences were seen in nutrient content, all grasses studied were low quality. This may be the result of harvesting when over mature with only two harvests per year. Farmer practices of harvesting were followed in the experiment to reflect the local feeding situation. Grasses are usually harvested after 6 to 8 weeks of growth to obtain better quality feed. In this study, forage materials from all the grass species had greater than 60% NDF which may result in low intake and digestibility in livestock. McDonald et al. (2002) reported that the primary chemical composition of feeds that determines the rate of digestion is NDF, which is itself a measure of cell-wall content; thus there is a negative relationship between the NDF content of feeds and the rate at which they are digested. Schroeder et al. (2012) also reported that as NDF percentages increase, dry-matter intake generally will decrease. *U. ruziziensis* accessions had ADF above the minimum recommended value (17–21 percent) for NRC (2001). This result suggests that *U. ruziziensis* species will have moderate digestibility compared to the other grasses evaluated in this experiment. Nussio et al. (1998) reported that forage with ADF content around 40% or more, shows low intake and digestibility. In this study forage materials from all the grass species had low CP below the 7% CP required for microbial protein synthesis in the rumen that can support at least the maintenance requirement of ruminants (Van Soest, 1994). IVDMD levels were low and Mugeriwa et al. (1973) reported that the IVDMD values greater than 65% indicate good feeding value and values below this threshold level result in reduced intake due to lowered digestibility.

Conclusions

Based on dry matter yield and crude protein yield data, *U. decumbens* 13205, *U. ruziziensis* 13332, *S. sphacelata* 6543 and Desho grass (variety Kulumsa) are recommended for the study area and similar agro-ecologies as alternative forage grasses.

Acknowledgments

The funds for this study were granted by the Ethiopian Institute of Agricultural Research. We are grateful to the technical and field assistants of the forage and pasture research program, Holetta Agricultural Research Center for data collection. We thank also the Holetta laboratory technicians and researchers working in animal nutrition for the laboratory analysis.

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(Note of the editors: All hyperlinks were verified 8 September 2021).

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(Received for publication 23 January 2021; accepted 2 September 2021; published 30 September 2021)

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(3):300–306
doi: 10.17138/TGFT(9)300-306

Research Paper

Is organic fertilizer application a viable alternative to synthetic fertilizer for Piatã grass?

¿Son los fertilizantes orgánicos una alternativa viable a fertilizantes sintéticos para el pasto Piatã?

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Abstract

Organic fertilizer in many cases can replace mineral fertilizers and in consequence reduce production costs and improve soil quality. Thus, the aim of this work was to evaluate productive, morphogenic and structural characteristics of Piatã grass (*Urochloa brizantha*) fertilized with urea, organic compost and biofertilizer throughout a year. The trial design was a block split-plot in time (seasons) design with 4 treatments (fertilizing with urea, organic compost, biofertilizer and Control) and 6 repetitions. The evaluated parameters were: dry matter production (DMP), leaf elongation rate (LER), leaf appearance rate (LAR), phyllochron (PHYL), leaf lifespan (LLS), pseudostem elongation rate (SER), final leaf length (FLL), number of live leaves (NLL) and number of tillers (NT). The highest LAR values were observed during summer and spring for the treatment with urea, which also produced the highest LER values. No difference was found in SER among the fertilizer treatments but all fertilized treatments were superior to Control. NT and DMP values were highest ($P<0.05$) in the treatment with urea, followed by biofertilizer, organic compost and Control. In conclusion, while the use of urea provided greatest forage production, applying biofertilizer gave superior yields to organic compost. Other benefits of organic fertilizers should be assessed as well as combinations of organic and inorganic fertilizers.

Keywords: Biofertilizer, nitrogen, organic compost, season, urea, *Urochloa brizantha*.

Resumen

La fertilización orgánica, en muchos casos, puede reemplazar a los fertilizantes minerales y, en consecuencia, reducir los costos de producción y mejorar la calidad del suelo. Así, el objetivo de este trabajo fue evaluar las características productivas, morfológicas y estructurales del pasto Piatã (*Urochloa brizantha*) fertilizado con urea, compuesto orgánico y biofertilizante durante un año. Para eso, se utilizó un diseño de bloques con parcelas divididas en el tiempo (estaciones), compuesto por cuatro tratamientos (fertilización con urea, compuesto orgánico, biofertilizante y control) y seis repeticiones. Los parámetros evaluados fueron: producción de materia seca (DMP), tasa de elongación de hojas (LER), tasa de aparición de hojas (LAR), filocrón (PHYL), vida útil de las hojas (LLS), tasa de elongación de pseudotallo (SER), longitud final de la hoja (FLL), número de hojas vivas (NLL) y número de macollas (NT). Los valores de LAR más altos se observaron durante el verano y la primavera para el tratamiento con urea, que también producjo los valores más altos de LER. No se encontró diferencia en el SER entre los fertilizantes probados, sin embargo, hubo una diferencia entre estos tratamientos y el control. Los valores de NT y DMP fueron mayores en el tratamiento con urea, seguido de biofertilizante, compuesto

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orgánico y control. Se puede concluir que el uso de urea brindó mayor rendimiento forrajero, sin embargo, la fertilización orgánica con biofertilizante resultó ser más ventajosa en comparación con el compuesto orgánico.

Palabras clave: Biofertilizante, compost orgánico, estaciones del año, nitrógeno, urea, *Urochloa brizantha*.

Introduction

The use of synthetic fertilizer is the most common way to restore soil nutrients; however, its use is becoming impractical due to high prices on the international market. According to data published by ANDA (2019), 36 million tonnes of fertilizer were applied in Brazil in 2019, and approximately 80% of this material was imported. It is possible that use of an alternative form of nutrients, e.g. organic fertilizer made from organic waste, could greatly reduce agricultural fertilizer costs in the country.

The utilization of organic fertilizer may be a viable alternative for fertilizing tropical pastures (Orrico Jr. et al. 2012). Organic wastes are cheaper than conventional inorganic fertilizers and contain additional nutrients important for forage growth. In the literature, there are several studies that prove the efficiency of using organic fertilizers on pastures (Orrico Jr. et al. 2013; Ryals et al. 2016; Grave et al. 2018; Orrico Jr. et al. 2018). However, there is a very wide variety of organic wastes with different chemical compositions, which may lead to a great variety of responses. Among the main waste treatment systems are composting (aerobic process producing solid fertilizer) and biodigestion (anaerobic process producing liquid fertilizer), which result in the production of organic compost and biofertilizer, respectively. Even when the same raw material (poultry litter for example) is used, the final chemical composition and its value as fertilizer may be markedly different between these two types of fertilizer, which could result in distinctly different responses (Bowden et al. 2007).

Therefore, this research aimed to determine if organic compost and biofertilizer produced different responses in growth and productivity of the pasture grass Piatã (*Urochloa brizantha*), and to assess differences in responses produced between these organic fertilizers and urea when applied to the grass.

Material and Methods

The trial was carried out in a greenhouse at the experimental area of Embrapa Agropecuária Oeste, Dourados, Mato Grosso do Sul, Brazil (22°16’30” S, 54°49’00” W). The climate in the region according to the Köppen classification is type Cwa (humid mesothermal, with hot summers and dry winters). The meteorological data recorded during the experiment are presented in Figure 1.

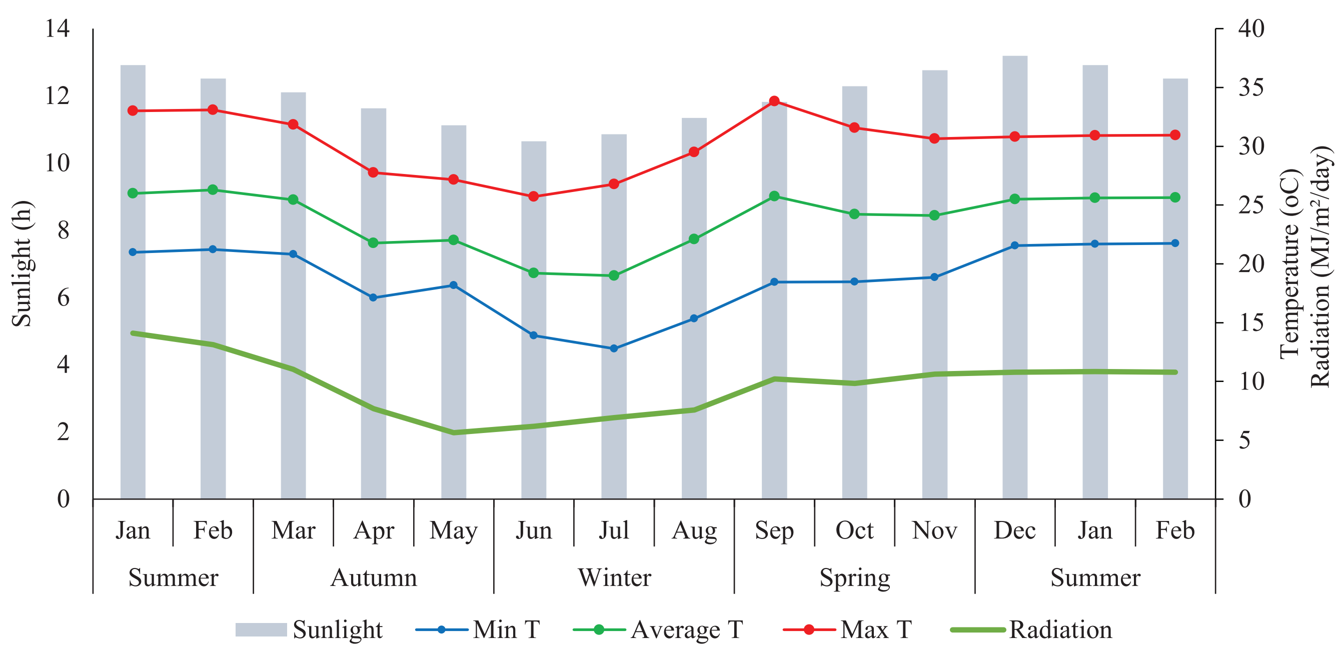

Figure 1. Average (average T), maximum (Max T) and minimum (Min T) air temperatures, hours of sunlight and radiation measured during the experimental period (2017–2018) at Dourados-MS, Brazil.

The soil used was an Oxisol of clay texture with the following characteristics: sand, 12.8%; silt, 10.7%; clay, 76.5%; pH in $CaCl_2$, 4.78; P, 5.14 mg/dm^3; K, 1.00 cmolc/dm^3; Ca^{2+}, 2.86 cmolc/dm^3; Mg^{2+}, 1.29 cmolc/dm^3; Al^{3+}, 0.15 cmolc/dm^3; H+Al, 6.08 cmolc/dm^3; cation-exchange capacity (CEC), 11.22 cmolc/dm^3; OM, 27.24 g/kg; and base saturation, 45.85%.

A block split-plot in time (seasons) design with 4 treatments, i.e. Control (= no fertilizer) and fertilization with urea, organic compost and biofertilizer, and 6 replicates, was used giving a total of 24 experimental units (40 L pots). All fertilized pots received 400 kg N/ha/year (0.8 g N/pot), applied in ten 40 kg N/ha applications. The concentrations of N, P, K, Mg, Ca and Na were 2.13, 1.77, 2.6, 0.41, 0.86 and 0.71 g/100 g in compost and 0.23, 0.19, 0.31, 0.05, 0.09 and 0.071 g/100 mL in biofertilizer, respectively.

Soil moisture in the pots was maintained at around 70% of field capacity throughout using an irrigation system. On 6 December 2016, 30 seeds of Piatã grass (*Urochloa brizantha*; syn. *Brachiaria brizantha*) were sown in each pot. Seven days after emergence, seedlings were thinned to retain the most vigorous 9 plants in each pot. A standardization cut was made 50 days after sowing (25 January 2017) at 20 cm from the soil surface (beginning of the experimental period). Subsequently, evaluation harvests were performed every 35 days at 20 cm from the soil. After each harvest, the next application of fertilizer was applied and a new data collection cycle began. Pots were irrigated when fertilizer was applied, to reduce nitrogen loss by urea volatilization. Ten harvests were made between 1 March 2017 and February 2018.

The total weight of green forage contained in the pots above a height of 20 cm from the soil was recorded at each harvest. The material collected was taken to the laboratory and placed in a forced-air oven at 65 °C for at least 72 h to determine the dry matter concentration according to the methodology described by AOAC (2005).

In order to assess forage morphogenic and structural characteristics, 3 tillers per pot were tagged with colored string after the standardization cut. The leaves and living and senescent parts were measured every 3 days with a rule and the data were used to calculate the following morphogenic and structural characteristics: (1) leaf appearance rate (LAR, leaves/tiller/day) - the number of leaves that appeared divided by the number of days of cycle evaluation; (2) phyllochron (PHYL, days) - the interval between the appearance of 2 consecutive leaves on a tiller, the opposite of LAR; (3) leaf elongation rate (LER, cm/tiller/day) - the difference between the final and initial lengths of leaf blades divided by the number of days of the evaluation period; (4) pseudostem elongation rate (SER, cm/tiller/day) - the difference between initial and final stem lengths divided by the number of days of evaluation; (5) leaf lifespan (LLS) - the number of live leaves multiplied by the phyllochron; (6) final leaf length (FLL, cm/tiller) - the mean leaf blade length of all expanded leaves present on a tiller; (7) number of live leaves (NLL) - the total number of green leaves on each tiller; and (8) number of tillers (NT) - the total number of green tillers in each pot.

The parameters were submitted to analysis of variance using the split-plot in time scheme (using the PROC MIXED procedure) to assess the effect of the main treatments (fertilizer types), secondary treatments (seasons) and their interaction (fertilizer type × season). The means of the treatments were compared by Tukey's test at 5% probability. The statistical analysis was performed through the software SAS 6.1.

Results

PHYL, LAR, LLS and NLL showed interactions ($P<0.01$) between season and type of fertilizer applied (Figure 2). LAR on treatments fertilized with urea during summer and spring was greater ($P<0.05$) than those for the remaining treatments. However, during autumn and winter differences in LAR between fertilized treatments were small and LAR on all fertilized treatments exceeded ($P<0.05$) those of Control. Overall highest LAR values occurred in summer and the lowest in winter ($P<0.05$). PHYL values followed the inverse behavior of LAR, with highest values being recorded in winter, the absolute highest value (29.9 days) for Control in winter and the lowest values for all treatments in summer.

Control had longer ($P<0.05$) LLS than fertilized treatments in autumn and winter but there were no differences between treatments in summer and spring ($P>0.05$). The highest LLS value was obtained for the Control during winter (176 days) with about 40 days in summer and spring. NLL values varied from 5 to 6.5 leaves/tiller between treatments tested, with no consistent difference between treatments.

There were no significant interactions between season and fertilizer type for other parameters, so main effect responses only are shown in Table 1. LER values were significantly affected by fertilizer type, being highest for the urea treatment (3.99 cm/d) and lowest for Control (2.49 cm/d) with compost and biofertilizer intermediate (mean 3.5 cm/d) ($P<0.01$). Pseudostem elongation rate

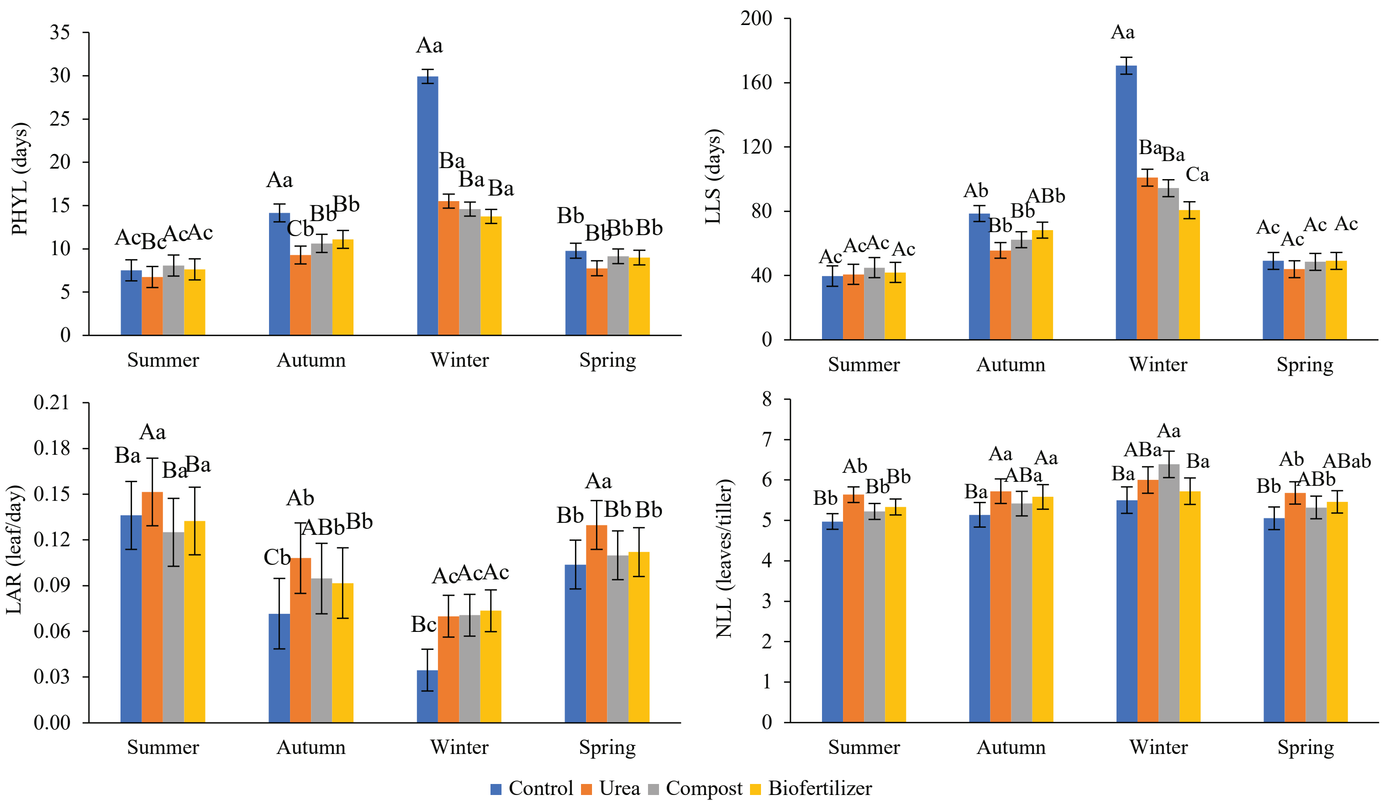

Figure 2. Effects of fertilizer type and season on phyllochron (PHYL), leaf appearance rate (LAR), leaf lifespan (LLS) and number of live leaves (NLL) of Piatã grass.
PHYL: effects of type of fertilizer (P<0.01), season (P<0.01) and interaction between type of fertilizer and season (P<0.01) (s.e.m. = 0.634).
LAR: effects of type of fertilizer (P<0.01), season (P<0.01) and interaction between type of fertilizer and season (P<0.01) (s.e.m. = 0.003).
LLS: effects of type of fertilizer (P<0.01), season (P<0.01) and interaction between type of fertilizer and season (P<0.01) (s.e.m. = 3.548).
NLL: effects of type of fertilizer (P<0.01), season (P<0.01) and interaction between type of fertilizer and season (P=0.03) (s.e.m. = 0.049).
Means for season with different lower-case letters differ by Tukey's test (P<0.05); and means for fertilizer type with different upper-case letters differ by Tukey's test (P<0.05).

was greater for all fertilizer treatments than for Control (P<0.01), resulting in final leaf length following the same pattern (P<0.01). Parameters with the greatest fertilizer effects were number of tillers/pot and DM yield/pot. The urea treatment produced the greatest number of tillers/pot followed by biofertilizer, compost and Control with significant differences between all treatments. This resulted in significant differences in DM yields/pot for all treatments, with the highest yield for urea (8.47 g DM/pot) and the lowest for Control (3.88 g DM/pot).

Both LER and SER were strongly affected by season with the following order: summer > spring > autumn > winter (Table 1; P<0.01). Leaf growth in winter was less than half of that in summer, while pseudostem growth virtually ceased in winter. Final leaf length in summer and spring exceeded those in autumn and winter (P<0.01). However, number of tillers per pot was greatest in summer with no difference between other seasons (P<0.01). As might be expected for tropical grasses, DM production was greatest in summer (9.16 g DM/pot) and lowest in autumn and winter (mean 4.59g DM/pot), with spring intermediate (mean 6.40 g DM/pot).

Table 1. Effects of fertilizer type and season on productive, morphogenic and structural characteristics of Piatã grass.

Parameter	Fertilizer				Season				s.e.m.	P value		
	Control	Urea	Compost	Biofertilizer	Summer	Autumn	Winter	Spring		F	S	F*S
LER (cm/d)	2.49C	3.99A	3.43B	3.56B	4.81a	2.95c	1.84d	3.88b	0.135	<0.01	<0.01	ns
SR (cm/d)	0.03A	0.03A	0.03A	0.02A	0.01a	0.02a	0.02a	0.01a	0.004	ns	ns	ns
SER (cm/d)	0.44B	0.58A	0.54A	0.58A	0.78a	0.61c	0.05d	0.70b	0.031	<0.01	<0.01	ns
FLL (cm)	14.6B	19.0A	17.4A	18.5A	18.2a	17.2b	16.3b	18.2a	0.288	<0.01	<0.01	ns
NT (no./pot)	75D	122A	89C	101B	117a	94b	95b	96b	1.859	<0.01	<0.01	ns
DMP (g DM/pot)	3.88D	8.47A	5.88C	6.50B	9.16a	4.47c	4.70c	6.40b	0.561	<0.01	<0.01	ns

LER = leaf elongation rate; SR = senescence rate; SER = pseudostem elongation rate; FLL = final leaf length; NT = number of tillers; DMP = dry matter production. Means for season with different lower-case letters differ by Tukey's test (P<0.05) and means for fertilizer type with different upper-case letters differ by Tukey's test (P<0.05).

Discussion

This glass-house study has provided valuable information on the variation in growth responses in Piatã grass to inorganic and organic fertilizers throughout the year. While urea produced a 118% increase in DM yield over the unfertilized Control, responses with biofertilizer were only 67% and with compost were 52%. These differences are probably a function of differences in the ready availability of the N in the various fertilizers. The published literature suggests there is a strong positive correlation between availability of N in the fertilizer applied and growth responses in tropical forages (Al-Solaimani et al. 2017; McRoberts et al. 2018). With synthetic fertilizers like urea, a high proportion of the N is readily available to plants and can be rapidly absorbed to produce rapid growth responses (Bowden et al. 2007). On the other hand, the availability of N in the organic fertilizers depends on a range of factors, including C:N ratio in the fertilizer, origin of the material and the treatment to which the waste has been subjected (Gutser et al. 2005). When organic residues are submitted to anaerobic biodigestion, NH^{4+}-N concentration in the effluents increases, which leads to a higher proportion of the N being available for plants (Gutser et al. 2005). Organic compost presents significant levels of N in organic form, which becomes more slowly available in the soil. This would help to explain why organic compost had the lowest values of dry matter production between the types of fertilizer tested.

Orrico Jr. et al. (2018) required a 4.8-fold higher dose of organic compost to reach the same forage production obtained by Orrico Jr. et al. (2013) with the use of biofertilizer. Although this comparison refers to separate studies, these were both pot studies performed by the same authors under very similar environmental conditions, i.e. type of forage, soil type and season.

The morphogenic and structural characteristics of Piatã grass that were measured supported the growth responses obtained, with leaf appearance rate (LAR) being greater for urea treatments than for other treatments in spring and summer in particular and LER and SER on all fertilized treatments exceeding those of Control. The superiority of urea over compost and biofertilizer in producing rapid growth responses was reflected in the higher LAR values for urea combined with the greater values for LER. Higher values of LAR and LER result, in most cases, in forages with a high proportion of leaves. Fertilizers that promote a high proportion of leaves also produce forage with high levels of crude protein. Mihret et al. (2018) also observed higher values of LAR, LER and CP in grasses fertilized with synthetic fertilizers (NPK) when compared with organic fertilizer. Unfortunately, we did not measure the CP concentration in the forage in this study.

Higher numbers of tillers per pot with urea were an important component of the superior responses produced with this fertilizer. According to Fagundes et al. (2006), ready availability of N leads to more rapid formation of axillary buds (due to greater LAR values), which, consequently, contributes to the emergence of new tillers. This greater density of young tillers in the pasture results in the improvement of forage productivity (Caminha et al. 2010).

Another variable that deserves to be highlighted in this work is the marked seasonality of production of forage even under controlled conditions in a pot trial. According to Araujo et al. (2018), one of the main characteristics of tropical forages is seasonality of production, with growth being greatest during summer and spring seasons. Although this study was carried out in pots, the forage was exposed to the same variations of regional photoperiod and temperatures as would be the case in a field study. Of the total annual production obtained (24.73 g DM/pot or equivalent to 12,376 kg DM/ha), proportions produced in different seasons were:

26, 37, 18 and 19% for spring, summer, autumn and winter, respectively. This highlights the important effects of both temperature and hours of daylight on growth of tropical pastures, in this case Piatã grass, since the study was conducted under conditions where soil moisture levels were maintained at 70% field capacity throughout. When seasonality of rainfall is taken into account, one might question whether or not N application in autumn and winter in the absence of irrigation is warranted and this aspect should be investigated in field studies.

These results are important because, on many farms, fertilizing of pastures with organic fertilizer is frequently practiced during all months of the year. Waste is produced daily and converted to compost or biofertilizer and farmers do not store the waste for lengthy periods (mainly biofertilizers, that are very diluted). Nitrogen applied during seasons when the grass cannot absorb it can lead to an excess of N in the soil, with possible contamination of groundwater (Blum et al. 2013); further investigations of this system seem warranted.

Conclusions

Application of synthetic fertilizer (urea) resulted in greater forage production than application of organic fertilizers. However, there are other benefits from applying organic fertilizer, such as increase in soil organic matter, improvement in soil structure, etc. While the fertilizer N in urea was readily available for plants, the slow release of N from the biofertilizers does not necessarily mean that the remaining N is lost from the system. This N could become available for plant use subsequently. Since waste is a by-product of agricultural systems and must be disposed of in an environmentally safe manner, application to fields to reduce the levels of inorganic fertilizer to be applied is a beneficial practice. These aspects warrant further investigation.

Acknowledgments

This research was carried out with the financial support of the Foundation for the Support to the Development of Teaching, Science and Technology of the State of Mato Grosso do Sul (Fundect grant number -0239/2014), and the Master's degree scholarship was provided by the Coordination for the Improvement of Higher Level Personnel (CAPES).

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(Received for publication 6 October 2020; accepted 24 June 2021; published 30 September 2021)

doi: 10.17138/TGFT(9)307-314

Research Paper

Evaluation of corn-soybean inter-cropping systems in southwestern Japan

Evaluación de sistemas de cultivos intercalados de maíz con soya en el suroeste de Japón

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Abstract

To assess the effects of inter-cropping corn and soybean under southwestern Japan's climatic conditions, 5 different treatments were compared, namely: CW (mono-cropped corn - weeded); CTW (corn + soybean cv. Tachinagaha - weeded); CT (corn + soybean cv. Tachinagaha - unweeded); CSW (corn + soybean cv. Suzukaren - weeded); and CS (corn + soybean cv. Suzukaren - unweeded). Parameters measured were plant height, yield, nutrient composition of corn and soybean and the numbers of Japanese beetles (*Popillia japonica*). Plant height of mono-cropped corn was significantly ($P<0.05$) greater than that of corn in most of the inter-cropped treatments. The number of Japanese beetles had increased dramatically, especially on unweeded inter-cropped treatments, at 55 DAS (days after sowing). Fresh and dry matter yields (FMY and DMY) of corn did not differ among treatments ($P>0.05$), while CTW treatment produced higher FMY and DMY for soybean ($P<0.05$) than in CSW and CS. Weeding tended to reduce the number of Japanese beetles on soybean plants, but it did not affect yield of soybean in this study. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations in corn cobs, whole corn plants and whole soybean plants did not differ among treatments ($P>0.05$), while crude protein (CP) concentration in whole corn plants in CTW exceeded ($P<0.05$) those for mono-cropped corn and CSW treatments. These results indicated that soybean can be successfully inter-cropped with corn in southwestern Japan. Soybean plants may be infested with Japanese beetles. It is advisable to control weeds in the stands to reduce the level of beetle infestation and to minimize competition for the planted crops.

Keywords: Corn-legume intercropping, crude protein, forage yield, nutrient composition, *Popillia japonica*.

Resumen

Para evaluar los efectos del cultivo intercalado de maíz y soja en las condiciones climáticas del suroeste de Japón, se compararon 5 tratamientos diferentes, a saber: CW (maíz monocultivo - desyerbado); CTW (maíz + soja cv. Tachinagaha - desyerbado); CT (maíz + soja cv. Tachinagaha - sin desyerbar); CSW (maíz + soja cv. Suzukaren - desyerbado); y CS (maíz + soja cv. Suzukaren - sin desyerbar). Los parámetros medidos fueron la altura, el rendimiento, la composición de nutrientes del maíz y la soja y el número de escarabajos japoneses (*Popillia japonica*). La altura de la planta de maíz monocultivo fue significativamente ($P<0.05$) mayor que la del maíz en la mayoría de los tratamientos entre cultivos. El número de escarabajos japoneses había aumentado drásticamente, especialmente en tratamientos de cultivos intercalados sin deshierbe, a los 55 DAS (días después de la siembra). Los rendimientos de materia fresca y seca (FMY y DMY) del

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maíz no difirieron entre los tratamientos (P>0.05), mientras que el tratamiento CTW produjo FMY y DMY más altos para la soja (P<0.05) que en CSW y CS. El deshierbe tendió a reducir el número de escarabajos japoneses en las plantas de soja, pero no afectó el rendimiento de la soja en este estudio. Las concentraciones de fibra detergente neutra (NDF) y fibra detergente ácida (ADF) en mazorcas de maíz, plantas enteras de maíz y plantas enteras de soja no difirieron entre tratamientos (P>0.05), mientras que se excedió la concentración de proteína cruda (CP) en plantas enteras de maíz en CTW (P<0.05) los de los tratamientos de maíz monocultivo y CSW. Estos resultados indicaron que la soja se puede intercalar con el maíz en el suroeste de Japón. Las plantas de soja pueden estar infestadas de escarabajos japoneses. Es aconsejable controlar las malas hierbas en los rodales para reducir el nivel de infestación de escarabajos y minimizar la competencia por los cultivos plantados.

Palabras clave: Composición nutricional, cultivos intercalados maíz-leguminosa, *Popillia japonica*, proteína cruda, rendimiento de forraje.

Introduction

Maize (*Zea mays* L.), also known as corn, is one of the most important cereal crops in the world, and is grown in a wide range of environments worldwide. It is being increasingly used for a range of purposes, such as human food, feed for livestock and a source of raw material for industrial products. The demand for corn as animal feed will continue to grow faster than the demand for its use as human food, particularly in Asia, where a doubling of production is expected to almost 400 million tonnes in 2030 (Paliwal et al. 2000). Although corn is high in digestible starch and water-soluble carbohydrates (WSC), making it a high-energy feed for ruminants (Masoero et al. 2006), it does not provide sufficient crude protein (8.8%) for high levels of production (National Research Council 2001). Therefore, supplementation with protein feeds is needed to fulfill the requirements of high-producing ruminants. In order to improve yields and forage quality of corn, alternatives are being sought.

One suitable alternative may be inter-cropping of corn with legumes such as soybean [*Glycine max* (L.) Merr.] (Ofori and Stern 1987; Carruthers et al. 1998). Legumes have long been recognized as a good source of crude protein (CP) (Anil et al. 2000). However, leguminous forage is highly difficult to ensile because of its high buffering capacity and low level of WSC (Maasdorp and Titterton 1997), but it is possible to ensile high-energy corn silage with protein-rich forages to obtain a better nutrient composition (Anil et al. 2000). Recently, several studies have found that inter-cropping of corn with legumes is a feasible option to increase CP concentration in forage produced (Prasad and Brook 2005; Contreras-Govea et al. 2009; Zhu et al. 2011; Costa et al. 2012). Herbert et al. (1984) and Putnam et al. (1986) reported that inter-cropping of corn with soybean increased CP concentration by 19–36 and 11–15 percentage units, respectively. However, inter-cropping of corn with soybean in southwestern Japan has not been reported, despite the corn-soybean inter-cropping system becoming popular worldwide.

Ishigaki et al. (2017) investigated the agronomic characteristics and yield of several soybean varieties as forage crops, and reported on the risk of insect damage during the cultivation period. The authors confirmed that leaf damage caused by scarabs, especially Japanese beetles (*Popillia japonica*; Coleoptera: Rutelidae), was remarkable. Since the cultivation period of forage soybeans and the time when scarabs are most prolific overlap, it is important to determine the impact of beetle infestation on production of forage soybeans. In addition, weeds can hamper soybean growth. However, there are no registered pesticides or herbicides for use in forage soybeans in Japan. Therefore, there is a need to document the occurrence of Japanese beetles under the corn-soybean inter-cropping system and the effects on forage yield and quality of corn in southwestern Japan, with the aim of developing a pest management strategy for forage soybeans that does not rely on the use of pesticides.

The present studies were conducted: (i) to assess the occurrence of Japanese beetle on soybean plants under mono-cropped and inter-cropped cultivation; and (ii) to evaluate the effects on growth, forage yield and nutrient composition of inter-cropping corn with 2 forage soybean varieties relative to mono-cropped corn.

Material and Methods

Experimental site

The field experiment was carried out at the Sumiyoshi Livestock Science Station, Field Science Education Research Center, Faculty of Agriculture, University of Miyazaki, Japan (31°55′ N, 131°28′ E; 10 masl), from April to July 2018. Meteorological conditions are shown in Table 1. Air temperature and precipitation during the experimental period were obtained from the database of the Geospatial Information Authority of Japan (jma-net.go.jp/miyazaki), and were recorded 16 km from the experimental site. The soil type of the experimental field was characterized as sand-dune Regosol with moderate organic matter (4.0%), 0.09% N, 0.3% P, 0.2% K and a soil pH of 6.2.

Table 1. Mean temperature and precipitation during April–July 2018 in the area of the field experiment in Miyazaki, southwestern Japan.

Month	Temperature (°C)	Precipitation (mm)
April	17.6	60
May	20.5	409
June	23.6	480
July	27.5	580

Plant material

In this experiment, a corn cultivar, NS118 Super (KANEKO seed company), which matures in a period of 118 days, and 2 soybean cultivars, Tachinagaha and Suzukaren, released by National Agriculture and Food Research Organization (NARO), were used. Tachinagaha is medium-late maturing and Suzukaren is late maturing.

Experimental design and treatments

The experimental plots were laid out in a randomized complete block design (RCBD) with 5 treatments and 3 replications for each treatment. The description of treatments is summarized in Table 2. Individual treatment plots were 12.8 m^2 (4 × 3.2 m) and there were 15 plots in all. The cropping systems were mono-crop corn and corn-soybean inter-cropping. Prior to planting, lime with 10% MgO at 800 kg/ha and compost (2.5% N, 4.0% P and 2.1% K) at 1,114 t/ha were added to the soil in March. After field preparation, corn seed was sown on 16 April 2018 with 5 rows per plot, at an inter-row spacing of 75 cm, and intra-row spacing of 25 cm. Sowing rate of corn was 68,571 viable seeds/ha and sowing depth was 4–5 cm. In inter-cropped treatments, a single row of soybean was sown 20 cm from a corn row, with an intra-row spacing of 6 cm, giving a sowing rate of 285,714 viable seeds/ha; sowing depth was 5–6 cm. No rhizobium was applied to the soybean seeds and no herbicides or insecticides were used. Basal N:P:K fertilizer (14:12:10) was applied at a rate of 400 kg/ha during corn planting and 80 kg N/ha in the form of urea (46% N) was applied on 17 May 2018. The crops were inspected manually and insects (*Popillia japonica*) were first observed feeding on soybean leaves in late May (24–30 May). The CW, CTW and CSW treatments were weeded by hand during the 2nd and 4th weeks after sowing.

Table 2. Description of experimental treatments and cropping systems.

Treatment	Cropping system	Description
CW	Mono-crop	Corn - weeded
CTW	Inter-crop	Corn + soybean cv. Tachinagaha - weeded
CT	Inter-crop	Corn + soybean cv. Tachinagaha - unweeded
CSW	Inter-crop	Corn + soybean cv. Suzukaren - weeded
CS	Inter-crop	Corn + soybean cv. Suzukaren - unweeded

Data collection and analysis

At 24, 45, 68 and 80 DAS (days after sowing), 3 plants (each of corn and soybean) were selected at random from each treatment for measuring plant height. All Japanese beetles (*Popillia japonica*) on all plants in each plot (4 × 3.2 m) were collected and counted at 49, 55, 57, 66, 70 and 78 DAS. Corn and soybean inter-crops were harvested by using sickles on 13 July 2018. All plants were cut at about 10 cm above the ground in the net plot area (3 × 1.7 m), excluding border rows. Fresh matter yield (FMY) was measured in each experimental plot. To determine dry matter (DM), 10 samples of corn and soybean plants were selected from each plot, weighed fresh and dried in an oven at 70 °C for 48 hours to calculate dry matter yield (DMY). The dried samples were ground to 1 mm for chemical analysis. N concentration was measured using the NC-Analyzer (model Sumigraph NC-220F, Sumika Chemical Analysis Service Ltd, Japan), allowing calculation of crude protein concentration (CP%) and crude protein yield (CPY) for each species and cultivar as well as total CPY/ha. Neutral detergent fiber (NDF) was measured using a modified Ankom Filter bag technique (Ankom Technology, ANKOM200 Fiber Analyzer, NY, USA) and acid detergent fiber (ADF) was run sequentially after NDF using the Ankom Filter bag technique.

Statistical analysis

One-way ANOVA was conducted to assess the effects of cropping system and treatment on growth traits and yield traits. Additionally, Tukey's test was conducted to assess the means of these traits. Percentage data were transformed into angular figures (Claringbold et al. 1953). All statistical analyses were carried out with the program R software (version 3.1.1, R Core Team 2014).

Results

Plant height

Plant height of mono-cropped corn (CW) at 24 DAS (days after sowing) was significantly (P<0.05) greater than that for CT treatment (Table 3) and by 45 DAS heights of corn for CW and CSW were greater (P<0.05) than those for CTW and CT. At 68 and 80 DAS, corn in CW was taller (P<0.05) than corn in most inter-cropped treatments (Table 3). Weeding had no significant (P>0.05) effect on plant height of corn. For soybean, plant heights at 24 and 45 DAS for CTW and CT were significantly (P<0.05) greater than those for CSW and CS (Table 3). Differences between cultivars declined with time and by 68 DAS only soybean in CTW was taller than soybean in CSW and CS, while by 80 DAS only CT was taller than CS. As for corn, weeding had no effect on height of soybean.

Table 3. Plant height (cm) of corn and soybean at different growth stages for the inter-crop and mono-crop treatments.

Treatment	24 DAS	45 DAS	68 DAS	80 DAS
Corn				
CW	36±1.3a	233±1.3a	282±1.2a	293±2.0a
CTW	34±1.4ab	217±2.9b	268±2.9b	282±2.4b
CT	30±0.9b	222±3.8b	271±2.2b	284±2.5b
CSW	31±1.5ab	234±2.0a	273±2.4b	287±1.9ab
CS	31±1.1ab	225±2.3ab	272±1.5b	284±1.8b
Soybean				
CTW	13±0.4a	53±2.5a	95±2.9a	92±1.7ab
CT	12±0.5a	53±1.2a	88±3.4ab	94±2.0a
CSW	10±0.5b	39±1.9b	81±4.1b	87±3.0ab
CS	10±0.5b	39±2.6b	76±3.1b	86±1.8b

Data are presented as mean ± s.e.; means within columns and crop type with different letters are significantly different (P<0.05) by Tukey-test. DAS: days after sowing. CW: mono-crop corn - weeded; CTW: corn + soybean cv. Tachinagaha - weeded; CT: corn + soybean cv. Tachinagaha - unweeded; CSW: corn + soybean cv. Suzukaren - weeded; CS: corn + soybean cv. Suzukaren – unweeded.

Insect occurrence

No signs of disease were observed. However, a number of insect pests were observed during the growing season. The most significant one was Japanese beetle (*Popillia japonica*) (Figure 1; Table 4), which was first observed feeding on soybean leaves in late-May (24–30 May) and was present throughout June, with a few individuals still observed in July. At 49 DAS, only low numbers of beetles were collected (0.7–7.1 individuals/plot, i.e. 4 × 3.2 m) with higher, but not significantly so (P>0.05), numbers on unweeded treatments. By 55 DAS, insect numbers had increased dramatically, especially on unweeded inter-cropped treatments. There were significant differences (P<0.05) between unweeded and weeded treatments for cv. Tachinagaha but not for cv. Suzukaren. The situation remained similar at 66 DAS. By 70 DAS and 78 DAS insect numbers had dropped to fewer than 3 individuals per plot on all treatments. Corn plants suffered only minimal damage from insect pests.

Figure 1. Adult Japanese beetles (*Popillia japonica*) feeding on soybean leaves.

Yield traits

Treatments applied had no significant effects (P>0.05) on fresh (FMY) or dry matter (DMY) yields of corn with FMY ranging from 63.3 to 59.8 t/ha and DMY from 21 to 17.5 t/ha (Table 5). However, failing to weed the inter-cropped treatments produced a non-significant decrease in both FMY and DMY of corn (mean 9.2%). Soybean yields varied from 4.5 to 6.6 t FM/ha and 1.0 to 1.5 t DM/ha, with weeded Tachinagaha outyielding other treatments in terms of DMY (P<0.05). Total DMY ranged from 18.7 to 22.5 t DM/ha with no significant differences between treatments (P>0.05; Table 5).

Table 4. Numbers of Japanese beetle (*Popillia japonica*) per plot (4 × 3.2 m) collected on soybean plants.

Treatment	49 DAS	55 DAS	57 DAS	66 DAS	70 DAS	78 DAS
CW	-	-	-	-	-	-
CTW	0.7±0.3a	1.8±0.4b	4.0±0.8ab	8.4±1.6b	2.9±1.0a	0.1±0.1a
CT	7.1±3.4a	23.2±7.6a	21.8±7.2a	22.0±6.3a	1.2±0.7ab	1.9±1.0a
CSW	0.7±0.4a	0.1±0.1b	1.0±0.8b	2.8±0.9b	0.2±0.1b	0.0±0.0a
CS	4.1±1.5a	16.2±5.5ab	19.0±6.5ab	12.1±2.6ab	1.1±0.4ab	0.4±0.2a

Data are presented as mean ± s.e.; means within columns with different letters are significantly different (P<0.05) by Tukey test. DAS: days after sowing. CW: mono-crop corn - weeded; CTW: corn + soybean cv. Tachinagaha - weeded; CT: corn + soybean cv. Tachinagaha - unweeded; CSW: corn + soybean cv. Suzukaren - weeded; CS: corn + soybean cv. Suzukaren - unweeded.

Table 5. Fresh and dry matter yields (t/ha) of corn and soybean.

Treatment	FMY		DMY		Total DMY
	Corn	Soybean	Corn	Soybean	
CW	62.7±3.5		19.6±1		19.6
CTW	63.3±1.1	6.6±0.1a	21.0±1.2	1.5±0.1a	22.5
CT	59.8±4.1	5.4±0.3ab	17.5±0.8	1.2±0.1b	18.7
CSW	67.3±2.6	4.7±0.3b	20.0±0.9	1.1±0.1b	21.1
CS	60.7±0.8	4.5±0.3b	18.7±0.3	1.0±0.1b	19.7

Data are presented as mean ± s.e.; means within columns with different letters are significantly different (P<0.05) by Tukey test. CW: mono-crop corn - weeded; CTW: corn + soybean cv. Tachinagaha - weeded; CT: corn + soybean cv. Tachinagaha - unweeded; CSW - corn + soybean cv. Suzukaren – weeded; CS: corn + soybean cv. Suzukaren – unweeded. FMY: fresh matter yield; DMY: dry matter yield.

Nutrient composition

Treatments applied had very little effect on chemical composition of forage produced (Table 6). The only significant effect was for CP concentration, where CP% of corn forage from the Tachinagaha inter-cropped weeded treatment exceeded that of whole corn forage grown as a sole crop (7.0 vs. 5.3%; P<0.05). As a result, CP yield from corn in the Tachinagaha inter-cropped weeded treatment exceeded (P<0.05) those of all other treatments except inter-cropped weeded corn + cv. Suzukaren, while CP yields of soybean of both Tachinagaha treatments exceeded those of Suzukaren (P<0.05; Table 7). Similarly, total CP yield of the inter-cropped weeded Tachinagaha treatment exceeded those of all other treatments (P<0.05). NDF concentration in whole corn ranged from 54 to 57.9%, while concentration in soybean ranged from 44.4 to 48.5%. On the other hand, ADF concentrations for corn ranged from 35.1 to 37.2% and for soybean from 36.9 to 42.1%.

Table 6. Nutrient composition (% DM basis) of corn and soybean.

Nutrient	Treatment				
	CW	CTW	CT	CSW	CS
NDF					
Corn - cobs	35.0±1.6	36.6±0.9	38.7±1.7	38.3±0.5	38.7±0.9
Corn – whole plant	56.1±0.4	54.0±1.5	57.9±2.1	54.0±1.1	55.2±0.4
Soybean - whole plant	-	44.4±1.8	47.0±0.4	48.5±0.4	47.6±0.8
ADF					
Corn - cobs	18.4±1.0	20.0±1.0	19.3±1.4	19.5±0.5	19.4±0.2
Corn – whole plant	37.2±0.3	35.1±0.3	37.1±1.4	36.9±0.8	36.3±0.9
Soybean – whole plant plant	-	36.9±2.3	39.6±1.0	42.1±0.7	40.0±1.7
CP					
Corn - cobs	6.3±0.2	5.4±0.2	5.6±0.3	5.9±0.2	6.1±0.2
Corn – whole plant	5.3±0.1b	7.0±0.3a	6.0±0.4ab	5.6±0.1b	5.8±0.2ab
Soybean – whole plant	-	17.8±0.7	18.9±0.9	17.6±0.9	16.6±0.9

Data are presented as mean ± s.e.; means within columns with different letters are significantly different (P<0.05) by Tukey test. CW: mono-crop corn - weeded; CTW: corn + soybean cv. Tachinagaha - weeded; CT: corn + soybean cv. Tachinagaha - unweeded; CSW: corn + soybean cv. Suzukaren - weeded; CS: corn + soybean cv. Suzukaren - unweeded.

Table 7. Crude protein yield (t/ha) of corn and soybean.

Treatment	Corn	Soybean	Total
CW	1.04±0.1b	-	1.04±0.1b
CTW	1.48±0.3a	0.27±0.03a	1.75±0.2a
CT	1.04±0.1b	0.23±0.03ab	1.27±0.1b
CSW	1.13±0.1ab	0.19±0.03b	1.32±0.1b
CS	1.08±0.1b	0.17±0.03b	1.25±0.1b

Data are presented as mean ± s.e.; means within rows with a different letter are significantly different (P<0.05) by Tukey test. CW: mono-crop corn - weeded; CTW: corn + soybean cv. Tachinagaha - weeded; CT: corn + soybean cv. Tachinagaha - unweeded; CSW: corn + soybean cv. Suzukaren - weeded; CS: corn + soybean cv. Suzukaren - unweeded.

Discussion

Plant height

The reduction in plant height of corn in inter-cropped treatments relative to the mono-cropped corn treatment would have been a response to increased competition for light, moisture and nutrients, where corn was sown with the legumes. Baker (1979) and Mbah et al. (2007) indicated that inter-cropped treatments have reduced growth relative to mono-crops because of competition for resources. This hypothesis is reinforced by the fact that differences were not significant during the early stages of growth but increased as plants matured and available resources were consumed. Surprisingly, weeding of inter-cropped treatments had no significant effect on plant height of corn, despite the fact that competition for resources in the unweeded treatments might have been expected to be greater than in weeded treatments. While Tachinagaha plants were significantly taller than Suzukaren plants in the early growth stages, differences were no longer significant at the end of the study, despite absolute values favoring Tachinagaha. These differences appear to be merely varietal differences as opposed to any superiority of Tachinagaha in terms of competitive ability. Unfortunately, we failed to record biomass yields of weeds in unweeded treatments, as Rose et al. (1984) reported up to 45% differences in weed biomass production when in competition with various soybean genotypes.

Occurrence of insects

The significant insect injury to soybean caused by Japanese beetle (*Popillia japonica*) was not surprising as this insect is a voracious feeder on soybean leaves and could pose problems for soybean growth and yield under corn-soybean inter-cropping systems in the study location. The higher populations of insects in unweeded treatments indicated that weeding has more advantages than merely reducing competition for resources. Our results suggest that weeding and physical insect control under corn-soybean inter-cropping systems should be carried out to prevent possible yield loss. However, in Japan, there are no registered pesticides for control of scarab beetles, including Japanese beetle. Thus, pesticide-free control methods, such as the use of light traps, should possibly be tested for control of this insect under southwestern Japan's climatic conditions.

Yield traits

The absence of significant differences in total dry matter yield (TDMY) between mono-cropped corn and corn-soybean inter-cropped treatments suggests that competition for resources prevented any marked increase in dry matter production from a given area regardless of species involved. However, absolute DMYs for the inter-cropped weeded treatments were greater than for mono-cropped corn, suggesting that more and larger studies should be conducted to verify these findings. Similarly, the consistent, though non-significant, reductions in yields of forage in unweeded treatments also suggest that more and larger studies seem warranted to verify these findings. Reta Sánchez et al. (2010) and Baghdadi et al. (2016) reported that total DMY of corn-soybean inter-cropped treatments was similar to that of mono-cropped corn. The higher DMYs for Tachinagaha than for Suzukaren suggest that the former cultivar might be more suitable for growing in this environment. Different competitive ability of the soybean cultivars could be a factor in the different yields displayed (Callaway 1992), although we did not have any pure legume treatments in this study. Gutu et al. (2015) in their experiment on corn-soybean inter-cropping also stated that forage and grain yields of soybean were significantly different for different soybean varieties.

Nutrient composition

The absence of significant differences in NDF and ADF of corn in the different treatments was not surprising, as all crops were harvested at the same maturity stage of corn plants as suggested by Mugweni et al. (2001). While CP concentration in corn forage in inter-cropped treatments was generally not significantly greater than that of mono-cropped corn, absolute values consistently favored the inter-cropped treatments. Added to this was the much higher CP concentration in the soybean forage than in the corn forage, so overall forage produced in inter-cropped treatments was of higher quality than the mono-cropped corn. As a result, animals fed the mixed forage would be expected to perform at a higher level than those fed mono-cropped corn. Numerous studies, e.g. Lithourgidis et al. (2006), and Eskandari et al. (2009), have reported similar results indicating that inter-cropping cereal crops with legumes significantly increased the CP yield per hectare.

In conclusion, the findings of this study clearly showed that corn and soybean can be inter-cropped with simultaneous sowing under southwestern Japan's climatic conditions without any deleterious effects on the corn. While dry matter yields of forage produced might not be significantly greater than that for mono-cropped corn, the overall quality of the forage would be superior in the inter-cropped system, especially in terms of CP concentration. It appears that cv. Tachinagaha might be superior to cv. Suzukaren for growing in this environment. Again, it appears that weeding may reduce the level of insect infestation on the soybeans and possibly increase yields. These findings need verification on a larger scale and in a range of seasonal conditions to ensure recommendations are soundly based. Further investigations are required to determine the most effective non-chemical control methods for insects (Japanese beetle) and for control of invasive weeds in corn-soybean inter-cropping systems under southwestern Japan's climatic conditions.

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(Note of the editors: All hyperlinks were verified 13 July 2021).

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(Received for publication 14 August 2020; accepted 12 July 2021; published 30 September 2021)

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(3):315–320
doi: 10.17138/TGFT(9)315-320

Research Paper

Quality properties of sunn hemp (*Crotalaria juncea* L.) and maize (*Zea mays* L.) silages

*Propiedades de calidad de ensilaje de Crotalaria (*Crotalaria juncea *L.) y maíz (*Zea mays *L.)*

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Abstract

Maize is an ideal forage crop for ensilage because of its high levels of fermentable carbohydrates, although it is low in protein. Sunn hemp is a legume with a high crude protein content with potential to be used in combination with maize to provide a silage with a higher protein content. Different percentages of sunn hemp-maize mixtures of 80-20, 60-40, 40-60 and 20-80 respectively were compared to silages of sole maize and sunn hemp. In the laboratory study, DLG classifications (color, smell, structure, total score and quality class), silage loss (%), silage pH, dry matter content, flieg score, crude protein content, crude ash content, NDF, ADF, metabolic energy (MJ kg/DM), dry matter intake, percent digestible dry matter and relative feed value were determined at the end of 60 days ensilage. The crude protein contents of silages increased as the sunn hemp ratio in the mixtures increased. In addition, pure sunn hemp silage and mixtures, especially 80% sunn hemp mixed with 20% maize, were found suitable for silage and it was concluded that sunn hemp and sunn hemp-maize silage mixtures could be used in animal husbandry.

Keywords: Ensilage, feed value, forage crop, legume mixture, protein supply.

Resumen

El maíz es un cultivo forrajero ideal para ensilaje por sus altos niveles de carbohidratos fermentables, aunque es bajo en proteínas. La crotalaria es una leguminosa con un alto contenido de proteína cruda con potencial para ser utilizada en combinación con el maíz para proporcionar un ensilaje con un mayor contenido de proteína. Se compararon diferentes porcentajes de mezclas de crotalaria-maíz de 80-20, 60-40, 40-60 y 20-80 respectivamente con ensilajes de sólo maíz y crotalaria. En el estudio de laboratorio, clasificaciones DLG (color, olor, estructura, puntaje total y clase de calidad), pérdida de ensilado (%), pH del ensilaje, contenido de materia seca, puntaje de flieg, contenido de proteína bruta, contenido de ceniza bruta, NDF, ADF, energía metabólica (MJ kg/MS), consumo de materia seca, porcentaje de materia seca digestible y el valor relativo del alimento se determinaron al final de los 60 días de ensilaje. El contenido de proteína cruda de los ensilajes aumentó a medida que aumentaba la proporción de crotalaria en las mezclas. Además, el ensilaje de crotalaria pura y las mezclas, especialmente el 80% de crotalaria mezclada con el 20% de maíz, resultaron adecuados para el ensilaje y se concluyó que la crotalaria y las mezclas de ensilaje de maíz y crotalaria se podrían utilizar en la cría de animales.

Palabras clave: Cultivo forrajero, ensilaje, mezcla de legumbres, suministro de proteínas, valor alimenticio.

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Introduction

The constant growth of the world population requires the development of productive and efficient agricultural practices to meet food demand. Turkey is very suitable for animal husbandry with the required natural resources and ecological conditions and the livestock sector is important for the general economy (Seydosoglu 2019). Roughages, one of the indispensable feed sources of livestock, are mainly obtained from meadow pasture areas and forage crop production in field agriculture (Moore et al. 2020). According to 2018 data, total hay production of these two sources in Turkey was 31 million tons, which is not sufficient for livestock needs (Acar et al. 2020).

Turkey's ecological conditions and topography allows the cultivation of many forage plants (Tan and Yolcu 2021). In addition to traditional forage crops, growing alternative forage crops will help to prevent feed shortages. Many studies reported that sunn hemp (*Crotalaria juncea* L.), which was previously not cultivated in Turkey, can be successfully grown in regions with Mediterranean climatic conditions (Demiroğlu Topçu and Özkan 2019).

Maize silage is one of the most important forage crops worldwide thanks to its high biomass yield, high energy values and the high content of non-structural carbohydrates that favor the fermentation process. Protein contribution to the ruminal system is low in maize silage (Colombini et al. 2010). Legume forage crops such as sunn hemp and soybean can be ensiled by mixing up to 50% with maize in order to increase the protein content in maize silage (Zavala et al. 2011; Sulas et al. 2012).

The aim of this study was to determine the sensory and chemical silage quality properties of silages prepared with different ratios of maize and sunn hemp under Mediterranean climate conditions.

Materials and Methods

Site description and agronomic details

This research was carried out in the experimental fields and silage laboratories of the Field Crops Department, Faculty of Agriculture, Ege University in Izmir, Turkey during the 2019 growth season. Izmir has typical Mediterranean climate conditions and a silty-clay loam soil with pH 7.8, organic matter (1.13%), salt (0.075%), total N (0.11%), available phosphorus (40 ppm) and available potassium (400 ppm). The climate and soil properties at Izmir, Turkey are favorable for sunn hemp cultivation.

The Tillage Sun cultivar of sunn hemp and C-955 cultivar of silage maize were used for the study. The plants were grown separately in the field on approximately 0.004 ha each. The plants were simultaneously sown at the beginning of July 2019 under second crop planting conditions. The sunn hemp was seeded in rows with 40 cm row spacing (50 kg/ha) and maize was sown in rows with 70 cm row spacing (95.238 plants/ha). Conventional agriculture practices were carried out during the growing season. Since there were no significant problems of pests, diseases or weeds in the study site, no chemical was applied.

Plant harvesting and ensiling process

Sunn hemp was harvested at the beginning of flowering and maize was harvested at the dough stage simultaneously from the middle rows of the plots. Plants were harvested by hand by cutting at soil level. Plants were chopped to about 2-3 cm in size and the chopped materials were thoroughly mixed to attain homogeneity. Different mixture ratios (sunn hemp %-maize %; 100-0, 80-20, 60-40, 40-60, 20-80, 0-100; on the basis of fresh weight) were used for preparing the silage in four replications with a randomized design (6 treatments x 4 replications = 24 silage samples).

Samples of 500±20 g of each silage mixture were placed in separate vacuum bags (thickness 110 microns or more), and after 99.9% of the air was removed by vacuum, bags were glued and closed (Johnson et al. 2005). The bagged silage samples were stored in a dark and cool environment at 24±4°C for 60 days.

Assessment of silage samples

Physical quality analysis for DLG classifications (DLG 1987) and chemical properties were examined after 60 days of fermentation. The silage loss was calculated according to Danley et al. (1973). The pH of the silage juice was measured by the HANNA HI 2211 pH/ORP pH meter (Hanna Instruments Ltd., USA). The silage samples were dried in an oven at 65°C for 48 hours, ground and passed through a 2 mm sieve to prepare for chemical analysis. Nitrogen content was determined by the Kjeldahl method and multiplied by the coefficient of 6.25 to obtain crude protein concentrations (AOAC 1990). Crude ash content was determined at 550ºC (Bulgurlu and Ergul 1978). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations were analysed by the sequential detergent analysis method (Van Soest et al. 1991). Flieg score, metabolizable energy (ME), estimated dry matter intake (DMI), digestible dry matter (DDM) and relative feed value (RFV) were determined by using the following commonly used formulas (Kirchgessner and Kellner 1977; Van Dyke and Anderson 2000; Morrison 2003).

Flieg score = 220 + (2 x % Dry Matter - 15) - 40 x pH

Dry Matter Intake (% of BW) = 120 / (% NDF)

Digestible Dry Matter (%) = 88.9 - (0.779 x % ADF)

Relative Feed Value = (DMI x DDM) / 1.29

Metabolizable Energy, MJ kg/DM = 14.70 - 0.150 x ADF

Statistical analysis

Statistical analyses were conducted using ANOVA, Statistical Analysis System version 7.0 (SAS Institute 1998) for a completely randomized design. The treatment means were compared by the LSD test described by Steel and Torrie (1980).

Results

Physical observation values (color, smell, structure) and DLG classifications of the silage samples are given in Table 1. The total DLG score obtained by summing the color, smell and structure scores is an indicator of the quality class of silage (20-18:very good, 17-14:good, 13-10:medium, 9-5:low, 4-0:deteriorated) (DLG 1987). In terms of sensory properties, all silage alternatives (sole crop and mixtures) were classed as high quality.

There were significant differences (P<0.05) in silage losses of fresh matter and silage pH among pure and mixed silages (Table 2). Silage pH values increased as the ratio of sunn hemp in the silage was increased. Significant differences (P<0.05) were found among silage types for dry matter content and Flieg score (Table 2). Dry matter content and Flieg score values of silages decreased as the ratio of sunn hemp increased. Crude protein and crude ash values were found to be significantly different (P<0.05) among the sunn hemp and maize silages (Table 2).

The silage NDF, ADF and metabolizable energy values were found to be statistically significantly (P<0.05) different in sole crop and different ratios of sunn hemp and maize mixtures (Table 3).

Significant differences (P<0.05) in dry matter intake, digestible dry matter content and relative feed value were observed among the silage mixtures (Table 3). The relative feed value of the silage decreased as the ratio of maize in the mixture decreased.

Table 1. Physical observation values and DLG classification of sunn hemp-maize silage.

Mixtures	Color (point)	Smell (point)	Structure (point)	DLG (point)	Quality Class
100% S	2.00	12.75	4.00	18.75	Very Good
100% M	2.00	14.00	4.00	20.00	Very Good
80% S + 20% M	2.00	13.25	4.00	19.25	Very Good
60% S + 40% M	2.00	13.75	4.00	19.75	Very Good
40% S + 60% M	2.00	14.00	4.00	20.00	Very Good
20% S + 80% M	2.00	14.00	4.00	20.00	Very Good

S=sunn hemp, M=maize

Table 2. Silage loss, pH, dry matter, flieg score, crude protein and crude ash contents of sunn hemp-maize silage

Mixtures	Silage Loss (%)	Silage pH	Dry Matter (g/kg)	Flieg Score (score)	Crude Protein (g/kg DM)	Crude Ash (g/kg DM)
100% S	3.06 a	4.44 a	273.4 e	81.95 d	163.8 a	70.7 a
100% M	2.47 d	3.93 d	339.0 a	115.59 a	82.2 f	62.1 d
80% S + 20% M	2.83 b	4.30 b	288.3 d	90.53 c	148.8 b	69.4 a
60% S + 40% M	2.60 c	4.13 c	306.4 c	101.09 b	130.1 c	66.9 b
40% S + 60% M	2.62 c	4.10 c	313.2 c	103.51 b	115.8 d	65.1 c
20% S + 80% M	2.52 cd	3.98 d	325.8 b	111.09 a	100.2 e	63.1 d
Mean	2.68	4.15	307.7	100.63	123.5	66.2
CV (%)	2.97	1.43	2.24	3.02	2.47	1.75
LSD (0.05)	0.12	0.09	10.4	4.58	4.6	1.7

S=sunn hemp, M=maize. Means followed by different letters are significantly different (P<0.05).

Table 3. Silage quality features of sunn hemp-maize silage.

Mixtures	NDF (g/kg DM)	ADF (g/kg DM)	ME (MJ kg^{-1} DM)	DMI (% of BW)	DDM (%)	RFV
100% S	603.8 a	422.7 a	8.36 f	1.99 d	55.97 d	86.23 e
100% M	520.8 d	318.7 f	9.92 a	2.30 a	64.08 a	114.46 a
80% S + 20% M	578.1 b	402.7 b	8.66 e	2.08 c	57.53 c	92.58 d
60% S + 40% M	572.6 b	387.6 c	8.89 d	2.10 bc	58.71 c	95.38 d
40% S + 60% M	555.3 c	356.6 d	9.35 c	2.16 b	61.12 b	102.44 c
20% S + 80% M	529.7 d	340.2 e	9.60 b	2.27 a	62.40 b	109.59 b
Mean	560.1	371.4	9.13	2.15	59.97	100.11
CV (%)	1.72	1.94	1.27	2.04	1.56	3.04
LSD (0.05)	14.5	1.08	0.17	0.07	1.41	4.58

S=sunn hemp, M=maize, DMI=Dry Matter Intake, DDM=Digestable Dry Matter, RFV=Relative Feed Value. Means followed by different letters are significantly different (P<0.05).

Discussion

The highest quality silage was determined as the mixture of 80% sunn hemp with 20% maize. The findings of this study are similar with other studies on different legumes with maize (Budakli Carpici 2016; Titterton and Maasdorp 1997). Loss of silage dry matter and decrease in the feed value of silage of 3-5% could occur due to respiration or fermentation (Buxton et al. 2003). Wang et al. (2009) reported that pure sunn hemp silage has a high pH value. The results of our study were in accordance with the previous research and pH values increased as the ratio of sunn hemp increased (Zavala et al. 2011).

The fermentation of silages may be adversely affected by dry matter. Panyasak and Tumwasorn (2015) reported that the dry matter content of well fermented silage should be between 25-40%. The high dry matter values obtained indicate that the soluble carbohydrate content per unit dry matter of silage was high and the lactic acid fermentation was also successful.

The Flieg score value provides a practical assessment of the chemical properties of silage. All ratios of sunn hemp with maize in this study were included in the "very good" quality class and Flieg scores were inversely proportional to silage pH as expected (Woolford 1984). Crude protein contents increased as the ratio of sunn hemp was increased. Budakli Carpici (2016) found that crude protein contents of mixtures of different silages varied between 7.08–17.43% and Martínez-García (2015) reported increased crude protein content as a result of increases in legume ratio in silage mixtures similar to that reported in this study. The NDF content is lower than found by Titterton and Maasdorp (1997) who reported a NDF value of 675 g kg/DM for sunn hemp silage. The results of the study indicated that as the sunn hemp ratio is increased in the mixtures, the NDF and ADF ratios decreased. The dry matter intake ratio of silage is negatively correlated with NDF and the digestible dry matter of silage is inversely related with ADF (Yucel et al. 2018). The metabolizable energy values of all silage samples examined in the study were at an acceptable level (Boguhn et al. 2003) and similar to the 8.2 MJ kg/DM reported for sunn hemp silage (Titterton and Maasdorp 1997). Relative feed value has been used to compare the quality of legume and legume/grass hays or silages (Jeranyama and Garcia 2004) and was positively correlated with dry matter intake and digestible dry matter contents of silage (Yucel et al. 2018). According to the quality classification of Rohweder et al. (1978), the sole sunn hemp and mixtures of sunn hemp-maize silage studied were of acceptable quality for use as feed.

Conclusions

Maize silage is used extensively in Turkish dairy rations to address the inadequacy of quality feed supply. The results of this study showed that sunn hemp could make good silage and improve the nutritive value of maize silage. Sole sunn hemp silage and the mixture of 80% sunn hemp and 20% maize were found suitable for making good quality silage and it was concluded that they could be used in animal husbandry.

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(Note of the editors: All hyperlinks were verified 6 September 2021).

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(Received for publication 19 January 2021; accepted 2 September 2021; published 30 September 2021)

doi: 10.17138/TGFT(9)321-336

Artículo científico

Criterios de uso y conservación de árboles en potreros basados en el conocimiento local de los ganaderos en una zona de bosque seco tropical en Colombia

Criteria for use and conservation of trees in pastures based on farmers' local knowledge in a tropical dry forest zone in Colombia

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Resumen

Se atribuye a los árboles un papel importante en las fincas ganaderas, cumpliendo diversas funciones. El estudio documenta la conservación de especies leñosas en fincas ganaderas con base en el conocimiento local y técnico en una región seca de la parte alta de la cuenca del rio Magdalena, Colombia. Se aplicaron 195 cuestionarios semiestructurados para identificar la percepción de los productores sobre la clasificación y usos de especies arbóreas, con base en criterios físicos, nutricionales, fenológicos y ambientales, como indicadores de conservación y uso de las especies en potreros. Con estos indicadores se construyeron índices que identificaron características importantes de las especies mencionadas por los ganaderos. Los datos se analizaron con estadística descriptiva, comparaciones de media y técnicas de análisis multivariados. Los productores aprecian a las especies con base en criterios de uso y funciones relacionadas con su actividad productiva. Seis especies altamente valoradas: *Gliricidia sepium*, *Guazuma ulmifolia*, *Pithecellobium dulce*, *Albizia guachapele*, *Acacia farnesiana* y *Albizia saman* coinciden con otros estudios de conocimiento local. Adicionalmente, el trabajo aporta información relevante de otras doce especies que no han sido reportadas en estudios previos. Se sugiere que el valor y uso potencial de estas especies para fincas ganaderas debe investigarse más a fondo.

Palabras clave: Árboles multipropósito, forraje, investigación participativa, reconocimiento, sistemas silvopastoriles, zonas secas.

Abstract

Trees are attributed an important role in livestock farms, fulfilling various functions. The study documents the retention of woody species in cattle farms from local and technical knowledge in the upper part of the Magdalena river basin, Colombia. 195 semi-structured questionnaires were applied to identify the perception of producers about the classification and uses of tree forage species, based on physical, nutritional, phenological and environmental criteria, as indicators of conservation and use of species in pastures. With these indicators, indices were constructed that identified important characteristics of the species mentioned by the ranchers. Data were analyzed with descriptive statistics, mean comparisons, and multivariate analysis techniques. It is recognized that producers appreciate species based on criteria of use and functions related to their productive activity. Six highly valued species *Gliricidia sepium*, *Guazuma ulmifolia*, *Pithecellobium dulce*, *Albizia guachapele*, *Acacia farnesiana* and *Albizia saman* coincide with other studies of local knowledge. The study provides relevant information on twelve species associated with livestock, which have not been reported in previous studies, so it suggests deepening and complementing with scientific knowledge to recognize and

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assess the use of these potential species for livestock production, allowing interaction between knowledge in a concerted technological system.

Keywords: Dry zones, forage, multipurpose trees, participatory research, silvopastoral systems.

Introducción

Uno de los principales problemas que enfrenta la ganadería del trópico es la limitada producción de forrajes en cuanto a cantidad y calidad de las gramíneas en épocas de sequía. Es el resultado de la explotación tradicional extensiva que ha llevado a bajas producciones y bajos ingresos de los productores, mostrando ser la alternativa menos viable de producción ganadera (López et al. 2009).

Villanueva et al. (2009) resaltan la importancia de conocer las especies forrajeras que puedan ser incluidas en sistemas silvopastoriles para asegurar una producción adecuada durante épocas de sequía. Por tanto, diferentes autores destacan la importancia de los árboles en los sistemas de producción ganadera, más aún si se parte del conocimiento que los productores han acumulado a través del tiempo (Pezo 2009). Dicho conocimiento no debe restringirse a la identificación de especies, sino también considerar las características, usos, limitantes y potencialidades de los árboles en sus fincas (Stokes 2001).

Diversas características de los árboles y arbustos han sido reconocidas por los productores al valorar tanto su potencial forrajero como su importancia como generadores de servicios ecosistémicos. Esto los clasifica como componentes multipropósito que han hecho contribuciones importantes en los sistemas de producción ganadera, al tiempo que contribuyen al equilibrio del medio ambiente y aportan a la economía familiar (Harvey et al. 2008; Pezo 2009).

Pérez-Almario et al. (2017) han demostrado que en Colombia existe un gran número de especies leñosas con alto potencial para la alimentación bovina, con contenidos nutricionales más altos que las gramíneas, y que a su vez contribuyen con servicios ambientales. Lo anterior ha llevado a que los productores encuentren en los árboles una opción como fuente forrajera, sobre todo en épocas críticas, y que hayan desarrollado criterios que les permiten seleccionar, adoptar y conservar las especies adaptadas a las condiciones de sus fincas (Mosquera 2010).

Varias investigaciones han identificado diferentes características de los árboles en las fincas (Sierra et al. 2017; Holguín et al. 2018). Sin embargo, los criterios más utilizados por los productores al momento de decidir sobre la conservación, es decir, la no tala, de los árboles en sus potreros son aquellos que de una u otra forma generan algún tipo de beneficio inmediato (Sirrine et al. 2010). El conocimiento de especies leñosas con múltiples funciones es más significativo en áreas secas, dada la función de sombra y oferta de alimento que estas especies pueden aportar a los animales en épocas de sequía prolongada (Serrano et al. 2014; Sierra et al. 2017).

Por tanto, el objetivo del estudio es documentar la percepción de los productores ganaderos sobre el uso y la clasificación de las especies leñosas presentes en los potreros de sus fincas en la zona de estudio, con base en las características de los árboles, y relacionarlas con los aportes de uso y funciones percibidos.

Materiales y Métodos

Zona de estudio

El estudio se realizó en la parte alta de la cuenca del río Magdalena y cubre siete municipios en el norte del Departamento del Huila y once en el centro y sur del Departamento del Tolima. Su área seca es influenciada por una zona desértica (‘La Tatacoa’) y tiene una extensión aproximada de 1,200,000 ha, en el denominado Valle Cálido del Alto Magdalena (Corpoica-Cortolima 2011) (Figura 1). Ecológicamente, la zona de estudio corresponde al bosque seco tropical (bs-T) y bosque muy seco tropical (bms-T), con áreas subhúmedas y semiáridas, respectivamente (Holdridge 1978). El rango de los promedios mínimo y máximo de precipitación anual es de 1,270 a 1,880 mm con distribución bimodal (abril–mayo y octubre–noviembre); es importante resaltar que en el año se presentan entre 240 y 265 días sin precipitación. La temperatura promedio oscila entre 26 y 30 °C y la humedad relativa entre 56 y 79% (Pérez-Almario et al. 2017). Parte de los suelos en la zona son bastante erosionados con afloramientos rocosos y fertilidad mediana a baja. Se han reportado Ultisoles, Alfisoles e Inceptisoles (Mantilla et al. 1998).

Muestreo

Se seleccionó una muestra de 195 fincas con ganado bovino en los 18 municipios de la zona de estudio con un rango altitudinal entre 300 y 1,000 msnm (Cuadro 1), aplicando criterios cualitativos y cuantitativos de las teorías de muestreo (Teddlie y Yu 2007). Las fincas se eligieron a partir de un muestreo estratificado donde los estratos correspondieron a las áreas de las fincas para definir los conglomerados del estudio. La importancia del método de muestreo consistió en lograr que el mayor número de fincas elegidas proporcionara la mayor información posible para profundizar sobre la pregunta de investigación (Martínez-Salgado 2012).

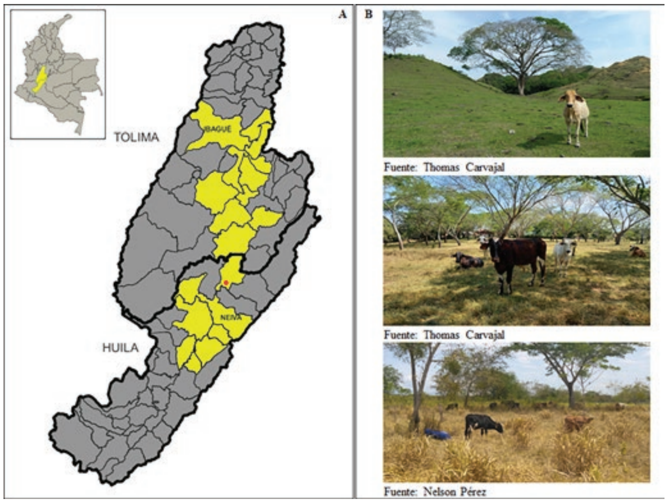

Figura 1. A) Zona de estudio en 18 municipios (amarillo) de los departamentos del Huila y Tolima, entre latitudes N 2°37' y 4°33', y longitudes O 74°53' y 75°24'. B) Árboles en fincas ganaderas de la zona de estudio. El punto rojo indica la ubicación del desierto La Tatacoa.

Los criterios para seleccionar la muestra fueron tres: fincas ganaderas con árboles; accesibilidad a las fincas; y saturación de la información. El primero fue un criterio '*sine qua non*' dada la naturaleza del objetivo de la investigación, mientras que el segundo dependió de la infraestructura vial y el costo de desplazamientos. El tercer criterio, el nivel de saturación de la información, es entendido como el punto en el cual se ha escuchado cierta diversidad de información, y con cada entrevista u observación adicional no aparecen nuevos elementos (Mayan 2009). Mientras siga apareciendo nueva información, la búsqueda no para, por lo cual este criterio es tanto punto de partida del diseño de muestreo como resultado: el criterio de suficiencia (saturación) en la información solo puede determinarse en el proceso y no *a priori*, dejando con el investigador la responsabilidad de determinar cuándo el nivel de saturación es lo suficientemente alto para declarar apropiado el muestreo (Martínez-Salgado 2012). La información obtenida con cierto nivel de repetibilidad o con nuevas especies o características que describen el nivel de uso y conservación de estas en las fincas por parte de los ganaderos, indica el nivel de saturación.

En las 195 fincas se realizaron entrevistas a los productores y se aplicó un cuestionario semiestructurado. La indagación correspondió a la caracterización de las fincas y a la identificación de especies leñosas, con las preferencias de uso de los ganaderos y la valoración de las especies. Estas se encuentran sobre todo como árboles o arbustos dispersos en potreros, producto de la regeneración natural (Figura 1B). Algunas especies también se encuentran como árboles en cercas vivas, sembrados por los ganaderos para tal fin o nacidos por regeneración natural.

Cuadro 1. Distribución de las fincas encuestadas por municipio en el sur del Tolima y norte del Huila, Colombia.

No.consecutivo	Municipio	Departamento	No. de fincas
1	Coyaima	Tolima	12
2	Coello	Tolima	1
3	Espinal	Tolima	9
4	Guamo	Tolima	13
5	Ibagué	Tolima	23
6	Natagaima	Tolima	8
7	Ortega	Tolima	20
8	Piedras	Tolima	6
9	Prado	Tolima	10
10	Saldaña	Tolima	4
11	San Luis	Tolima	7
12	Campoalegre	Huila	8
13	Hobo	Huila	10
14	Neiva	Huila	11
15	Palermo	Huila	24
16	Rivera	Huila	7
17	Villavieja	Huila	16
18	Yaguará	Huila	6
Total de fincas			195

Características del cuestionario estructurado

Con un cuestionario semiestructurado, diseñado por Ospina y Pérez-Almario (2013), se indagó sobre el tamaño de las fincas, distribución del uso de la tierra, las características productivas de las fincas y los usos de los árboles (véase Anexo).

Para clasificar los usos de las especies se establecieron tres categorías: 1) uso forrajero (ramoneo directo, cercas vivas, corte y acarreo); 2) confort animal (sombrío); y 3) otros usos (madera y mejoramiento del suelo). Las variables de cada categoría fueron calificadas como de uso alto (A), medio (M) y bajo (B), para cada especie.

Para valorar las especies, se registraron las frecuencias (porcentajes) con que los ganaderos usaron los juicios de valor previamente identificados en las diferentes categorías de criterios, para decidir si una especie debe conservarse en la finca.

Los cuatro grupos de criterios evaluados fueron:

Criterios físicos (9): 1. Hojas suaves para el ganado; 2. No tiene espinas; 3. Hojas pequeñas; 4. Tiene hojas en la punta de la rama; 5. Hojas duras; 6. Hojas dispersas en las ramas; 7. Tienen espinas; 8. Hojas grandes; 9. Tiene raíz profunda.

Criterios nutricionales (8): 1. Altamente nutritivo; 2. El ganado se engorda; 3. Aumenta la leche; 4. Es rico en calcio y fósforo; 5. Controla parásitos externos; 6. Muy digestible; 7. Controla enfermedades y/o parásitos internos; 8. Le gusta al ganado y no es amargo.

Criterios fenológicos (5): 1. Retiene parte de las hojas; 2. No se caen las hojas; 3. Se caen todas las hojas; 4. Se caen los frutos; 5. No se caen todos los frutos.

Criterios ambientales (5): 1. Tolera sequía; 2. Tolera encharcamiento y sequía; 3. Se encuentra en varias alturas (m.s.n.m.); 4. Se encuentra en varios tipos de suelo; 5. Produce alta sombra y confort.

Para cuantificar la información obtenida mediante el cuestionario se construyó una base de datos en Excel y se procedió a estandarizar los datos usando el método de estandarización (z-score) según el protocolo de Schuschny y Soto (2009). Este consiste en ajustar la variable original, restándole a cada valor la media y dividiendo este entre la desviación estándar. El resultado para cada cálculo fue denominado Índice de Diferencia Ajustada (I), cuyos resultados se obtuvieron mediante la fórmula:

$$I = \sum \frac{(x_i - \overline{X}_{x_i})}{\sigma_{x_i}}$$, donde:

I es el valor del índice;

x_i es el valor de cada unidad de la variable del criterio;

$\overline{X}_{xi}$ es el valor promedio de la variable usado para el ajuste;

σ_{xi} es el valor de la desviación estándar de cada variable usado para el ajuste.

Análisis estadístico

Se aplicaron análisis descriptivos, comparaciones de medias usando la diferencia mínima significativa (LSD de Fisher) y análisis multivariados [componentes principales, conglomerados (método de Ward)], mediante los cuales se agruparon las fincas según su área, uso de la tierra, número y área de potreros, grupos de animales y número de estos. Las variables de las especies incluidas en cada criterio de uso y conservación se describieron con el índice (I) anteriormente mencionado. Sumando los índices de los cuatro criterios para diferenciar las especies de mayor importancia se obtuvo un índice consolidado. La distribución de las especies en función de los criterios se representó mediante un gráfico Biplot; para ello se usó el paquete estadístico InfoStat (Di Rienzo et al. 2018).

Resultados

Características productivas de las fincas

La mayor proporción de las fincas analizadas son pequeñas y medianas. El área difiere (P>0.05) entre los tres grupos resultantes del análisis de conglomerados. Mientras que el conglomerado C2 presenta mayor número de fincas de tamaño mediano, el conglomerado C1 (fincas más pequeñas) ocupa un segundo lugar en cuanto a número de fincas, y el conglomerado C3, con el menor número de fincas, muestra las propiedades de mayor superficie. Las fincas grandes poseen mayor número de potreros (pasturas) cuya área promedio (32 ha) es además muy superior al de los potreros en las fincas medianas y pequeñas (Cuadro 2).

La distribución y los usos de la tierra en las fincas difieren entre conglomerados. La proporción del área dedicada a potreros es mayor en las fincas pequeñas y medianas (69 y 70%, respectivamente) comparadas con la observada en fincas grandes (47%). Sin embargo, las fincas grandes tienen una importante proporción del área dedicada a conservar los bosques ribereños y áreas de barbecho (27.7 y 25.5 ha, respectivamente). De otro lado, la conservación de los árboles difiere con el tamaño de las fincas, pues las pequeñas conservan en promedio 98 árboles, las medianas 515 y las grandes 2,223 (Cuadro 3).

Descripción de usos de los árboles

Se reportaron 31 especies leñosas que los ganaderos relacionaron con diferentes usos en sus fincas. Los resultados sugieren que hay una alta proporción de especies con usos múltiples diferentes al forrajero. Se reportaron: 16 especies usadas para el consumo por ramoneo directo, de las cuales 7 se consideran de uso alto, 2 de uso medio y 7 de uso bajo; 10 especies para corte y acarreo en bancos forrajeros (8 de uso alto y 2 de uso medio); 31 especies para el confort de los animales (13 de uso alto, 5 de uso medio y 13 de uso bajo); 8 especies para uso en cercas vivas (6 de uso alto y 2 de uso medio); y 26 especies para madera usada en la finca (10 de uso alto, 4 de uso medio y 12 de uso bajo). Todas las especies fueron mencionadas con alto uso como mejoradores de suelos (Cuadro 4).

Cuadro 2. Número y área de las fincas por conglomerado y número y área media de potreros en las fincas (medias ± error estándar).

Conglomerado	No. de fincas	Área promedio (ha)	No. de potreros[1]/finca	Área promedio de potrero (ha)
C1	76c	39.4c ± 8.29	5.0b ± 0.37	5.4b
C2	99b	61.0b ± 7.11	9.5b ± 0.75	4.5b
C3	20a	400.0a ± 10.90	17.6a ± 3.61	31.5a

Medias en una misma columna seguidas por una letra común no difieren significativamente (P>0.05).
[1]Incluye potreros con y sin árboles.

Cuadro 3. Distribución de áreas de uso de la tierra en las fincas por conglomerado (medias + error estándar).

Uso de la tierra	C1	C2	C3
Cultivo anual/transitorio (ha)	7.7b ± 3.4	5.8b ± 1.5	143.4a ± 23.4
Pasturas sin árboles (ha)	21.7b ± 7.1	27.8b ± 3.9	103.6a ± 15.9
Pasturas arboladas (ha)	5.4b ± 0.8	14.7b ± 2.4	82.0a ± 32.5
Pastos corte (ha)	0.2a ± 0.05	0.9b ± 0.2	1.0b ± 0.3
Cultivos permanentes (ha)	0.7b ± 0.3	1.8b ± 1.6	11.9a ± 6.5
Barbecho (ha)	1.3b ± 0.5	4.4b ± 0.9	25.5a ± 10.6
Bosque ribereño (ha)	2.1b ± 0.4	3.2b ± 0.5	27.7a ± 12.04
Bosque/parche bosque (ha)	0.5a ± 0.2	1.0b ± 0.3	1.5b ± 0.7
Sistema silvopastoril intensivo (ha)	0.1b ± 0.04	0.4a ± 0.4	0.6a ± 0.5
Huertos familiares (ha)	0.3b ± 0.06	1.04b ± 0.6	2.8a ± 2.25
Número de árboles por finca	98.5c ± 21.6	515.0b ± 27.6	2,223.4a ± 69.7

Medias en una misma fila seguidas por una letra común no difieren significativamente (P>0.05).

Índices para el uso y la conservación de las especies

Los criterios individuales representados en los índices o valores de importancia mostraron seis especies con índice más alto, pero con diferente orden en los criterios físicos, nutricionales, fenológicos y ambientales: Matarratón (*G. sepium*), Guácimo (*G. ulmifolia*), Payandé (*P. dulce*), Iguá (*A. guachapele*), Pelá (*A. farnesiana*) y Samán (*A. saman*) (Cuadro 5). Para estas especies, los índices (número de veces que fueron mencionadas) de los respectivos criterios oscilaron entre 11 y 19 (criterios físicos), 12 y 23 (nutricionales); 8 y 16 (fenológicos); y 9 y 17 (ambientales), respectivamente. Sin embargo, al consolidar (sumar) los índices de cada criterio para las mismas especies se encontraron valores de importancia alta (75.3; 65.2; 52.5; 48.1; 48.0; y 42.8) para Matarratón (*G. sepium*), Guácimo (*G. ulmifolia*), Payandé (*P. dulce*), Iguá (*A. guachapele*), Pelá (*A. farnesiana*) y Samán (*A. saman*), respectivamente (Cuadro 5).

Características de uso y conservación de mayor importancia para los ganaderos

El nivel de saturación para este estudio es alto, debido a que la información obtenida en los cuatro criterios incluye un importante número de especies leñosas con información 'nueva' (distintos usos) que aún no ha sido reportada para el sector ganadero en zonas secas de Colombia; entre ellas: Acacia amarilla, Ambuco, Angarillo, Bayo, Cachingo, Carbonero, Chaparro, Ciruelo, Dinde, Gomo, Palma real, Tachuelo, Tamarindo y Vainillo.

Criterios físicos. Las variables de mayor importancia para los ganaderos fueron 'hojas suaves para el ganado' (25.8%); 'no tiene espinas' (23.8%); 'hojas pequeñas' (13.2%); 'hojas dispersas en las ramas' (12.5%); y 'hojas duras' (9.6%), las cuales acumularon el 84.9% de la información suministrada.

Criterios nutricionales. Las variables nutricionales más importantes fueron 'altamente nutritivo' (21.7%); 'el ganado se engorda' (19.5%); 'aumenta la leche' (19.4%); 'es rico en calcio y fósforo' (18.9%); y 'controla parásitos externos' (15.5%), las cuales acumularon el 95% de la información.

Criterios fenológicos. Para el grupo fenológico, las variables importantes fueron 'retiene parte de las hojas' (33.4%); 'no se caen las hojas' (28.3%); y 'se caen todas las hojas' (16.7%), acumulando el 78.5% de la información. Aquí la percepción de los productores sobre la importancia de la conservación de las hojas en los árboles es entendida como forraje disponible para el consumo animal en la época seca.

Criterios ambientales. Las variables de este grupo que mostraron importancia alta para los ganaderos fueron 'muy tolerantes a sequía' (25.7%); 'tolerantes a encharcamientos y sequías' (23.1%); 'se encuentran en diferentes alturas sobre el nivel del mar' (18.9%); y 'en diferentes tipos de suelos' (18.4%). Entre estas, acumularon el 86% de la información registrada.

Cuadro 4. Nombres, usos e intensidad de uso de las especies reportadas por los productores.

Nombre común (local)	Nombre científico[1]	Uso forrajero			Confort animal	Otros usos		No. productores que mencionan la especie
		Ramoneo	Cerca viva[2]	Corte y acarreo	Sombrío	Madera	Mejora suelo	
Acacia amarilla	*Senna siamea* (Lam.) H.S. Irwin & Barneby(Fabaceae)				A	B	A	1
Ambuco	*Acacia canescens* (Britton & Killip) García-Barr. (Fabaceae)				B	A	A	10
Angarillo	*Chloroleucon mangense* var. vincentis (Benth.) Barneby & J.W. Grimes(Fabaceae)	B			B	A	A	50
Bayo	*Albizia niopoides* (Benth.) Burkart (Fabaceae)	A			A	M	A	10
Botón de oro	*Tithonia diversifolia* (Hemsl.) A. Gray (Compositae)	B		A	B		A	3
Cachingo	*Erythrina fusca* Lour. (Fabaceae)	M	A	A	M	B	A	15
Carbonero	*Calliandra riparia* Pittier (Fabaceae)	A			M	B	A	38
Chaparro	*Curatella americana* L.(Dilleniaceae)				B	B	A	14
Ciruelo	*Spondias purpurea* L. (Anacardiaceae)		A	A	B		A	16
Cují	*Prosopis juliflora* (Sw.) DC.(Fabaceae)				B	A	A	25
Dinde	*Maclura tinctoria* (L.) D. Don ex Steud. (Moraceae)				A	A	A	27
Gomo	*Cordia alba* (Jacq.) Roem. & Schult. (Boraginaceae)	A	M	A	M	B	A	14
Guácimo	*Guazuma ulmifolia* Lam. (Malvaceae)	A		A	M	B	A	163
Gualanday	*Jacaranda caucana* Pittier (Bignoniaceae)				A	A	A	5
Guayaba	*Psidium guajava* L. (Myrtaceae)	M			B	A	A	5
Iguá	*Albizia guachapele* (Kunth) Dugand (Fabaceae)	A			A	A	A	128
Leucaena	*Leucaena leucocephala* (Lam.) de Wit (Fabaceae)	A		A	M	B	A	56
Matarratón	*Gliricidia sepium* (Jacq.) Walp. (Fabaceae)	B	A	A	B	B	A	179
Moringa	*Moringa oleifera* Lam. (Moringaceae)		A	A	B		A	1
Nacedero	*Trichanthera gigantea* (Humb. & Bonpl.) Nees (Acanthaceae)		A	M	B		A	15
Neem	*Azadirachta indica* (A. Juss.) (Meliaceae)				A	M	A	22
Orejero	*Enterolobium cyclocarpum* (Jacq.) Griseb. (Fabaceae)				A	B	A	4
Palma real	*Attalea butyracea* (Mutis ex L. f.) Wess. Boer(Arecaceae)				B		A	10
Patevaca	*Bauhinia variegata* L.(Fabaceae)	A			A	B	A	7
Payandé	*Pithecellobium dulce* (Roxb.) Benth. (Fabaceae)	B	A		A	M	A	117
Pelá	*Acacia farnesiana* (L.) Willd. (Fabaceae)	B			B	A	A	109
Samán	*Albizia saman* (Jacq.) Merr.(Fabaceae)	B			A	B	A	94
Tachuelo	*Zanthoxylum rhoifolium* Lam. (Rutaceae)				B	A	A	1
Tamarindo	*Tamarindus indica* L. (Fabaceae)				A	M	A	16
Totumo	*Crescentia cujete* L. (Bignoneaceae)	B	M		A	A	A	20
Vainillo	*Senna spectabilis* (DC.) H.S. Irwin & Barneby(Fabaceae)				A	B	A	3

A = Uso alto; M = Uso medio; B = Uso bajo. [1]Nomenclatura científica según The World Flora Online (worldfloraonline.org); [2]Uso forrajero por ramoneo y corte y acarreo.

Cuadro 5. Valor de los índices de las especies según el orden de importancia para los productores en cada criterio

Especie	Físico	Especie	Nutricional	Especie	Fenológico	Especie	Ambiental
Guácimo	19.1	Matarratón	23.1	Matarratón	16.6	Matarratón	17.0
Payandé	19.1	Guácimo	21.5	Guácimo	9.9	Guácimo	14.7
Matarratón	18.6	Iguá	14.4	Pelá	8.7	Iguá	12.0
Pelá	15.3	Pelá	14.4	Samán	8.7	Payandé	11.0
Iguá	13.7	Payandé	13.9	Payandé	8.5	Samán	10.7
Samán	11.4	Samán	12.1	Iguá	8.0	Pelá	9.6
Angarillo	8.7	Ciruelo	8.6	Leucaena	4.9	Carbonero	5.0
Cují	8.2	Totumo	5.1	Angarillo	4.0	Ciruelo	4.4
Carbonero	7.1	Carbonero	4.8	Ciruelo	3.5	Leucaena	2.9
Ciruelo	6.6	Angarillo	4.2	Carbonero	3.4	Angarillo	2.4
Cachingo	6.0	Neem	4.0	Cují	2.6	Totumo	2.4
Totumo	6.0	Chaparro	3.3	Neem	2.3	Nacedero	2.0
Leucaena	5.0	Leucaena	3.2	Dinde	1.8	Cachingo	2.0
Dinde	4.4	Cují	2.6	Gomo	1.7	Dinde	1.8
Neem	3.7	Cachingo	2.3	Totumo	1.3	Cují	1.8
Nacedero	3.5	Gomo	2.3	Tamarindo	1.3	Chaparro	1.3
Chaparro	3.3	Palma real	2.2	Ambuco	1.1	Bayo	1.3
Ambuco	3.1	Dinde	2.1	Cachingo	1.1	Palma real	1.2
Gomo	2.9	Nacedero	1.3	Nacedero	1.0	Neem	0.9
Palma Real	2.1	Bayo	1.3	Chaparro	0.8	Gomo	0.6
Bayo	1.7	Ambuco	1.3	Palma real	0.8	Tamarindo	0.6
Tamarindo	1.2	Orejero	1.0	Bayo	0.6	Patevaca	0.5
Patevaca	1.1	Botón de oro	0.8	Patevaca	0.4	Botón de oro	0.5
Gualanday	0.9	Guayaba	0.7	Gualanday	0.3	Orejero	0.4
Botón de oro	0.6	Gualanday	0.6	Moringa	0.3	Gualanday	0.3
Orejero	0.6	Patevaca	0.6	Botón de Oro	0.2	Ambuco	0.3
Acacia amarilla	0.1	Tamarindo	0.5	Orejero	0.2	Acacia amarilla	0.1
Tachuelo	0.1	Moringa	0.3	Vainillo	0.1	Vainillo	0.09
Guayabo	0.1	Acacia amarilla	0.3	Acacia amarilla	0.05	Moringa	0.05
Vainillo	0.04	Vainillo	0.07	Tachuelo	0.05	Guayaba	0.04
Moringa	0.02	Tachuelo	0.03	Guayaba	0.02	Tachuelo	0.01

Grupos de especies con similitud entre criterios

En la Figura 2 se presenta una gráfica *biplot* como una herramienta estadística del análisis de componentes principales, para ilustrar la forma cómo se relacionan y agrupan características multivariables. Se observa que de los cuatro grupos, los criterios físicos constituyen el vector de la mayor distancia desde el origen y por tanto representa la variable que más contribuye a la variabilidad de las observaciones. En los Cuadrantes I y IV se encuentran las especies que más reconocen los ganaderos (las mencionadas con más frecuencia) mientras que los Cuadrantes II y III incluyen las especies mencionadas con menor frecuencia y valoración.

Las especies observadas en el Cuadrante I incluyen a Pelá (*A. farnesiana*), Guácimo (*G. ulmifolia*) y Payandé (*P. dulce*) mientras que el Cuadrante IV muestra a Iguá (*A. guachapele*), Samán (*A. saman*) y Matarratón (*G. sepium*). Estas seis especies presentan similitud entre sí y se asocian con los criterios nutricional y ambiental al encontrarse cerca al eje que los divide. Sin embargo, especies como Angarillo (*C. mangense var. vincentis*), Carbonero (*C. riparia*) y Ciruelo (*S. purpurea*) están en los mismos cuadrantes, lo cual sugiere similitud entre ellas, pero no se asocian claramente con los criterios físico, nutricional y ambiental.

En los Cuadrantes II y III se encuentran las especies Orejero (*E. cyclocarpum*), Dinde (*M. tinctoria*), Totumo (*C. cujete*), Vainillo (*S. spectabilis*), Neem (*A. indica*), Gomo (*C. alba*), Nacedero (*T. gigantea*), Bayo (*A. niopoides*), Moringa (*M. oleifera*), Ambuco (*A. canescens*), Cují (*P. juliflora*), Palma real (*A. butyracea*), Cachingo (*E. fusca*), Tachuelo (*Z. rhoifolium*), Patevaca (*B. variegata*), Tamarindo (*T. indica*), Chaparro (*C. americana*), Leucaena (*L. leucocephala*) y Gualanday (*J. caucana*). Estas especies muestran similitud entre sí al encontrarse ligadas al eje que divide sus cuadrantes, pero no se asocian a ningún criterio de selección.

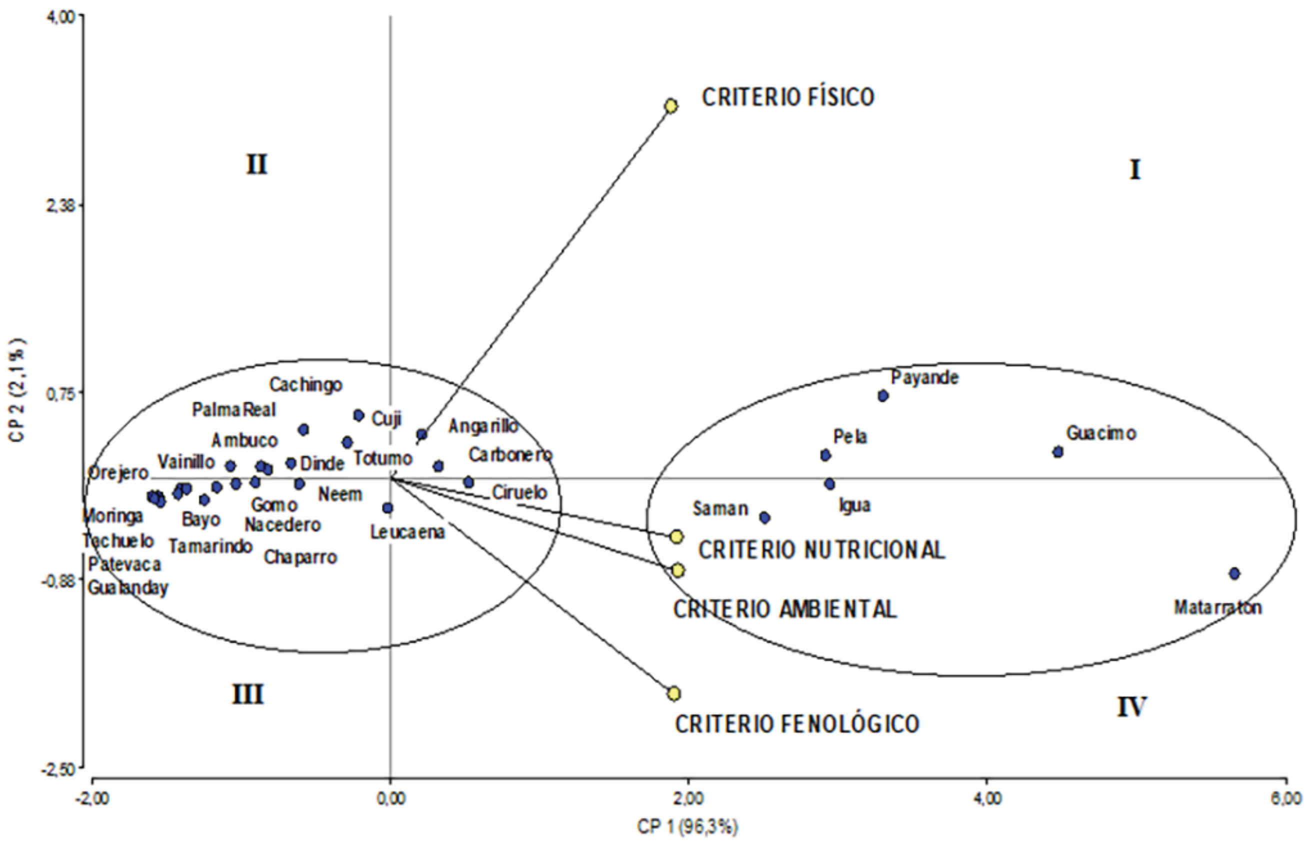

Figura 2. *Biplot* de criterios y especies arbóreas según menciones de los ganaderos de las fincas en la zona de estudio.

Discusión

Caracterización de las fincas

La muestra de fincas analizada constituye un reflejo de la estructura agraria de la región Alto Magdalena, en la cual es evidente una concentración de la propiedad en pocos latifundios y un fraccionamiento de las fincas de áreas medianas y pequeñas que representan la mayor proporción de la muestra (hasta 90%). Esto coincide con otros estudios (Mora-Delgado et al. 2014; Medina-Ríos 2019) que describen una alta concentración de las fincas ganaderas en el Tolima al tiempo que observan un fraccionamiento de propiedades pequeñas, caracterizadas por problemas de sobrepastoreo. Este estudio evidencia la presencia de amplias áreas de conservación para usos de bosque en las fincas grandes (13.7%) en comparación con 10.4 y 6.8% en las fincas pequeñas y medianas, respectivamente; esto es concordante con lo reportado por Medina-Ríos et al. (2016). Las fincas grandes tienen una mayor posibilidad de diversificar la producción agropecuaria, mientras que los propietarios de predios pequeños manifiestan que sus opciones para establecer actividades agrícolas son limitadas, dada la evidente escasez de agua en la zona del estudio. Olaya et al. (2000) reportan un déficit hídrico durante gran parte del año, lo cual es limitante para la agricultura si no se cuenta con riego, siendo la ganadería extensiva la actividad más rentable, por su baja inversión. Por tanto, la mayor parte de una finca pequeña es destinada a pasturas y las actividades agrícolas se limitan a pequeñas áreas para el autoconsumo. Hallazgos similares fueron reportados por Rodríguez (2020).

Una característica interesante en esta tipología de predios es que, durante la apertura de la frontera agrícola para el establecimiento de pasturas, no arrasaron totalmente con los árboles; aún se conservan especies de árboles dispersos en potreros, cercas vivas y bosques ribereños. Varios autores, que han estudiado las formas de conservación de especies leñosas en fincas ganaderas de la región, dan cuenta de la retención de los árboles (Serrano et al. 2014; Sierra et al. 2017; Herrera 2020), una evidencia del potencial de estos sistemas para la conservación de la diversidad florística (Harvey et al. 2008).

Descripción de usos de los árboles según la percepción de los ganaderos

Gran parte de los criterios utilizados por los ganaderos para identificar las especies arbóreas se relacionan con la identificación de las especies más conocidas por su calidad nutritiva; de hecho, a esta relación se llega por indicadores empíricos como el aumento en la cantidad de leche y o ganancia de peso observada. Por otra parte, las percepciones sugieren una apreciación de las especies leñosas por su tolerancia a variables del clima como las sequías prolongadas. Estas percepciones ratifican la capacidad de los productores para usar modelos causales para la identificación y conservación de árboles importantes frente a la estacionalidad forrajera, el valor alimenticio y la tolerancia a sequías. Similitudes con estos criterios fueron reportados por Muñoz (2004) y Esquivel et al. (2011), quienes advierten que tales relaciones empíricas no constituyen evidencia científica, sino expresiones de la experiencia basada en la observación. Sin embargo, sus juicios van más allá de la simple percepción o la sola identificación de especies (Mora-Delgado et al. 2014; Vásquez et al. 2014) en la medida que los criterios y relaciones son establecidos con base en la comprobación empírica repetida en él tiempo. Los criterios están relacionados con el aprovechamiento de recursos forrajeros no convencionales, pero también son usados en arreglos espaciales no planeados ofreciendo productos como madera y servicios ecosistémicos como el confort animal y mejoramiento del suelo (Pérez-Almario et al. 2017).

Varias especies registradas en este estudio son reportadas también en trabajos fuera de Colombia, respecto a sus características de consumo y calidad nutritiva. Por ejemplo, en México (Pinto-Ruiz et al. 2010; Olivares-Pérez et al. 2016) y Costa Rica (Esquivel et al. 2011), destacaron a *G. ulmifolia*, *C. alba* (*sin. C. dentata*), *P. dulce, Erythrina* sp. y *G. sepium*. Para Costa Rica, Stokes (2001) y Muñoz (2004) reportaron que *G. sepium*, *G. ulmifolia*, *Erythrina* sp. *A. saman*, *L. leucocephala*, *E. cyclocarpum* y *Crescentia* sp. fueron utilizados por el 87% de los ganaderos para suplementación animal durante las épocas de sequía.

Una de las características mencionadas por todos los ganaderos respecto a las especies estudiadas corresponde a los aportes de estas al mejoramiento del suelo, entre las que se destacan las raíces profundas que ayudan a “descompactar” el suelo y los nutrientes que mejoran la fertilidad. Esto es atribuido a la caída de las hojas de los árboles por ser deciduos, los que contribuyen a incrementar la materia orgánica y, en el caso de leguminosas, además aportes importantes de nitrógeno.

La percepción de los productores respecto a la tolerancia de los árboles a factores de clima, y la plasticidad de estos para permanecer en diferentes tipos de suelo, también ha sido reportada por Muñoz (2004) y Mora-Delgado et al. (2014) quienes asociaron la presencia de árboles con indicadores de “suelos buenos” o “suelos malos”. Conceptos como el que el follaje de un árbol “es rico en calcio y fosforo”, mencionados por los ganaderos, son valoraciones que hace el productor sin base experimental, pero son usados para tomar decisiones. Al respecto hay que reconocer que estudios de etnobotánica han validado algunas plantas como fuentes ricas en calcio, fósforo y otros nutrientes (Earle 2001), lo cual sugiere la necesidad de ampliar la investigación en estas relaciones para darles soporte científico.

Los resultados sugieren que el aprecio hacia los árboles en los predios ganaderos no se da de forma aleatoria, sino que depende de características funcionales que facilitan la provisión de bienes y servicios para el productor, que pueden resultar en ingresos adicionales, convirtiéndose en un factor determinante para el mantenimiento de algunas especies en las fincas.

Características de las especies según los criterios definidos

Entre las características importantes de las especies sobresalen la calidad nutritiva del forraje asociada al incremento de la producción ganadera; la textura y el tamaño de las hojas; y el control de parásitos externos. Otras características determinantes en la decisión de los ganaderos sobre la conservación o no de las especies en los potreros son la percepción de la permanencia de las hojas en los árboles y la tolerancia a diferentes tipos de estrés.

El proyecto FRAGMENT (p.ej., Restrepo-Sáenz et al. 2004) coincide con nuestros resultados al reportar que *G. ulmifolia*, *G. sepium*, y *A. saman* son las especies forrajeras mejor calificadas, según la percepción de los ganaderos en estudios desarrollados en Centroamérica. En dicho estudio además incluyeron *L. leucocephala*, pero no obtuvo una buena calificación en este estudio. Lo anterior es debido a altos consumos y aportes significativos a la producción, calidad nutricional y palatabilidad del forraje (Pérez-Almario 2011; Joya et al. 2004).

Conclusiones

Se confirma el conocimiento sobre el valor multiuso de árboles en fincas ganaderas, especialmente en el arreglo de árboles dispersos en potreros. Esto ratifica la importancia de indagar a los ganaderos sobre sus saberes y prácticas como una premisa esencial para que el ejercicio de la investigación de leñosas sea relevante para sus necesidades, y por ende para sus sistemas ganaderos.

Este estudio sugiere la importancia de diferentes funciones y roles de los árboles que ocupan diferentes estratos en fincas ganaderas. Se requiere continuar procesos experimentales agronómicos y zootécnicos que reconozcan el potencial productivo y el valor ecológico de estas especies.

Agradecimientos

A la Corporación Colombiana de Investigación Agropecuaria – Agrosavia; Ministerio de Agricultura y Desarrollo Rural – MADR; Universidad del Tolima.

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(Nota de los editores: Los enlaces se verificaron el 19 de agosto de 2021).

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(Recibido para publicación 27 agosto 2020; aceptado 5 agosto 2021; publicado 30 septiembre 2021)

Anexo. Criterios de selección de especies forrajeras con potencial para sistemas silvopastoriles

Guía de Encuesta

Fecha	Municipio
No. Encuesta	Nombre del encuestador
Nombre del productor(a)	Coordenadas geográficas
Departamento	Revisado por:

I. Datos generales de la unidad de produccion			
A. Residencia del productor			
¿Ud. vive en la finca?			
1. De forma permanente		3. Ocasionalmente	
2. Por temporada		4. Otro	
B. Información productiva del hato			
Número de bovinos en el hato			
Vacas en producción: ____ Vaca horra/escotera: ____ Novilla levante: ____ Novilla vientre: ____ Novillos: ____			
Crías hembras: ____ Crías machos: ____ Toros: ____		Sistema de monta: Natural ____ Artificial ____ Cuál ____	
Producción de leche diaria: ____ Por vaca día: ____		Vende la leche: Si ____ No ____	
Litros de leche vendidos ____ Autoconsumo ____		Procesa la leche: Si ____ No ____	
En cuajada ____ Queso ____ Otros ____			
Litros de leche transformada ____ % de la producción que comercializa ____ Autoconsumo ____			
Costo de transporte para la comercialización: ____			
Donde comercializa el producto: en la finca ____ pasteurizadora ____ otro ____			
Cuál ____ Precio por kilo o litro ____			
Fuente de agua:			
Las fuentes de agua de la finca son propias: Si ____ No ____			
El agua de uso en la finca procede de quebradas ____ Ríos ____ Nacimientos ____ # de nacimientos ____			

II. Sistema de produccion y limitaciones tecnológicas			
A. Uso de la tierra en la finca			
Registre la tierra ocupada físicamente durante el año pasado en cada finca de su propiedad			
Conceptos	Área total (ha)	Conceptos	Área total (ha)
Cultivos anuales/transitorios (ha)		Rastrojos/barbecho (ha)	
Pasturas sin árboles (ha)		Bosque ribereño/galería (ha)	
Pasturas con árboles (ha)		Sistemas silvopastoriles intensivos (ha)	
Pastos de corte (ha)		Huertos familiares (ha)	
Plantaciones permanentes (ha)		Número divisiones potreros	
		Total área de la finca (ha)	
¿Qué labores o actividades realiza en el año para mantener a sus animales?			
Labores	Frecuencia de la labor (mes)	Insumos unidad (mes)	Cantidad (unidad)
1. alimentación			
2. vitaminación			
3. vacunación			
4. desparasitación. Externa			
5. desparasitación. Interna			
6. otro (especifique)			

B. Árboles en potreros

¿Como tiene distribuidos los potreros en la finca?

	Especies leñosas en el sistema de pastoreo	Nombre del pasto	Densidad especies de árboles y/o arbustos predominantes (Clave 1)	¿Cuántos árboles/ha? (Clave 2)	¿Qué tipo de sombra tiene? (Clave 3)	¿Árboles de regeneración natural o establecidos? (Clave 4)
1	Pasturas naturales sin árboles					
2	Pastos naturales con alta densidad de árboles					
3	Pastos naturales con mediana densidad de árboles					
4	Pastos naturales con baja densidad de árboles					
5	Pastos mejorados con alta densidad de árboles					
6	Pastos mejorados con mediana densidad de árboles					
7	Pastos mejorados con baja densidad de árboles					
Total						

	Clave 1	Clave 2	Clave 3	Clave 4
Pasto natural	1. Alta densidad	1. mayor a 100 árboles/ha	1. Copa no definida	1. Naturales
	2. Mediana densidad	2. 50 a 99 árboles/ha	2. Copa ancha	2. Establecidos
	3. Baja densidad	3. 1 a 49 árboles/ha	3. Copa cerrada	3. Ninguno
Pasto mejorado	4. Alta densidad	4. mayor a 100 árboles/ha	4. Copa cónica	
	5. Mediana densidad	5. 50 a 99 árboles/ha	5. Copa abierta	
	6. Baja densidad	6. 1 a 49 árboles/ha	6. Ninguna	

Especies leñosas forrajeras encontradas en la finca

Nombre especies	Densidad/ha	Orden de preferencia	Criterios físicos	Criterios nutricionales	Criterios fenológicos	Criterios ambientales y otros
1.						
2.						
3.						
~						
20.						

Clave de densidad	Clave 1	Clave 2	Clave 3	Clave 4
1. Alta	1. Hojas suaves para el ganado	1. Altamente nutritivo	1. Retiene parte de las hojas	1. Tolerantes a sequía
2. Media	2. No tiene espinas	2. El ganado se engorda	2. No se caen las hojas	2. Tolera encharcamiento y sequía
3. Baja	3. Hojas pequeñas	3. Aumenta la leche	3. Se caen todas las hojas	3. Se encuentra en varias alturas (m.s.n.m.)
	4. Tiene hojas dispuestas en la punta de la rama	4. Es rico en calcio y fósforo	4. Se caen los frutos	4. Se encuentra en varios tipos de suelo
	5. Hojas duras	5. Controla parásitos externos	5. No se caen todos los frutos	5. Produce alta sombra y confort
	6. Hojas dispersas en la rama	6. Muy digestible		
	7. Tienen espinas	7. Controla enfermedades y/o parásitos internos		
	8. Hojas grandes	8. Le gusta al ganado y no es amargo		
	9. Tiene raíz profunda			

Uso de las especies forrajeras

Nombre especies	Uso forrajero			Confort animal	Otros usos				Importancia
	Ramoneo	Cerca viva	Corte-acarreo	Árbol sombrío	Madera	Mejora suelos			
						Descompacta suelo	Aporta M.O	Reduce temperatura dosel	(Alto, medio, bajo)
1.									
2.									
3.									
~									
20.									

C. Sistema de manejo de los forrajes

Clave	Aplicaciones	# de veces o pases	Tipo insumos	Cosecha
Preparación del suelo	Clave 1			
Siembra semilla	Clave 2			
Fertilización	Clave 3			
Control malezas	Clave 4			
Poda	Clave 5			
Cosecha de forraje	Clave 6			
Riego	Clave 7			

Clave 1
1. Roza y quema
2. Control químico
3. Mecanización
4. Otro (especifique)

Clave 2
1. Al voleo
2. Manual en surcos
3. Siembra directa con estolones
4. Mecanizada
5. Renovación
6. Otro (cuál)

Clave 3
1. química
2. orgánica
3. orgánica y química
4. ninguna

Clave 4
1. química
2. biológica
3. manual
4. mecánica
5. ninguna

Clave 5
1. cada 6 meses
2. cada año
3. otro especifique
4. nunca
5. ninguno

Clave 6
1. pastoreo directo
2. rotacional
3. alterno
4. corte y acarreo

Clave 7
1. gravedad
2. aspersión
3. gravedad y aspersión
4. ninguno
5. otro (especifique)

D. Principales limitaciones tecnicas de la ganaderia (rumiantes)

¿Sobre cada uno de los parámetros siguientes, está Ud. satisfecho, o piensa que algo le impide lograr mejores resultados?

Parámetros	¿Satisfecho? 1. Si __ 2. No __	¿De dónde proviene el problema? Clave 1	¿Cómo podría mejorar? Clave 2	¿Por qué no lo hace? Clave 3
Área forrajera				
Carga animal				
Incremento de peso				
Edad del primer parto				
Intervalo entre partos				
Duración de la lactación				
Producción diaria de leche de una vaca en invierno				
Producción diaria de leche de una vaca en verano				
Mortalidad				
Costos de producción				
Mejoramiento genético de su hato				
Otro: Cuál__________________				

Clave 1:
1. Manejo alimentación
2. Manejo reproducción
3. Condiciones de manejo del ganado
4. Precios de los productos
5. Manejo de la sanidad
6. Genética
7. Otro (especifique)

Clave 2:
1. Mejorando la sanidad
2. Cambiando el tamaño de potreros
3. Cambiando el tipo de pasto
4. Utilizando pastos de corte
5. Mejorando la raza
6. Mejorando las condiciones de vida del ganado
7. Otro (especifique)

Clave 3:
1. No sabe hacerlo
2. No sería rentable
3. Falta de recursos financieros
4. Otro (especifique)

E. Manejo tecnico en otras actividades pecuarias

Mencione: _____________________________ no. Animales ____

F. Principales limitaciones tecnicas de las actividades pecuarias (rumiantes)

¿Sobre cada uno de los parámetros siguientes, está Ud. satisfecho, o piensa que algo le impide tener mejores resultados?

Parámetros	¿Satisfecho? 1. Si _2. No_	¿De dónde proviene el problema? Clave 1	¿Cómo podría mejorar? Clave 2	¿Por qué no lo hace? Clave 3
Incremento de peso				
Edad del primer parto				
Intervalo entre partos				
Producción semanal de huevos				
Mortalidad				
Costos de producción				
Mejoramiento genético de sus animales				
Otro:				

Clave 1	Clave 2	Clave 3
1. Manejo alimentación	1. Mejorando la sanidad	1. No sabe hacerlo
2. Manejo reproducción	2. Mejorando la alimentación	2. No sería rentable
3. Condiciones de vida del ganado	3. Mejorando el potencial genético	3. Falta de recursos financieros
4. Precios de los productos	4. Mejorando las condiciones de vida	4. Otro (especifique)
5. Manejo de la sanidad	5. Otro (especifique)	
6. Genética		
7. Otro (especifique)		

G. Cambios y limitaciones en cuanto a comercializacion y almacenamiento de los productos pecuarios

COMERCIALIZACION

A. ¿Ha habido cambios en las condiciones de comercialización de sus productos pecuarios en el último año?

B. ¿Hay problemas en cuanto a ello? 1. Si (llenar cuadro) 2. No. (Pasar a columna 8 del cuadro)

	A						B		
Nombre del producto	¿Cuáles han sido los cambios? Clave 1	Fuente del cambio Clave 2	¿Qué resultado ha tenido este cambio? Clave 3	Cuantifique este resultado% Clave 3 (1 a 5)	¿Está satisfecho de este cambio? 1. si; 2. no	¿Está satisfecho de las condiciones de comercialización? 1. si; 2. no	¿Cuál es el problema? (si existe) Clave 4	¿Qué habría que hacer para resolver este problema? Clave 5	¿Por qué no se hace? Clave 6
Leche cruda									
Leche transformada									
Carne									
Crías									

Clave 1	Clave 2:	Clave 3:	Clave 4:	Clave 5	Clave 6:
1. El agente comercializador	1. modificación de las condiciones socioeconómica.	1. Ha mejorado el precio	1. Precio muy bajo (en general)	1. Mejorando la calidad	1. No sabe hacerlo
2. El lugar de venta del producto	2. innovación propia	2. Ha disminuido el tiempo de venta	2. Se vende a una época en que el precio es bajo	2. Organizarse varios productores	2. No sería rentable
3. La forma de llevar el producto	3. por imitación directa	3. Disminuido perdidas de producto	3. El costo de acceso al mercado es muy alto	3. Que haya más comerciantes	3. Falta de recursos financieros
4. La presentación del producto	4.inducida por Consejo técnico, sin que haya una demanda	4. Ha aumentado las ventas	4. Variabilidad de precio de un año al otro	4. Que se mejoren las vías	4. Otro (especifique)
5. La calidad del producto	5.Cambio técnico p/respuesta a una demanda	5. Otro (especifique)	5. Otro (especifique)	5. Disminuyendo costos transporte	
6. Otro producto transformado	6.demanda canalizada por una OP			6. No sabe	
7. El momento de venta	7. inducido por razones no técnicas (especifique)			7. Otro (especifique)	
8. Otro (especifique)	8= otro (especifique)				

doi: 10.17138/TGFT(9)337-347

Research Paper

Physiological responses of Bajra-Napier hybrids and a tri-specific hybrid to salinity stress

Respuestas fisiológicas de los híbridos Bajra-Napier y de un híbrido tri-específico al estrés por salinidad

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Abstract

Physiological responses of 3 Bajra-Napier (*Cenchrus* spp., syn. *Pennisetum* spp.) hybrid varieties, viz. BNH-3, BNH-6, BNH-10, and 1 tri-specific hybrid (TSH) were tested under different gradients of soil salinity, i.e. Control, 4, 6 and 8 dS/m electric conductivity (ECe), in a pot trial. The experiment was laid out in a factorial completely randomized design with 3 replications. Shoot dry weight, root dry weight, root:shoot ratio and chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations were reduced with increasing salinity level as compared with Control. However, the concentration of Na^+ in leaves increased and K^+ concentration decreased with increasing salinity level. Physiological parameters, i.e. relative water content (RWC), membrane stability index (MSI), chlorophyll stability index, carotenoid stability index and K^+: Na^+ ratio, in leaves tended to be higher in the BNH-3 variety than in other varieties. Shoot dry weight showed highly positive significant correlation with RWC, MSI, K^+ concentration and K^+:Na^+ ratio, while it was negatively correlated with Na^+ concentration ($P<0.01$). All BN hybrid varieties and the tri-specific hybrid studied were susceptible to salinity stress, showing marked reductions in growth as the level of salinity increased above 4 dS/m. However, even at salinity levels producing EC of 8 dS/m these varieties still produced 25–44% DM yields. There are prospects for improving forage yields from saline soils by planting these hybrids but further breeding studies are warranted to identify germplasm with greater tolerance of saline conditions if these soils are to be utilized effectively to contribute more to supplying forage to support the world's ruminant population.

Keywords: *Cenchrus americanus*, *Cenchrus purpureus*, *Cenchrus squamulatus*, dry matter yields, *Pennisetum* hybrids, salt-tolerance, tropical grasses.

Resumen

Se examinaron las respuestas fisiológicas de 3 variedades híbridas de Bajra-Napier (*Cenchrus* spp., syn. *Pennisetum* spp.), a saber, BNH-3, BNH-6, BNH-10, y 1 híbrido ttri-específico (TSH) bajo diferentes gradientes de salinidad del suelo: Control, 4, 6 y 8 dS/m de conductividad eléctrica (EC), en un ensayo en macetas. El experimento se realizó en un diseño factorial completamente al azar con 3 repeticiones. El peso seco del brote, el peso seco de la raíz, la relación raíz:brote y las concentraciones de clorofila a, clorofila b, clorofila total y carotenoides se redujeron con el aumento del nivel de salinidad en comparación con el Control. Sin embargo, la concentración de Na^+ en las hojas aumentó y la de K^+ disminuyó con el aumento del nivel de salinidad. Los parámetros fisiológicos: contenido relativo de agua (RWC), índice de estabilidad de la membrana (MSI), índice de estabilidad de la clorofila, índice de estabilidad de los carotenoides y la

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relación K^+: Na^+, en las hojas tendieron a ser más altos en la variedad BNH-3 que en otras variedades. El peso seco de los brotes mostró una correlación significativa altamente positiva con el RWC, el MSI, la concentración de K^+ y la relación K^+:Na^+, mientras que se correlacionó negativamente con la concentración de Na^+ (P<0.01) Todas las variedades híbridas BN y el híbrido tri-específico estudiado fueron susceptibles al estrés por salinidad, mostrando marcadas reducciones en el crecimiento a medida que el nivel de salinidad aumentaba por encima de 4 dS/m. Sin embargo, incluso a niveles de salinidad que producían una EC de 8 dS/m, estas variedades seguían produciendo un rendimiento de 25–44% de materia seca. Hay perspectivas de mejorar los rendimientos de forraje de los suelos salinos mediante la siembra de estos híbridos, pero se justifica la realización de más estudios de mejoramiento para identificar el germoplasma con mayor tolerancia a las condiciones de salinidad si se quiere utilizar estos suelos de manera eficaz para contribuir más al suministro de forraje para mantener a la población mundial de rumiantes.

Palabras clave: *Cenchrus americanus*, *Cenchrus purpureus*, *Cenchrus squamulatus*, gramíneas tropicales, híbridos de *Pennisetum*, rendimiento de materia seca, tolerancia a la sal.

Introduction

Salinity is one of the major abiotic stresses of arid and semi-arid regions that affect crop growth, development and productivity (Pons et al. 2011). About 20% of the world's cultivated area and about half of the world's irrigated lands are affected by salinity stress (Sairam and Tyagi 2004). More than 800 million hectares of land throughout the world are adversely affected by high salinity (Munns and Tester 2008). In India, salt-affected soils occupy an area of about 6.73 Mha of which saline and sodic soils constitute about 40 and 60%, respectively (Singh et al. 2010).

The physiological responses of a plant to salinity are often complex and multi-faceted, which makes experiments difficult to design and interpret (Negrão et al. 2017). Salinity poses two major threats to plant growth, i.e. osmotic stress and ionic stress (Flowers and Colmer 2008). The responses to these changes are often accompanied by a variety of symptoms, such as a decrease in leaf area, an increase in leaf thickness and succulence, abscission of leaves, necrosis of roots and shoots and a decrease in internode lengths (Parida and Das 2005). Roots, being a primary organ, are directly exposed to saline environments, but their growth is less vulnerable to salinity than that of shoots (Picchioni et al. 1990). The accumulation of Na^+ in roots is an adaptive response used by various woody species to avoid its toxicity in shoots (Picchioni et al. 1990; Gucci and Tattini 1997).

Livestock production is the backbone of Indian agriculture and it has been projected that the livestock population will increase to around 286.5 million adult cattle units by 2050 (IGFRI Vision 2050). The major concern is to ensure sufficient green fodder is available throughout the year, as there is a deficiency of green fodder and concentrate feed (Semple et al. 2003). Cultivation of cereals and cash crops has resulted in the reduction in the area of land for fodder production for livestock, which is the major constraint in green fodder production. There is a need to use degraded lands, particularly saline soils, by identifying salt-tolerant crops and grasses, which could be used as fodder for grazing livestock (Kumar and Sharma 2020).

Bajra-Napier (BN) hybrid is an interspecific hybrid between bajra [*Cenchrus americanus* (L.) Morrone, the name currently accepted by the GRIN taxonomy (npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch) for *Pennisetum glaucum*) and Napier grass (*Cenchrus purpureus* (Schumach.) Morrone, syn. *Pennisetum purpureum*). Bajra-Napier hybrid and tri-specific hybrid (*Cenchrus americanus* × C. *purpureus* × C. *squamulatus*; syn. *Pennisetum glaucum* × *P. purpureum* × *P. squamulatum*) are perennial, multi-cut forage grasses with high biomass and high nutritional quality coupled with high palatability (Singh et al. 2018). BN hybrids can withstand drought for a short spell and currently about one hundred thousand hectares are grown in India. Considering the adverse effects of salt stress on crop growth and productivity, the development of salt-tolerant genotypes and more particularly salt-tolerant BN hybrids and tri-specific hybrids could play a major role in sustaining livestock production in the salt-affected lands and would also be helpful in future breeding programs. We hypothesize that these hybrids are salt-tolerant and should produce well in saline soils. Keeping in view the above facts, the present experiment was conducted to evaluate the physiological responses in 3 BN hybrids and 1 tri-specific hybrid (TSH) grown under saline conditions in a glasshouse.

Materials and Methods

Experimental design

This pot study was conducted at Crop Improvement Division of ICAR - Indian Grassland and Fodder Research

Institute, Jhansi (25°45’ N, 78°58’ E; 243 masl), during Rabi (winter season, October–March) 2018 in a complete randomized block design. Root slips of 4 varieties, viz. BNH-3, BNH-6, BNH-10 and TSH were collected from ICAR-IGFRI Technology Demonstration Block and planted in pots containing 6 kg of soil at 4 different (Control, 4, 6 and 8 dS/m) levels of salinity and 3 replications. The initial properties of the collected soil were: slightly alkaline with pH 7.62; electrical conductivity (ECe) 1.12 dS/m; and low in organic carbon (0.49%). The total nitrogen, available phosphorus and potassium concentrations in the soil were 213, 13.8 and 191 kg/ha, respectively. Saline conditions were created by adding a mixture of NaCl, Na_2SO_4, $MgCl_2$ and $CaSO_4$ (in ratio 13:7:1:4) to pots to provide electrical conductivity of treated soils of 4, 6 and 8 dS/m at 30 days after transplanting with a Control (1.12 dS/m) for comparison. Plants were harvested at 30 and 55 days after stress was imposed.

Shoot dry weight and root dry weight

At each harvest, i.e. at 60 and 85 days of age, above-ground material was removed, placed in paper bags and oven-dried at 45 °C until a constant weight was reached after about 72 hours to determine shoot dry weight (SDW). At the 85-day harvest, roots were also collected and dried (RDW). Root:shoot ratio (RSR) was determined based on the shoot and root values measured.

Physiological parameters

The acetone method was applied to green leaf samples (200 mg fresh weight) from the 3rd leaf from top portion to extract chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl) and carotenoid (Car) and 100 mg leaf (green leaf) samples were used to determine membrane stability index (MSI) (3rd leaf from top portion) according to the method of Premachandra et al. (1990). The relative water content (RWC) of 100 mg leaf samples (3rd leaf from top portion) was analyzed by the method of Weatherley (1950). Sodium (Na^+) and potassium (K^+) concentrations in 1 g dry leaf (sampled from young shoot leaves) samples were determined by the flame photometer method of Jackson (1973). Chlorophyll stability index (CHSI) was calculated by following the method described by Sairam et al. (1997) using the formula: (Total chlorophyll in salt-stressed plants/Total chlorophyll in Control plants) × 100; a similar formula was used to determine carotenoid stability index (CARSI).

Reduction in performance relative to Controls (%ROC) was calculated as follows:

$$\%ROC = \frac{\text{Value for Control-Value for stressed plants}}{\text{Value for Control}} \times 100$$

Statistical analysis

The study was conducted as a factorial experiment based on a completely random design with 3 replications. The data were analyzed by Microsoft Excel and SAS 9.3 statistical analytical tool and the significance of differences between treatment means was checked with Duncan's multiple range test at $P<0.05$.

Results

Significant to highly significant interactions were found between variety and level of salinity for SDW and RWC at the first harvest and for MSI and carotenoids at the second harvest, whereas highly significant interactions were found for K^+ and Na^+ concentrations and K^+:Na^+ ratio at the first harvest and for SDW, RDW, RWC, Chl a, Chl b, Total Chl, K^+ and Na^+ concentrations at the second harvest (Table 1).

Effects of salt stress on shoot dry weight, root dry weight and root:shoot ratio

Shoot dry weight (SDW), root dry weight (RDW) and root:shoot ratio (RSR) declined for all varieties as level of salinity increased (Table 2). While an ECe level of 4 dS/m had no significant effect on growth at the first harvest, at the highest salinity level reduction in SDW over Controls ranged from 56% for BNH-3 to 75% for BNH-6, and at the second harvest from 61% for BNH-3 to 72% for BNH-10. Reductions in RDW over the Controls at the second harvest were more pronounced than for SDW with reductions of 19–33% at 4 dS/m and 71–78% at 8 dS/m. As a result, RSR declined from 0.42–0.54:1 for Controls to 0.33–0.39:1 at the highest salinity level (Table 2). At the first harvest, SDW showed positive significant correlations ($P<0.01$) with RWC, MSI, K^+ and K^+:Na^+ ratio and negative correlations with Chl a, Chl b, Total Chl, carotenoid and Na^+ concentrations. At the second harvest, SDW indicated positive significant correlations with RDW, RSR, RWC, MSI and Chl b, Total Chl, carotenoid and K^+ concentrations, while Na^+ concentrations showed negative significant correlations ($P<0.01$) with RSR, MSI and K^+:Na^+ ratio (Table 6).

Table 1. ANOVA results of the effects of salt stress on SDW, RDW, RWC, MSI, Chl a, Chl b, Total Chl, Car, K^+, Na^+ and K^+:Na^+ ratio of *Cenchrus* hybrid varieties.

		First Harvest Mean square											
Variable	df	SDW	RWC	MSI	Chl a	Chl b	Total Chl	Carotenoids	Chl a+b	K^+	Na^+	K^+:Na^+ ratio	
V	3	4.43*	90*	301**	0.18**	0.10**	0.55**	0.008**	0.004NS	0.069**	0.268**	0.081**	
ECe	3	164.45**	569**	363**	0.02*	0.02*	0.09*	0.002NS	0.001NS	1.859**	0.085**	0.765**	
V×ECe	9	1.59*	7*	2NS	0.004NS	0.003NS	0.004NS	0.001NS	0.08NS	0.027**	0.075**	0.010**	
Error	30	0.726	74.564	46.657	0.008	0.005	0.021	0.001	0.045	0.004	0.004	0.002	
		Second Harvest Mean square											
		SDW	RDW	RWC	MSI	Chl a	Chl b	Total Chl	Carotenoids	Chl a+b	K^+	Na^+	K^+/Na^+ ratio
V	3	4.14*	2.99**	85**	110*	0.15**	0.07**	0.4**	0.02**	0.03NS	0.183**	0.035**	4.123 NS
ECe	3	146.75**	41.60**	4669**	159*	0.40**	0.21**	1**	0.01**	0.03NS	17.843**	0.575**	1237.96 NS
V×ECe	9	1.50**	0.14**	48**	14*	0.01**	0.01**	0.04**	0.0005*	0.01NS	0.170**	0.014**	0.860 NS
Error	30	0.155	0.005	0.826	36.355	0.002	0.0012	0.006	0.0001	0.018	0.004	0.003	1.779

SDW - shoot dry weight
RDW - root dry weight
RWC - relative water content
MSI - membrane stability index
Chl a - chlorophyll a
Chl b - chlorophyll b
Total Chl -total chlorophyll
Car - carotenoid
K^+ - potassium
Na^+ - sodium
K^+:Na^+ ratio - potassium to sodium ratio
V - variety
ECe - electrical conductivity of the extract of a saturated soil-paste.

Table 2. Effects of salt stress on shoot dry weight (g/pot), root dry weight (g/pot) and root:shoot ratio of *Cenchrus* hybrid varieties at 60 days (1st harvest) and 85 days (2nd harvest) of age.

Variety/	Shoot dry weight (1st harvest)				Shoot dry weight (2nd harvest)				Root dry weight (2nd harvest)				Root:shoot ratio			
Treatment	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10
Control	11.3±0.6	12.0±0.9	11.2±0.5	12.4±0.6	10.8±0.1	10.0±0.5	10.1±0.2	12.4±0.2	5.5±0.1	4.8±0.0	6.0±0.1	5.2±0.0	0.51	0.48	0.54	0.42
ECe4	11.0±0.5	11.5±0.2	10.9±0.3	12.0±0.3	9.6±0.2	9.5±0.1	8.7±0.1	11.2±0.4	4.5±0.0	3.2±0.0	4.7±0.0	4.1±0.0	0.47	0.34	0.43	0.36
ROC%	2	4	2	3	11	5	14	10	19	33	22	21				
ECe6	6.2±0.1	5.7±0.0	7.8±0.1	6.8±0.2	5.1±0.1	4.2±0.1	5.8±0.0	5.6±0.1	1.9±0.0	1.4±0.0	2.9±0.0	1.9±0.0	0.37	0.33	0.37	0.34
ROC%	45	53	30	45	52	58	42	55	65	71	52	63				
ECe8	4.5±0.1	3.0±0.1	4.9±0.0	4.0±0.1	3.7±0.1	3.3±0.0	3.9±0.0	3.4±0.0	1.4±0.0	1.1±0.0	1.7±0.0	1.1±0.0	0.39	0.33	0.35	0.33
ROC%	60	75	56	68	66	68	61	72	74	78	71	79				

Mean (n = 3)
ROC% - per cent reduction over Control for ECe of 4, 6 and 8 dS/m.

Effects of salt stress on Relative water content and Membrane stability index

Relative water content (RWC; %) and Membrane stability index (MSI; %) were considered reliable parameters to assess the salt stress and tolerance of crop species. RWC of leaf declined in all varieties with increasing salinity, with percentage reduction relative to Controls at the highest salinity level ranging from 48 to 63% for the different varieties at the first harvest and from 50 to 69% at the second harvest (Table 3). Membrane stability index (MSI) for all varieties also declined with increasing salinity at first ($P<0.01$) and second ($P<0.05$) harvests. RWC showed highly significant positive correlations with SDW, MSI, K^+ and K^+:Na^+ ratio at the first harvest, and highly significant positive correlations with SDW, RDW, MSI and K^+ and moderately significant correlation with K^+:Na^+ ratio at the second harvest (Table 6). MSI showed highly significant positive correlations with SDW, RWC, K^+ and K^+:Na^+ ratio at the first harvest, and with SDW, RDW, RSR, RWC, K^+ and K^+:Na^+ ratio at the second harvest.

Effects of salt stress on photosynthetic pigments and Chlorophyll stability index and Carotenoid stability index

Data in Table 4 show that chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations decreased as salinity increased at both harvests with the main part of the decline occurring between 6 and 8 dS/m. Chlorophyll stability index (CHSI) and carotenoid stability index (CARSI) also declined as salinity level increased, with the major reduction occurring between 6 and 8 dS/m (Table 5). Photosynthetic pigments (Chl a, Chl b and Total Chl) showed significant positive correlations with each other and carotenoid concentrations at both first and second harvests (Table 6).

Effects of salt stress on K^+ and Na^+ concentrations and K^+:Na^+ ratio in leaves

Potassium concentrations in leaves at the first and second harvests declined as salinity levels increased (Table 7) but differences failed to reach significance ($P>0.05$) despite reductions in concentrations at 8 dS/m ECe being about 52 and 70%, respectively. In contrast, sodium concentrations showed little consistent response at the first harvest ($P>0.05$) but increased markedly for TSH, BNH-3 and BNH-6 and decreased for BNH-10 at the second harvest with again no significant responses ($P>0.05$). In general K^+:Na^+ ratio declined as level of salinity increased at both harvests with the effect being much more pronounced at the second harvest (except for BNH-10) but again differences were not significant ($P>0.05$).

In addition to correlations mentioned earlier, K^+ concentrations showed significant positive correlations with K^+:Na^+ ratio at the first and second harvests, while Na^+ concentration showed significant negative correlations with K^+:Na^+ ratio in first and second harvests (Table 6).

Table 3. Effects of salt stress on Relative water content and Membrane stability index in *Cenchrus* hybrid varieties.

Variety/ Treatment	Relative Water Content (%)							
	1st Harvest				2nd Harvest			
	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10
Control	82.7±1.21a	78.4±2.40a	74.6±1.70a	78.7±2.04a	77.1±2.23a	70.0±2.31bc	68.0±1.73cd	72.0±1.79b
4 dS/m	78.7±1.91ab (5)	70.0±2.31b (11)	73.0±1.15a (2)	74.0±1.73a (6)	67.0±1.73d (13)	61.0±2.31e (13)	60.0±1.15e (12)	66.0±0.58d (8)
6 dS/m	52.0±2.19c (37)	49.0±2.48c (37)	60.0±2.19a (20)	56.0±1.50c (29)	44.0±1.15g (43)	38.0±1.73h (46)	48.0±1.04f (29)	48.0±1.44f (33)
8 dS/m	38.0±1.44d (54)	29.0±2.31de (63)	39.0±0.92d (48)	36.0±1.73d (54)	24.0±1.33k (69)	23.0±2.31k (67)	34.0±1.73i (50)	30.0±2.19j (58)
Variety/ Treatment	Membrane Stability Index (%)							
	1st Harvest				2nd Harvest			
	TSH	BNH-6	BNH-3	BNH-10	TSH	BNH-6	BNH-3	BNH-10
Control	65.0±1.44a	54.2±1.54ab	64.7±1.20a	58.3±0.96a	53.0±3.18a	48.0±2.6a	50.0±2.89a	49.0±3.76a
4 dS/m	57.7±1.50a (11)	46.2±2.17abc (15)	61.5±1.17a (5)	53.3±1.80ab (9)	33.09±1.8b (26)	32.0±2.5b (33)	37.0±1.73b (26)	35.0±1.2b (28)
6 dS/m	42.73±1.92abc (34)	31.72±2.26c (41)	42.11±0.87abc (35)	38.16±1.25bc (35)	34±1.62c (37)	30±2.48bc (38)	35±1.45bc (30)	32±2.37bc (35)
8 dS/m	24.21±1.45c (63)	19.31±2.13c (64)	28.32±1.97c (56)	24.71±1.84c (58)	26±1.82d (51)	14±2.23d (70)	26±1.51cd (48)	25±1.62d (49)

Means within column(s) followed by the same letter(s) are not significantly different ($P>0.05$). N=3. Values in parenthesis depict per cent reduction over control (ROC%).

Table 4. Effects of salt stress on chlorophyll and carotenoid concentrations (mg/g fresh weight) in *Cenchrus* hybrid varieties

Variety/Treatment		First harvest				Second harvest			
		Chl a	Chl b	Total Chl	Car	Chl a	Chl b	Total Chl	Car
TSH	Control	0.60+0.0 4abc	0.47+0.048 abc	1.06+0.004 cbd	0.22+0.03 a	0.56+0.02 bcd	0.43+0.017 edf	0.99+0.038 ed	0.16+0.009 bcd
BNH-6		0.81+0.05 ab	0.65+0.028 ab	1.46+0.083 ab	0.19+0.02 ab	0.75+0.06 a	0.59+0.021 ab	1.34+0.076 ab	0.11+0.028 fhig
BNH-3		0.88+0.05 a	0.69+0.038 a	1.57+0.008 a	0.25+0.02 a	0.78+0.06 a	0.61+0.035 a	1.39+0.052 a	0.22+0.030 a
BNH-10		0.62+0.03 abc	0.49+0.042 abc	1.11+0.076 abcd	0.19+0.02 ab	0.57+0.04 bcd	0.45+0.015 cde	1.03+0.023 cde	0.15+0.007 cdef
TSH	4 dS/m	0.56+0.04 bc (7)	0.42+0.0 4bc (9)	0.99+0.00 4cbd (7)	0.20+0.018 ab (7)	0.51+0.02 cde (10)	0.37+0.015 efg (14)	0.88+0.03 efg (11)	0.15+0.008 cde (8)
BNH-6		0.73+0.05 ab (9)	0.57+0.02 ab (12)	1.33+0.078 ab (9)	0.17+0.016 ab (9)	0.66+0.05 abc (13)	0.49+0.017 cd (22)	1.14+0.07 bcd (15)	0.10+0.006 hifg (15)
BNH-3		0.84+0.04 a (5)	0.65+0.03 a (6)	1.50+0.007 a (5)	0.23+0.023 a (6)	0.71+0.03 ab (8)	0.54+0.018 abc (12)	1.25+0.05 ab (10)	0.20+0.016 ab (9)
BNH-10		0.58+0.03 abc (6)	0.46+0.03 abc (7)	1.04+0.072 bcd (6)	0.18+0.016 ab (7)	0.51+0.03 cde (11)	0.40+0.013 defg (14)	0.91+0.02 edf (12)	0.12+0.006 b (18)
TSH	6 dS/m	0.46+0.05 c (23)	0.39+0.016 bc (17)	0.85+0.031 cd (17)	0.18+0.01 ab (16)	0.35+0.03 fg (38)	0.30+0.023 g (29)	0.65+0.052 g (34)	0.13+0.012 cdefg (20)
BNH-6		0.61+0.04 abc (25)	0.49+0.038 abc (25)	1.10+0.006 abcd (25)	0.15+0.01 b (20)	0.47+0.01 def (38)	0.41+0.023 defg (30)	0.88+0.037 efg (34)	0.08+0.005 fhig (28)
BNH-3		0.72+0.05 a (18)	0.58+0.029 ba (17)	1.30+0.076 ab (17)	0.23+0.02 a (8)	0.65+0.03 abc (16)	0.49+0.040 bcd (19)	1.14+0.068 bcd (18)	0.17+0.004 bc (22)
BNH-10		0.49+0.03 bc (21)	0.41+0.017 bc (16)	0.90+0.018 cd (16)	0.18+0.01 ab (8)	0.40+0.02 ef (30)	0.34+0.012 fg (24)	0.74+0.029 fg (28)	0.13+0.012 defg (10)
TSH	8 dS/m	0.30+0.03 c (50)	0.26+0.016 c (44)	0.56+0.019 d (44)	0.14+0.01 ab (36)	0.21+0.01 gh (62)	0.20+0.007 h (53)	0.41+0.020 h (59)	0.08+0.003 hij (51)
BNH-6		0.32+0.03 c (60)	0.28+0.029 c (57)	0.60+0.054 d (57)	0.1+0.01 b (37)	0.12+0.02 h (84)	0.12+0.030 h (80)	0.24+0.029 h (82)	0.04+0.020 j (64)
BNH-3		0.54+0.02 bc (39)	0.43+0.019 bc (38)	0.97+0.005 bcd (38)	0.2+0.02 b (35)	0.46+0.00 def (41)	0.35+0.023 fg (43)	0.81+0.027 efg (41)	0.12+0.014 defg (46)
BNH-10		0.31+0.03 c (50)	0.25+0.019 c (49)	0.56+0.010 d (49)	0.1+0.02 b (37)	0.21+0.02 gh (63)	0.19+0.004 h (58)	0.43+0.026 h (58)	0.09+0.004 hig (39)

Means in column (s) followed by the same letter (s) are not significantly different (P>0.05). N=3. Values in parenthesis depict per cent reduction over control (ROC%).

Table 5. Effects of salt stress on chlorophyll stability index and carotenoid stability index in *Cenchrus* hybrid varieties.

Variety/ Treatment	Chlorophyll stability index (%)					
	1st harvest			2nd harvest		
	EC4	EC6	EC8	EC4	EC6	EC8
TSH	93	80	53	88	66	41
BNH-6	91	75	41	85	66	18
BNH-3	95	82	61	91	83	87
BNH-10	94	81	50	94	79	21
Variety/ Treatment	**Carotenoid stability index (%)**					
	1st harvest			2nd harvest		
	EC4	EC6	EC8	EC4	EC6	EC8
TSH	93	84	64	92	80	49
BNH-6	91	80	63	85	72	36
BNH-3	94	92	65	91	78	54
BNH-10	93	92	63	82	90	61

Table 6. Correlations among different parameters in *Cenchrus* hybrid varieties subjected to salinity stress.

PM	1st Harvest												
	SDW	RWC	MSI	Chl a	Chl b	Total_Chl	Car	Chl a:b	% K^+	% Na^+	K^+:Na^+ ratio		
SDW	—												
RWC	0.977***	—											
MSI	0.930***	0.964***	—										
Chl a	-0.139	-0.041	-0.059	—									
Chl b	-0.130	-0.033	-0.042	0.996***	—								
Total Chl	-0.130	-0.036	-0.054	0.999***	0.998***	—							
Car	-0.173	-0.034	-0.027	0.801***	0.792***	0.791***	—						
Chl a:b	-0.261	-0.179	-0.243	0.682**	0.622*	0.660**	0.633**	—					
% K^+	0.796***	0.817***	0.841***	-0.312	-0.310	-0.314	-0.004	-0.251	—				
%Na^+	-0.178	-0.223	-0.300	0.337	0.331	0.341	0.056	0.269	-0.368	—			
K^+:Na^+ ratio	0.720**	0.751***	0.799***	-0.351	-0.349	-0.355	0.007	-0.258	0.976***	-0.548*	—		
PM	2nd Harvest												
	SDW	RDW	RSR	RWC	MSI	Chl a	Chl b	Total Chl	Car	Chl a:b	% K^+	% Na^+	K^+:Na^+ ratio
SDW	—												
RDW	0.927***	—											
RSR	0.651**	0.858***	—										
RWC	0.963***	0.936***	0.710**	—									
MSI	0.808***	0.880***	0.802***	0.874***	—								
Chl a	-0.258	-0.194	0.016	-0.123	-0.139	—							
Chl b	-0.264	-0.184	0.032	-0.133	-0.150	0.993***	—						
Total Chl	-0.274	-0.201	0.018	-0.140	-0.152	0.998***	0.997***	—					
Car	-0.424	-0.287	0.040	-0.264	0.020	0.734**	0.717**	0.732**	—				
Chl a:b	-0.260	-0.194	0.039	-0.120	-0.074	0.839***	0.782***	0.815***	0.695**	—			
% K^+	0.779***	0.838***	0.764***	0.786***	0.816***	-0.199	-0.189	-0.203	-0.151	-0.158	—		
% Na^+	-0.596*	-0.679**	-0.598*	-0.714**	-0.614*	0.132	0.161	0.148	0.243	0.001	-0.379	—	
K^+:Na+ ratio	0.572*	0.732**	0.857***	0.663**	0.778***	-0.041	-0.076	-0.060	0.115	0.200	0.654**	-0.711**	—

PM - parameters; SDW - shoot dry weight; RDW - root dry weight; RSR - root:shoot ratio; RWC - relative water content; MSI - membrane stability index; Chl a - chlorophyll a; Chl b - chlorophyll b; Total Chl - total chlorophyll; Chl a:b - Chl a:Chl b ratio; Car - carotenoid; K^+ - potassium; Na^+ - sodium; K^+:Na^+ ratio - potassium: sodium ratio.

Table 7. Effects of salt stress on Na^+ and K^+ concentrations in leaves of *Cenchrus* hybrid varieties over 2 harvests.

Variety	Treatment	1st Harvest			2nd Harvest		
		% K^+	%Na^+	K^+:Na^+ ratio	% K^+	% Na^+	K^+:Na^+ ratio
TSH	Control	1.81±0.06bd	1.67±0.03ca	1.09±0.02bd	1.93±0.03cd	0.08+0.00a	24.08+0.40bd
	ECe4	1.12±0.02bc	1.90±0.05c	0.59±0.01bc	1.53±0.02c	0.23±0.01ab	6.71±0.19bc
	ECe6	0.95±0.03b	1.80±0.06cb	0.53±0.03b	0.51±0.02bc	0.56±0.01ac	0.92±0.06b
	ECe8	0.88±0.03ab	1.83±0.03cb	0.48±0.01ab	0.26±0.01ac	0.67±0.01ad	0.39±0.01ab
BNH-6	Control	1.67±0.05ad	1.65±0.03ad	1.01±0.05ad	1.22±0.01ad	0.05±0.005ab	24.48±2.18ad
	ECe4	1.00±0.07ac	1.98±0.01cd	0.50±0.03ac	0.65±0.01ac	0.23+0.01b	2.78+0.10ac
	ECe6	0.82±0.01ab	2.00±0.06bd	0.41±0.02ab	0.59±0.01ab	0.59±0.02bc	1.01±0.02ab
	ECe8	0.76±0.02a	2.27±0.04bd	0.34±0.00a	0.49±0.02a	0.70±0.01bd	0.70±0.04a
BNH-3	Control	1.80±0.04ad	1.64±0.03a	1.10±0.04d	1.82±0.01bd	0.09±0.00a	20.90±0.78abd
	ECe4	1.13±0.01ac	1.64±0.04ac	0.69±0.01cd	0.76±0.02bc	0.20±0.01ab	3.70±0.36abc
	ECe6	1.12±0.01ab	1.70±0.02ab	0.66±0.00bd	0.67±0.01b	0.30+0.01ac	2.21+0.17ab
	ECe8	0.93±0.08a	1.50±0.03ab	0.62±0.04ad	0.58±0.01ab	0.46±0.01ad	1.27±0.03aab
BNH-10	Control	1.62±0.03bd	1.70±0.02ab	0.95±0.03bd	2.31±0.01ac	0.59±0.01ab	3.92±0.05abd
	ECe4	1.33±0.03bc	1.95±0.03bc	0.68±0.02bc	0.68±0.02c	0.23±0.01b	3.00±0.05abc
	ECe6	0.99±0.01b	1.67±0.04b	0.59±0.01b	0.61±0.01bc	0.29±0.02bc	2.13±0.18ab
	ECe8	0.75±0.02ab	1.60±0.04b	0.47±0.004ab	0.48±0.02ac	0.30±0.02bd	1.60±0.04ab

Means in columns followed by the same letter (s) are not significantly different (P>0.05), where letter “a” represents the least value. N = 3.

Discussion

Salinity stress affects growth and productivity in plants by altering physiological mechanisms like water relations, metabolism, ion accumulation, nutrient imbalance and Reactive Oxygen Species (ROS) generation. While salinity tolerance in annual forages and plants is well defined (Roy and Chakraborty 2014; Munns et al. 2020a, 2020b; Rahimi et al. 2021), this is not the case for perennial grasses and plants. Salts are common and necessary components of soil and many salts (e.g. sodium nitrate, potassium carbonate, bicarbonate and potassium chloride) are essential plant nutrients at low concentrations.

Grasses are quite variable in their tolerance of salinity in terms of growth (Khan et al. 1999; Hester et al. 2001; Muscolo et al. 2003; Joshi et al. 2004). Muscolo et al. (2003) reported that the biomass of kikuyu grass (*Cenchrus clandestinus* formerly *Pennisetum clandestinum*) leaves and roots was affected by 150 mM NaCl and extensively reduced at high concentration of NaCl (200 mM) compared with Control, while growth was little affected at lower concentrations of NaCl (50 mM).

Our results showed that shoot dry weight, root dry weight and root:shoot ratio declined for all varieties as the level of salinity increased, while the low level of 4 dS/m had very little or no effect on growth and dry matter yield. These results agreed with Al-Ghumaiz et al. (2017), who reported that dry fodder yield declined at high levels of salinity (8,000 ppm NaCl) with very little or no effect on growth and dry fodder yield at the low level of salinity (4,000 ppm NaCl) in perennial ryegrass, tall fescue and orchard grass.

As a macronutrient, potassium (K^+) mostly contributes to a plant’s survival when exposed to various environmental stresses such as drought, salinity and cold (Wang and Wu 2013). The positive role of K^+ in the response to salinity is due to: (1) its competitiveness with sodium (Na^+) for binding sites and maintaining relative water content (RWC) in plants (Capula-Rodríguez et al. 2016); and (2) its ability to regulate the balance between ROS and antioxidants to adjust protein synthesis and stomatal function, thereby improving a plant’s photosynthetic status (Wang et al. 2013). Moreover, foliar spraying of perennial ryegrass with KNO_3 (10 mM) enhanced growth, chlorophyll concentration and K:Na ratio when grown under saline conditions. The decrease in RWC under saline conditions can be attributed to a reduction of soil water potential in the root zone (Munns et al. 2006). Sairam and Tyagi (2004) and Singh et al. (2020) suggested that reduced shoot height, leaf area and number of leaves in sensitive genotypes under saline conditions may be due to their leaves having lower relative water content and membrane stability index. In addition, the accumulation of Na^+ and Cl^- ions can lead to the production of ROS which, in turn, increases the permeability of the cell membrane and decreases MSI (Nazar et al. 2011). RWC and MSI are good indicators of leaf water status and stability of membranes and are successfully used to determine stress resistance or tolerance in many crop plants (Bangar et al. 2019;

Rahimi et al. 2021). Many reports reveal that RWC and MSI are reduced under drought and salinity (Bangar et al. 2019; Rahimi et al. 2021) and those plants that maintain high RWC and MSI under extreme stress are regarded as being more stress-tolerant (Bangar et al. 2019; Rahimi et al. 2021). In our study, the reductions in RWC at the first harvest at the highest salinity level ranged from 48 to 63% and at the second harvest from 50 to 69%, while reductions in MSI ranged from 56 to 64% at the first harvest and from 48 to 71% at the second harvest. This indicates that, while these varieties can tolerate low salinity levels, impacts on these parameters at higher levels of salinity are quite significant. In our study, shoot dry weight (SDW) was positively correlated with RWC and MSI at both harvests. The highest reductions in SDW relative to Controls at both first and second harvests occurred at the highest salinity level and ranged from 56 to 75% at the first harvest and from 61 to 72% at the second harvest, which are of comparable magnitude to the reductions in RWC and MSI. Our results are in conformity with Rahimi et al. (2021), who reported that RWC and MSI were significantly and positively correlated with K^+: Na^+ ratio and K^+ concentration in shoots and roots of rye grass under salinity stress.

Chlorophyll has been proposed as a useful biochemical indicator of salt tolerance in different plants (Akram and Ashraf 2011) as chlorophyll and carotenoids are involved in the primary step concerning energy production during photosynthesis. Since salinity affects chlorophyll and carotenoid levels, it is not surprising that the growth of plants is inhibited when grown in saline situations. Salt stress increases the activity of chlorophyllase, which promotes degradation of chlorophyll and reduces chlorophyll concentration in plants (Yang et al. 2011). Although salt stress can reduce chlorophyll concentration, the extent of the reduction depends on the salt tolerance of the particular plant species. Differences in reductions in chlorophyll concentrations between the different varieties in our study suggest that the degree of tolerance of salinity by the various varieties was relatively similar, although BNH-3 did display lower reductions relative to Control than other varieties as salinity level increased. Carotenoids play an important role as a precursor in signalling during plant development under abiotic stress as they protect the membranes from oxidative damage (Verma and Mishra 2005). While all varieties demonstrated reductions in carotenoid concentrations relative to Controls with increasing salinity, at the higher salinity levels BNH-10 showed a tendency to suffer less reduction than other varieties. These results corroborate other studies that indicate that plants subjected to increased salinity levels show decreased photosynthetic pigments (Aghaleh et al. 2009; Jampeetong and Brix 2009; Al-humaiz et al. 2017).

Numerous studies have shown that salt tolerance is ultimately manifested in plants through several physiological processes including Na^+ uptake and exclusion, in homeostasis, especially between K^+:Na^+ ratio and partitioning (Ren et al. 2005). Various studies have shown that plants increase Na^+ uptake and reduce K^+ uptake under salt stress (Horie et al. 2001; Zhu 2003). The K^+ ions are beneficial to plants and by increasing K^+ concentration, plants can reduce the absorption of Na^+ ions to a certain extent, thus improving the K^+: Na^+ ratio.

Generally, the data in Table 7 indicate that the mean percentages of Na^+ in leaves of all varieties increased with increase in salinity, while K^+ concentration declined because Na^+ effectively competes with K^+ for uptake in a common transport system, i.e. the Na^+ concentration in saline environments is usually greater than that of K^+ (Gorham et al. 1990). In other words, the decrease in K^+ resulted from the presence of excessive Na^+ in the growth medium because high external Na^+ concentrations are known to have an antagonistic effect on K^+ uptake in plants (Sarwar et al. 2003). Interestingly K^+ concentration in leaf tissue of Controls was relatively similar for both harvests, while Na^+ concentration was much lower at the second than the first harvest.

Conclusions

This study has shown that the varieties of BN hybrids and the tri-specific hybrid studied were all susceptible to salinity stress, showing marked reductions in growth as the level of salinity increased above 4 dS/m. However, dry matter yields obtained at high salinity level (ECe of 8 dS/m) were still at the range of 25–44%. There are prospects for improving forage yields from saline soils by planting these hybrids but further breeding studies are warranted to identify germplasm with greater tolerance of saline conditions if these soils are to be utilized effectively to contribute more to supplying forage to support the world's ruminant population.

Acknowledgments

The authors acknowledge Indian Council of Agricultural Research (ICAR) and ICAR–Indian Grassland and Fodder Research Institute, Jhansi for financial support for conducting the experiment.

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(Note of the editors: All hyperlinks were verified 1 September 2021).

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(Received for publication 20 October 2020; accepted 24 August 2021; published 30 September 2021)

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(3):348–358
doi: [10.17138/TGFT(9)348-358](https://doi.org/10.17138/TGFT(9)348-358)

Genetic Resources Communication

Clearing confusion in *Stylosanthes* taxonomy. 3. *S. hamata* sensu stricto vs. *S. hamata* sensu lato

Aclarando confusiones en la taxonomía de Stylosanthes. *3.* S. hamata *sensu stricto vs.* S. hamata *sensu lato*

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Abstract

Stylosanthes hamata (L.) Taub., a suffruticose leguminous species with spreading prostrate or ascending stems, is widely distributed in the Caribbean region. It was originally described as *Hedysarum hamatum* by Linnaeus and later transferred to *Stylosanthes* by Taubert. To date, chromosome analysis of accessions of *S. hamata* originating from the Caribbean islands has revealed all to be diploids (2*n*=20). An accession of a morphologically similar *Stylosanthes* species, collected near Maracaibo in Venezuela in 1965 and subsequently misidentified as *S. hamata,* has found application as sown forage on low fertility soils in the subhumid to dry tropics since its registration as cultivar Verano in Australia in 1975. This morphotype has been shown to be tetraploid, and has been referred to in the literature as "tetraploid *S. hamata*" or "*S. hamata* sensu lato". More recent work has demonstrated that the tetraploid is in fact an allotetraploid with *S. hamata* sensu stricto and *S. humilis* Kunth as the putative diploid progenitors. Various authors have recommended that the allotetraploid be treated as a separate species. We support this recommendation and suggest that, based on the information provided in this paper, the new species be described and validly published following examination of a more exhaustive range of specimens.

Keywords: Cytology, Fabaceae, molecular markers, morphology, phylogeny.

Resumen

Stylosanthes hamata (L.) Taub. es una leguminosa subarbustiva con tallos postrados a ascendentes. Es ampliamente distribuida en la región del Caribe y fue originalmente descrita por Linnaeus como *Hedysarum hamatum* y después transferida por Taubert a *Stylosanthes*. Con base en análisis de cromosomas quedó evidente que todas las accesiones de *S. hamata* originarias de las islas del Caribe son diploides (2*n*=20). Una accesión de una especie de *Stylosanthes* morfológicamente similar, colectada en 1965 cerca de Maracaibo, Venezuela y erróneamente identificada como *S. hamata*, llegó a ser ampliamente usada como forraje sembrado en suelos de baja fertilidad en regiones tropicales secas a subhúmedas, después de su registro como cultivar Verano en Australia en 1975. Se estableció que este morfotipo es tetraploide y en la literatura se le encuentra denominado "*S. hamata* tetraploide" o "*S. hamata* sensu lato". En un estudio más reciente se demostró que el tetraploide es en realidad un alotetraploide con *S. hamata* sensu stricto y *S. humilis* Kunth como supuestos progenitores diploides. Varios autores han recomendado que el alotetraploide sea tratado como una especie separada. Apoyamos esta recomendación y sugerimos que, con base en la información recopilada en este documento, la nueva especie sea descrita y válidamente publicada.

Palabras clave: Citología, Fabaceae, filogenética, marcadores moleculares, morfología.

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Introduction

As with previous papers in this series (Cook and Schultze-Kraft 2019; Schultze-Kraft et al. 2020), we draw attention to an issue involving two related chromosomal races of *Stylosanthes*, one diploid and one tetraploid. In this case the two broad karyotypic groups are mostly referred to under the same species epithet, hamata. The taxonomic confusion arose following the decline of large areas of naturalized stands of *S. humilis* Kunth in northern Australia in the early 1970s due to the spread of anthracnose, a serious disease caused by the fungus, *Colletotrichum gloeosporioides*. Research identified an accession of *Stylosanthes* originating from near Maracaibo in Venezuela as having potential to replace *S. humilis*. This accession, catalogued as *S. hamata* CPI 38842, was released as cultivar 'Verano' in Australia (McKay 1975), with a similar accession, CPI 55822, from the same Maracaibo region, released as 'Amiga' (Edye 1997), both appearing in the registration statement as *Stylosanthes hamata* (L.) Taub., with a tetraploid chromosome complement of 2*n*=40. Previous work had shown *S. hamata* to be diploid, 2*n*=20 (Cameron 1967). Although plants of 'Verano' and 'Amiga' are similar to diploid morphotypes, there is now strong evidence that the cultivars more correctly belong to a new tetraploid species.

The recommendation to revise *S. hamata*, providing clear taxonomic distinction between the diploid and tetraploid types, is not novel, having already been raised over a number of years by Stace and Cameron (1987), Maass and Sawkins (2004), and Calles and Schultze-Kraft (2016). This paper serves to reiterate the urgency for taxonomic revision of *S. hamata* sensu lato by presenting current cytological evidence supported by morphological, geographical, genetic, and rhizobiological evidence that *S. hamata* sensu lato actually comprises two distinct species.

Taxonomy

Stylosanthes hamata was described by Linnaeus (1758) as *Hedysarum hamatum* based on Sloane's illustration of a specimen from Jamaica and Burman's illustration of a specimen from Sri Lanka. Taubert (1891) provided a more detailed description in a monograph of *Stylosanthes* and transferred the species to *Stylosanthes*.

The Maracaibo tetraploid has been referred to in the literature as "tetraploid *Stylosanthes hamata*" or "*Stylosanthes hamata* sensu lato", neither being strictly appropriate. The former is incorrect because there is published scientific evidence showing it to be an allotetraploid with *S. hamata* as one of the putative genome donors, and an allopolyploid should not be assigned to the taxon of one of the putative parents (pers. comm. M. Schori, USDA ARS). The latter term should not be used because it is imprecise, implicitly embracing both types. Stace and Cameron (1984) addressed the issue by referring to the tetraploid as *Stylosanthes* sp. nov. (2*n*=40), which also lacks specificity. Stace (pers. comm. to R. Schultze-Kraft, June 1984) suggested the allotetraploid be called "*Stylosanthes maracaibensis*" in reference to the geographical origin of the species, or "*S. hemihamata*" in reference to its alloploid origin. However, neither of the proposed epithets has been validly published as prescribed by the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2018). We will therefore refer to the Maracaibo allotetraploid as *Stylosanthes* sp. nov. throughout the remainder of this paper.

The genus *Stylosanthes* can be divided into two taxonomic sections on the basis of presence or absence of a small feathery appendage at the base of the flower and loment, possibly a small rudimentary secondary floral axis often referred to as the axis rudiment. *Stylosanthes hamata* possesses the axis rudiment and is accordingly placed in section *Stylosanthes*, while the other genome donor of *Stylosanthes* sp. nov., *S. humilis*, which lacks the axis rudiment, is assigned to section Astyposanthes (Hert.) Mohl. *Stylosanthes* sp. nov., which possesses the axis rudiment in the lower flowers only, may best remain unassigned by virtue of its intersectional origins.

Cytology and morphology

The existence of both diploid (Cameron 1967) and tetraploid (Brolmann 1979; Stace and Cameron 1984, 1987) accessions assigned to *S. hamata* in various genetic resource collections has been long recognized. In his pioneer chromosome work, Cameron (1967) showed two Caribbean island accessions, CPI 33205 from Guadeloupe and CPI 33231 from Puerto Rico, to be diploid (2*n*=20). The Jamaican specimen from which the lectotype Sloane illustration was prepared was most probably also diploid since current understanding suggests that the diploid race only is native in Jamaica and other Caribbean islands. Recognition of a tetraploid race within the northern South American populations of *S. hamata* was first mentioned by Stace and Cameron (1984). During 1986, the collection of *Stylosanthes* sp. nov. was substantially increased, following collecting expeditions, primarily to Venezuela but also to Colombia (Edye 1986), targeting collection of "tetraploid *S. hamata*".

S. hamata has been described by a number of authors since Taubert (1891): Mohlenbrock (1957); Costa (2006); Costa et al. (2008); Calles and Schultze-Kraft (2010); and Vanni (2017). However, none of these provides any morphological distinction between diploids and tetraploids, even though several may unknowingly have included tetraploid specimens from within the possible geographic distributional range of *Stylosanthes* sp. nov. Similarly, Burt's (1983) observation that there was considerable variability among the many accessions held as *S. hamata* in the Australian tropical forages collection may also be confounded, since by 1983 there were already more than 20 tetraploid accessions in the CSIRO collection. It is therefore conceivable that some characters or dimensional range extremes may be attributable to tetraploid specimens in those descriptions, but any morphological differences were considered to fall within the species circumscription.

The only published description of *Stylosanthes* sp. nov. (Edye and Topark-Ngarm 1992) nominates a number of features that could separate this species from *S. hamata* (L.) Taub., but does not identify the specimens observed in compiling the description. A similar description prepared by Stace (unpublished 1987) was based on the examination of only two specimens of *Stylosanthes* sp. nov. from each of Venezuela and Colombia. While both nominate a number of relative morphological differences between the two species, the most consistent field differences in both descriptions are the presence in *Stylosanthes* sp. nov. of the axis rudiment in the lower part of the inflorescence only and of a long terminal bristle on the tips of the stipules and bracts. We believe that these differences need to be confirmed through examination of a wider range of identified herbarium specimens for a comprehensive description of a new species.

Table 1 below highlights some of the currently recognized key differences and similarities among the three species, drawing on information from Edye and Topark-Ngarm (1992) and various sources relating to *S. hamata* and *S. humilis*.

Table 1. Comparison of key features of *Stylosanthes* sp. nov. and its putative parent species.

Feature	*S. hamata*	*Stylosanthes* sp. nov.	*S. humilis*
Ploidy	2*n*=20	2*n*=40	2*n*=20
Life cycle	Short-lived perennial	Short-lived perennial	Obligate annual
Stem hairs	Line of fine hairs along alternating sides of internodes	Line of fine hairs along alternating sides of internodes	Line of fine hairs along alternating sides of internodes
Stem bristles	Absent	Absent	Abundant
Stipule bristles	Absent	On tips of teeth	On sheath and teeth
Bract bristles	Absent	On tips of teeth	Abundant
Axis rudiment	Present	In lower flowers only	Absent
Loment beak	Uncinate; beak ≤ upper articulation	Uncinate, slightly coiled; beak ≥ upper articulation	Uncinate to coiled; longer than other two spp., beak ≥ upper articulation
Seed color	Mostly cream, yellow to light brown	Frequently tan to dark maroon, ± mottled	Mostly brown to black

Geographic distribution

The diploids are geographically more widespread than the tetraploids, being found from about 28° N in Florida, USA, through much of the Caribbean island region to about 8° N in Venezuela, with adventive populations between about 3° and 9° S in the north-eastern Brazilian states of Ceará and Pernambuco (Edye and Maass 1997).

Three distinct tetraploid populations have been identified (Edye and Maass 1997):

- Venezuela-Colombia population occurring between 9° N and 11°30' N found sympatrically with diploid *S. hamata*. This is the group that has been the target of forage collection expeditions and provided two forage cultivars. It is also the group that has contributed to taxonomic confusion.
- Guatemala population represented in the Australian Pastures Genebank by two accessions, APG 57426 (=CPI 46587) and APG 57837 (=CPI 46588), occurring around 16° N.
- USA population at four separate sites along the southeast coast of Florida between about 26° N and 27° N; distinguished by short curved beak on the upper articulation; possibly a separate species; sympatric with the more widespread diploids (Brolmann 1979).

A disjunct population of a species identified as *S. hamata* but of undetermined ploidy occurs at about 21° S in the landlocked state of Mato Grosso do Sul, Brazil (Costa et al. 2008).

Reference to tabulated collection data for a large range of diploid and tetraploid accessions held in the Australian forage germplasm collection as *S. hamata* (Date 2010), shows that average annual rainfall was (300–)500–1,000(–1,600) mm in areas where diploid accessions were collected and (250–)500–800(–2,200) mm for tetraploids. Soil pH at collection sites was mostly in the range of (6.2–)6.5–7.5(–8.5) for diploid and (5.4–) 6.0–7.5 for tetraploid accessions. Both diploids and tetraploids are commonly found at lower elevations, but collections of both have been made at elevations >1,000 masl. Lists of diploid and tetraploid accessions with the Australian CPI and CIAT equivalent accession numbers are shown in the Appendix. Stace and Cameron (1987) also included genomic structure along with ploidy in the list of CPI accessions held as *S. hamata* by CSIRO in 1981. This work revealed that the two Guatemalan tetraploids have different genomic structure from that of *Stylosanthes* sp. nov. that they refer to as the "Maracaibo tetraploid".

Rhizobiology

Date (2010) noted that with few exceptions, *S. hamata* and *Stylosanthes* sp. nov. fell into different pairs of groups produced from analysis of extensive accession × *Bradyrhizobium* effectiveness experiments. The former showed a high level of specificity in respect to effectiveness of nodulation by bradyrhizobia, and the latter showed the typical rhizobial response patterns of promiscuity for tetraploid accessions. In this screening, *Bradyrhizobium* strains CB2126 and CB3050 were selected as suitable for *S. hamata* and the wide-spectrum strains CB756 and CB1650 for *Stylosanthes* sp. nov. CB2126 and/or CB3050 were also effective on many of the more promiscuous *Stylosanthes* sp. nov. accessions (Eagles and Date 1999).

Molecular biology and phylogeny

The dearth of stable morphological characters means that classification of *Stylosanthes* at the species level is extremely difficult. However, various cytological and molecular-level procedures have facilitated phylogenetic analysis that irrefutably separates *Stylosanthes* sp. nov. from *S. hamata*. Stace and Cameron (1984, 1987), using alcohol dehydrogenase (ADH) isozyme analysis, first demonstrated that *Stylosanthes* sp. nov. (2*n*=40) comprises *S. hamata* (2*n*=20) and *S. humilis* (2*n*=20) genomes, implying that *Stylosanthes* sp. nov. is an allotetraploid product of the two diploid species. They also noted that *Stylosanthes* sp. nov. should not be confused with another taxon known at the time as *Stylosanthes* sp. aff. *hamata* that has since been identified as *S. scabra* Vogel. Curtis et al. (1995), using restriction fragment length polymorphism (RFLP) analysis of genomic DNA from representative accessions of *S. humilis*, *S. hamata* and *Stylosanthes* sp. nov. ('Verano'), presented molecular evidence that the two diploids are progenitors of 'Verano'. Gillies and Abbott (1996) undertook detailed analysis of chloroplast DNA restriction fragment length variation to reconstruct the maternal phylogeny of a range of *Stylosanthes* species. They concluded that *S. humilis* is the likely maternal parent of *Stylosanthes* sp. nov., and *S. hamata*, by inference from previously published findings, the likely paternal progenitor. Further evidence on the origin and individuality of *Stylosanthes* sp. nov. is provided by Vander Stappen et al. (1999a, 1999b, 2002).

Conclusion

This paper presents clear evidence that the tetraploid taxon to which the widely used forage cultivars 'Verano' and 'Amiga' belong is not only cytologically and to some extent morphologically different from the diploid *S. hamata* sensu stricto, but can conclusively be separated from that species on the basis of its phylogeny as determined from molecular studies. It is clearly not an autotetraploid derived solely from *S. hamata* (L.) Taub., but an allotetraploid derived from *S. hamata* (L.) Taub. and *S. humilis* Kunth. We strongly and respectfully suggest that future authors desist from using taxon names such as "tetraploid *S. hamata*" and "*S. hamata* sensu lato" in reference to the above cultivars and conspecific accessions, but in the absence of a validly published name, the allotetraploid be referred to in the first instance as *Stylosanthes* sp. nov. (Maracaibo allotetraploid).

Acknowledgments

We are extremely grateful to Arsenio Ciprián for his help in the arduous task of creating the consolidated accessions list with CPI and CIAT identifiers, and to Dr Melanie Schori for valuable advice in preparation of this paper.

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(Note of the editors: All hyperlinks were verified 24 August 2021).

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(Received for publication 21 March 2021; accepted 13 August 2021; published 30 September 2021)

Appendix: Range of *Stylosanthes hamata* and related tetraploid species germplasm, as far as ploidy levels have been assessed, with Australian (CPI) and CIAT forage genebank identifiers.

A: Diploid accessions [*Stylosanthes hamata* (L.) Taub.]				Source[2,3]
CPI No.	CIAT No.	Country[1]	State/Department	
33205	1010	GLP	Port Louis	1, 2, 3, 4(*)
33231	12299	PRI	San Juan	1, 2, 3, 4(*)
36046	12316	USA	Florida	2, 3, 4(*)
37037	12318	DOM		2, 3
37038	12319	DOM		2, 3, 4
38843	12322	USA	Florida	2, 3
40264	12339	BRA	Pernambuco	2, 3, 4
40268	12332	BRA	Ceará	2, 3
40275	12333	BRA	Ceará	2, 3
49080	12346	COL	Atlántico	2, 3
50997	12356	VEN		2, 3, 4
50998	not reg.	VEN		4
56211	not reg.	GLP		2, 3
57247	12389	VEN	Falcón	2
57248	12390	VEN	Falcón	2, 3
61623	12404	VEN	Nueva Esparta	2
61624	not reg.	VEN	Nueva Esparta	2
61669	not reg.	VEN		2, 3
61670	12406	VEN		2, 3, 4
61671	12407	VEN		2, 3
61672A	12408	VEN		3
62160	87	COL	Atlántico	2
62162	not reg.	VEN	Nueva Esparta	4
65361	58	COL	Atlántico	2
65363	87	COL	Atlántico	2, 4
65364	88	COL	Atlántico	4
65369	141	COL	Atlántico	2
65370	142	COL	Atlántico	2
70358	not reg.	ATG	St. George	2
70359	12426	ATG	St. John	2
70360	12427	ATG	St. John	2, 4(*)
70361	12428	ATG	St. John	2
70362	12429	ATG	St. John	2
70363	not reg.	LCA		2
70364	12430	ATG	St. George	2
70365	12431	ATG	St. Phillip	2
70366	12432	ATG	St. George	2, 4(*)
70367	12433	ATG	St. George	2
70368	not reg.	ATG	St. George	2
70369	12434	CUB	Matanzas	2
70370	12435	ATG	St. George	2, 4(*)
70371	12436	ATG		2, 4(*)
70372	not reg.	ATG		2, 4(*)
70373	not reg.	ATG		2
70374	12437	ATG		2, 4(*)
70375	not reg.	ATG		2
70376	12438	ATG	St. George	2
70377	not reg.	ATG		2
70520	12439	USA	Florida	2
70523	12440	USA	Florida	4(*)

Continued

A: Diploid accessions [*Stylosanthes hamata* (L.) Taub.]				Source[2,3]
CPI No.	CIAT No.	Country[1]	State/Department	
70524	12441	USA	Florida	4
70525	12442	USA	Florida	2, 4
70526	12443	USA	Florida	2
70527	12444	USA	Florida	2
72850	12446	USA	Florida	4
72852	12447	USA	Florida	4
72854	12448	USA	Florida	4
72959	12449	USA	Florida	2, 4
73484	12450	ATG	St. George	4(*)
73485	12451	ATG	St. George	2
73486	12452	ATG	St. George	4(*)
73487	12453	ATG	St. George	4(*)
73488	12454	ATG	St. John	4(*)
73490	not reg.	ATG	St. Phillip	2
73491	not reg.	ATG	St. George	4(*)
73497	not reg.	KNA	St. Kitts	4(*)
73498	not reg.	ATG	St. Paul	4(*)
73499	not reg.	ATG	St. Paul	4(*)
73501	12457	ATG	St. George	4(*)
73505	not reg.	ATG		4(*)
73506	not reg.	ANT	Curaçao	4(*)
73507	not reg.	ANT	Curaçao	4(*)
73509	not reg.	ANT	Curaçao	4(*)
73511	1475	CUB	Matanzas	4
73513	12459	KNA	Nevis	4(*)
73514	12460	KNA	Nevis	4(*)
73515	12461	KNA	Nevis	4(*)
73517	1465	KNA	Nevis	4(*)
73519	1466	KNA	Nevis	4(*)
73523	12462	ANT	Curaçao	4(*)
82313	not reg.	CUB	Santiago de Cuba	2
94130	12666	USA		4
94443	12674	USA		4
99670	12680	USA	Florida	4
99675	12685	PRI	Corozal	4
105678	not reg.	BRA	Bahia	4(*)
109305	11194	COL	Atlántico	4
109307	11196	COL	Atlántico	4
109308	11197	COL	Atlántico	4
109310	11199	COL	Atlántico	4
109312	11201	COL	Atlántico	4
109314	11203	COL	Atlántico	4
109315	11204	COL	Atlántico	4
109316	11205	COL	Magdalena	4
109346	11237	COL	Guajira	4
110066	12534	VEN	Zulia	4
110067	12535	VEN	Zulia	4
110077	12539	VEN	Falcón	4
110083	12542	VEN	Falcón	4
110084	12543	VEN	Falcón	4
110087	12544	VEN	Falcón	4
110090	12547	VEN	Falcón	4

Continued

A: Diploid accessions [*Stylosanthes hamata* (L.) Taub.]				Source[2,3]
CPI No.	CIAT No.	Country[1]	State/Department	
110099	12553	VEN	Lara	4
110108	12558	VEN	Lara	4
110110	11779	VEN	Cojedes	4
110114	11781	VEN	Nueva Esparta	4
110119	not reg.	VEN	Sucre	4
110125	not reg.	VEN	Aragua	4
110171	not reg.	VEN	Lara	4
110173	12586	VEN	Lara	4
110174	11793	VEN	Lara	4
110176	12587	VEN	Lara	4
110179	12588	VEN	Trujillo	4
110181	11795	VEN	Trujillo	4
110185	11796	VEN	Lara	4
110186	12590	VEN	Lara	4
110190	12593	VEN	Mérida	4
110207	not reg.	VEN	Distrito Capital	4
110311	124	COL	Atlántico	4

B: Tetraploid accessions [*Stylosanthes* sp. nov. (Maracaibo allotetraploid)]				Source[2,3]
CPI No.	CIAT No.	Country[1]	State/Department	
38842	1	VEN	Zulia	2, 3
38842	1953	VEN	Zulia	2
55812	12371	VEN	Zulia	2, 3
55820	12372	VEN	Zulia	2, 3
55821	12373	VEN	Zulia	2, 3, 4
55822	12374	VEN	Zulia	2, 3, 4
55823	12375	VEN	Zulia	2, 3
55824	12376	VEN	Zulia	2, 3
55825	12377	VEN	Zulia	2
55826	12378	VEN	Zulia	2, 3, 4
55827	12379	VEN	Zulia	2, 3
55828	12380	VEN	Zulia	2, 3
55830	12381	VEN	Zulia	2, 3
55831	12382	VEN	Zulia	2, 3
61672B	12408	VEN		2, 3
61672BB	12408	VEN		4
65365	114	VEN	Zulia	2, 4
65367	120	VEN	Zulia	2
65368	122	VEN	Zulia	2, 4
65371	147	VEN	Guárico	2, 4
65962	12412	COL	Magdalena	4
65965	12415	COL	Magdalena	2, 4
68837	167	COL	Guajira	2, 4
68838	174	COL	Magdalena	2, 4
68840	1039	COL	Magdalena	2, 4
109320	11209	COL	Magdalena	4
109325	11214	COL	Magdalena	4
109326	11215	COL	Magdalena	4
109331	11221	COL	Magdalena	4
109332	11222	COL	Magdalena	4
109344	11235	COL	Guajira	4
109347	11238	COL	Guajira	4

Continued

B: Tetraploid accessions [*Stylosanthes* sp. nov. (Maracaibo allotetraploid)]				Source[2,3]
CPI No.	CIAT No.	Country[1]	State/Department	
109349	11240	COL	Cesar	4
109350	11241	COL	Cesar	4
110024	12409	VEN	Zulia	4
110025	12509	VEN	Zulia	4
110026	12510	VEN	Zulia	4
110027	12511	VEN	Zulia	4
110028	12512	VEN	Zulia	4
110029	12513	VEN	Zulia	4
110030	12514	VEN	Zulia	4
110033	12515	VEN	Zulia	4
110035	12516	VEN	Zulia	4
110036	11761	VEN	Zulia	4
110037	11762	VEN	Zulia	4
110038	12517	VEN	Zulia	4
110039	12518	VEN	Zulia	4
110040	12519	VEN	Zulia	4
110041	12520	VEN	Zulia	4
110042	12521	VEN	Zulia	4
110043	11763	VEN	Zulia	4
110044	12522	VEN	Zulia	4
110045	12523	VEN	Zulia	4
110046	11764	VEN	Zulia	4
110048	12525	VEN	Zulia	4
110049	12526	VEN	Zulia	4
110050	11765	VEN	Zulia	4
110051	11766	VEN	Lara	4
110057	12529	VEN	Zulia	4
110068	12536	VEN	Zulia	4
110069	12537	VEN	Zulia	4
110070	11770	VEN	Zulia	4
110095	11778	VEN	Lara	4
110098	12552	VEN	Lara	4
110104	12555	VEN	Trujillo	4
110109	12559	VEN	Lara	4
110116	11782	VEN	Táchira	4
110134	12568	VEN	Zulia	4
110135	12569	VEN	Zulia	4
110138	11787	VEN	Zulia	4
110162	12580	VEN	Zulia	4
110166	12582	VEN	Aragua	4
110168	12584	VEN	Yaracuy	4
110205	12596	VEN	Miranda	4
110206	12597	VEN	Distrito Capital	4
110209	12598	VEN	Aragua	4
110316	179	COL	Magdalena	4
110317	182	VEN	Zulia	4

C: Tetraploid accessions (Guatemala and Florida)				Source[2,3]
CPI No.	CIAT No.	Country[1]	State/Department	
46587	12343	GTM	Alta Verapaz	2, 3, 4
46588	12344	GTM		2, 3
94444	12675	USA	Florida	4

[1]Country abbreviations: ANT = Netherlands Antilles; ATG = Antigua and Barbuda; BRA = Brazil; BHS = Bahamas; CUB = Cuba; COL = Colombia; DOM = Dominican Republic; GLP = Guadeloupe; GTM = Guatemala; LCA = Saint Lucia; KNA = Saint Kitts and Nevis; PRI = Puerto Rico; USA = United States of America; VEN = Venezuela.
[2]Sources: 1 - Cameron (1967); 2 - H. Stace pers. comm. (1984); 3 - Stace and Cameron (1987); 4 - Date (2010).
[3]Source with (*): accession is mentioned as "presumed diploid".

doi: 10.17138/TGFT(9)359-370

Genetic Resources Communication

Genetic diversity and population structure of *Heteropogon contortus* L. germplasm collected from diverse agro-climatic regions in India and development of a core germplasm set

Diversidad genética y estructura poblacional de germoplasma de Heteropogon contortus *L. provenientes de regiones agro-climáticas diversas en India y desarrollo de una colección núcleo*

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Abstract

Heteropogon contortus, an important constituent of major grasslands of India, Australia and many countries in Africa, Asia and the Americas, is important for pasture and grassland productivity. Hence genetic improvement of the grass needs attention. A genetic variability study, including development of a core subset, was carried out by evaluating 235 accessions collected from different agro-ecological zones of India. The study, based on 16 metric and 14 non-metric traits along with 8 nutritional parameters, indicated that considerable genetic variability existed among the germplasm and selection could result in identification of suitable types for target environments. Clustering and subclustering was performed to select 35 accessions to form a core subset. The statistical analysis indicated that the core subset captured almost all the variability present in the entire germplasm. The study will help researchers to focus future studies on this core subset in developing genetic improvement programs.

Keywords: Black spear grass, forage grass, grassland, plant genetic resources, rangeland.

Resumen

Heteropogon contortus es una pastura altamente utilizada en la India, Australia y muchos países de África, Asia y América; muy importante para la productividad forrajera. Por tanto, es necesario prestar atención al mejoramiento genético de la hierba. Se llevó a cabo un estudio de variabilidad genética, incluido el desarrollo de un subconjunto central, mediante la evaluación de 235 accesiones recolectadas de diferentes zonas agroecológicas de la India. El estudio, basado en 16 rasgos métricos y 14 no métricos junto con 8 parámetros nutricionales, indicó que existía una variabilidad genética considerable entre el germoplasma y que la selección podría resultar en la identificación de tipos adecuados para los ambientes objetivo. Se realizaron agrupaciones y subgrupos para seleccionar 35 accesiones para formar un subconjunto central. El análisis estadístico indicó que el subconjunto central capturó casi toda la variabilidad presente en todo el germoplasma. El estudio ayudará a los investigadores a centrar los estudios futuros en este subconjunto central en el desarrollo de programas de mejora genética.

Palabras clave: Barba negra, gramínea forrajera, pasturas, recursos genéticos, variabilidad.

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Introduction

Heteropogon contortus (L.) P. Beauv. ex Roem. & Schult. (Poaceae: Andropogoneae) is an important perennial pasture grass. It is commonly known as black spear grass or bunch spear grass (Australia), tangle head (United States), pilli grass (Hawaii), assegai grass (Zimbabwe) and Lampa ghas (India). The grass is native to Africa, southern Asia, northern Australia and parts of Oceania and is naturalized in tropical and subtropical regions of the Americas, East Asia and Oceania. Commonly found in tropical Africa (Soromessa 2011), it is also very common in other tropical, subtropical and warm temperate regions of the world, particularly in the Indian subcontinent, Burma, North Africa, Australia and the Pacific as a perennial range grass. The grass is frequently present in major grasslands of India such as *Sehima-Dichanthium, Dichanthium-Cenchrus-Lasiurus, Themeda-Arundinella* and Peninsular India grasslands (Dabadghao and Shankarnarayan 1973; Malaviya et al. 2018; Malaviya and Roy 2021) and establishes well around tropical and subtropical grasslands of the world (Carino 1999).

H. contortus is an important shrub-layer grass of moist deciduous forests (Rhind 2010) dominated by *Shorea robusta* in the upper Gangetic plains (Singh 2012). Historically, Hawaiians used fire to manage grasslands dominated by *H. contortus* (Hoffmann 2008; Daehler and Goergen 2005), although Goergen and Daehler (2001) reported the grass to be only lowly tolerant of clipping and burning. It has also been found to be an effective live mulch in tropical agricultural systems (Moata 2009). *H. contortus*, the dominant grass in northern Australia savanna, is a valued species for cattle, but noxious for sheep because of its awns and a weed in sown grass-legume pastures (FAO 2007).

H. contortus prefers areas with rainfall less than 800 mm, is adapted to coarse-textured soils with pH 6–8 and has moderate to low tolerance of drought, high temperatures, soil salinity and low soil fertility (Moata 2009). Fang and Xiong (2015) and Wang et al. (2016) indicated that, although the grass is drought-tolerant, severe water stress inhibits its growth. Due to its tolerance of soil nutrient deficiencies and limited soil moisture, it is a valuable species for vegetation restoration (Goergen and Daehler 2001; 2002). Owing to its high nutritional and medicinal value, Daehler and Goergen (2005) considered ethnobotanical and ecological research was important for restoration of *H. contortus*-dominated grasslands. *H. contortus* is tolerant of limited shading and often dominates the under-storey of woodlands in tropical and subtropical Australia.

The grass is a nutritious fodder which can be easily conserved as hay (Bor 1960). It is highly palatable during the vegetative phase. However, its robust awns shed easily and are considered a negative factor since at maturity they are pointed sharp and intermingle, causing injury in the mouth and stomach of grazing animals. It spreads naturally and is considered an aggressive invasive species because its seeds survive even after burning of rangelands/grasslands by burying themselves (Roy et al. 2019a).

In India, livestock production is primarily based on rangeland and grassland grazing. About 40% of the country is available for grazing of livestock as pasture lands, forest lands, cultivated wastelands, fallow lands, non-agricultural lands, miscellaneous tree crops and groves etc. In such grazing lands in tropical, subtropical, arid, semi-arid and lower hills of India and isoclimatic conditions in the world, *H. contortus* is widely adapted. However, to increase pasture productivity it is essential to introduce high-yielding accessions in such grazing lands, especially in dry areas. There is a deficit in demand and supply of green and dry forages for livestock in India (Roy et al. 2019b) and perennial grasses like *Heteropogon* are important in bridging the gap.

At an international level, genetic diversity of the grass is conserved in germplasm collections at the Southern Regional Plant Introduction Station, Griffin, Georgia of the USDA/ARS National Genetic Resources Program, and in Indonesia, Ethiopia and Zimbabwe; however, there is negligible representation of germplasm from the Indian subcontinent. ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India (ICAR-IGFRI) has collected a wide diversity of this grass through a series of explorations from various agro-climatic conditions in the country. The germplasm is maintained at ICAR-IGFRI and ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. Knowledge about the existing genetic variability is a prerequisite for any genetic improvement program, and studies of available natural variation are helpful in developing a genetic improvement strategy. Hence, the present study was undertaken to critically evaluate available germplasm, collected from diverse parts of India, to enable breeders to utilize the genetic divergence for isolating promising types. Additionally, development of a core germplasm subset was envisaged, to allow researchers to focus on a limited range of germplasm for further studies and also to accelerate breeding programs.

Material and Methods

A study of variability was conducted on 235 accessions of *H. contortus* collected from different agro-ecological zones of India and conserved at ICAR-IGFRI, Jhansi, India. A list of accessions along with their place of collection is given in Table 1. The soil and climatic conditions of the place of collection following agro-ecological zones defined by Ahmad et al. (2017) and ICAR (2018), are presented in Table 2.

Seeds of the accessions were taken from the gene bank of IGFRI and planted in a nursery. Six-week-old seedlings were transplanted in 3 m-long paired rows, spaced at 75 cm, accommodating 6 tussocks in each row. Rows of plants were 1 m apart. Data on morphological traits were recorded at 50% flowering stage on 3 randomly-selected plants, excluding border plants at the ends of the rows. Seed-related observations were recorded at maturity.

Data on 16 quantitative metric traits were recorded, i.e. length of main tiller (cm); number of tillers/tussock; fresh weight (g) of single tussock (average of 3) harvested at 50% flowering; main tiller diameter (cm); internodal length (cm); number of nodes on main tiller; leaf blade length (cm); leaf blade width (cm); leaf sheath length (cm); leaf sheath width (cm); flag leaf blade length (cm); flag leaf blade width (cm); flag leaf sheath length (cm); flag leaf sheath width (cm); ligule length (mm); and inflorescence length (cm). Length of the main tiller was measured from ground-level to the tip of the last emerged leaf. The fourth leaf from the top was considered for measuring leaf width and length. Internodal length was measured between 3rd and 4th nodes.

Data were also recorded for 14 non-numeric traits, i.e. growth (overall vegetative growth by visual observation as very poor, poor, medium, good, very good), habit (prostrate, decumbent, erect), leaf hairiness (glabrous, light hairy, hairy), leaf blade color (light green, green, bluish green, greenish blue, greenish violet), leaf sheath color (light green, green, greenish violet), tiller internode color (light green, greenish violet, light violet, violet), node anthocyanin coloration (light violet, violet, dark violet), node color (green, greenish violet, violet, dark violet), node hairiness (light hairy, non-hairy), anthocyanin coloration on leaf sheath (absent, weak, strong), anthocyanin coloration on leaf blade (absent, weak, medium, strong), spike color (light green, green, greenish violet), awn stature (hard, soft) and awn pubescence distribution (pubescence up to 50% awn length, pubescence on full awn length i.e. 100%).

Nutritional quality parameters were analyzed for 167 accessions in terms of: ash (%); organic matter (%); neutral detergent fiber (NDF) (%); acid detergent fiber (ADF) (%); hemicellulose (%); cellulose (%); lignin (%); and crude protein concentrations (%). AOAC (1980) methods were followed for estimation of dry matter, crude protein and ash, whereas the Goering and Van Soest (1970) method was followed for NDF and ADF. In vitro dry matter digestibility (IVDMD) % was estimated following Tilley and Terry (1963) for 81 accessions. For these analyses, samples were collected on different dates, so as to match a uniform 50% flowering stage (i.e. 50% of the inflorescence in the row having spikelets at the anthesis stage). The whole plant was harvested from 10 cm above ground and the samples were oven dried at 60 °C and ground.

Statistical analysis

The metric traits data (excluding ligule length) were averaged over replications and analyzed statistically using Non–Hierarchical Euclidian Cluster Analysis of grouping of accessions (Sparks 1973). The computer software 'Statistical Tool for Agricultural Research (STAR)' (IRRI 2020) was used for computation. The accessions per cluster for the core subset were decided upon following Brown (1989a). The number of accessions for a core subset was kept at 15% of the total germplasm following Brown (1989a). Accessions from each cluster were selected as per the formula below, following Roy et al. (2020) and the logarithmic strategy suggested by Brown (1989b). Accessions for the core subset from each cluster were randomly selected.

$$s = (\log p^i / m\Sigma^{t=1} \log p) \times n$$

where:

s = number of accessions selected in a character;

p = size of cluster;

p^i = proportion of ith cluster;

n = number of accessions to be selected for core (15% of base collection); and

m = total number of clusters.

For larger clusters, a second level of clustering was done for further assortment of accessions, to constitute the core subset.

Correlation among various quantitative traits was studied using Microsoft Excel program (MS-Excel). Key characters contributing to diversity were identified using Principal Component Analysis. Scree plot analysis was performed to determine the number of principal components to be retained as per Cattell (1966). The mean value for different traits, as obtained in the core value, was compared with that of total germplasm by student's t-test using MS-Excel.

Table 1. List of accessions of *Heteropogon contortus* and their place of collection (state in India).

SN	Accession	State	SN	Accession	State	SN	Accession	State	SN	Accession	State
1	Bundel var-1	MP	60	IG02-635	Cg	119	IG95-13	UP	178	IG97-167	MP
2	Hc-13	UP	61	IG02-636	Cg	120	IG95-15	UP	179	IG97-181	MP
3	Hc-15	UP	62	IG02-637	Cg	121	IG95-17	UP	180	IG97-182	MP
4	Hc-17	UP	63	IG02-639	Cg	122	IG95-21	UP	181	IG97-183	MP
5	Hc-18	UP	64	IG02-640	Cg	123	IG95-23	UP	182	IG97-183	MP
6	Hc-23	UP	65	IG02-641	Mh	124	IG95-242	MP	183	IG97-183	MP
7	Hc-5/18	UP	66	IG02-641	Mh	125	IG95-242	MP	184	IG97-209	MP
8	IG01-513	MP	67	IG02-642	Mh	126	IG95-25	UP	185	IG97-209	MP
9	IG01-514	MP	68	IG02-643	Mh	127	IG95-258	MP	186	IG97-224	MP
10	IG01-516	MP	69	IG02-644	Mh	128	IG95-26	UP	187	IG97-253	MP
11	IG01-517	MP	70	IG02-645	Mh	129	IG95-270	MP	188	IG99-210	Rj
12	IG01-519	MP	71	IG02-646	Mh	130	IG95-271	MP	189	IG99-210	Rj
13	IG01-520	MP	72	IG02-647	Mh	131	IG95-274	MP	190	IG99-219	Rj
14	IG01-520	MP	73	IG02-649	Mh	132	IG95-277	MP	191	IG99-311	Rj
15	IG01-522	MP	74	IG02-650	Mh	133	IG95-279	MP	192	IG99-312	Rj
16	IG01-522	MP	75	IG02-651	Mh	134	IG95-280	MP	193	IG99-313	Rj
17	IG02-129	UP	76	IG02-652	Mh	135	IG95-283	MP	194	IG99-314	Rj
18	IG02-185	UP	77	IG02-653	Mh	136	IG95-284	MP	195	IG99-314	Rj
19	IG02-190	UP	78	IG02-655	Mh	137	IG95-284	MP	196	IG99-315	Rj
20	IG02-191	UP	79	IG02-655	Mh	138	IG95-286	MP	197	IG99-316	Rj
21	IG02-195	UP	80	IG02-657	Mh	139	IG95-287	MP	198	IG99-317	Rj
22	IG02-204	UP	81	IG02-657	Mh	140	IG95-289	MP	199	IG99-318	Rj
23	IG02-205	UP	82	IG02-658	MP	141	IG95-290	MP	200	IG99-319	Rj
24	IG02-209	UP	83	IG02-658A	MP	142	IG95-292	MP	201	IG99-319	Rj
25	IG02-291	HP	84	IG02-659	MP	143	IG95-293	MP	202	IG99-320A	Rj
26	IG02-293	HP	85	IG02-660	MP	144	IG95-328	MP	203	IG99-321	Rj
27	IG02-342	MP	86	IG02-661	MP	145	IG95-340	MP	204	IG99-322	Rj
28	IG02-343	MP	87	IG02-663	MP	146	IG95-341	UP	205	IG99-323	Rj
29	IG02-344	MP	88	IG02-665	MP	147	IG95-341	UP	206	IG99-325	Rj
30	IG02-345	MP	89	IG02-666	MP	148	IG95-343	UP	207	IG99-326	Rj
31	IG02-346	MP	90	IG02-668	MP	149	IG95-344	UP	208	IG99-327	Rj
32	IG02-347	MP	91	IG02-670	MP	150	IG95-344	UP	209	IG99-329	Rj
33	IG02-348	MP	92	IG02-671	MP	151	IG95-345	UP	210	IG99-330	Rj
34	IG02-349	MP	93	IG02-679	MP	152	IG95-346	UP	211	IG99-333	Rj
35	IG02-350	MP	94	IG03-361	TN	153	IG95-346	UP	212	IG99-335	Rj
36	IG02-351	MP	95	IG03-371	Kl	154	IG95-347	UP	213	IG99-336	Rj
37	IG02-352	MP	96	IG03-371	Kl	155	IG95-348	UP	214	IG99-337	Rj
38	IG02-353	MP	97	IG03-376	Kl	156	IG95-349	UP	215	IG99-338	Rj
39	IG02-354	MP	98	IG03-377	Kl	157	IG95-350	UP	216	IG99-338	Rj
40	IG02-355	MP	99	IG2000-101	UP	158	IG95-352	UP	217	IG99-345	MP
41	IG02-356	MP	100	IG2000-73	UP	159	IG95-352	UP	218	IG99-346	MP
42	IG02-357	UP	101	IG2000-73A	UP	160	IG95-363	UP	219	IG99-349	MP
43	IG02-358	UP	102	IG2000-74	MP	161	IG95-366	UP	220	IG99-50	Cg
44	IG02-359	UP	103	IG2000-93	MP	162	IG95-367	UP	221	IG99-51	Cg
45	IG02-362	UP	104	IG2000-98	UP	163	IG95-368	UP	222	IGO2-184	UP
46	IG02-363	J&K	105	IG95-101	MP	164	IG95-369	UP	223	IGO2-186	UP
47	IG02-364	J&K	106	IG95-103	MP	165	IG95-369	UP	224	IGO2-187	UP
48	IG02-371	MP	107	IG95-104	MP	166	IG95-371	UP	225	IGO2-188	UP
49	IG02-375	MP	108	IG95-104-1	MP	167	IG95-374	UP	226	IGO2-189	UP
50	IG02-487	MP	109	IG95-104-2	MP	168	IG95-374	UP	227	IGO2-191	UP
51	IG02-624	MP	110	IG95-104A	MP	169	IG95-7	UP	228	IGO2-192	UP
52	IG02-625	MP	111	IG95-104B	MP	170	IG95-99	MP	229	IGO2-193	UP
53	IG02-626	Mh	112	IG95-105	MP	171	IG96-164	Kt	230	IGO2-200	UP
54	IG02-627	Mh	113	IG95-105A	MP	172	IG96-167	Kt	231	IGO2-201	UP
55	IG02-629	Mh	114	IG95-106	MP	173	IG96-21	TN	232	IGO2-281	HP
56	IG02-630	Mh	115	IG95-108	MP	174	IG96-97	TN	233	IGO2-281 A	HP
57	IG02-631	Mh	116	IG95-109	MP	175	IG97-163	MP	234	IGO2-288	HP
58	IG02-632	Mh	117	IG95-110	MP	176	IG97-165	MP	235	IGO2-294	HP
59	IG02-633	Mh	118	IG95-111	MP	177	IG97-166	MP			

Cg = Chhattisgarh; HP = Himachal Pradesh; J&K = Jammu & Kashmir; Kl = Kerala; Kt = Karnataka; MP = Madhya Pradesh; Mh = Maharashtra; Rj = Rajasthan; TN = Tamil Nadu; UP = Uttar Pradesh.

Table 2. Soil and climate conditions in the Indian states where accessions of *Heteropogon contortus* were collected [Source: Ahmad et al. (2017); ICAR (2018)].

SN	State	Climate	Precipitation (mm/yr)	PET (mm)	CGP	Soil
1	Rajasthan	Hot arid/semi-arid with desert	<300–800	1,500–2,000	<90	Saline, alluvium-derived
2	Maharashtra	Hot semi-arid to hot subhumid	600–1,000	1,600-1,800	90–180	Shallow and medium (with inclusion of deep) black; black & red
3	Madhya Pradesh	Hot semi-arid to hot subhumid	500–1,000	1,400–2,000	90–180	Alluvium-derived soils or medium and deep black soils
4	Karnataka	Hot subhumid to semi-arid	600–1,000	1,300–1,600	90–150	Coastal alluvium-derived soils;shallow and medium (with inclusion of deep) black, red loamy
5	Himachal Pradesh	Warm subhumid to humid, also with some perhumid zone	1,600–2,000	800–1,300	180–210+	Brown forest and podzolic soils
6	Jammu & Kashmir	Warm subhumid to humid also with some perhumid zones	1,600–2,000	800–1,300	180–210+	Brown forest and podzolic soils
7	Chhattisgarh	Hot subhumid	1,200–1,600	1,400–1,500	150–180	Red and yellow soils
8	Tamil Nadu	Hot semi-arid	600-1,000	1.300–1,600	90–150	Red loamy soils
9	Uttar Pradesh	Hot subhumid	1,000–1,500	1,300–1,500	90–180	Red and black soils, alluvium-derived
10	Kerala	Hot humid, perhumid	2,000–3,200	1,400–1,600	90–210+	Red, lateritic and alluvium-derived soils

PET = Potential Evapotranspiration; CGP = Crop growing period (no. of days)

Results

Observations were recorded on 235 accessions of *H. contortus* for 16 numeric and 14 non-numeric morphological traits and on 167 accessions for 9 nutritional quality parameters in order to characterize the germplasm being maintained at the IGFRI gene bank, and to further develop a core subset of germplasm. Clustering of accessions using 15 metric traits (excluding ligule length) resulted in formation of 6 distinct clusters (Figure 1, Table 3). Clusters 3 and 4 were big clusters consisting of 101 and 94 accessions, respectively, whereas Cluster 1 was of moderate size comprising 30 accessions. Clusters 2, 5 and 6 were small clusters, comprising 3, 5 and 2 accessions, respectively. In Cluster 6, the 2 accessions were vigorous with high values for fresh weight, tiller height, tiller diameter, leaf length and leaf width. These two accessions originated from north and central India, representing tropical climate conditions. The accessions of Cluster 2 also showed high values for agronomic traits. Other clusters showed moderate values for various agronomic traits. Collections from the State of Uttar Pradesh were from the semi-arid districts near Jhansi and the majority of these 63 accessions grouped in Clusters 3 and 4, with a few in Clusters 1, 5 and 6. Similarly, 29 accessions from the arid climate of Rajasthan (western Indian state) grouped mostly in Clusters 3 and 4. Barring 2 accessions, the remaining accessions were from temperate environments, i.e. Himachal Pradesh and Jammu and Kashmir, grouped in Cluster 3; however, this cluster included also accessions from the hot arid climates of central, western and northern India.

Principal Component Analysis revealed that the first 6 principal components accounted for more than 80% of the cumulative variability (Table 4). Scree plot analysis revealed that the first 2 principal coordinates or up to 6 principal components can be retained for explaining most of the diversity (Figure 2).

Height of the plants ranged from 36 to 110 cm (mean 74 cm) (Table 4). Accessions with poor tillering and shorter plant heights were generally annual types. Robust accessions possessed as many as 265 tillers, whereas the minimum tiller number per tussock was 9 only. These 2 traits contribute significantly to total biomass, which was well reflected in fresh biomass, which ranged from 20 to 435 g. The highest fresh biomass per tussock was noted for Cluster 6 (263 g) with 2 accessions only. The second highest average fresh biomass per tussock occurred in accessions of Cluster 3 (143 g), followed by that of Cluster 4 (125 g). Morphological traits, which showed highly significant ($P<0.01$) positive correlation with fresh biomass, were plant height (0.299), number of tillers (0.758) and leaf length (0.289). Tiller diameter with 0.130 and leaf width with 0.140 correlation coefficients were also positively significant ($P<0.05$). However, internodal length was not correlated with fresh

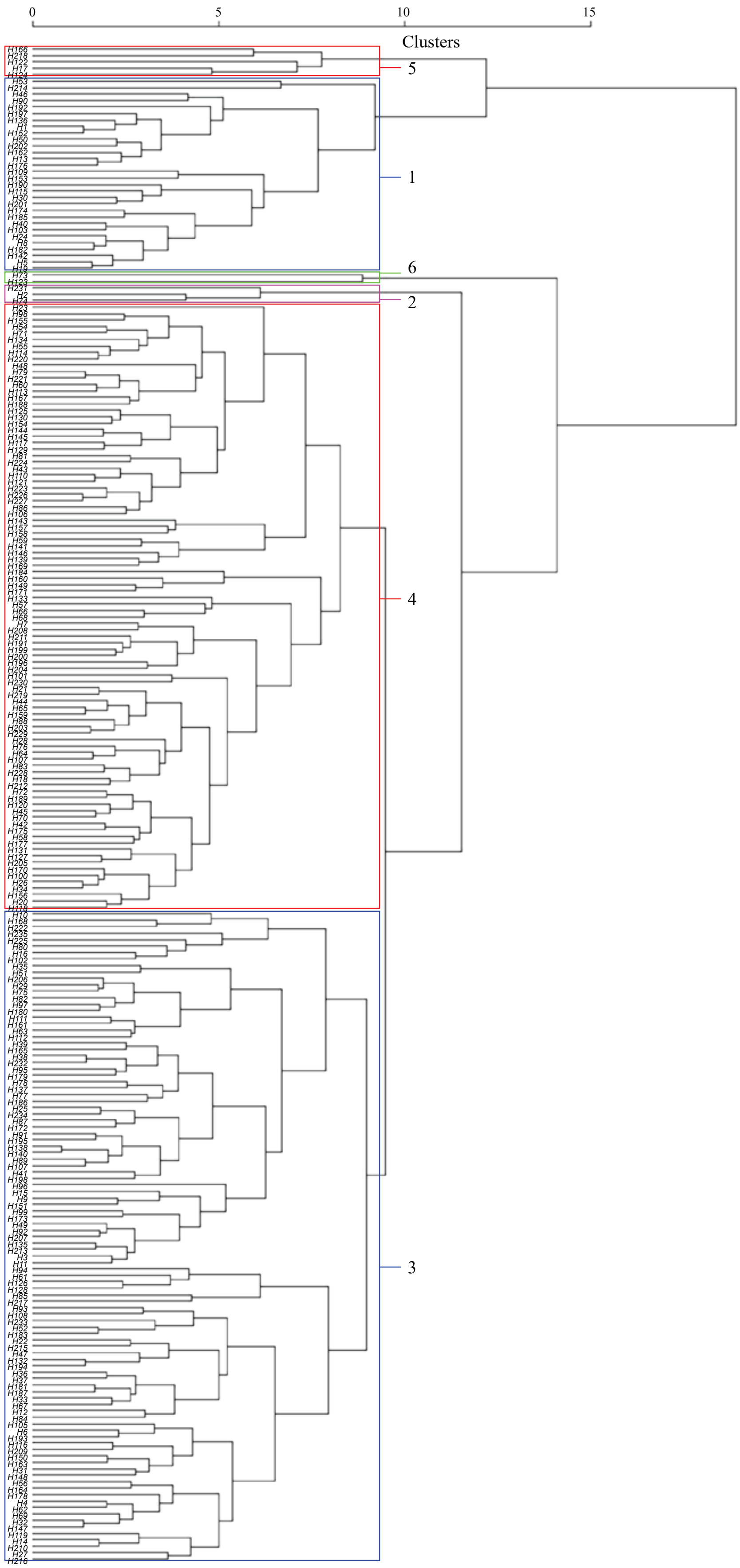

Figure 1. Clustering of *Heteropogon contortus* accessions (dendrogram using agglomerative clustering method).

Table 3. Accessions of *Heteropogon contortus* belonging to different clusters.

Cluster	Accessions (as per SN in Table 1)
1	1, 5, 8, 13, 19, 24, 30, 40, 46, 50, 53, 90, 103, 109, 115, 136, 142, 152, 153, 162, 174, 176, 182, 185, 190, 192, 197, 201, 202, 214
2	2, 74, 231
3	3, 4, 6, 9, 10, 11, 12, 14, 15, 16, 22, 25, 27, 29, 31, 32, 33, 35, 36, 37, 38, 39, 41, 47, 49, 51, 52, 56, 61, 62, 63, 67, 69, 75, 77, 78, 80, 82, 84, 85, 87, 89, 91, 92, 93, 94, 95, 96, 97, 99, 102, 105, 107, 108, 111, 112, 116, 119, 126, 128, 132, 135, 137, 138, 140, 147, 148, 150, 151, 161, 163, 164, 165, 168, 172, 173, 178, 179, 180, 181, 183, 186, 187, 193, 194, 195, 198, 206, 207, 209, 210, 213, 215, 216, 217, 222, 225, 232, 233, 234, 235
4	7, 18, 20, 21, 23, 26, 28, 34, 42, 43, 44, 45, 48, 54, 55, 57, 58, 59, 60, 64, 65, 66, 68, 70, 71, 72, 76, 79, 81, 83, 86, 88, 98, 100, 101, 104, 106, 110, 113, 114, 117, 118, 120, 121, 125, 127, 129, 130, 131, 133, 134, 139, 141, 143, 144, 145, 146, 149, 154, 155, 156, 157, 158, 159, 160, 167, 169, 170, 171, 175, 177, 184, 188, 189, 191, 196, 199, 200, 203, 204, 205, 208, 211, 212, 219, 220, 221, 223, 224, 226, 227, 228, 229, 230
5	17, 122, 124, 166, 218
6	73, 123

Table 4. Descriptive statistics of 15 quantitative metric traits among *Heteropogon contortus* accessions, cluster mean performances and principal components.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
Descriptive statistics of traits among total accessions															
Min	36.2	9.00	20.0	0.10	3.73	3.00	3.50	0.30	3.27	0.30	1.07	0.10	3.90	0.10	2.67
Max	110.7	265.0	435.0	0.34	14.1	13.0	29.0	1.03	9.00	1.03	10.7	0.63	12.0	0.50	12.0
average	74.3	74.3	125.6	0.19	8.32	5.39	14.1	0.60	5.96	0.60	6.50	0.35	7.16	0.35	5.64
SD	12.61	45.38	72.93	0.04	1.91	1.43	3.64	0.11	0.92	0.11	1.51	0.06	1.14	0.06	0.98
Kurtosis	0.23	2.32	2.51	2.13	-0.09	5.34	1.69	1.25	1.10	1.25	0.82	2.17	1.49	1.02	12.78
Skewness	0.22	1.28	1.26	0.44	-0.13	1.83	0.72	0.25	0.56	0.26	-0.26	-0.16	0.36	-0.53	2.11
Cluster means of traits															
I	61.0	51.8	69.8	0.16	8.17	4.67	9.63	0.48	5.16	0.48	5.15	0.30	6.51	0.30	5.06
II	95.3	66.1	109.7	0.22	9.17	5.89	18.9	0.70	7.27	0.70	6.88	0.36	8.36	0.37	11.10
III	72.6	90.1	143.1	0.18	8.73	4.93	13.7	0.57	5.87	0.57	6.26	0.34	7.02	0.34	5.48
IV	79.1	65.8	124.9	0.21	7.89	6.00	15.5	0.67	6.17	0.67	7.38	0.38	7.35	0.38	5.85
V	72.2	56.5	77.1	0.17	9.22	5.00	14.6	0.51	6.92	0.51	2.39	0.18	9.67	0.21	5.49
VI	107.0	72.5	262.5	0.34	7.00	11.5	28.7	0.90	8.35	0.90	6.65	0.35	6.85	0.35	4.8
Principal Components															
SD	2.23	1.46	1.35	1.17	0.99	0.93	0.83	0.73	0.71	0.63	0.49	0.44	0.42	0.31	0.07
VP	0.33	0.14	0.12	0.09	0.07	0.06	0.05	0.04	0.03	0.03	0.02	0.01	0.01	0.01	0.00
CP	0.33	0.48	0.60	0.69	0.75	0.81	0.86	0.89	0.93	0.95	0.97	0.98	0.99	1.00	1.00
EV	4.99	2.14	1.83	1.38	0.97	0.86	0.69	0.53	0.51	0.40	0.24	0.19	0.18	0.10	0.01
Descriptive statistics of traits among core subset															
Min	42.4	11.0	20.0	0.10	3.73	3.00	3.50	0.30	3.27	0.30	1.07	0.10	3.90	0.10	2.67
Max	110.0	265.0	435.0	0.34	14.07	13.00	29.00	1.03	9.00	1.03	9.57	0.45	12.00	0.45	11.95
average	75.0	83.6	136.9	0.19	8.47	5.71	14.31	0.59	6.04	0.59	5.90	0.32	7.27	0.32	5.52
SD	16.03	59.24	100.1	0.05	2.52	2.06	5.52	0.17	1.28	0.17	1.94	0.08	1.58	0.07	1.72
Kurtosis	0.13	1.69	1.97	2.82	-0.41	3.53	1.20	0.75	0.49	0.58	0.23	0.76	1.75	1.44	6.42
Skewness	0.297991	1.28	1.26	0.44	-0.13	1.83	0.72	0.25	0.56	0.26	-0.26	-0.16	0.36	-0.53	2.11
t test*	0.406	0.190	0.262	0.425	0.375	0.195	0.427	0.337	0.369	0.381	0.045	0.021	0.341	0.025	0.349

C1 = length of main tiller (cm); C2 = number of tillers/tussock; C3 = fresh weight/tussock (g); C4 = tiller internode diameter (cm); C5 = 4th inter-nodal length (cm); C6 = number of nodes on main tiller; C7 = 4th leaf blade length (cm); C8 = 4th leaf blade width (cm); C9 = 4th leaf sheath length (cm); C10 = 4th leaf sheath width (cm); C11 = flag leaf blade length (cm); C12 = flag leaf blade width (cm); C13 = flag leaf sheath length (cm); C14 = flag leaf sheath width (cm); C15 = inflorescence length (cm); EV = Eigen Values; VP = variance proportion; CP = cumulative proportion; * t test core subset vs. all accessions (shows P values).

yield (-0.076). A difference of almost 3 times was noted for tiller internode diameter, which ranged from 1 to 3 mm. Internodal length between the 3rd and 4th nodes varied from 3 to 14 cm with an average of 8.32 cm. Number of nodes per tiller varied from 3 to 13. Leaf blade and leaf sheath lengths varied from 3 to 29 cm and 3 to 9 cm, respectively. Leaf blade and leaf sheath widths both varied from 0.3 to 1 cm. Such variation was also noted for flag leaf length and width. Flag leaf blade length differed among accessions from 1 to 10 cm, whereas flag leaf sheath length differed from 3 to 12 cm. Width of flag leaf blade and sheath varied from 0.1 to 0.6 cm and 0.1 to 0.35 cm, respectively. Inflorescence length also varied significantly, i.e. 2 to 11 cm.

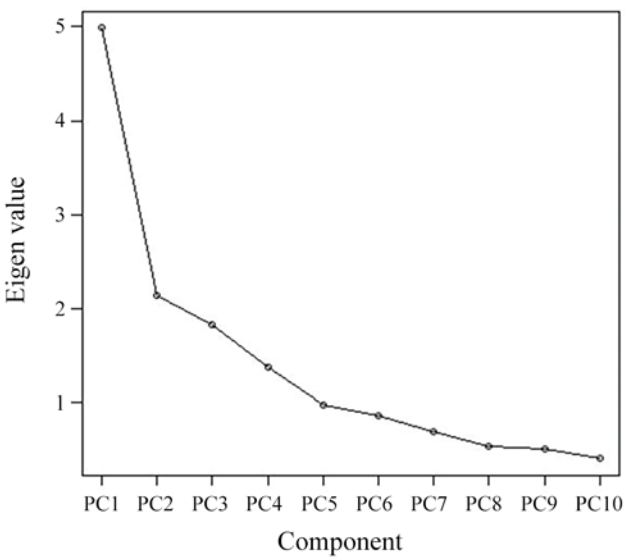

Figure 2. Scree plot of Heteropogon contortus accessions.

Visual observation of non-metric traits revealed that 97 accessions were poor to very poor in growth, whereas 72 accessions were good to very good (Table 5). As regards plant habit, 157 accessions were decumbent, 76 were erect and only 2 were prostrate. Tiller internode color varied from violet to green with some mixed shades. Greenish-violet tiller was predominant with 114 accessions, whereas violet and light violet figured in 54 accessions. Sixty-seven accessions had light green tillers. Nodes were mostly greenish-violet to dark violet, except in 16 accessions, which possessed green nodes. Nodes were predominantly non-hairy and only 60 accessions possessed scant hair on nodes. Leaf surface of 164 accessions was glabrous, while 71 accessions had medium or scant hairs. Light green and green were the most dominant leaf blade colors with 170 and 53 accessions, respectively. Two accessions were blue-green and 6 accessions had greenish-blue leaf blades. Green leaf sheath color was also dominant with 171 accessions being green or light green. Sixty-four accessions were noted with greenish-violet leaf sheaths. Anthocyanin coloration on leaf surfaces was less common than on leaf sheath surfaces and nodes. Spike color was green among all accessions but varied in intensity. Except for 2 accessions which possessed soft awns, accessions had hard awns. The soft-awned accessions were short-lived annual types. Ligule length ranged from 0.5 to 1 mm among accessions, with the majority being 1 mm.

One of the important nutritional parameters, crude protein concentration, varied from 2 to 10% with an average of 5.7%, whereas IVDMD varied from 31 to 59% with an average of 46.2% (Table 6). Variation in fiber concentrations ranged from 74 to 87% (average

Table 5. Variation for non-metric traits and the ligule length in 235 *Heteropogon contortus* accessions.

Growth		Habit		Leaf hairiness		Leaf blade color		Leaf sheath color	
Good	39	Decumbent	157	Glabrous	164	BG	2	Green	101
Medium	66	Erect	76	Light hairy	65	G	53	GV	64
Poor	61	Prostrate	2	Hairy	6	GV	4	LG	70
Very Good	33					GB	6		
Very Poor	36					LG	170		
Node color		Node hairiness		Tiller internode color		Anthocyanin coloration on leaf blade		Anthocyanin coloration on leaf sheath	
DV	60	LH	60	GV	114	Absent	151	Absent	88
G	16	NH	175	LG	67	Medium	46	Strong	30
GV	104			LV	14	Strong	29	Weak	117
V	55			V	40	Weak	9		
Node anthocyanin coloration		Spike color		Awn pubescence distribution		Awn stature		Ligule length (mm)	
Absent	2	G	185	50%	203	Hard	233	0.05	42
DV	72	GV	30	100%	32	Soft	2	0.1	193
LV	32	LG	20						
V	129								

BG = bluish-green; G = green; GV = greenish-violet; LG = light green; LV = light violet; V = violet; GB = greenish-blue; DV = dark violet; LH = Light hairy; NH = Non-hairy; Number against traits are number of accessions.

Table 6. Nutritional parameters of 167 accessions of *Heteropogon contortus*.

	Ash %	OM %	CP %	NDF %	ADF %	Hemi-cellulose %	Cellulose %	Lignin %	IVDMD[1] %
Among accessions									
Min	5.35	87.9	2.14	74.4	38.2	23.72	22.9	2.70	31.5
Max	12.10	94.7	10.10	87.1	59.0	44.89	51.1	21.41	59.9
Average	8.58	91.4	5.67	81.7	48.0	33.70	36.9	7.39	46.2
Mean values among clusters									
Cluster 1	8.60	91.4	5.93	81.8	47.1	34.7	36.2	7.18	46.3
Cluster 2	7.67	92.3	5.06	81.7	47.1	34.6	36.5	6.49	44.9
Cluster 3	8.69	91.3	5.52	81.9	47.6	34.3	36.2	7.76	46.0
Cluster 4	8.48	91.5	5.76	81.6	48.7	32.8	37.9	7.20	46.4
Cluster 5	9.00	91.0	5.57	81.4	47.0	34.4	35.7	6.48	51.8
Cluster 6	9.56	90.4	5.37	78.9	47.6	31.3	33.9	7.57	48.6
Among core subset accessions									
Min	5.87	89.45	3.81	78.6	41.8	28.0	31.3	2.70	36.0
Max	10.55	94.13	7.79	85.7	52.8	39.1	41.7	8.04	52.5
Average	8.67	91.33	5.69	81.7	47.6	34.1	36.4	6.58	46.6
t test[2]	0.375	0.375	0.462	0.470	0.230	0.264	0.214	0.004	0.381

OM = organic matter; CP = crude protein concentration; NDF = neutral detergent fiber concentration; ADF = acid detergent fiber concentration; Cell = cellulose; Lig = lignin; IVDMD = *in vitro* dry matter digestibility.

[1]81 accessions analyzed.

[2]t test core subset vs. all accessions (shows P values).

Table 7. Core subset of *Heteropogon contortus* germplasm.

Cluster	Original size	Log value	Proportion of log value	No. of accessions to be selected	No. of second-level cluster formed	No. of accessions selected from each cluster	Accession number (as per SN in Table 1)
1	30	1.477	0.21	8	8	8	5, 13, 46, 53, 90, 115, 153, 214
2	3	0.477	0.07	2	2	2	74, 231
3	101	2.004	0.29	10	10	10	9, 22, 35, 85, 91, 105, 126, 147, 207, 222
4	94	1.973	0.28	10	10	10	20, 23, 54, 66, 101, 121, 158, 160, 184, 229
5	5	0.699	0.10	3	3	3	17, 122, 218
6	2	0.301	0.04	2	1	2	73, 123
Total	235	6.931		35		35	

81.7%) for NDF and 38 to 58% (average 48.0%) for ADF. Differences for lignin were much greater and ranged from 2.7 to 21.4%. Ash concentration ranged from 5 to 12% and organic matter from 87 to 94%. Accessions showed variation for hemicellulose and cellulose, which varied from 23 to 44% and 22 to 51%, respectively.

A subset of 35 accessions was identified as a core germplasm set, representing 15% of the total germplasm evaluated (Table 7). This subset represented 8, 2, 10, 10, 3 and 1 accessions from Clusters 1 to 6, respectively. Almost all variability was captured in the core subset (Tables 4 and 6). Student's t-test for various morphological traits of the core subset against the total accessions showed non-significant variation, except for flag leaf blade length and width and flag leaf sheath width (Table 4). Similarly, t-test for various nutritional parameters of the core subset against the total accessions showed non-significant variation, except for lignin concentration (Table 6).

Discussion

Analysis of data recorded on various morphological quantitative metric and non-metric traits, as well as nutritive parameters established high variability among *Heteropogon contortus* accessions. The germplasm represented tropical semi-arid climates (126 accessions from Chhattisgarh, Madhya Pradesh and Maharashtra), tropical arid climate (29 accessions from Rajasthan), tropical subhumid climate (9 accessions from Tamil Nadu, Karnataka and Kerala), subtropical semi-arid climate (63 accessions from Uttar Pradesh) and subtemperate to temperate climate (8 accessions from Himachal Pradesh, Jammu and Kashmir). A high degree of genetic variation was also noted for non-metric traits, growth and habit along with nutritional parameters. Clustering of accessions collected from different places indicated that these accessions either originated from a common source or moved from one place to other, resulting in lesser inter-population differences. Grouping of material based on statistical numerical procedures helps in understanding of variability, which further becomes the basis for identification of core germplasm, which can be exploited in breeding programs. Development of a core subset is effective when it is truly representative of the variation present in the germplasm collection. A comparison of the data shows that almost all the variability has been captured in the core subset.

The study established high genetic variability and correlation of some traits with biomass production. Tiller number, high nodal number and longer leaf in *H. contortus* were reported to be associated with forage yield (Roy 2004). Morphological traits like plant height, number of tillers and leaf length were also reported to be associated with forage yield and considered as important traits in constructing selection criteria for forage yield in the perennial grass *Sehima nervosum* (Roy et al. 1999). Earlier studies involving tropical perennial grasses indicated a wide range of diversity for different characters and the clustering pattern was observed to be independent of their geographical distribution in *Dichanthium* (Agarwal et al. 1999; Chauhan et al. 2007), *Sehima nervosum* (Roy et al. 1999), *H, contortus* (Roy 2004; Bhat and Roy 2007; 2014) and Guinea grass (Jain et al. 2003a, 2003b, 2006; Roy et al. 2020). The annual accessions, with soft awns, were quite low in biomass. In fact, these accessions were short-lived perennials and could not survive in the harsh climate of Jhansi, India (max temp 45 °C during May and June). Such annual types have been described from India earlier (Soromessa 2011). In earlier studies, evaluation of accessions of *H. contortus* collected from different parts of India revealed high values for heritability, genetic advance and genotypic and phenotypic coefficients of variation for tiller number/tussock, green fodder yield and dry matter yield (Roy 2004), and were considered to be useful for selection of accessions. An isozymic study on *H. contortus* accessions indicated high genetic intra-species diversity; however, clustering could not be correlated with the geographical origin of the accessions (Bhat and Roy 2014).

The present study identified the wide range of genetic variation among the germplasm set evaluated, which is in congruence with earlier reports of high genetic variation. The presence of genetic variability in the germplasm collection of this apomictic grass can be attributed to recombinations taking place owing to residual sexuality among some plants. The grass is an aposporous apomict with 2n = 20, 40, 44, 60 and 80 (Fedorov 1974; Srivastava and Purnima 1990). Although it is primarily apomictic in nature, it shows high genetic variability in subhumid dry regions of south India, probably due to the presence of residual sexuality in a few accessions. Genetic diversity in the species has been reported by Carino and Daehler (1999) utilizing molecular studies within and among Hawaiian populations of the species. Roy (2004) and Bhat and Roy (2007; 2014) have also reported high genetic variability among germplasm based on morphological and isozyme studies. A considerable amount of localized variation was reported in the early botanical literature because of occasional sexual reproduction in *H. contortus* (Soromessa 2011). The lack of uniformity among individuals in Hawaiian populations of this apomictic grass, based on RAPD studies, indicated frequent sexual reproduction (Carino and Daehler 1999). Diverse forms collected from the same location (Bhat and Roy 2007) also indicate presence of some recombinations taking place through residual sexuality among some plants.

Other factors could also be responsible for such variation. Introduction of germplasm from other countries over different time periods is one potential source of variation, but there are no documented reports of this occurring. However, dispersal of seed through other biotic and abiotic sources cannot be ruled out. One plausible explanation of seed dispersal may be its hard awns, which get stuck in animal or human bodies or coats.

The collections represent different agro-ecological zones of the country and variation was found within local populations as well as between populations, which indicates a distinct possibility of sexual reproduction taking place, unless the species was introduced into India from more than one source. Further domestication and recombination might have contributed more to the variability. The presence of among-population differentiation but lack of between-island differentiation was considered to indicate that *H. contortus* was an early Polynesian introduction to the Hawaiian Islands (Carino and Daehler 1999). Hence, further molecular studies involving Indian germplasm and germplasm from some other countries, particularly neighboring ones, may give some idea on possible movement of germplasm. The study will help researchers to focus future studies on this core subset in developing genetic improvement programmes.

Acknowledgments

Authors are thankful to National Bio-resource Development Board, Department of Biotechnology, Government of India for financial support. Authors are also thankful to Indian Council of Agricultural Research, New Delhi, India.

Conflicts of interest

The authors declare that they have no conflict of interest.

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(Note of the editors: All hyperlinks were verified 30 July 2021).

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(Received for publication 4 September 2020; accepted 8 July 2021; published 30 September 2021)

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(3):371–375
doi: 10.17138/TGFT(9)371-375

Short Communication

What drives the adoption of fodder innovation(s) in a smallholder dairy production system? Evidence from a cross-sectional study of dairy farmers in India

¿Qué impulsa la adopción de nuevas opciones forrajeras en un sistema de producción de leche a pequeña escala? Evidencia de un estudio transversal entre productores de leche en India

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Abstract

The study in India involving 384 households found that 42.7% of dairy farmers adopted new forage varieties when varieties were released. The farmer's resources, their caste, access to markets for milk and price received for milk had positive effects on the decision to adopt. Management of farms by women-headed households had negative effects on the adoption decision. Increased forage yield and ease of propagation and establishment were important reasons for adoption of varieties, e.g. the relative advantage of pearl millet × Napier grass (*Cenchrus americanus* × C. *purpureus*) vs. hedge lucerne (*Desmanthus virgatus*). Thus, researchers need to address these issues when developing new germplasm, if farmers are to readily adopt new varieties, especially in the case of resource-poor farmers.

Keywords: Forage attributes; surveys; smallholder dairying; tropical forages; variety acceptance.

Resumen

El estudio en India que involucró a 384 hogares encontró que el 42.7% de los productores de leche adoptaron nuevas variedades de forrajes cuando se liberaron. Los recursos del agricultor, su casta, el acceso a los mercados de la leche y el precio recibido por la leche tuvieron efectos positivos en la decisión de adoptar. Las mujeres enfrentaron más dificultades para adoptar las nuevas opciones forrajeras en sus fincas. El aumento del rendimiento del forraje y la facilidad de propagación y establecimiento fueron razones importantes para la adopción de variedades, p.ej. la ventaja relativa del mijo perla × pasto elefante (*Cenchrus americanus* × *C. purpureus*) frente al frijolillo (*Desmanthus virgatus*). Por lo tanto, los investigadores deben abordar estos problemas al desarrollar nuevo germoplasma, para que los agricultores adopten fácilmente nuevas variedades, especialmente en el caso de agricultores de escasos recursos.

Palabras clave: Aceptación de variedad; atributos del forraje; encuestas; forrajes tropicales; lechería en pequeña escala.

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Introduction

Attributes (characteristics) of an innovation are considered to be drivers of its adoption or rejection by end-users, explaining 49–87% of the variance in adoption (Rogers 2003). These attributes include: relative advantage over the existing technology; compatibility with values, lifestyle and needs; ease of use; trialability on a small scale; and results/benefits and risk/uncertainty easily seen; these attributes influence the decision to adopt or not (Rogers 2003; Trope and Liberman 2003; Castaño et al. 2008). Socio-demographic factors also impact on decisions to adopt (Arts et al. 2011). These inferences are based mainly on adoption of consumer goods, such as durable goods, fast-moving consumer goods, fashion etc. and have been used to modify and design innovations and/or reposition them in the market. However, research on factors driving adoption of improved fodder varieties by smallholder dairy-farmers, who account for 90% of milk produced and are associated with 80 million rural households in India, is limited.

Since 1970, various stakeholders have made attempts to enhance productivity of dairy animals through improvement in feed and fodder resources, inter alia. These innovations encompassed: enrichment of crop residues; promotion of concentrate feeding; and fodder cultivation. However, livestock are still under-nourished and there is an estimated 35.6% deficit of green fodder in India (Indian Grass and Fodder Research Institute 2013). Furthermore, for the year 2025, Singh et al. (2013) predicted the deficit to increase to 65% for green fodder and 25% for dry fodder (residues of cereal and pulse crops). This scenario holds good for Tamil Nadu state, a tropical region and one of the leading milk-producing states of India. For a substantial period, various stakeholders, including Tamil Nadu Agricultural University (TNAU), have been addressing the shortage of feed resources and between 1976 and 2019 TNAU released 22 fodder varieties/hybrids (Table 1).

Mean annual yields of pearl millet × Napier, multi-cut sorghum and hedge lucerne are 80, 49 and 20 tonnes dry matter (DM)/hectare, respectively, while the nearest competitor, single-cut sorghum, yields about 10 tonnes DM/ha. From 2010 onwards, Animal Husbandry Department of Tamil Nadu intensively promoted and propagated perennial forage germplasm, namely: pearl millet × Napier, multi-cut forage sorghum and hedge lucerne as mixed fodder crops/individual crops through various incentive programs across the state (Government of Tamil Nadu 2018). Continuous efforts of the various stakeholders resulted in an increase in forage cropping. A micro-study by Thirunavukkarasu et al. (2014) reported that the area under pearl millet × Napier had increased from 0.01 ha to 0.08 ha during the period 2001–2011 at an individual household level. Thirunavukkarasu et al. (2011a; 2011b) reported wide variation in availability of green fodder and deficits of dry fodder across the state. To provide base data for planning future fodder development programs, a deeper understanding of the socio-demographic factors which affect adoption of new forage varieties was needed. We conducted a study of dairy farmers to clarify the situation.

Table 1. Major fodder varieties developed in Tamil Nadu Agriculture University between 1976 and 2019.

Fodder	Year of release	Purpose
Hedge lucerne (*Desmanthus virgatus*)	1976; 2019	Introduced as a perennial multi-cut crop to meet the protein requirements and minimize costs of protein supplementation. In 2019 for the first time mutational breeding was carried out and an improved variety was released.
Pearl millet × Napier grass (*Cenchrus americanus* × *C. purpureus*)	1982–2012[1]	To replace cereal-based green crop residue; to reduce grazing dependence; and as a perennial fodder. Five varieties released.
Multi-cut fodder sorghum (*Sorghum bicolor* × *S. sudanense*)	2001–2014[1]	To replace single-cut sorghum as perennial green and dry fodder. Two varieties released.
Guinea grass (*Panicum maximum, now Megathyrsus maximus*)	1993–2009[1]	Introduced as a perennial multi-cut crop; shade-tolerant. Three varieties released.
Lucerne (*Medicago sativa*)	1980–2013[1]	Early-maturing leguminous fodder crop. Two varieties released.
Cowpea (*Vigna unguiculata*)	1986–2004[1]	Introduced as leguminous fodder crop; resistant to root-rot and cowpea yellow mosaic virus. Two varieties released.

[1]Periodic releases of improved varieties/hybrids with higher yields and better attributes.

Material and Methods

A sample of 384 dairy-farming households, proportionally representing different farming systems of Tamil Nadu, were selected using stratified multi-stage random sampling (D. Thirunavukkarasu unpublished data). The selected farmers were interviewed regarding their status in relation to adoption of new forage varieties and socio-demographic factors were recorded. During the course of interviewing, fodder adoption status, membership in farmers' collectives, caste and gender role were captured at a categorical level and other socio-demographic factors were measured at a continuous level. For the purpose of triangulation of the collected data, to improve understanding and obtain additional information, 'focus group' discussions were organized in villages. In this exercise, participants, including farmers (both female and male) and the village-level animal health service providers, identified the reasons for planting or not planting new varieties.

To understand the socio-demographic differences between adopters and non-adopters, the chi square (for categorical variables) and Mann-Whitney U test (for continuous variables) were used taking account of nature of variables and non-normal distribution of data. Binary logistic regression was used to understand the causal factors that promote adoption of new varieties along with descriptive statistics. In performing binary logistic regressions, if a farmer adopted any of the promoted fodders, the farm was coded as 1, with 0 for non-adopters. The binary logit model was as follows:

Fodder adoption status = $\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + [...] + \beta_n x_n$
where:
x_1, x_2, x_3 [...] x_n represent independent variables;
β_0 = constant;
β_1, β_2 are logistic variables;
regression coefficients (estimates)
Adoption status = 0, (adoption ≤ 0)
Adoption status = 1, (adoption >0).
The above statistical analysis was carried out using Statistical Package for Social Sciences and spread sheets at stat-help.com/spreadsheets.html

Results and Discussion

Mean age of respondents was 46 years, while land holdings were marginal (20% of respondents were landless) for sustaining their family needs. Respondents were mostly (58% of households) the third lowest caste in the Indian hierarchy ('caste' is a social stratification prevailing in India). Fifty-three percent of respondents belonged to the rural middle economic class and owned 1–3 adult dairy animals, producing around 10 L milk/day/household. Family women operated more than one-third of the farms (35%) on their own with occasional support of men, while remaining farms were operated by men or men plus women. Fifty-eight percent of respondents reared dairy animals primarily, to supply their household needs, with any surplus sold as market milk. The majority (53.1%) of farmers were members of farmers' collectives (farmers' producer organizations). About 40% of farmers were able to market milk through either co-operative dairies or privately-owned dairy processors; 19.3% of households had provisions to market milk through both co-operatives and private processors; 25.5% were able to market milk only through a milk vendor; and the remainder had no marketing opportunities. On average farmers produced 3,613 L milk annually, for which they received 22 INR/L (1 USD = 75 INR). These farmers access mass media and mass contact programs (exhibitions, campaigns, etc.) to obtain information. In addition, farmers interact with the extension system (propagators of livestock-related innovations), including veterinarians, para-veterinarians and other associated stakeholders at village level.

The data indicated that 42.7% of the dairy-farming households have adopted at least 1 or more improved fodder varieties promoted through the Animal Husbandry Department and others. Adopters and non-adopters differed significantly in terms of land holdings and animal numbers, socio-economic class, reasons for dairying, gender role, caste, milk production, access to markets, price received for milk, availability of mass media and extension agency contact but differences were not necessarily influential in terms of adoption of new varieties. On average households cultivated 0.1 ha (range 0–1 ha) of fodder on their own or leased land. Among the adopting farmers (164 farmers), 87.8, 22.0 and 1.2 % of farmers planted pearl millet × Napier, multi-cut fodder sorghum and hedge lucerne, respectively. Of the total cultivated area devoted to fodder production, pearl millet × Napier accounted for 64.8% and fodder sorghum 35.2%, with very little under hedge lucerne.

Among the above discussed variables, those which differentiated between adopters and non-adopters were checked for bivariate relationships. Animal numbers and socio-economic class, which had highly significant bivariate correlation with land, caste and annual milk production, were excluded for understanding the role of predictor variables in logit regression. Among the selected variables, land, milk sale price, annual milk production, market opportunity, gender role and caste explained about 47.3% (Pseudo R2 = 0.473) of the variance in fodder adoption among the farmers (Table 2). Even though many variables differed between adopters and non-adopters, the only ones displaying positive relationships with fodder adoption were: land size, annual milk production and access to milk marketing opportunities (P<0.001), caste (P<0.05) and price received for milk (P<0.10). For one unit changes in acreage of land, liters of annual milk produced and marketing opportunity score (ranged from 0 to 3), the estimated odds of adoption (Odds ratio) are multiplied by 1.3, 1 and 2.876, respectively. Members of the third lowest caste were 2.2 times as likely to adopt as those in the second lowest caste, while gender had a significant negative relationship (P<0.05) with adoption. Women-operated farms were only 0.49 times as likely to adopt fodder innovations as male-operated farms, i.e. women-operated farms were less likely to adopt fodder varieties than farms operated by men only or men and women.

Thus, farmers with better resources (relatively large land holdings) and access to commodity markets (markets plus good milk price), high milk production and higher in caste hierarchy are more likely to adopt new forage varieties. Crossing of Indian-origin Zebu cattle breeds with European origin dairy breeds for increasing milk productivity demanded improved feeding strategies, such as feeding of cultivated fodder. Thus, in tandem, cattle breeding programs and adoption of fodder varieties might have improved milk production. Large landholders have the option to divert some land from cropping to green forage production. Better marketing opportunities in the form of better access to markets and higher milk prices (incentives) act as extrinsic motivational factors for adoption. Even landless farmers had acted to lease land for cultivation of fodder. These findings are in agreement with observations by other researchers that incentives or extrinsic motivational factors drive adoption of innovations (Donkor et al. 2018).

Women-operated farms were less likely to have cultivated fodder than those operated by men only or men plus women. Mobility restriction of women, on account of cultural factors and limited access to transport systems, limits their access to extension services, technological solutions and external inputs, which may limit their adoption of innovations (Theis et al. 2018). Castes that are higher in social stratification tend to adopt fodder innovations more than lower castes, which may be related to limited access to extension services and quality information among the lower castes (Krishna et al. 2019). Nguthi (2007) suggested that an indirect inference for rejection by non-adopters may be a lack of fit with existing livelihood assets, available options and activities for resource-poor farmers. Similarly, poor market access is a disincentive (due to market disparities) to adoption.

At the same time all new varieties of fodder are not uniformly adopted by farmers and group discussion with farmers and others revealed the following facts: While hedge lucerne was released earlier than pearl millet × Napier and multi-cut forage sorghum, the latter 2 are certainly preferred. Biomass yield of pearl millet × Napier is higher (a relative advantage) than that of hedge lucerne, and planting materials (vegetative setts) are readily available through exchange between farmers (less complex). Collection of planting material (seeds) of hedge lucerne is a tedious, laborious process and seed is not readily available. In addition, hedge lucerne seeds need seed treatment prior to sowing to break seed dormancy. Therefore, relative ease of obtaining planting materials, nature of planting material and forage yields are obvious reasons for greater adoption of pearl millet ×Napier and multi-cut sorghum than hedge lucerne.

Table 2. Estimated coefficients of logistic regression for factors influencing adoption of new fodder varieties (n=384).

Variable	Estimated coefficient	Standard error	Odds ratio[1]
Land size	0.246	0.053	1.279***
Price received for milk	0.052	0.031	1.054#
Annual milk production	0.000	0.000	1.000***
Access to milk marketing opportunities	1.056	0.162	2.876***
Gender role (women-operated farms coded as 1; otherwise as 0)	-0.705	0.289	0.494*
Caste (lowest ranked in caste hierarchy–Schedule caste coded as 0)			
Most backward caste (second lowest in caste hierarchy coded as 1)	0.408	0.388	1.053
Backward caste (third lowest in caste hierarchy coded as 2)	0.799	0.404	2.223*
Constant	-0.5480	0.910	0.004

Pseudo R^2 = 0.473; Log likelihood = 357.4

***P<0.001; **P<0.01; *P<0.05; #P<0.10

[1]Increased odds of adoption from a unit increase in the variable.

Conclusions

Similar to the situation with consumer goods, socio-cultural factors and forage attributes influence the adoption/rejection of new forage varieties. These varieties are more readily adopted by farmers with adequate land, producing larger volumes of milk and with ready access to milk markets paying acceptable milk prices. New forages with higher yields and ease of propagation are also more readily adopted. When developing new germplasm and propagating new varieties, researchers need to consider these issues by focusing on material that does not require complex processes to establish and manage. Extension professionals need to improve access to the extension system and technological inputs by marginalized sections within the dairy-farming community, if all sectors of the community are to take advantage of the new resources, especially resource-poor farmers.

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(Note of the editors: All hyperlinks were verified 15 July 2021).

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(Received for publication 2 May 2020; accepted 6 July 2021; published 30 September 2021)

Tropical Grasslands-Forrajes Tropicales (2021) Vol. 9(3):376–382
doi: 10.17138/TGFT(9)376-382

Nota Técnica

Uso de sensores remotos en la determinación del forraje disponible de *Urochloa humidicola* cv. Llanero bajo pastoreo en la Altillanura colombiana

Use of remote sensors to determine forage availability in grazed pastures of Urochloa humidicola *cv. Llanero in the Colombian Altillanura*

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Resumen

La modernización de los sistemas pastoriles basados en pasturas del género *Urochloa* en los Llanos Orientales de Colombia requiere de técnicas que usan sensores remotos desde plataformas satelitales para estimar la oferta de forraje. En el C.I. Carimagua de la Corporación Colombiana de Investigación Agropecuaria (Agrosavia) se evaluó una pastura de *Urochloa humidicola* cv. Llanero con imágenes Landsat 8 y Sentinel 2A. Se utilizaron los índices de vegetación NDVI, SAVI, EVI y GNDVI, calculados a partir de las bandas azul, verde, rojo e infrarrojo cercano. Los resultados fueron analizados con el software de estadística R y se compararon con el aforo (= mediciones en campo) del forraje disponible bajo pastoreo en época seca. El aforo fluctuó entre 290 y 656 kg MS/ha y los índices de vegetación fueron, para los sensores Landsat 8 y Sentinel 2A, respectivamente: NDVI = 0.67 (±0.037) y 0.69 (±0.061); SAVI = 0.48 (±0.048) y 0.41 (±0.046); EVI = 0.70 (±0.052) y 0.41 (±0.047); y GNDVI = 0.60 (±0.028) y 0.70 (±0.034). La relación entre los índices de vegetación con la oferta de forraje fue lineal directa; para la valoración de los modelos predictivos se usaron los criterios coeficiente de determinación R^2 (0.56–0.72) y el error cuadrático medio (RMSE) (63.95–80.16) de las ecuaciones de regresión. Se concluye que para las condiciones del estudio el EVI (para Landsat 8) y el NDVI (para Sentinel 2A) son índices apropiados para predecir la oferta forrajera del pasto Llanero.

Palabras clave: EVI, imágenes satelitales, índices de vegetación, NDVI.

Abstract

Modernization of pastoral systems based on the use of *Urochloa* species in the Colombian Eastern Llanos need the use of remote sensing techniques from satellite platforms to estimate amount of offered forage. In the Carimagua Research Centre of the Colombian Corporation for Agricultural Research (Agrosavia), an *Urochloa humidicola* cv. Llanero pasture was evaluated using Landsat 8 and Sentinel 2A images. The NDVI, SAVI, EVI y GNDVI vegetation indices were determined by using the blue, green, red and near infrared bands and the results analyzed with the R free software, to relate those indices with forage availability field measures taken during the dry season. Forage availability ranged between 290 and 656 kg DM ha^{-1} and the vegetation indices for the Landsat 8 and Sentinel 2A sensors were: NDVI = 0.67 (±0.037) and 0.69 (±0.061); SAVI = 0.48 (±0.048) and 0.41 (±0.046); EVI = 0.70 (±0.052) and 0.41 (±0.047); y GNDVI = 0.60 (±0.028) and 0.70 (±0.034), respectively. The relationships between vegetation indices and forage availability were linear. The Coefficient of Determination (R^2= 0.56–0.72) and the Mean Square Error (MSR =63.95–80.16) of the prediction equations were used. In conclusion, under the conditions of the study, the EVI for Landsat 8 and NDVI for Sentinel 2A were considered adequate for estimating forage availability of *Urochloa humidicola* cv. Llanero.

Keywords: EVI, satellite images, NDVI, vegetation indexes.

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Introducción

Para el monitoreo de grandes extensiones de tierra donde están incorporados los sistemas ganaderos, se propone el uso de información proveniente de plataformas satelitales (Beaulieu et al. 2007). Con esta información, que es cada vez más detallada en términos de resolución espectral, espacial y temporal, es posible caracterizar la cobertura herbácea y derivar prácticas de manejo de las áreas pastoriles (Beaulieu et al. 2006).

Los índices de vegetación (IV) derivados de imágenes satelitales, como Landsat 8 y Sentinel 2A, son combinaciones algebraicas de varias bandas espectrales, diseñadas para destacar el vigor de la vegetación y sus propiedades (biomasa del dosel, radiación absorbida, contenido de clorofila, entre otros) (Bannari et al. 1995). Los IV más usados en la evaluación de pasturas y praderas son: NDVI (*Normalized Difference Vegetation Index*); SAVI (*Soil Adjusted Vegetation Index*); EVI (*Enhanced Vegetation Index*); y GNDVI (*Green Normalized Difference Vegetation Index*) (Huete 1988; Candiago et al. 2015; Hoffmann 2018).

Para áreas pastoriles se han aplicado estas técnicas de uso de sensores remotos en los Pirineos españoles (Barrachina et al. 2009, 2010); la Pampa argentina (Cristiano 2010; Irisarri et al. 2013); la región Este de Uruguay (Baeza et al. 2011); el departamento de Antioquia en Colombia (Ramírez 2014; Padilla 2017); y el estado de Sao Paulo en Brasil (Cisneros et al. 2020).

Estudios realizados anteriormente en los Llanos del Orinoco en Colombia por Girard y Rippstein (1994), usando radiometría terrestre e imágenes SPOT, y por Serna-Isaza (2001), usando sensores AVHRR de NOAA, el sensor MSS/TM de Landsat 4 y 5 y los sensores HRV a bordo de SPOT 3 y 4, han mostrado el valor de los datos de la teledetección en plataformas satelitales para la cartografía de pasturas mejoradas y sabanas nativas, bajo diferentes manejos. En los llanos del Orinoco en Venezuela, Chacón (2004) realizó mapeos de los ecosistemas de sabana utilizando imágenes multitemporales del satélite NOAA, apoyándose en el conocimiento experto e índice NDVI.

En la región de los llanos de Colombia es limitada la información sobre el uso de sensores remotos para la estimación de la oferta forrajera, pese a que la región es típicamente ganadera con una población bovina de 5.75 millones de cabezas (ICA 2020) y un área estimada en pasturas mejoradas, especialmente del género *Urochloa*, de más de dos millones de hectáreas (Álvarez y Rincón 2010).

Por tanto, el objetivo de este trabajo fue estimar la oferta de forraje en una pastura de *Urochloa humidicola* cv. Llanero a partir de imágenes multiespectrales provenientes de Landsat 8 y Sentinel 2A, en la subregión de la altillanura de los Llanos Orientales de Colombia.

Materiales y Métodos

La investigación se realizó en el Centro de Investigación Carimagua de la Corporación Colombiana de Investigación Agropecuaria – Agrosavia, ubicado en el municipio de Puerto Gaitán, Meta, en la subregión de la altillanura plana de los Llanos Orientales de Colombia (Figura 1). El promedio anual de las precipitaciones es de 2,000 mm; su distribución es monomodal, correspondiendo la época seca al período noviembre–febrero. Los suelos en el área de estudio son Oxisoles y Ultisoles ácidos, isohipertérmicos (Álvarez y Rincón 2010).

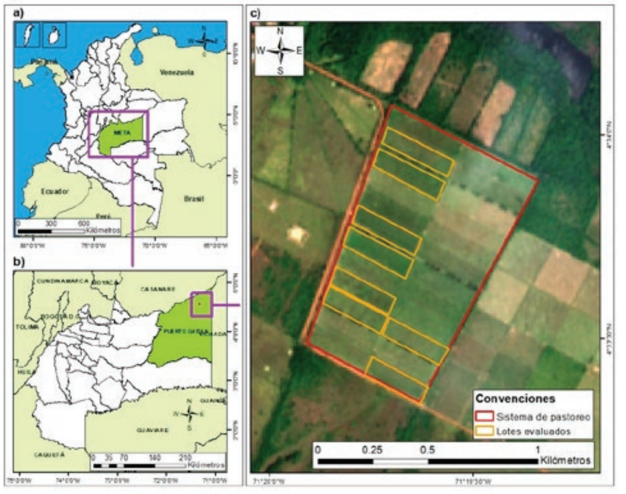

Figura 1. Ubicación del área de pastoreo en el C.I. Carimagua: a) Departamento del Meta en la República de Colombia; b) Municipio de Puerto Gaitán en el Departamento del Meta; c) Área de pastoreo y lotes evaluados.

Los aforos de forraje y las imágenes corresponden al día 8 de diciembre de 2015 (época seca). Las áreas bajo pastoreo por bovinos utilizadas son de pasturas de *Urochloa humidicola* CIAT 6133 cv. Llanero (antes considerado como *Brachiaria dictyoneura*; Rincón et al. 2018) con manejo rotacional. El aforo se realizó en ocho potreros de 2.61 ha (±0.15) cada uno: En cada potrero (unidad experimental) se tomó en 20 puntos una muestra con un marco de 0.25 m^2 cortando con hoz a 15 cm de altura, se pesó el forraje verde con una balanza electrónica y se secaron las muestras a una temperatura de 70 ºC durante tres días para determinar la materia seca.

Las imágenes Landsat 8 y Sentinel 2A fueron obtenidas del portal del Servicio Geológico de los Estados Unidos (earthexplorer.usgs.gov). Las correcciones atmosféricas y el cálculo de los IV se realizaron con el módulo SCP (*Semi-Automatic Classification Plugin*) y la calculadora ráster en el Software libre Q-GIS. En el Cuadro 1 se presentan los IV y las bandas espectrales utilizados en este trabajo.

Cuadro 1. Fórmulas de cálculo de los índices de vegetación con base en las bandas espectrales utilizadas.

Índice espectral de vegetación	Referencia
NDVI=(IR – R) / (IR + R)	Rouse et al. (1974)
SAVI= 1.5 × (IR – R) / (IR + R + 0.5)	Huete (1988)
EVI =2.5 × ((IR - R) / (IR + 6 × R – 7.5 x A + 1))	Jiang et al. (2008)
GNDVI = (IR-V) / (IR+V)	Wang et al. (2007)

NDVI = Normalized Difference Vegetation Index; SAVI = Soil Adjusted Vegetation Index; EVI = Enhanced Vegetation Index; GNDVI = Green Normalized Difference Vegetation Index. A = banda espectral del azul; V = banda espectral del verde; R = banda espectral del rojo; IR = banda espectral del infrarrojo cercano.

A partir de las imágenes de Landsat 8 y Sentinel 2A se calcularon los IV según las fórmulas en el Cuadro 1 y se procedió a establecer las ecuaciones de regresión lineal que explican la relación entre el IV y la oferta forrajera para cada una de las imágenes. Una vez obtenidos los modelos que estiman la producción de forraje se determinó cuál es el de mejor ajuste, para lo cual se utilizaron los criterios raíz del error cuadrático medio (*root mean square error*, RMSE) y el R2 de las ecuaciones. Adicionalmente se estableció, mediante la prueba t de Student, si había diferencias entre los valores estimados por las ecuaciones de regresión para cada uno de los sensores. Para todos los análisis se usó el software libre R (R Core Time 2016).

Resultados

La oferta de forraje determinada en campo varió entre 290 y 656 kg MS/ha para el día de la evaluación, con un promedio de 501 kg MS/ha. Los valores de los índices que se obtuvieron de las imágenes de Landsat 8 y Sentinel 2A se muestran en el Cuadro 2.

Cuadro 2. Valores medios y dispersión de los índices de vegetación obtenidos de los dos sensores.

Índice	Landsat 8	Sentinel 2A
NDVI	0.67 ± 0.037	0.69 ± 0.061
SAVI	0.48 ± 0.048	0.41 ± 0.046
EVI	0.70 ± 0.052	0.41 ± 0.047
GNDVI	0.60 ±0.028	0.70 ± 0.034

En las Figuras 2, 3, 4 y 5 se presentan las rectas de regresión de ajuste lineal entre los IV y la oferta de forraje (biomasa de las pasturas).

Con respecto a los índices obtenidos por Landsat 8, el EVI presentó el valor de R^2 más alto (0.721) y el RMSE más bajo (63.95) (Cuadro 3), por lo cual con base en los resultados experimentales es razonable inferir que este es el IV de elección para estimar la oferta forrajera. Con relación a los índices calculados a partir de Sentinel 2A, el NDVI es el más apropiado por cuanto alcanza el valor de R^2 más alto (0.712) y RSME más bajo (64.89) en la estimación de la oferta forrajera (Cuadro 3).

En la Figura 6 se muestra la distribución espacial de la biomasa obtenida para cada sensor.

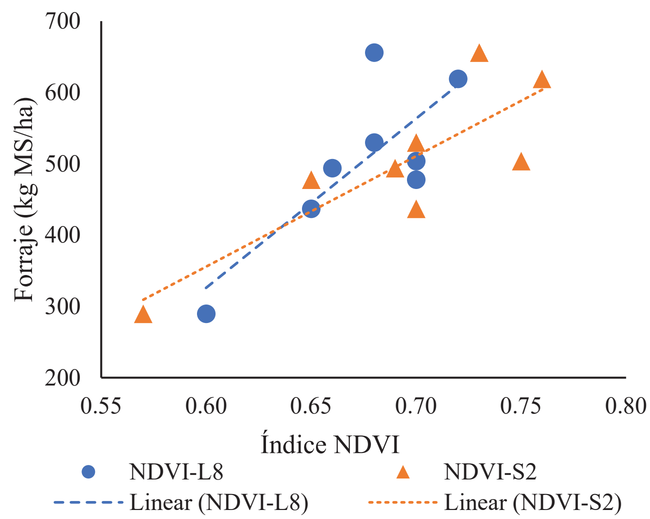

Figura 2. Rectas de regresión de ajuste lineal para el índice NDVI obtenido por los sensores de Landsat 8 (NDVI-L8) y Sentinel 2A (NDVI-S2) y la oferta de forraje.

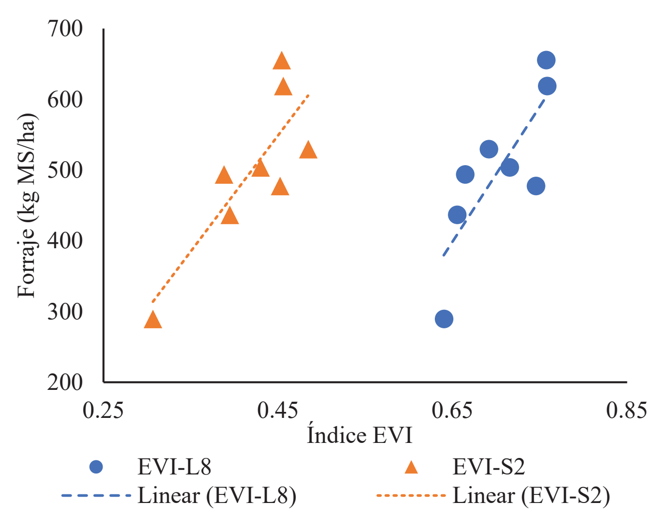

Figura 3. Rectas de regresión de ajuste lineal para el índice EVI obtenido por los sensores de Landsat 8 (EVI-L8) y Sentinel 2A (EVI-S2) y la oferta de forraje.

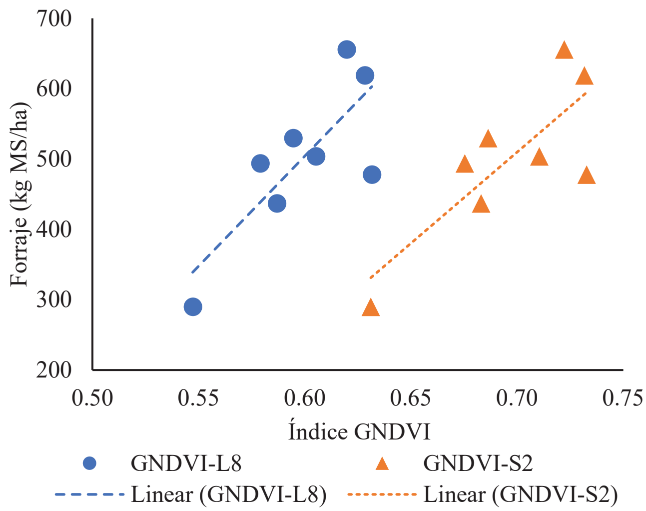

Figura 4. Rectas de regresión de ajuste lineal para el índice GNDVI obtenido por los sensores de Landsat 8 (GNDV-L8) y Sentinel 2A (GNDVI-S2) y la oferta de forraje.

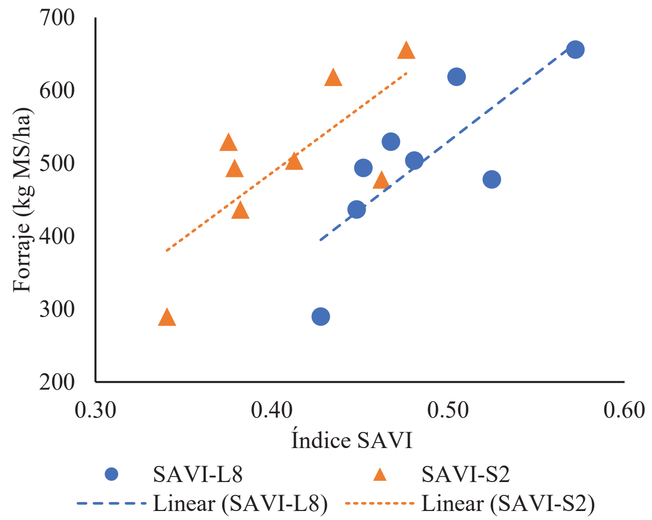

Figura 5. Rectas de regresión de ajuste lineal para el índice SAVI obtenido por los sensores de Landsat 8 (SAVI-L8) y Sentinel 2A (SAVI -S2) y la oferta de forraje.

Cuadro 3. Ecuaciones de regresión lineal y los criterios de selección R2 y RMSE para los sensores e índices utilizados.

Índice	Sensor	Ecuación	R2	RMSE
NDVI	Landsat 8	Y= 2,376.5 X - 1,100.2	0.628	73.84
	Sentinel 2A	Y= 1,547.7 X - 572.71	0.712	64.89
EVI	Landsat 8	Y = 1,912.6 X - 844.28	0.721	63.95
	Sentinel 2A	Y = 1,638 X - 187.98	0.655	71.08
GNDVI	Landsat 8	Y = 3,131.5 X – 1,375.1	0.628	73.81
	Sentinel 2A	Y = 2,596.3 X – 1,307.6	0.645	72.15
SAVI	Landsat 8	Y = 1,861 X - 400.93	0.648	71.83
	Sentinel 2A	Y = 1,788.2 X - 228.29	0.561	80.16

RMSE = error cuadrático medio.

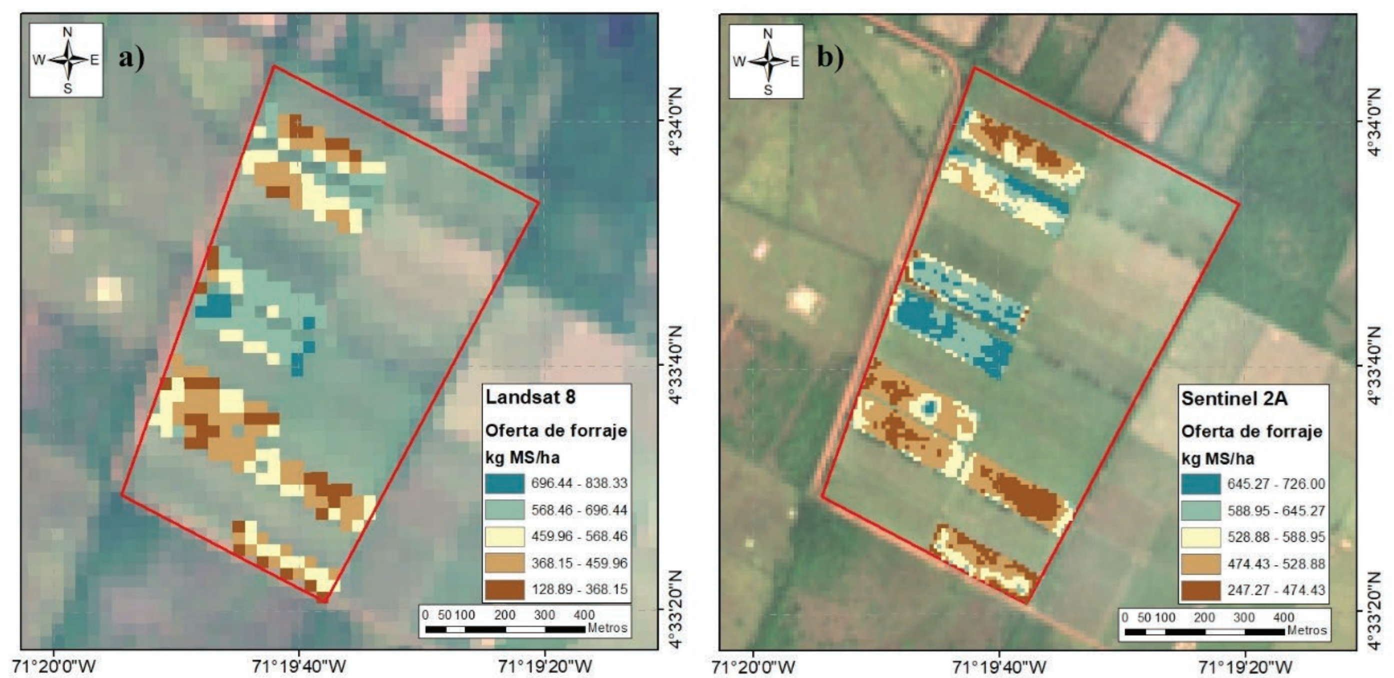

Figura 6. Representación gráfica de la biomasa estimada en el área de estudio. a) oferta de forraje estimada con la ecuación lineal a partir del EVI obtenido con Landsat 8; b) oferta de forraje estimada con la ecuación lineal a partir del NDVI obtenido con Sentinel 2A.

Discusión

La oferta promedio de forraje del pasto Llanero obtenida en esta investigación (501 kg MS/ha) fue inferior a los valores reportados por Pérez et al. (2019) para el mismo pasto, también en época seca y en el C.I. Carimagua (645.7 kg MS/ha con periodos de descanso de 35 a 40 días), Carulla et al. (1991) en el Piedemonte llanero del Meta (669 kg MS/ha) y Pardo y Pérez (2010), también en el Piedemonte llanero (1,365 kg MS/ha para el mismo pasto y 770 kg MS/ha para *Brachiaria humidicola*).

Las diferencias en los valores de biomasa que se observan en la Figura 6 pueden atribuirse a factores como las propiedades del suelo, la microtopografía, la humedad y temperatura las cuales pueden ocurrir aún en áreas relativamente pequeñas (Tamme et al. 2016).

Respecto a los valores de oferta forrajera estimados con base en Landsat 8 y Sentinel 2A para cada uno de los índices, no se encontraron diferencias significativas ($P>0.05$) según la prueba t de Student. Los modelos utilizados muestran su potencial para predecir el aforo de pastizales, pero deben tomarse con precaución sobre todo los obtenidos con Landsat 8, considerando los R^2 entre 0.56 y 0.72 y RMSE entre 63.95 y 80.16, para Landsat 8 y Sentinel 2A, respectivamente.

Mientras que los cuatro índices mostraron relaciones positivas con el aforo de los pastos (Cuadro 3), es necesario reconocer que las observaciones realizadas a partir de Sentinel 2A tiene un mayor detalle respecto a la variabilidad espacial de la oferta forrajera, debido a las características de la resolución espacial en comparación con Landsat 8. Esto permite una mejor estimación de la oferta de forraje, para áreas de estudio ≥2 hectáreas, tal como es evidente en la Figura 6.

La estimación de la oferta forrajera del pasto debe ser contextualizada con la magnitud del área a evaluar: Desde luego estudios a una escala mayor (1:2,500) (potrero o predio) exigen sensores cuya resolución espacial corresponda a tamaños de pixeles relativamente pequeños (10 m × 10 m) como es el caso de Sentinel 2A, mientras que en el caso de trabajo a escala menor (1:50,000; regional, nacional) pueden ser utilizados sensores como Landsat 8 (30 m × 30 m), con tamaños de pixeles mayores. Para una escala aún menor (1:100,000), Baeza et al. (2011) y Ramírez (2014) han usado exitosamente el sensor MODIS con pixeles de 250 m × 250 m, para estudios de sabanas tropicales y subtropicales.

Conclusiones

Con la información obtenida a partir de los sensores de Landsat 8 y Sentinel 2A fue posible establecer, en forma preliminar, un modelo de relación lineal directa entre los índices EVI y NDVI con el forraje disponible de *U. humidicola* cv. Llanero bajo pastoreo en la Altillanura colombiana. El uso de Landsat 8 y Sentinel 2A tiene el potencial para convertirse en una herramienta predictiva para el manejo de praderas en sistemas de producción pastoriles de esta región.

Estudios futuros deben considerar la validación de la información de las plataformas satelitales para la gestión de áreas pastoriles mayores, e incorporar en ellos tanto series multitemporales de imágenes asociadas a los ciclos hidrometeorológicos como para otras especies de pastoreo.

Agradecimientos

Los autores agradecen al Ministerio de Agricultura y Desarrollo Rural de Colombia por el apoyo financiero y al personal administrativo y operativo de los Centros de Investigación Carimagua y La Libertad de Agrosavia, quienes con su apoyo hicieron posible el desarrollo de las actividades del proyecto.

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(Nota de los editores: Los enlaces se verificaron el 2 de agosto de 2021).

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(Recibido para publicación 28 septiembre 2020; aceptado 27 julio 2021; publicado 30 septiembre 2021)

doi: 10.17138/TGFT(9)383-390

Short Communication

The effects of increasing concentrations of *Trichanthera gigantea* leaves in pellets on the nutritive value and short-term intake of diets of grass plus pellets offered to lambs reared under tropical conditions in the Caribbean

Efectos de las concentraciones crecientes de hojas de Trichanthera gigantea *en pélets sobre el valor nutritivo y la ingesta a corto plazo de dietas de pasto más pélets ofrecidas a corderos criados en condiciones tropicales en el Caribe*

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Abstract

There is currently limited information on the benefits of increasing the concentration of *Trichanthera gigantea* leaves in pelleted diets offered to lambs reared under tropical conditions in the Caribbean. Twelve crossbred Barbados Blackbelly rams aged 5 months were used to determine the effects of increasing the concentrations of *T. gigantea* in pelleted diets, on the nutritive value and intake of grass forage plus pellets offered to lambs. Animals were randomly assigned to a basic diet (4 kg) of chopped *Cenchrus purpureus* plus 1 of 6 pelleted diets (500 g) comprised of either 100% intact commercial pellets or a pelleted mixture of ground commercial pellets and ground (dry fallen) *T. gigantea* leaf in the following ratios (*T. gigantea* leaves:ground commercial pellets): 20:80 (T20); 40:60 (T40); 60:40 (T60); 80:20 (T80); and 100:0 (T100). Total intakes of forage and pellets (TPI) were measured at the end of each day during a period of 7 days, and the average daily nutrient intakes of the different treatment diets were calculated. Overall, there was no significant difference in the intakes of pellets containing 0 to 80% *T. gigantea* leaves (P>0.05) but intakes of pellets comprising 100% *T. gigantea* leaves were significantly lower (P<0.0001). Both CP and soluble protein intakes declined progressively as the percentage of *T. gigantea* leaves in the pellets increased. While level of *T. gigantea* leaves in pellets fed to lambs did not generally affect total intakes of pellets, grass, or grass+pellets, animal performance on these various rations cannot be assumed to be similar until longer-term feeding studies have been performed, as reduced protein and energy concentrations in the pellets could significantly lower weight gains as level of leaf in the pellets increased.

Keywords: Barbados Blackbelly sheep, multi-purpose trees, pellet feeding.

Resumen

Actualmente, existe información limitada sobre los beneficios de aumentar la concentración de hojas de *Trichanthera gigantea* en las dietas peletizadas que se ofrecen a los corderos criados en condiciones tropicales en el Caribe. Se utilizaron doce carneros Barbados Blackbelly mestizos de 5 meses de edad para determinar los efectos del aumento de las concentraciones de *T. gigantea* en dietas peletizadas, sobre el valor nutritivo y la ingesta de forraje de pasto más pélets ofrecidos a los corderos. Los animales fueron asignados aleatoriamente a una dieta básica (4 kg) de *Cenchrus purpureus* picado más 1 de 6 dietas peletizadas (500 g) compuestas por pélets comerciales 100% intactos o una mezcla peletizada

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de pélets comerciales molidos y hojas secas de *T. gigantea* en las siguientes proporciones (hojas de *T. gigantea*:pélets comerciales molidos): 20:80 (T20); 40:60 (T40); 60:40 (T60); 80:20 (T80); y 100:0 (T100). Se midieron las ingestas totales de forraje y pélets (TPI) al final de cada día durante un período de 7 días, y se calcularon las ingestas promedio diarias de nutrientes de las diferentes dietas de tratamiento. En general, no hubo diferencias significativas en la ingesta de pélets que contenían de 0 a 80% de hojas de *T. gigantea* (P> 0.05), pero la ingesta de pélets compuestos 100% de hojas de *T. gigantea* fue significativamente menor (P <0.0001). Tanto la ingesta de proteína cruda como la de proteína soluble disminuyeron progresivamente a medida que aumentaba el porcentaje de hojas de *T. gigantea* en los pélets. Si bien el nivel de hojas de *T. gigantea* en los pélets dados como alimento a los corderos generalmente no afectó la ingesta total de pélets, pasto o pasto+pélets, no se puede suponer que el rendimiento de los animales en estas diversas raciones sea similar hasta que se hayan realizado estudios de alimentación a más largo plazo, ya que las concentraciones reducidas de proteína y energía en los pélets podrían reducir significativamente las ganancias de peso a medida que aumenta el nivel de hojas en los pélets.

Palabras clave: Alimentación con pellets, árboles polivalentes, oveja de Barbados Blackbelly.

Introduction

Trichanthera gigantea is a common non-leguminous multi-purpose tree species (MPT) used in small ruminant production systems in the Caribbean (Heuzé et al. 2017). The nutritive value of fresh intact *T. gigantea* leaves is attributed to its high protein concentration, which ranges between 150 and 220 g/kg DM (Rosales 1997; Rosales and Rios 1999). In addition, the presence of hydrolyzable tannins in *T. gigantea* may increase rumen undegradable or bypass protein, which can be a direct benefit to ruminants when consumed (Rosales 1997; Edwards et al. 2012). Compared with other MPTs at the same stage of maturity, *T. gigantea* is typically higher in non-structural and storage carbohydrates and lower in structural carbohydrate, which results in its high rumen degradability (Rosales and Rios 1999). Further, *T. gigantea* has cystoliths on leaf and stem surfaces, which result in high ash concentration and a large percentage of calcium, which is typically greater than 20% DM (Benton and Benton 1963; Barahona 1999). The higher ash concentration may be used to improve the mineral concentrations in the diets of livestock in the tropics, where mineral deficiencies in tropical pastures often occur (McDowell and Arthington 2005).

Apart from fresh leaves, leaf fall may be a potential dry season feed for animals, despite the possible lower nutritive value relative to intact *T. gigantea* leaves as a result of senescence (Charlton et al. 2003). During periods of prolonged drought, there is often an abundance of biomass available as leaf fall (Wright and Cornejo 1990). This may be significant, particularly in the Caribbean, where prolonged severe dry periods are frequent and are predicted to become more common (Lallo et al. 2017). Though there are several studies focused on the use of fresh intact *T. gigantea* leaves, there is no known study on the use of fallen leaves as a prospective feed ingredient for lambs. Further, there is currently no information on the nutritive value of dry fallen leaves of *T. gigantea* and effects of feeding them to lambs in the Caribbean. Therefore, the objective of this study was to determine the effects on nutritive value and intake of pellets of increasing concentrations of dry fallen *T. gigantea* leaves in pellets offered to lambs with grass forage.

Materials and Methods

The effects of including dry ground fallen *T. gigantea* leaf in commercial pellets at 0 (T0), 20 (T20), 40 (T40), 60 (T60), 80 (T80) and 100% (T100) fed with a fresh grass forage basal diet on intake by lambs were examined over 2 periods: Period 1 (10–15 May 2019) and Period 2 (22–28 May 2019). Due to limitations with the facilities (spacing), all 6 treatments could not be compared at the same time so intakes of treatments T0, T20 and T40 were measured during Period 1 and intakes of Treatments T60, T80 and T100 were measured during Period 2. The study was conducted at the Eastern Caribbean Institute for Agriculture and Forestry (ECIAF) – University of Trinidad and Tobago (10.56° N, 61.32° W).

Harvesting and pelleting material

Dry fallen *T. gigantea* leaves (mature flowering stage; approximately 88% DM) were collected one week prior to the study period from the plantation at the "Up the Hill Farms", which is located in Moruga, Trinidad (10.11° N, 61.29° W).

Dry leaves and a commercial ration were the primary ingredients used to produce the pellets examined in this study. On the Control diet (T0, i.e. 100% commercial pellets) intact commercial pellets made of 80% (DM basis) wheat middlings, 20% (DM basis) corn and a vitamin and mineral mix were fed with fresh grass forage (see below). Pellets fed in the other treatments included mixtures of ground commercial pellets and ground dry fallen *T. gigantea* leaves in the following ratios (*T. gigantea* leaves:ground commercial pellets): 20:80 (T20); 40:60 (T40); 60:40 (T60); 80:20 (T80); and 100:0 (T100). Firstly, the commercial pellets and dry *T. gigantea* leaves were ground separately to pass through a 0.635 cm screen (screen was initially 2.54 cm and modified to 0.635 cm) of a Craftsman shredder-hammer mill (Model 247.776380). The ground materials were weighed according to the ratios for the different pellet treatments. After weighing the respective ratios for the different treatment groups, the ground materials were mixed manually for 10–15 minutes and pelleted using a Changchai-ZS1115 Pellet Mill (22 Horse-Power Diesel Engine) with a die length and diameter of 2.54 and 1.27 cm, respectively. Prior to Periods 1 and 2, a single batch of pellets for each treatment group was produced and fed to the respective treatments throughout the respective periods.

In addition to the pellets, mature (6–8 weeks regrowth and 1.5 m high) *Cenchrus purpureus* (syn. *Pennisetum purpureum*) grass was manually harvested with a machete each day from the Eastern Caribbean Institute for Agriculture and Forestry Campus – University of Trinidad and Tobago (ECIAF-UTT) according to Gemeda and Hassen (2014). *C. purpureus* was used as the basal diet for both Periods 1 and 2. Once harvested, the *C. purpureus* (including leaves and stem) was manually chopped to lengths of about 5–10 cm according to Schnaider et al. (2014), for daily feeding.

Animals and diets

The same 12 crossbred (Barbados Blackbelly × West African) intact rams, aged 5 months, were used in both periods (Periods 1 and 2) to measure the intakes of the treatment diets. Mean live weight at the commencement of Period 1 was 22 ± 2.2 kg and for Period 2 was 27 ± 2.4 kg. Before Period 1 commenced, the lambs were subjected to a 19-day adaptation period, where they were examined, treated for internal parasites, fed a diet of 4 kg of chopped *C. purpureus* (including leaves and stem) plus 500 g of commercial pellets and allowed to become familiar with their enclosures. The 12 lambs were randomly assigned on the basis of live weight to 3 groups of 4 animals, which were allocated to 1 of 3 diets (T0, T20 and T40) and intakes were recorded for 7 days. Lambs were then returned to the same diet as fed prior to Period 1 for 5 days. Period 2 then commenced, where the concentrations of *T. gigantea* in pellets were 60, 80 and 100%. Groups of lambs fed T0, T20 and T40 diets in Period 1 were assigned to dietary treatments T60, T80 and T100, respectively. This was done to minimize between-lamb variation within treatment groups. During the experiment, all lambs were confined to well-ventilated individual pens (1.22 × 1.22 m) and had unrestricted access to water and a mineral block (Alphablock), which contained: 55,000 IU Vitamin A; 27,500 IU Vitamin D3; 300 IU Vitamin E; 30,000 mg calcium; 5,000 mg magnesium; 1,800 mg iron; 2,500 mg manganese; 50 mg cobalt; 1,500 mg zinc; 10 mg selenium; and 35 mg iodine per kg DM.

Experimental procedure and design

Animals were fed twice daily at 09:00 h (4 kg forage) and 15:00 h (500 g pellets). Total forage and total pellets offered and refused for each animal were recorded daily to calculate intake of each component of the diet. At 06:30 h daily, both total forage intake (TFI) and total pellet intake (TPI) were recorded. Total dry matter intake (TDMI) was calculated as the sum of TPI and TFI.

Sampling and analytical procedures

Feed samples (forage and pellets) were collected at the end of each week for DM determination and chemical analysis. The pellet samples included 2 subsamples from a total of 2 batches used for feeding. Forage on offer during the week was consistent and a representative sample was selected to determine nutritive value. The total nutrient concentration in the diets was calculated by determining the concentrations of each nutrient (on a DM basis) in both forage and pellets fed, from which total daily intakes on the various treatments were calculated.

Chemical analysis

Samples were dried at 60 °C for 72 h and ground to pass through a 2 mm sieve using a Thomas Scientific mill. These were then packaged (package included Export permit no. 139517 for Research) and exported to Cumberland Valley Analytical Services (CVAS;

Waynesboro, PA, USA) for further analysis. Dry matter of *C. purpureus* (modified method) was determined by drying samples at 105 °C for 3 h (National Forage Testing Association 2002). Dry matter concentrations for pellets and *T. gigantea* were determined by drying samples at 35 °C for 2 h (method no. 930.15, AOAC 2000). ADF was determined using a Whatman 934-AH glass micro-fiber filter with 1.5 µm particle retention in place of a fritted glass crucible (modification of method no. 973.18, AOAC 2000). NDF was obtained using Whatman 934-AH glass micro-fiber filters with 1.5 µm particle retention used in place of a fritted glass crucible (a modification of Van Soest et al. 1991). Ash was determined using 0.35 g sample, which was ashed for 4 h at 535 °C (a modification to method no. 942.05, AOAC 2000).

Statistical analysis

Statistical analysis was conducted using R environment for statistical computing and visualization (R Core Team 2013). Intake measurements obtained from each lamb at different times were treated as repeated measures. Package nlme (Pinheiro et al. 2018) was used to apply a linear mixed effect model to the intake data. The model consisted of treatment, day and day × treatment interaction as fixed effects and animal as the random effect. An analysis of variance (Anova) from Package car (Fox and Weisberg 2011) and Agricolae (de Mendiburu 2019) was used to obtain the P-value for the model differences. Means and superscripts were generated using the R package emmeans (Lenth et al. 2019) and multcomp (Hothorn et al. 2016), which helps in separating significantly different means using Tukey's multiple comparison test. Differences were considered statistically significant if $P<0.05$.

Results

Chemical composition of the dry fallen *T. gigantea* leaves used, *C. purpureus* and all pelleted feeds offered to lambs in the current study is presented in Table 1. The crude protein (CP) concentration of commercial pellets (181 g/kg DM) and *C. purpureus* (150 g/kg DM) was higher than those reported for *T. gigantea* pellets (98–145 g/kg DM) and *T. gigantea* leaves (81 g/kg DM). Actual CP% in feed consumed could be higher than these data suggest as only about 75% of the grass offered was eaten. Since sheep are very selective and chopped material of whole plants was offered, one could assume that the lambs selected for leaf and rejected the stem, which would have much lower CP concentration than the average figures quoted for the grass. Unfortunately we did not analyze the forage rejected by the lambs to clarify this point. Soluble protein of *C. purpureus* was up to 20 g/kg DM more than that of the commercial pellets and more than twice the average value of 21.4 g/kg DM, reported for the pellets containing *T. gigantea* leaf. ADF and NDF concentrations of the feed components ranged between 109 and 425 g/kg DM and 302 and 660 g/kg DM, respectively, for all feeds. *Cenchrus purpureus* had the highest concentrations of both ADF and NDF.

The average feed and nutrient intakes for the different treatment groups are presented in Table 2. Total forage intake (TFI) and total dry matter intake (TDMI) were comparable across all treatment groups ($P>0.05$), ranging between 0.770 and 0.795 kg DM/hd/d for TFI and 1.13 and 1.21 kg DM/hd/d for TDMI, while total pellet intake (TPI) of the T100 group was lower ($P<0.0001$) than that of all other treatments.

Treatment had a significant effect on intakes of nutrients (Table 2). CP intake declined progressively from 194 g CP/hd/d for T0 group to 148 g CP/hd/d for T100 group ($P<0.0001$). Similarly, intake of soluble protein (SP) declined from 55 g SP/hd/d for T0 to 45 g SP/hd/d for T100 ($P<0.0006$). The average ADF intake for Groups T0, T20 and T40 (380 g/hd/d) was less than that for Groups T60, T80 and T100 (448 g/hd/d) ($P<0.0001$).

Total pellet intake/day (TPI) did not vary throughout the study for T0, T20, T40, T60 and T80, nor did it vary between these treatments ($P>0.05$) but increased from Day 1 to Day 5 for T100, before declining again (Table 3). TPI on T100 was lower ($P=0.0001$) than on other treatments on all days except Days 3 and 5.

Table 1. Chemical composition (g/kg DM) including crude protein, soluble protein, acid detergent fiber, neutral detergent fiber, ash and organic matter for *Cenchrus purpureus*, dry fallen *Trichanthera gigantea* leaves (TGL) and pellets fed in various treatments (T0, T20, T40, T60, T80 and T100).

Parameter	*C. purpureus*	TGL[1]	T0[2]	T20	T40	T60	T80	T100
Dry matter (g/kg)	270	878	870	876	871	854	843	833
Crude protein	150	81	181	145	143	103	109	98
Soluble protein	53	19	33	25	28	21	20	13
Acid detergent fiber	425	308	109	142	152	295	287	340
Neutral detergent fiber	660	430	351	302	316	365	432	459
Ash	132	194	64	85	88	151	159	159
Organic matter	138	684	806	791	783	703	684	674

[1]TGL: *Trichanthera gigantea* (ground dry fallen leaves of *T. gigantea*).
[2]Commercial pellets were offered intact with grass forage for the Control group (T0). Other treatments (T20, T40, T60 and T80) comprised ground commercial pellets mixed with increasing proportions of ground dry fallen *T. gigantea* leaf; T100 represents pellets with ground *T. gigantea* leaves as the sole ingredient.

Table 2. Average daily feed and nutrient intakes for the different treatment groups (n=4 lambs per treatment).

Parameter	T0[1]	T20	T40	T60	T80	T100	s.e.	P-value
Forage intake (kg DM/hd/d)	0.770	0.738	0.762	0.764	0.767	0.795	0.023	0.678
Pellet intake (kg DM/hd/d)	0.435a	0.438a	0.435a	0.423a	0.417a	0.330b	0.013	<0.0001
Total intake (kg DM/hd/d)	1.21	1.18	1.20	1.19	1.18	1.13	0.028	0.4049
Crude protein (kg/hd/d)	0.194a	0.179ab	0.172bc	0.162cd	0.161cd	0.148d	0.003	<0.0001
Soluble protein (kg/hd/d)	0.055a	0.052ab	0.051ab	0.051ab	0.049bc	0.045c	0.001	0.0006
Acid detergent fiber (kg/hd/d))	0.374a	0.389a	0.378a	0.459b	0.447b	0.439b	0.010	<0.0001
Neutral detergent fiber (kg/hd/d)	0.660	0.640	0.621	0.673	0.689	0.659	0.015	0.0707
Ash (kg/hd/d)	0.129a	0.139a	0.135a	0.168b	0.168b	0.154b	0.003	<0.0001

[1]T: *Trichanthera gigantea* (ground dry fallen leaves); T0: Commercial pellets (Control group fed grass forage + 100% commercial pellets); T20 group fed forage + pellets comprised of 20% ground dry fallen *T. gigantea* leaf and 80% commercial; T40 group fed forage + pellets comprised of 40% dry fallen *T. gigantea* leaf and 60% commercial; T60 group fed forage + pellets comprised of 60% dry fallen *T. gigantea* leaf and 40% commercial; T80 group fed forage + pellets with 80% dry fallen *T. gigantea* leaf and 20% commercial; and T100 fed forage + pellets comprised of 100% dry fallen *T. gigantea* leaf.
Means within rows with the same letters are not significantly different (P>0.05).

Table 3. Total pellet intake (TPI) (kg DM/day) of lambs fed rations made up of *Cenchrus purpureus* forage plus commercial pellets or forage plus pellets made of a mixture of ground commercial pellets and ground dry fallen leaves of *Trichanthera gigantea* in varying proportions (n=4 lambs per treatment).

	Treatment						P value			
Day	T0[1]	T20	T40	T60	T80	T100	s.e.	Treatment	Day	Treatment × Day
1	0.435ax[2]	0.438ax	0.435ax	0.402ax	0.418ax	0.319bx	0.0179	0.0001	0.6092	0.8518
2	0.435ax	0.438ax	0.435ax	0.426ax	0.421ax	0.316bx				
3	0.435ax	0.438ax	0.435ax	0.427abx	0.421abx	0.354bxy				
4	0.435ax	0.438ax	0.435ax	0.427ax	0.419ax	0.322bxy				
5	0.435ax	0.438ax	0.435ax	0.427ax	0.411ax	0.376ay				
6	0.435ax	0.438ax	0.435ax	0.427ax	0.412ax	0.316bx				
7	0.435ax	0.438ax	0.435ax	0.427ax	0.414ax	0.309bx				

[1]T: *Trichanthera gigantea* (ground dry fallen leaves); T0: Grass forage + commercial pellets (Control group fed pellets comprised of 100% commercial ingredients); T20 group fed grass forage + pellets comprised of 20% ground dry fallen *T. gigantea* leaf + 80% commercial; T40 group fed grass forage + pellets comprised of 40% ground dry fallen *T. gigantea* leaf and 60% commercial; T60 group fed grass forage + pellets comprised of 60% ground dry fallen *T. gigantea* leaf and 40% commercial; T80 group fed grass forage + pellets with 80% ground dry fallen *T. gigantea* leaf and 20% commercial; and T100 fed grass forage + pellets comprised of 100% ground dry fallen *T. gigantea* leaf.
[2]Means followed by the same letters (a,b,c,d) within rows are not significantly different (P>0.05) and means followed by the same letters (x,y,z) within columns are not significantly different (P>0.05).

Discussion

The inclusion of MPTs above 50% in ruminant diets is often associated with reduced intake as a result of anti-nutritional factors inherent to these species (Reed 1995; Min et al. 2003). However, in the current study, the total intakes of pellets with up to 80% *T. gigantea* (T80) were comparable with that of the commercial pellets which lambs were accustomed to being fed. Unlike many other multi-purpose tree species, there is no known report of anti-nutritional factors that limit the intake of *T. gigantea* (Barahona 1999; Wanapat 2009). This may explain the comparable intakes of pellets comprising up to 80% *T. gigantea* leaves.

Trichanthera gigantea is typically reported as having a moderate to low palatability because of the hirsute nature of its leaves (Mejía and Vargas 1993); however the TPI was generally high for all pellet treatments except T100. This may be as a result of the pelleting process, which is often associated with higher levels of palatability and the presentation of a more favorable form of the feed (Wallace et al. 1961; Dobie 1975). For instance, the smaller unit size of pellets makes it more prehensile and easier to ingest compared with the bulkier form of unprocessed forage. This smaller denser form of feed is also associated with more rapid flow of feed through the gastro-intestinal tract resulting in characteristically higher intakes when compared with bulkier unprocessed forage (Blaxter and Graham 1956; Minson 1963). In addition, the pelleting process involves the drying, grinding, mixing and compression of leaves with more favorable ingredients, which is often associated with reduced selection and increased intake (Wanapat et al. 2013). There are no current studies on the impact of pelleting on the intake *T. gigantea* leaves in small ruminants; however according to Beardsley (1964), pelleting can increase intake of forage feeds by up to 25%. Therefore, pelleting may provide an opportunity for improving the intake of and therefore performance on *T. gigantea*.

Daily CP intake by the Control group barely satisfied the CP requirement for finishing lambs (4–7 months of age) weighing 30 kg and growing at a daily rate of 295 g/d (191 g CP/d) (NRC 1985), while those of groups fed pellets containing *T. gigantea* leaf would not support gains of this magnitude. According to Hoover and Miller (1996) the amount of soluble protein (SP), that fraction of the rumen degradable protein that is immediately available for utilization by rumen microbes, should represent about 35% of feed protein in order to optimize rumen function. SP in this study was less than 35% of CP consumed and, based on CP intakes, the daily intakes of SP barely reached the minimum required amounts for growing lambs (0.05–0.07 kg SP/h/d) in all treatment groups. An adequate supply of degradable and bypass protein from diets is associated with increased efficiency of microbial fermentation; improved digestion; increased throughflow from the rumen; and therefore increased intake and improved performance (Lazzarini et al. 2009; Sampaio et al. 2009).

While total intakes of dry matter were not affected by amount of *T. gigantea* leaves included in the pellets, performance of animals on the different rations could vary substantially as CP concentrations in the different rations were quite different. Before any conclusions are drawn about appropriate levels of *T. gigantea* leaves to incorporate in pellets, longer-term feeding studies with animals where liveweight gains are recorded need to be conducted.

Acknowledgments

The authors are thankful to the Caribbean Agricultural Research and Development Institute (CARDI) and the School of Agriculture and Environment (SAE), Massey University for providing the funding support required to undertake the research. The authors are also thankful to the University of Trinidad and Tobago (UTT) for donating animals, granting access to housing facilities, forage banks and the provision of technical support and access to laboratory equipment required to conduct the studies. The donation of animals and forage from the Trinidad and Tobago Goat and Sheep Society is also gratefully acknowledged.

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(Note of the editors: All hyperlinks were verified 26 August 2021).

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(Received for publication 3 November 2020; accepted 13 August 2021; published 30 September 2021)

doi: 10.17138/TGFT(9)391-396

Short Communication

Effects of plant spacing and fertilizer level on forage yield and chemical composition of hybrid *Urochloa* cv. Mulato II grass during the first 150 days of growth under irrigation supplementation, in Chagni Ranch, Awi Zone, Ethiopia

Efectos del espaciamiento de las plantas y el nivel de fertilizante sobre el rendimiento del forraje y la composición química de la gramínea híbrida Urochloa *cv. Mulato II durante los primeros 150 días de crecimiento bajo riego suplementario, en Chagni Ranch, Awi Zone, Etiopía*

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Abstract

A study was conducted to evaluate the effects of plant spacing and N fertilizer application on dry matter yield and chemical composition of *Urochloa* hybrid cv. Mulato II grass for the first 150 days after planting. A factorial experiment with 3 urea fertilizer levels (0, 50 and 100 kg/ha) and 4 spacings between plants and rows (20 × 20, 30 × 40, 40 × 60 and 50 × 80 cm) with 3 replications was used. Data collected were dry matter yield (DMY), leaf:stem ratio and chemical analyses, i.e. crude protein (CP), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations. Results indicated that DMY, leaf:stem ratio, CP%, NDF% and ADF% were significantly ($P<0.05$) affected by interactions between plant spacing and fertilizer level. However, ash and ADL were significantly ($P<0.05$) affected only by main effects. The highest DMYs (9.18 t/ha and 8.93 t/ha) were recorded for narrowest plant spacing (20 × 20 cm) with higher urea fertilizer level (100 kg/ha) and narrowest plant spacing (20 × 20 cm) with medium urea fertilizer level (50 kg/ha), respectively. CP% ranged from 14.6 to 20% and leaf:stem ratio from 1.12 to 1.82:1. Similar studies need to be conducted over longer periods to determine to what extent these findings relate to performance over the life of a permanent pasture.

Keywords: Dry matter yield, N fertilizer, nutrient composition, spacing, urea.

Resumen

Se realizó un estudio para evaluar los efectos del espaciamiento de las plantas y la aplicación de fertilizantes nitrogenados sobre el rendimiento de materia seca y la composición química del híbrido *Urochloa* cv. Pasto Mulato II durante los primeros 150 días después de la siembra. Se utilizó un experimento factorial con 3 niveles de fertilizante de urea (0, 50 y 100 kg / ha) y 4 espaciamientos entre plantas y surcos (20 × 20, 30 × 40, 40 × 60 y 50 × 80 cm) con 3 repeticiones. Los datos recopilados fueron el rendimiento de materia seca (DMY), la relación hoja: tallo y los análisis químicos, como las concentraciones de proteína cruda (CP), ceniza, fibra detergente neutra (NDF), fibra detergente ácida (ADF)

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y lignina detergente ácida (ADL). Los resultados indicaron que el DMY, la relación hoja: tallo, CP%, NDF% y ADF% se vieron afectados significativamente (P <0.05) por las interacciones entre el espaciamiento de las plantas y el nivel de fertilizante. Sin embargo, las cenizas y las ADL se vieron afectadas significativamente (P <0.05) solo por los efectos principales. Los DMY más altos (9.18 t/ha y 8.93 t/ha) se registraron para el espaciamiento de plantas más estrecho (20 × 20 cm) con un nivel de fertilizante de urea más alto (100 kg/ha) y el espaciamiento de plantas más estrecho (20 × 20 cm) con medio nivel de fertilizante de urea (50 kg/ha), respectivamente. El porcentaje de CP varió de 14.6 a 20% y la relación hoja: tallo de 1.12 a 1.82:1. Es necesario realizar estudios similares durante períodos más prolongados para determinar en qué medida estos hallazgos se relacionan con el rendimiento durante la vida de una pastura permanente.

Palabras clave: Composición de nutrientes, espaciado, fertilizante N, rendimiento de materia seca, urea.

Introduction

Livestock are an important component of nearly all farming systems in Ethiopia, providing milk, meat, draught power, transport, manure, hides and skins and serve as a source of cash income (Funk et al. 2012). The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the Agricultural GDP. It also contributes 15% of export earnings and 30% of agricultural employment. The livestock subsector currently supports and sustains livelihoods for 80% of the total rural population (Leta and Mesele 2014). Despite the importance of livestock in the country, productivity is low (Gebremariam et al. 2010). One of the major constraints leading to such low productivity is shortage of feed in terms of both quantity and quality, especially during the dry season (Hassen et al. 2010), combined with high feed prices (Gebremariam et al. 2010).

In order to solve the shortage of feed and increase livestock production, introduction and cultivation of high-quality forages with high yielding ability and adaptation to the biotic and abiotic environmental stresses may be an option (Kahindi et al. 2007). Improved grasses, many of African origin, have greater palatability and productivity than other indigenous species and are therefore desirable additions to pastures and common grazing areas (Mengistu 2002). Among the improved forage crops introduced into Ethiopia, Mulato II grass, which is the result of crosses of *Urochloa ruziziensis*, *U. brizantha* and *U. decumbens*, is claimed to have the capacity to provide a significant amount of quality forage (CIAT 2006).

The optimization of production and nutritive value of grass can be achieved by planting on fertile soils and utilizing forage management tools such as plant spacing and utilizing when at high nutritive value (Yiberkew et al. 2020). Nitrogen (N) fertilizer application is a common practice, since this nutrient is found to be one of the most limiting factors influencing yield and chemical composition of grass pasture including crude protein (CP) concentration and digestibility, increases in which improve livestock production (Marques et al. 2017). Nevertheless, information regarding the effects of fertilizer levels and plant spacing on biomass yield and chemical composition of Mulato II grass is scarce in our country and specifically in the study area. We hypothesized that planting the grass more densely and fertilizing with N would produce more dry matter (DM) more rapidly than when planted at wide spacing and not fertilizing. We conducted the present study in order to generate information on yield and chemical composition of Mulato II grass during the first 150 days at different plant spacings with different rates of nitrogen fertilizer.

Materials and Methods

Description of the study area

The experiment was conducted in Chagni Ranch, Guangua Woreda, Awi Zone, Amhara National Regional State, Ethiopia (10°57 ′N, 36°30′ E; 1,583 masl). The area has average annual rainfall of 1,689 mm and mean minimum and maximum annual temperatures of 23 °C and 30 °C, respectively.

Experimental layout, Design and Treatments

The study was conducted using a 3 × 4 factorial arrangement in a randomized complete block design (RCBD) with 3 replications. The factors were 3 levels of urea fertilizer (0, 50 and 100 kg/ha) and 4 spacings (20 × 20, 30 × 40, 40 × 60 and 50 × 80 cm; S1, S2, S3 and S4, respectively) between plants and rows, respectively, giving 12 treatment combinations and 36 experimental plots. Control treatment was regarded as the unfertilized treatment at each plant spacing.

Each plot was 3 × 3.2 m and the total experimental area was 12.6 × 41.5 m (522.9 m^2). The spacings between plots and replications were 0.5 and 1.5 m, respectively. Treatments were randomly assigned to plots within each replication.

Soil samples were taken by auger from the center and corners of the experimental site prior to planting to a depth of 15 cm and analyses revealed the following: organic matter (OM) – 5.88%; organic carbon (OC) – 3.41%; total N – 0.30%; available P – 4 ppm; and pH – 5.6.

Land preparation and Experimental management

Land was oxen-ploughed and harrowing and bed preparation were carried out before planting manually. Root splits of Mulato II grass were collected from Finota Selam grass nursery site at an age of 7 months regrowth and planted at the experimental site on 6 September 2017. Urea was applied by split application with half applied at planting and the remainder at 30 days after planting with different levels based on treatment. Weeding was done manually during the experimental period. The experiment was irrigated once a week when rain was limited, with precautions taken to avoid contamination of treatments by cross flooding.

Sample collection and Dry matter yield determination

Data on dry matter yield (DMY) and chemical composition of Mulato II grass were recorded at harvesting time, 150 days after planting. On 6 February 2018, leaf:stem ratio was determined from 10 randomly selected plants in each plot by separating leaf and stem portions, air-drying the leaves and stems and weighing separately.

DMY per plot was determined by hand-harvesting plants in inner rows, i.e. excluding border rows, with sampling areas of 7.28 m^2 for S1, 5.76 m^2 for S2, 4.4 m^2 for S3 and 2.4 m^2 for S4 at a height of 5 cm from ground level. Fresh weight of forage was measured immediately after harvesting, before the forage was thoroughly mixed and a 0.5 kg fresh subsample was taken from each sample for DMY determination. The samples were oven-dried and DMY/ha was calculated.

Chemical analysis of forage

Following mixing of the forage a second 0.5 kg fresh subsample was taken from each plot for chemical analysis and dried in a forced-draft oven at a temperature of 105 ºC for 24 hours. The dried material was ground to pass through a 1 mm sieve for chemical analysis and preserved in plastic bags pending analysis at Debre Birhan Agricultural Research Center Animal Nutrition Laboratory. Ash and N were determined according to the procedures described by AOAC (1990) and neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to the procedures described by Van Soest (1985).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS 2007). Differences among treatment means were determined using Duncan's Multiple Range Test (DMRT) at P<0.05. The statistical model used was:

$$Y_{ijk} = \mu + B_i + F_j + S_k + (FS)_{jk} + e_{ijk}, \text{ where:}$$

Y_{ijk} = the response variable;
μ = overall mean;
B_i = ith block effect;
F_j = jth main factor effect (fertilizer level);
S_k = kth main factor effect (spacing);
$(FS)_{jk}$ = jkth interaction effect (fertilizer level × spacing); and
e_{ijk} = random error.

Results

Overall, there were significant interactions between the effects of the main treatment variables (plant spacing and urea level) on DMY, leaf:stem ratio and chemical composition.

Dry matter yield and leaf:stem ratio

DMY per hectare was significantly (P<0.01) affected by both plant spacing and urea fertilizer level (Table 1). Increasing plant spacing reduced DMY of forage at all fertilizer levels (P<0.05) and urea application increased DMY at all plant spacings but differences were significant (P<0.05) at only the narrowest plant spacing. Highest yields were obtained at the narrowest plant spacing with urea applied (P<0.05).

Leaf:stem ratio was increased by plant spacing at all fertilizer levels but differences were significant for only the unfertilized Control and 100 kg urea/ha treatments (P<0.05; Table 1). Similarly, urea application increased leaf:stem ratio at all plant spacings but differences were significant (P<0.05) for only the wider 2 spacings (S3 and S4).

Table 1. Total dry matter yield and leaf:stem ratio of *Urochloa* Mulato II at 150 days after planting as influenced by combinations of urea fertilizer level and plant spacing.

Fertilizer level	Plant spacing			
	S1	S2	S3	S4
Total dry matter yield (t/ha)				
F1	6.5b	5.17bcd	4.22de	2.91e
F2	8.93a	5.68bc	4.51cd	4.01de
F3	9.18a	6.46b	4.61cd	3.80e
Leaf:stem ratio				
F1	1.12e	1.37bcde	1.16de	1.42bcd
F2	1.32bcde	1.27cde	1.38bcde	1.48bc
F3	1.38bcde	1.44bcd	1.59ab	1.82a

S1 = 20 × 20 cm; S2 = 30 × 40 cm; S3 = 40 × 60 cm; and S4 = 50 × 80 cm spacing between plants and rows; F1= 0 kg urea/ha; F2 = 50 kg urea/ha; and F3 = 100 kg urea/ha.
Means for different treatments with different letters are significantly different (P<0.05).

Chemical composition

The significant effects of N fertilizer level, plant spacing and their interactions on crude protein percentage (CP) are indicated in Table 2. CP concentration increased (P<0.05) with increase in row spacing at all fertilizer levels and urea application increased CP% at all plant spacings but differences were significant (P<0.05) at only the narrowest and widest spacings. Highest CP% (20.0%) was recorded where 100 kg urea/ha was applied at the widest plant spacing and the lowest (14.6%) for the unfertilized Control treatment at the narrowest plant spacing.

Table 2. CP, NDF and ADF concentrations of *Urochloa* Mulato II at 150 days after planting as affected by combinations of urea fertilizer level and plant spacing.

Spacing	Fertilizer level			
	S1	S2	S3	S4
Crude protein (%)				
F1	14.6h	16.6fg	17.7cdefg	18.5bcd
F2	16.2g	16.6fg	18.3bcde	19.5ab
F3	17.2defg	18.1bcdef	19.1abc	20.0a
Neutral detergent fiber (%)				
F1	52.8a	51.0ab	48.3abc	45.5bcde
F2	48.5abc	47.8bcd	43.6cde	46.2bcde
F3	45.1cde	42.1e	46.6bcde	42.3de
Acid detergent fiber (%)				
F1	39.3a	38.2ab	36.3bcd	34.4bcde
F2	36.9abc	36.3abcd	30.8de	33.3bcde
F3	33.7bcde	32.1cde	31.0de	30.1e

S1 = 20 × 20 cm; S2 = 30 × 40 cm; S3 = 40 × 60 cm; and S4 = 50 × 80 cm spacing between plants and rows; F1= 0 kg urea/ha; F2 = 50 kg urea/ha; and F3 = 100 kg urea/ha.
Means for different treatments with different letters are significantly different (P<0.05).

Both NDF and ADF concentrations declined as fertilizer level increased but differences were significant (P<0.05) for only the narrower 2 plant spacings (Table 2). Similarly, NDF% declined as plant spacing increased but differences were significant (P<0.05) in the unfertilized Control treatment only. ADF% also declined as plant spacing increased but there were no consistent significant differences. Overall trends were for highest values for both NDF and ADF concentrations to occur in the Control (unfertilized) at the narrowest plant spacing and the lowest values with the higher urea level at the widest plant spacing.

Discussion

The higher DMY at narrower spacing with application of N fertilizer were to be expected as plant population was greater, plants were taller and soil fertility was improved with application of urea fertilizer. A combination of increased tiller numbers and number of leaves per plant could have contributed to increased photosynthetic activity and hence higher dry matter production (Damry et al. 2009). Those authors reported that increasing level of urea fertilizer application increased Mulato tiller numbers and DM production plus CP and NDF concentrations. Responses to urea in this study also confirm those of CIAT (2006) that *Urochloa* Mulato II is highly responsive to N fertilizer. Similarly, Bouathong et al. (2011) reported a trend for hybrid *Urochloa* grass yield components to increment as the level of N fertilizer application increased with no significant benefit of adding N at levels above 40 kg/ha. Similarly, Marques et al. (2017) reported DMY of Mulato II significantly increasing with increasing rates of N fertilizer. Yiberkew et al. (2020) reported that DMY of Mulato II hybrid was significantly affected by plant spacing where yields from spacings of 15 × 50 and 30 × 50 exceeded those at 45 × 50 cm at 3 months after planting. Buamool and Phakamas (2018) also reported that DMY of tropical grasses like Mulato II, Ruzi grass (*Urochloa ruziziensis*), Purple guinea (*Megathyrsus maximus* TD 58) and Mombasa guinea (*Megathyrsus maximus* cv. Mombasa) were higher following urea application than with ammonium sulphate or non-fertilized grasses.

Leaves are a good nutritional quality parameter for forage grass species. The application of N fertilizer increases soil fertility sufficiently to produce more leaves and make the plant grow vigorously. In addition, at the wider spacings, the plants receive more light, which could be used for leaf formation but in grass grown at narrower spacings there could be shading effects, resulting in the formation of fewer lateral shoots. Wider spacing reduces competition for light, nutrients and moisture so plants can grow more vigorously, which is stimulated by N application. Relatively lower leaf:stem ratios were recorded for narrower spacings due to competition among plants, which resulted in increased stem growth (early maturity) rather than leaf development. These results support the findings of Yiberkew et al. (2020) that leaf:stem ratio was higher at wider spacing (45 × 50 cm) than at intermediate (30 × 50 cm) and narrow plant spacing (15 × 50 cm) (1.39, 1.1 and 0.97, respectively).

While Mulato II showed good response to N fertilizer, depending on the level of soil fertility, one or more maintenance applications may be required to maintain high yields of good quality forage. Nutritive value of forage, i.e. concentrations of CP, ADF, NDF and digestibility, depends on soil fertility and stage of maturity. Planting Mulato II at wider plant spacings with higher N fertilizer level produced excellent nutritional value, particularly CP%, which is often a limiting nutrient in forages. Forage produced at all plant spacings with urea fertilizer application had CP concentration well above the level required for effective rumen function (7.5%) (Van Soest 1982) and for lactation (15%) (Norton 1982). This is a clear indication of the value of this particular grass as a forage for livestock. Factors contributing to the higher CP percentage at higher fertilizer level and wider plant spacing would be higher N uptake by individual plants, plus enhancement of leafiness and leaf:stem ratio of grass. This would agree with findings of Marques et al. (2017), who reported that application of N produced a significant increase in forage production and a linear increase in CP% of Mulato II.

NDF and ADF concentrations all declined as N fertilizer level and spacing increased. This would be a function of reduced stem percentage and reduced lignification at the wider spacing and with greater N availability in fertilized treatments. At lower fertilizer levels and narrower plant spacing, competition between plants for resources forces plants to prioritize development of structural components to cope with the environmental conditions. The current result is in line with Marques et al. (2017), who reported that higher fertilizer doses led to higher protein and lower NDF and ADF concentrations in Mulato II, which should increase nutritive value of grass and its intake. Increase in cell wall contents (NDF) is a very important limiting factor in terms of nutritive value of feeds (Van Soest 1985). NDF values greater than 60% result in decreased voluntary feed intake, increased rumination time and decreased conversion efficiency of metabolizable energy (Reed and Goe1989). All forage produced in this study had NDF concentrations below the critical value of 60%.

The current results revealed that DMY of Mulato II during the first 150 days of growth can be improved by urea fertilizer application. More specifically application of 50 kg urea/ha produced good responses in both DMY and CP concentration. While higher urea levels produced further increases in both parameters, the financial return was unlikely to be positive given the lower response with the extra amount of fertilizer applied. Similarly closer spacing of plants, i.e. closer planting, resulted in higher yields of forage at reduced CP%.

It must be remembered that this study covered only the first 150 days of growth, which is a very short time in the life of a perennial pasture. While narrow spacing resulted in additional forage growth in this early stage, it would be expected that, as the stand matured, differences in yield between different spacings would disappear. Future studies should be continued for at least 2 years to test this, while a range of harvest frequencies and maintenance fertilizer levels should be examined. Repeating these studies on a range of soil types and under differing environmental conditions will determine how applicable the results are over a range of conditions, while the true benefits of growing this grass will only be known when performance of animals consuming the forage is assessed.

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(Note of the editors: All hyperlinks were verified 31 August 2021).

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(Received for publication 23 December 2019; accepted 19 August 2021; published 30 September 2021)

Tropical Grasslands
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