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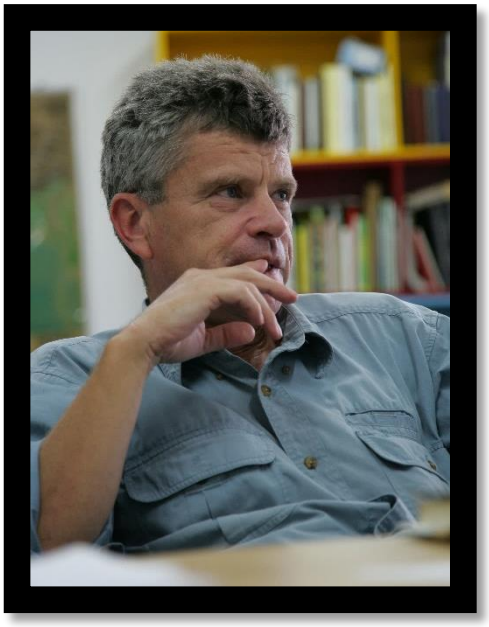
TGFT Editorial Team
A.A. 6713, Km 17 Recta Cali-Palmira, Cali, Valle del Cauca, Colombia.
Phone: +57 2 4450100 Ext. 3084
Email: CIAT-TGFT-Journal@cgiar.org

This issue is dedicated to the memory of **Michael Blümmel** (7 July 1955 – 10 October 2020), German animal nutritionist who, after working at USDA-ARS, joined the International Livestock Research Institute (ILRI) as Principal Scientist in 2001. At the time of his untimely passing he was Deputy Program Leader of ILRI´s Feed and Forage Development program and was a member of the Editorial Board of *Tropical Grasslands-Forrajes Tropicales* since the journal´s inception. His friends and colleagues in the tropical forages and feed scientific community will remember him for his pioneer role as scientist in the area of integrating crop improvement and livestock production, his involvement in developing decentralized small-scale business enterprises focused on feed production and transaction, his thorough scientific thinking and his pleasant personality.



Principal Contacts

Rainer Schultze-Kraft
The Alliance of Bioversity International and CIAT
Colombia
Phone: +57 2 4450100 Ext. 3036
Email: CIAT-TGFT-Journal@cgiar.org

Technical Support
José Luis Urrea Benítez
The Alliance of Bioversity International and CIAT
Colombia
Phone: +57 2 4450100 Ext. 3354
Email: CIAT-TGFT-Journal@cgiar.org

Table of Contents

Research Papers

Beef cattle production on Piatã grass pastures in silvopastoral systems **1–12**

Mariana Pereira, Maria da Graça Morais, Patrick Bezerra Fernandes, Valéria Ana Corvalã dos Santos, Sarah Glatzle, Roberto Giolo de Almeida

Height and mowing of pasture at the end of winter modulate the tillering of Marandu palisadegrass in spring **13–22**

Bruno Humberto Rezende Carvalho, Lilian Elgalise Techio Pereira, André Fischer Sbrissia, Gabriel de Oliveira Rocha, Manoel Eduardo Rozalino Santos

Urochloa brizantha cultivated in aluminum-toxic soil: Changes in plant growth and ultrastructure of root and leaf tissues **23–33**

Lucas Aparecido Manzani Lisboa, Gustavo Henrique de Oliveira Dias, Hiago Augusto Amaral Sacco, João Vitor Rodrigues Padovan, Gabriel Banos Rodrigues, Kauê Barbarotto Ribeiro, Gabriel Geminiano da Silva, Alan dos Santos Cardoso, Leandro Barradas Pereira, Paulo Alexandre Monteiro de Figueiredo

Chemical composition, fermentation profile, microbial population and dry matter recovery of silages from mixtures of palisade grass and forage peanut **34–42**

Françoise Mara Gomes, Karina Guimarães Ribeiro, Igor Alexandre de Souza, Janaina de Lima Silva, Mariele Cristina Nascimento Agarussi, Vanessa Paula da Silva, Thiago Carvalho da Silva, Odilon Gomes Pereira

Dry matter yields and quality parameters of ten Napier grass (*Cenchrus purpureus*) genotypes at three locations in western Oromia, Ethiopia **43–51**

Abuye Tulu, Mekonnen Diribsa, Worku Temesgen

Fermentation quality and aerobic stability of Napier grass ensiled with citric acid residue and lactic acid bacteria **52–59**

Xuxiong Tao, Chongwen Ji, Sifan Chen, Jie Zhao, Siran Wang, Junfeng Li, Fuxin Sun, Tao Shao

Selection for resistance to fungal diseases and other desirable traits in kikuyu grass (*Cenchrus clandestinus*) **60–69**

William J. Fulkerson, Nathan R. Jennings, Mark Callow, Karen J. Harper, Percy T.W. Wong, Peter M. Martin

Effects of a nitrogen-based supplement on intake, live weight and body energy reserves in breeding *Bos indicus* cross cows **70–80**

Rob M. Dixon, Robert J. Mayer

Comparison of forage production and nutritive value of 10 *Grona* spp. accessions in Danzhou, Hainan, China **81–88**

Linling Yan, Rongshu Dong, Wenqiang Wang, Sabine Douxchamps, Mary Atieno, Guodao Liu, Yiming Liu

Varietal differences in yield and nutritional quality of alfalfa (*Medicago sativa*) accessions during 20 months after planting in Ethiopia **89–96**

Tessema Atumo, Christopher Stephen Jones

Evaluation of sainfoin accessions exposed to powdery mildew disease at four locations in Iran **97–108**

Mohammad Ali Alizadeh, Ali Ashraf Jafari, Karam Sepahvand, Saied Davazdahemami, Mohammad Rahim Moeini, Farid Normand Moaied, Bita Naseri

Salinity tolerance of *Avena sativa* fodder genotypes **109–119**

Ajoy Kumar Roy, Devendra Ram Malaviya, Anjali Anand, Rang Nath Choubey, Mirza Jaynul Baig, Kuldeep Dwivedi, Pankaj Kaushal

How does agro-pastoralism affect forage and soil properties in western Serengeti, Tanzania? **120–133**

Pius Yoram Kavana, Ephraim J. Mtengeti, Anthony Sangeda, Christopher Mahonge, Robert Fyumagwa, Bukombe John

Short Communications

Chlorophyll concentration and production of *Urochloa decumbens* treated with diazotrophic bacteria and thiamine in the Brazilian Cerrado **134–137**

Eduardo Pradi Vendruscolo, Paulo Ricardo de Oliveira, Aliny Heloísa Alcântara Rodrigues, Sávio Rosa Correia, Luiz Fernandes Cardoso Campos, Alexsander Seleguini, Sebastião Ferreira de Lima

Hybrids of *Paspalum plicatulum* × *P. guenoarum*: Selection for forage yield and cold tolerance in a subtropical environment **138–143**

Karla M. Saraiva, Miguel Dall'Agnol, Eder A.M. da Motta, Emerson A. Pereira, Cleber H.L. de Souza, Carine Simioni, Roberto L. Weiler, Maurício M. Kopp, Raquel Schneider-Canny, Marlon R. Barbosa

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Research Paper

Beef cattle production on Piatã grass pastures in silvopastoral systems

Producción de ganado de carne en pasturas de Urochloa brizantha *cv. BRS Piatã en sistemas silvopastoriles*

MARIANA PEREIRA[1,3], MARIA DA GRAÇA MORAIS[1], PATRICK BEZERRA FERNANDES[1], VALÉRIA ANA CORVALÃ DOS SANTOS[2], SARAH GLATZLE[3] AND ROBERTO GIOLO DE ALMEIDA[4]

[1]*Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.* ufms.br
[2]*Escola Superior de Agricultura "Luiz de Queiroz", ESALQ, Universidade de São Paulo, São Paulo, Brazil.* esalq.usp.br
[3]*Institute of Agricultural Sciences in the Tropics, Hohenheim University, Stuttgart, Germany.* uni-hohenheim.de
[4]*Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Corte, Campo Grande, MS, Brazil.* cnpgc.embrapa.br

Abstract

Tropical beef cattle production involving animals grazing in a shaded and biologically diverse environment, surrounded by high-quality edible biomass, is achievable through silvopastoral systems (SPSs). However, it is necessary to assess the effects of the presence of trees on forage and animal performance over time. In the Brazilian Cerrado, we evaluated the effects of 2 densities of eucalyptus trees in 2 SPSs (8 years of age - SPS22: 227 trees/ha; SPS14: 357 trees/ha) on forage morphology, production and nutritive value of *Urochloa brizantha* cv. BRS Piatã grass plus performance of Nellore heifers, compared with a grass-only pasture, over a year from 2015 to 2016. On the one hand, SPSs improved (P<0.001) forage nutritive value as reflected in higher crude protein and digestibility and lower neutral and acid detergent fiber concentrations compared with a grass-only pasture. On the other hand, the grass-only pasture had higher (P<0.001) herbage mass and accumulation rate than the SPSs. Forage growth and animal production decreased with higher tree density. Increasing competition from trees with age could be a serious issue limiting pasture and animal production and should be monitored. The suitability of eucalyptus trees for planting in SPSs may be questionable after the 8th year of establishment and further studies are warranted.

Keywords: Agroforestry systems, eucalyptus, grazing systems, ruminant production, shading, tree density.

Resumen

Los sistemas silvopastoriles (SPS) en zonas tropicales permiten la producción de carne con animales en pastoreo en ambientes sombreados y biológicamente diversos. No obstante es necesario evaluar los efectos de la presencia de árboles en la pastura y el rendimiento de los animales a través del tiempo. En el período 2015–2016 se evaluó en el Cerrado brasileño el efecto de dos densidades de eucalipto (*Eucalyptus urograndis*) en dos SPS (SPS22 = 227 árboles/ha; SPS14 = 357 árboles/ha) en algunas características morfológicas, la producción y el valor nutritivo del forraje de *Urochloa brizantha* cv. BRS Piatã, y en el rendimiento de novillas Nellore, en comparación con la pastura sin árboles (Testigo). Los SPS mejoraron (P<0.001) el valor nutritivo del forraje en términos de proteína cruda y digestibilidad más altas, y concentraciones de fibra detergente neutra y ácida más bajas que el Testigo. El Testigo presentó mayor masa de forraje y una mayor tasa de acumulación de este (P<0.001) que los SPS. El crecimiento del forraje y la producción animal disminuyeron con mayor densidad de árboles. El aumento de la competencia de los árboles con la edad podría ser una limitante seria para la producción del pasto y de los animales, y por tanto debe ser monitoreado. La aptitud de los eucaliptos para uso en SPS puede ser cuestionada después del octavo año de establecimiento y necesita mayor investigación.

Palabras clave: Densidad de árboles, producción de rumiantes, sistemas agroforestales, sistemas de pastoreo, sombreado.

Correspondence: Mariana Pereira, Institute of Agricultural Sciences in the Tropics, Hohenheim University, 70593 Stuttgart, Germany.
Email: mariana.pereira@uni-hohenheim.de

Introduction

Silvopastoral systems (SPSs) are among the most recent agricultural developments in Brazil. Such SPSs provide livestock and forest products from the same area of land in rotation, consortium or succession, over a defined period. The systems have been promoted as a valuable option of sustainable intensification, by increasing food production while maintaining or improving environmental quality and preserving natural biodiversity (Costa et al. 2018).

The interaction between these components has raised many questions, highlighting the need for further investigations of the benefits of recovering pasture and degraded land, particularly in fragile ecosystems. For instance, livestock are the mainstay of livelihoods, which often leads to pasture degradation due to uncontrolled and poor grazing management practices – as commonly observed in the Brazilian Cerrado (Peron and Evangelista 2004; Garcia and Ballester 2016).

While tropical forages have high growth rates and good persistence, while enhancing soil cover, they require high incident light to reach maximum levels of production (Taiz and Zeiger 2010). In SPSs the presence of trees limits the amount of incident light reaching the sward. Dry matter (DM) yields of many tropical grasses and legumes, such as *Urochloa* spp., *Megathyrsus maximus*, *Paspalum notatum*, *Arachis pintoi*, *Neustanthus phaseoloides* (syn. *Pueraria phaseoloides*) and *Stylosanthes* spp., to name but a few, are greatly reduced as shade levels increase compared with sunny environments (Andrade et al. 2004; Martuscello et al. 2009; Sousa et al. 2010; Araújo et al. 2017). Moreover, in SPSs with eucalyptus and pastures, Pezzopane et al. (2015) observed soil moisture removal near the tree rows was greater than in the inter-row space, which was attributed to increased extraction by tree roots which penetrated to greater depths than the grass.

Nevertheless, several authors have reported an improvement in forage nutritive value under shading, particularly an increase in protein concentration levels (Baruch and Guenni 2007; Sousa et al. 2010; Paciullo et al. 2016), which enhances animal performance with average daily bodyweight (BW) gains per animal similar to those in sunny environments (Oliveira et al. 2014; Gamarra et al. 2017).

Amongst tropical forage grasses, the Piatã cultivar (*Urochloa brizantha* cv. BRS Piatã), adapted to medium fertility and well-drained soils, has been considered by researchers as a suitable option for planting in SPSs (Gamarra et al. 2017; Geremia et al. 2018). It is also an alternative for pasture diversification, showing high herbage mass production in Brazilian Cerrado soils (Euclides et al. 2008).

One of the grazing management practices used in sunny environments under continuous stocking is based on predetermined canopy heights (Martuscello et al. 2009; Pontes et al. 2016). However, there have been few recommendations for grazing management under shading (Baldissera et al. 2016).

As mentioned above, despite promoting a series of benefits, the presence of trees in pastures might decrease herbage mass and soil cover, along with reduction in soil moisture, and hence, constrain animal production over time – due to the continuous growth of trees with increased competition for light, water and nutrients. Therefore, the spatial arrangement of tree rows should match the intended objective, whether emphasis be on forest or livestock production. Long-term studies are required to avoid a decline in forage and animal production (Paciullo et al. 2011).

The systems evaluated in the present study were planted in 2008, aiming at recovering a degraded pasture. The 2 tree arrangements were designed to favor livestock production instead of forest production, following the majority of systems assembled at that time, with a short distance between the tree rows (Andrade et al. 2008; Devkota et al. 2009; Paciullo et al. 2008, 2011).

We hypothesize that herbage mass and animal growth rates are likely to decrease with time due to shading and other competitive effects in silvopastoral systems with eucalyptus trees, whereas in systems with lower eucalyptus tree density, BW gains per animal and per ha could be similar to those in a grass-only pasture as a result of improved forage nutritive value. Since the systems under study were in their 8th year since establishment, we measured pasture yield and quality plus animal performance at 2 densities of trees in comparison with a grass-only pasture.

Materials and Methods

Study site

Establishment of the experimental area was previously described in detail by Pereira et al. (2014). The experiment was carried out at Embrapa Beef Cattle, located in Campo Grande, Mato Grosso do Sul state, Brazil (20º27' S, 54º37' W; 530 masl), from June 2015 to May 2016. All procedures were approved by the Ethics and Animal Use Commission of Embrapa Beef Cattle under protocol no. 014/2014. According to the Köppen classification, climate of the experimental area falls in the transition between Cfa and humid tropical Aw, with

average annual rainfall of 1,560 mm. The dry season occurs in the coldest months (May–September) and the rainy season in the hottest months (October–April). Air ambient temperature and precipitation data during the trial were collected from Embrapa Beef Cattle meteorological station, and along with mean rainfall data for the last 30 years for the Campo Grande region from the National Institute of Meteorology (A756 – INMET), are depicted in Figure 1.

The soil at the experimental site was a Dystrophic Red Latosol, with clay texture, characterized by low pH, low base saturation and medium aluminum concentration. Mean values from soil chemical analyses performed in 2013 in the 0–20 cm layer showed that the area was relatively uniform, with clay contents of 41 ± 5%; P (Mehlich-1) 0.29–0.42 mg/dm^3; base saturation 26–34%; and aluminum saturation 10–23%.

In August 2008, soil preparation was performed through a heavy disking, subsoiling and applications of 3,000 kg lime/ha, 1,000 kg gypsum/ha and 300 kg fertilizer/ha as N:P:K (5:25:15) broadcast over the pasture canopy, followed by leveling with disk harrows. The 18-ha experimental area was divided into 12 paddocks each of 1.4 ha with 4 paddocks (experimental units) for each system evaluated. Three systems were established: grass-only system without trees, representing the Control treatment (CON); and 2 silvopastoral systems with eucalyptus trees, i.e. SPS22 with an arrangement of 22 m spacing between tree rows × 2 m tree spacing within rows, totaling 227 eucalyptus trees/ha; and SPS14 with an arrangement of 14 × 2 m, totaling 357 eucalyptus trees/ha. *Eucalyptus urograndis* clone (*E. urophylla* × *E. grandis*) was planted in single lines oriented at –20.41º south and –54.71º west in relation to the east-west axis. All systems followed the same management, involving a crop rotation strategy every 4 years, i.e. cultivation of soybean as a crop for one year, followed by 3 years with solely *Urochloa brizantha* cv. BRS Piatã grass.

The present experiment was carried out in the third year after pasture establishment in the second rotation cycle. In January 2016, the pasture received maintenance fertilizer of 50 kg N/ha, in the form of urea, plus 300 kg N:P:K fertilizer/ha (0:20:20).

The eucalyptus trees had reached average heights of 27 and 25 m in the SPS14 and SPS22 systems, respectively. In order to characterize the level of luminosity reaching the Piatã grass canopy, the photosynthetically active radiation (PAR) was recorded at canopy height at 5 points in each SPS paddock, and at a point under full sun conditions immediately before and at the end of SPS

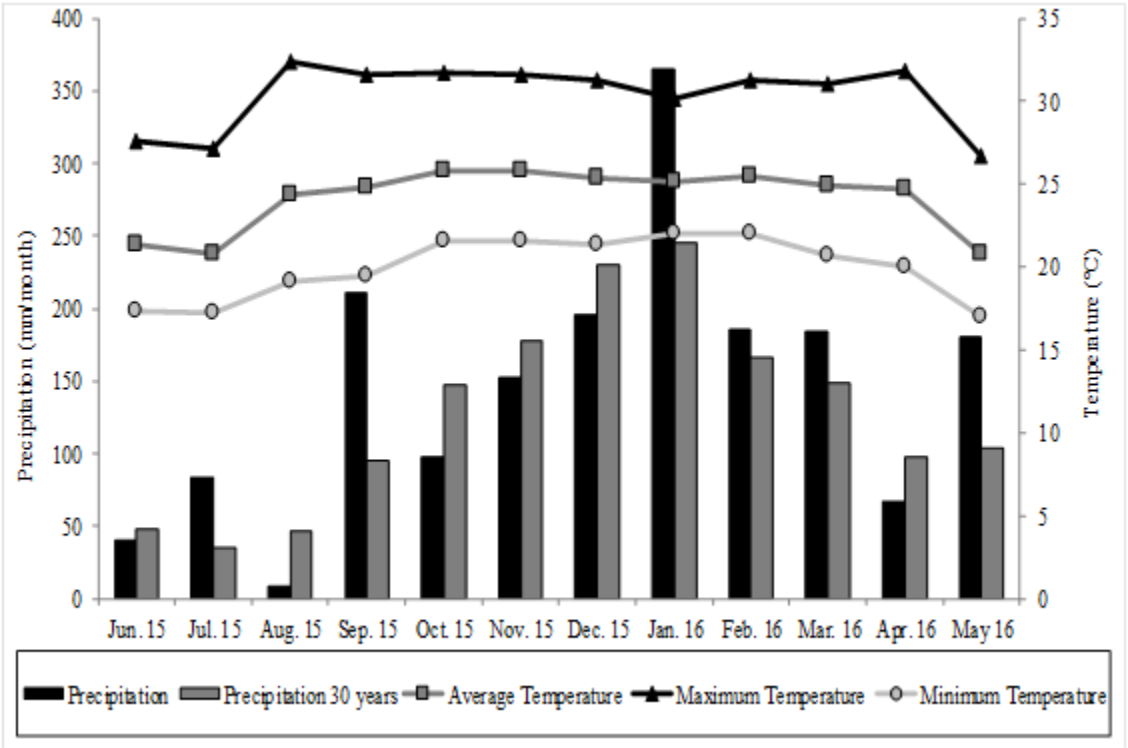

Figure 1. Maximum, average and minimum air ambient temperatures and precipitation from June 2015 to May 2016, and mean rainfall data for the last 30 years for the Campo Grande region, Mato Grosso do Sul. Winter: June–August; spring: September–November; summer: December–February; autumn: March–May.

recordings. All readings were taken in the morning and afternoon on one sunny day each month using a plant canopy analyzer (Accupar Ceptometer Model LP-80, METER Group Inc., Pullman, USA). Means of PAR were used to calculate the percentage of shading at grass canopy level in the SPS14 and SPS22 for each month, as a ratio between PAR reaching the canopy at the sampling points and PAR under full sun conditions at the corresponding measurement time.

The experimental design utilized was a randomized block (3 systems with 4 replications). The pastures were continuously grazed, with stocking rates (SR) intentionally varied over time to achieve predetermined pasture heights, according to Mott and Lucas (1952). For autumn and winter, height ranged from 30 to 35 cm, and for spring and summer from 35 to 40 cm.

Measurements of forage and animal parameters

A total of 80 Nellore (*Bos indicus*) heifers (initial mean body weight, BW = 290.8 ± 26.1 kg, ± SD) were randomly allocated to the paddocks and an extra area for SR management. Heifers had ad libitum access to water and a commercial mineral supplement, which was replenished weekly on the basis of 140 g/animal/d, and unrestricted access to their particular pasture area. Animals were weighed every 28 days in the morning, on a weigh scale (MRG Campo, Toledo, São Bernardo do Campo, Brazil; precision of 0.5 kg), following a 16 h fast from feed but with access to water. All heifers were vaccinated according to the official health calendar and dewormed at the beginning of the experiment. Over-grazing of SPS14 paddocks occurred in winter and animals were removed on 23 June 2015 and were returned to the paddocks on 9 December 2015. A similar situation occurred on SPS22 in spring and stock were removed on 15 September 2015 and were returned on 9 December 2015.

Average daily BW gain (ADG) was calculated by dividing the difference between the initial and final BW of animals by the number of days between weighings. Monthly SR (animal unit, AU = 450 kg) was the product of average BW and the period for which the animals remained on the paddocks. Animal BW gain per hectare (AWG) was calculated by multiplying ADG by the number of animals per hectare per month.

Canopy height measurements were taken from ground level to the top surface of the pasture leaf canopy, using a 1-m rule graduated in cm, every 2 weeks at 50 random points per paddock. Forage sampling was performed every 28 days, along 2 transects sited at right angles between the tree rows; along each transect, samples were taken at 5 points defined as 1 m from the north tree row, 1 m from the south tree row, 6 m from the north tree row, 6 m from the south tree row, and at the central point between the tree rows, 11 m from each row, in SPS22 (Figure 2). For SPS14, sites were 1 m from the north tree row, 1 m from the south tree row, 3 m from the north tree row, 3 m from the south tree row, and at the central point between the tree rows, 7 m from each. In CON, the points were chosen at random. Ten samples per paddock were harvested at 0.4 m from ground level using a coastal harvester, within a metallic frame of 1 × 1 m. Conjointly, forage accumulation was measured using the exclusion cage technique, according to Davies (1993) and Stuth et al. (1981), by placing 5 cages of 1 × 1 m per paddock along the canopy height transects between the tree rows, following the same procedure as used for the sampling points described above. The exclusion cages were moved to a new location every 28 days, and comparable points were always harvested to simulate the growth of the pasture.

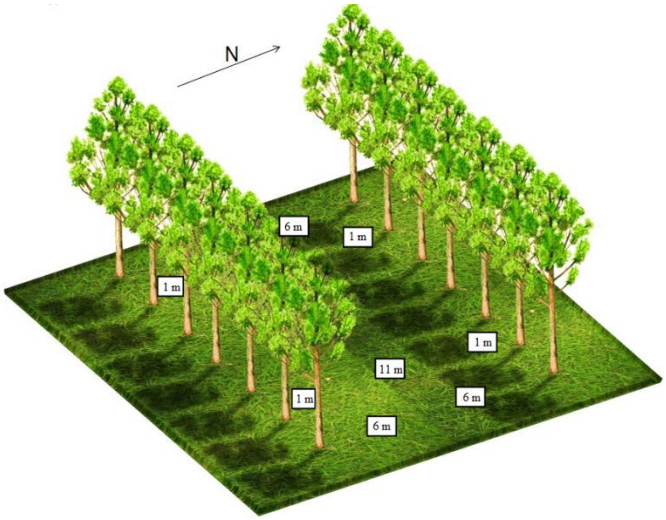

Figure 2. Schematic representation of forage sampling points along 2 transects sited at right angles to the eucalyptus rows in a silvopastoral system (SPS22: grass + 227 trees/ha). The distance separating each point from the trees rows is represented by the boxes. (Adapted from Oliveira et al. 2019).

All forage samples collected were individually weighed after harvesting. For each harvest, subsamples for each sampling point, for instance, along each transect, and from inside and outside the cages, were separately pooled and taken, put into paper bags, and dried in a forced-air oven at 65 ºC until constant mass for determination of dry matter (DM). Another subsample from each sampling point was selected and separated into its morphological components – leaf blade, stem with sheath and senescent material. Likewise, the morpho-

logical components were weighed and subsequently dried in a forced-air oven at 65 ºC for DM determination. Herbage mass (HM) was the average of measurements from all sampling points. The morphological component proportions were calculated as percentage of the total HM. Herbage accumulation rate (HAR) was the difference between the HM within the cages and outside the cages at previous harvest, i.e. when the cage was repositioned, divided by the days between samplings.

Canopy bulk density was calculated by dividing HM by canopy height at the sampling point where HM was measured. To determine herbage allowance (HAL, kg DM/100 kg BW), HM was summed up to the HAR, and divided by the SR transformed in kg BW in the area and season.

Dried leaf and stem samples were ground in a Wiley mill to pass a 1-mm screen. Crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations and in vitro digestibility of organic matter (IVDOM) were assessed through the proximal infrared reflectance spectrophotometry system (NIRS) according to Marten et al. (1985). Forage nutritive value was measured monthly and means determined seasonally. However, it was not possible to assess the chemical composition in autumn.

Statistical analysis

Data were grouped by season as follows: winter, results for June to August 2015 inclusive; spring, for September to November 2015; summer, for December 2015 to February 2016; and autumn, for March to May 2016. Data were analyzed by PROC GLM procedure through SAS V9.4 program (SAS Institute Inc., Cary, NC, USA), with a model including effects of block, system, season and their interactions. Means were compared using LSMEANS by Tukey test, and considered different when P<0.05.

Results

The PAR levels reaching the sward in CON, SPS22 and SPS14 are depicted in Figure 3, which shows that the amount of light reaching the grass canopy was highest in spring and declined as tree density increased. Amount of incident light reaching the grass canopy in SPS14 in winter, summer and autumn was 16–22% of that in full sun, while in spring 44% of light penetrated the trees. In SPS22, 45–50% of light reached the pasture throughout the year. There was an interaction of system × season (P<0.01) for pasture canopy height, HM, leaf and stem proportion and leaf:stem ratio. Canopy height in CON was taller (P<0.001) than in SPS14 in winter, whereas in spring, when both SPSs were destocked, canopy height in CON was shorter (P<0.001) than in both systems with trees, SPS22 and SPS14 (Table 1). In CON, HM was greater (P<0.001) than in both systems with trees for all seasons (Table 1). However, HM did not differ (P>0.05) between the systems with trees, despite the amount of PAR reaching the canopy in SPS14 being little more than half of that in SPS22.

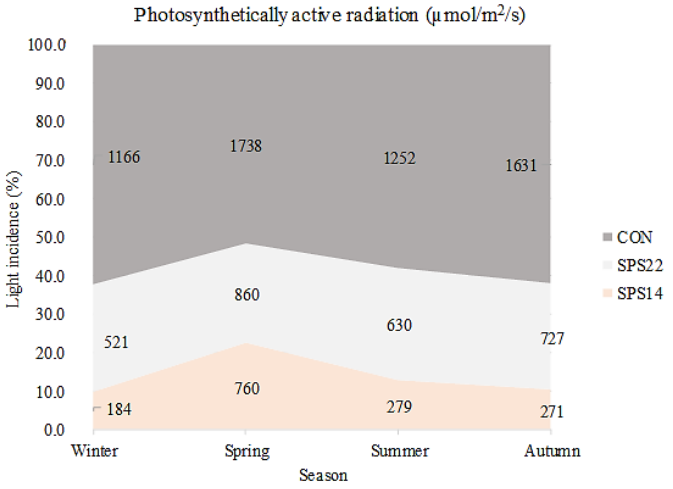

Figure 3. Light incidence reaching the sward in the silvopastoral systems (CON: grass-only, SPS22: grass + 227 trees/ha, SPS14: grass + 357 trees/ha), in the different seasons.

During winter, summer and autumn, leaf proportion in available forage was similar (P>0.05) in all systems (Table 1). In spring, when the SPSs were destocked, leaf proportion in available forage was higher in SPS22 and SPS14 than in CON (P<0.001; Table 1). In all systems, leaf proportion in available forage in spring and summer was greater than in winter and autumn. Similarly, summer had the highest stem proportion in available forage for all systems (P<0.001). Leaf:stem ratio in available forage was highest in spring (P<0.001) for all systems. Differences between systems were inconsistent, with no differences observed between systems in winter and summer (P>0.05), while significant differences occurred in spring and autumn (P<0.001).

Regarding forage nutritive value of leaf and stem, CP, NDF, ADF and IVDOM were affected (P<0.01) by a system × season interaction. Leaf CP varied from 84 to 145 g/kg DM across systems and seasons, with SPS14 and SPS22 having higher leaf CP than CON (P<0.001; Table 2). Leaf CP was generally higher in winter than in summer. Similarly, stem CP ranged from 45 to 104 g/kg DM with higher values for SPS14 and SPS22 than for CON (P<0.001) and higher values in spring than in winter and summer (P<0.001).

Table 1. Canopy height, herbage mass (HM), leaf proportion, stem proportion and leaf:stem ratio of Piatã grass in the systems CON, SPS22 and SPS14 during different seasons.

Season (S)	System (T)			s.e.m.	P-value	
	CON	SPS22	SPS14		T	T × S
			Canopy height (cm)			
Winter	31Ba	25Bab	20Cb[1]	0.03	0.188	<0.001
Spring	36Bb	45Aa[1]	45Aa[1]			
Summer	46Aa	49Aa	45Aa			
Autumn	34Ba	27Ba	29Ba			
			Herbage mass (kg DM/ha)			
Winter	2,466Ca	901Bb	626Bb[1]	306.5	<0.001	0.006
Spring	2,000Ca	1,032Bb[1]	642Bb[1]			
Summer	5,107Aa	2,772Ab	1,969Ab			
Autumn	3,370Ba	1,510Bb	908Bb			
			Leaf proportion (g/100 g DM)			
Winter	17.4ABa	10.8Ba	14.2Ca[1]	4.64	<0.001	<0.001
Spring	28.2Ab	41.1Aa[1]	59.9Aa[1]			
Summer	28.4Aa	30.6Aa	31.8Ba			
Autumn	10.6Ba	13.3Ba	13.0Ca			
			Stem proportion (g/100 g DM)			
Winter	17.4 Ba	7.3Bb	13.6BCa[1]	3.14	<0.001	<0.001
Spring	7.9Cb	10.7Bb[1]	22.9Ba[1]			
Summer	27.9Ac	36.5Ab	45.4Aa			
Autumn	18.8Ba	13.5Ba	14.4Ca			
			Leaf:stem ratio			
Winter	1.1Ba	1.7Ba	1.2Ba[1]	0.34	0.003	0.006
Spring	3.8Aa	4.1Aa[1]	2.9Ab[1]			
Summer	1.1Ba	0.8Ba	0.7Ba			
Autumn	0.6Bb	1.2Ba	1.0Bab			

Means followed by the same upper-case letters within columns and parameters and lower-case letters within rows do not differ (P>0.05) by the Tukey test. CON: grass-only, SPS22: grass + 227 trees/ha, SPS14: grass + 357 trees/ha.
[1]Pastures destocked.

On the contrary, fiber concentrations in both leaf and stem were sometimes higher for CON than for systems with trees. For leaf NDF, no differences between seasons were found for CON or SPS22, whereas leaf NDF was higher in winter than in spring and summer for SPS14. The effects of season on stem NDF were variable across systems. Similarly, leaf ADF followed variable and inconsistent patterns.

Leaf IVDOM ranged from 579 to 757 g/kg DM. In general, the highest leaf IVDOM was observed in SPS14, where it was higher in spring than in winter and summer. In contrast, leaf IVDOM in CON and SPS22 was lower in summer than in winter and spring. Stem IVDOM ranged from 464 to 659 g/kg DM, and the superiority of systems with trees over CON was observed only in spring (P<0.01).

There was significant interaction between systems × seasons for canopy density and HAR (P<0.05). In all seasons, CON had denser canopy than SPS22 and SPS14 (P<0.05; Table 3). In general, canopy density was higher in summer and autumn than in winter and spring.

The HAR in winter and spring did not differ between systems (P>0.05), whereas in summer and autumn, pasture in CON grew faster than in SPS22 and SPS14 (P<0.01; Table 3). Overall, Piatã grass grew faster in spring than in other seasons (P<0.001). No differences between systems were observed for HAL. However, when comparing seasons, HAL in summer was greater than in winter and autumn.

Overall, SR in CON was greater than in SPS22 and SPS14 (Table 4). As previously mentioned, destocking of both systems containing trees occurred during the winter-spring period. The systems showed similar ADG. Nevertheless, as a result of superior SR, CON had higher AWG than SPS22 and SPS14, except for during summer, when gains on SPS22 were similar to those on CON.

Table 2. Nutritive value of Piatã grass leaf and stem in the systems CON, SPS22 and SPS14 during different seasons.

Season (S)	System (T)			s.e.m.	P-value	
	CON	SPS22	SPS14		T	T × S
Leaf						
Crude protein concentration (g/kg DM)						
Winter	110Ab	132Aa	137Aa[1]			
Spring	97ABc	123ABb[1]	145Aa[1]	0.38	<0.001	0.001
Summer	84Bb	118Ba	131Aa			
Neutral detergent fiber concentration (g/kg DM)						
Winter	686Aa	682Aa	687Aa[1]			
Spring	693Aa	689Aa[1]	651Bb[1]	1.01	<0.001	0.004
Summer	697Aa	676Ab	642Bc			
Acid detergent fiber concentration (g/kg DM)						
Winter	306Ba	292Ba	306Aa[1]			
Spring	313ABab	321Aba[1]	290Ab[1]	0.6	0.007	0.001
Summer	339Aa	323Ab	309Ab			
In vitro organic matter digestibility (g/kg DM)						
Winter	642Ab	682Aab	696Ba[1]			
Spring	629Ac	699Ab[1]	757Aa[1]	2.26	<0.001	0.004
Summer	579Bc	625Bb	684Ba			
Stem						
Crude protein concentration (g/kg DM)						
Winter	48Bb	60Ba	67Ba[1]			
Spring	63Ab	96Aa[1]	104Aa[1]	0.26	<0.001	<0.001
Summer	45Bc	55Bb	65Ba			
Neutral detergent fiber concentration (g/kg DM)						
Winter	766Aa	771Aa	767Aa[1]			
Spring	758Aa	729Bb[1]	714Bb[1]	0.7	<0.001	0.004
Summer	771Aa	775Aa	743Ab			
In vitro organic matter digestibility (g/kg DM)						
Winter	478Ba	489Ba	485Ba[1]			
Spring	557Ab	618Aa[1]	659Aa[1]	2.91	0.002	0.001
Summer	490Ba	464Ba	493Ba			

Means followed by the same upper-case letter within columns and parameters and lower-case letters within rows do not differ (P>0.05) by the Tukey test. CON: grass only, SPS22: grass + 227 trees/ha, SPS14: grass + 357 trees/ha. [1]Pastures destocked.

Table 3. Canopy bulk density, herbage accumulation rate (HAR) and herbage allowance (HAL) in the systems CON, SPS22 and SPS14 during different seasons.

Season (S)	System (T)			s.e.m.	P-value	
	CON	SPS22	SPS14		T	T × S
Canopy density (kg DM/m^3)						
Winter	0.52Ba	0.23Cb	0.20ABb[1]	0.02	<0.001	0.032
Spring	0.60Ba	0.28BCb[1]	0.14Bb[1]			
Summer	1.12Aa	0.56Ab	0.42Ab			
Autumn	1.00Aa	0.51ABb	0.35ABb			
HAR (kg DM/ha/d)						
Winter	6.9Ba	-15.8Ba	-11.0Ba	0.92	<0.001	0.01
Spring	51.7Aa	48.4Aa	33.4Aa			
Summer	63.1Aa	16.8Bb	3.9Bb			
Autumn	62.8Aa	13.9Bb	-1.4Bb			
HAL (kg DM/100 kg BW/d)						
Winter	4.49Ca	2.23Ba	D[1]	0.99	<0.001	0.22
Spring	8.64AB	D	D			
Summer	11.22Aa	7.94Aa	8.25Aa			
Autumn	4.85BCa	3.93Ba	1.19Ba			

Means followed by the same upper-case letter within columns and parameters and lower-case letters within rows do not differ (P>0.05) by the Tukey test. CON: grass only, SPS22: grass + 227 trees/ha, SPS14: grass + 357 trees/ha. [1]SPS14 was destocked in winter and spring and SPS22 was destocked in spring.

Table 4. Stocking rate (SR), average daily BW gain (ADG) and animal BW gain per hectare (AWG) in the systems CON, SPS22 and SPS14 during different seasons.

Season (S)	System (T)			s.e.m.	P-value	
	CON	SPS22	SPS14		T	T × S
		SR (AU/ha)		0.3	<0.001	0.09
Winter	1.3Aa	0.7Aa	D[1]			
Spring	1.1A	D	D			
Summer	2.6Aa	2.4Aab	1.5Ab			
Autumn	2.6Aa	1.2Ab	0.9Ab			
		ADG (kg BW/animal/d)				
Winter	0.160Ba	0.058Ba	D	0.13	<0.001	<0.001
Spring	0.525A	D	D			
Summer	0.588Aa	0.783Aa	0.648Aa			
Autumn	0.373ABa	0.238Ba	0.435Aa			
		AWG (kg BW/ha)				
Winter	17Ba	3Ba	D	19.78	<0.001	<0.001
Spring	54Ba	D	D			
Summer	169Aa	186Aa	93Ab			
Autumn	136Aa	39Bb	25Bb			

Means followed by the same upper-case letter within columns and parameters and lower-case letters within rows do not differ (P>0.05) by the Tukey test. AU = 450 kg BW. CON: grass only, SPS22: grass + 227 trees/ha, SPS14: grass + 357 trees/ha. [1]D = destocked. BW = Body weight.

Discussion

This study has shown the huge impact of established eucalyptus trees on pasture growth in a silvopastoral system. During summer and autumn, when pastures in full sunlight (CON) grew at 63 kg DM/ha/d, those in SPSs grew at 15 (SPS22) and 1 (SPS14) kg DM/ha/d. Reductions in growth of this magnitude must raise the issue of the suitability of eucalyptus for planting in these silvopastoral systems. Not only do they produce shade but also have a well-developed root system which competes strongly for moisture (Ferraz et al. 2019; Mattos et al. 2019) and nutrients in the soil.

The systems with trees, SPS22 and SPS14, are likely to be more negatively affected by the winter season than CON. During water stress conditions, as in the winter, plants cannot compensate for the limited PAR by triggering mechanisms to increase radiation use efficiency. Leaf stomata must open to allow carbon dioxide diffusion into the leaf to utilize PAR. However, during water shortage, leaf stomata close or partially close to reduce water loss, which hampers carbon dioxide uptake (Feldhake 2009). This would result in negative HAR for SPS22 and SPS14 in winter, low canopy height, HM, leaf and stem proportions, canopy density and consequently limited animal production. During this season, only a few heifers continued to graze in SPS22 due to the canopy height, even though canopy height might not be an appropriate criterion for determining when to graze SPSs.

Canopy height is a grazing management target applied in grazing systems in the tropics, as it is highly correlated with HM (Martuscello et al. 2009; Pontes et al. 2016). Nantes et al. (2013) and Euclides et al. (2016) recommended a canopy height within the range 15–45 cm for Piatã grass under continuous stocking in full sun. Our findings suggest that canopy height is not an appropriate criterion for grazing management in shaded environments, because of large oscillations in the amounts of available biomass despite having a similar height, as a result of different structure of pasture in shade. Pontes et al. (2016) also reported an over-grazing condition in cool-season pastures under trees, showing low HM under >50% shade, when the systems were managed using a canopy height target defined for full-sun conditions.

It has been reported that shading imposed on forage grasses increases canopy height by stem and leaf elongation and leaf length enlargement, as a mechanism to improve light capture by the plant. The higher stem proportion in SPS14 during spring and summer, where incident light was severely restricted, supports this hypothesis. In contrast, tiller density declines at low radiation, which in turn decreases available HM (Castro et al. 2009; Gastal and Lemaire 2015; Baldissera et al. 2016).

Even though SPS22 and SPS14 had a taller pasture canopy than CON in the spring, their low HM (1,032 and 642 kg DM/ha for SPS22 and SPS14, respectively) was insufficient to support animals grazing during this season. Hodgson (1990) indicated a minimum HM of 2,000 kg

DM/ha to avoid restricting forage intake. Critically, HM in SPS22 surpassed this threshold only in summer, whereas SPS14 barely achieved it in summer, regardless of the absence of animals in winter and spring. Animals were returned to SPS14 in summer based on HAL of 8 kg DM/100 kg BW, as a result of the onset of rains in September coupled with an increase in PAR and the absence of animals in spring.

The HAR in CON in the winter is in good agreement with 6.0 kg DM/ha/d of Piatã grass growing in a full-sun condition, reported by Euclides et al. (2016) in the dry season, which occurs in winter. Subsequently, rainfall in September boosted HAR in spring for all systems, and HAR in CON closely matched the 64.1 kg DM/ha/d reported by Euclides et al. (2016) and the 64.5 kg DM/ha/d by Santos et al. (2016) during the rainy season. Rather than grouping data by winter, spring, summer and autumn, those authors grouped their data according to rainy and dry seasons. Santos et al. (2016) also evaluated HAR of Piatã grass in systems with eucalyptus trees. Despite having low values ranging from 20.0 to 31.1 kg DM/ha/d in the rainy season and 7.3 to 10.0 kg DM/ha/d in the dry season, the HAR was not negative as found in our study. Those authors affirmed that PAR declined by approximately 22 and 40% for their systems with lower and higher tree density, respectively, whereas PAR in our study declined on average by 53 and 75% for SPS22 and SPS14, respectively. Additionally, minimum air temperature from May to July reached 17 °C, the threshold for the growth of the pasture, as the temperature base for *Brachiaria brizantha* is 17.2 °C, the temperature at which HAR is zero (Cruz et al. 2011).

The HAR in SPS22 and SPS14 in summer declined dramatically, whereas in CON it increased by about 25%. Competition for nutrients and incident radiation by trees obviously prevented grass from accumulating forage at a greater rate, highlighting the effects of radiation on the growth of tropical grasses of C_4 metabolism (Taiz and Zeiger 2010).

The PAR in summer for all systems was considerably lower than in spring, with levels in SPS14 being quite low. Since PAR declined dramatically in the CON system as well as SPSs, cloud cover may have contributed to reduced levels of incident light overall, and change in sun position relative to the configuration of the trees possibly had a significant impact on the amount of light reaching the grass canopy in the SPSs.

Regardless of the lack of statistical differences for HM and HAR between SPS22 and SPS14 throughout the study, SPS22 constantly showed higher absolute values than SPS14, which ensured animals were retained in SPS22 during winter.

A morphological change influenced by shading is leaf elongation, which in turn increases leaf proportion (Baldissera et al. 2016). However, the general preference for leaves by animals could limit leaf accumulation in the forage canopy of the systems with trees. Due to low HM in both SPS22 and SPS14, removal of leaves by the animals would be expected to be more pronounced in those systems than in CON, despite the fact that HALs on all systems showed no significant difference. The only period when SPS22 and SPS14 had higher leaf proportion than CON was in spring, when the systems with trees were destocked. Animals preferentially select leaf when grazing forage, resulting in increased animal performance due to its highest nutrient concentrations (Geremia et al. 2018).

As expected, forage nutritive value in SPSs was superior to that in CON, mainly with regard to higher CP in shaded environments (Paciullo et al. 2016; Lima et al. 2018). Even though reports in the literature for effects of shading on NDF, ADF and IVDOM are inconsistent, several authors have recorded reductions in NDF (Paciullo et al. 2008, 2016; Lima et al. 2018) and ADF (Lima et al. 2018) in shade, which was attributed to higher numbers of sclerenchyma cells and thicker secondary walls under greater light incidence (Kephart and Buxton 1993; Deinum et al. 1996). A delay in morphological maturation within shaded environments compared with full sun conditions has also been claimed (Neel et al. 2016).

Despite the lower HM and higher stem proportion in SPS22 and SPS14, ADGs of animals in all systems were similar during summer and autumn, when HAL in all systems was similar. Systems with trees also had a higher forage nutritive value in summer than the grass-only system. However, as a consequence of lower SR in SPS14, its AWG was lower than that in CON and SPS22. Likewise, SPS22 provided lower AWG than CON in autumn due to a drop in SR.

Studies carried out in the area in previous years allowed grazing in all the systems during the whole experimental period (Oliveira et al. 2014; Gamarra et al. 2017). Moreover, in the third year after pasture establishment for the first rotation cycle (2011–2012), CON produced 537 kg BW/ha/yr, SPS22 459 kg BW/ha/yr and SPS14 334 kg BW/ha/yr (Oliveira et al. 2014). In the corresponding period of the second cycle in our study (2015–2016), BW production was appreciably lower, i.e. 376 kg BW/ha/yr, 228 kg BW/ha/yr and 118 kg BW/ha/yr in CON, SPS22 and SPS14, respectively. While a range of factors could have contributed to this reduction in animal production, continuous tree growth could have had an important impact, especially in the

SPSs. Eucalyptus trees may have a root system which spreads out a horizontal distance about 20 m from the trunk of individual trees (Zohar 1985). Tree root zone as a sink removes water arriving at the soil surface within the radius of its root zone, influencing the soil water movement and availability (Stirzaker et al. 1999; Bosi et al. 2019). Since these trees were 8 years old and were 25–27 m tall at the commencement of the study, and plot size was 1.4 ha, competition for water and nutrients in the grass-only pasture from adjacent trees cannot be dismissed. Eucalypts are very competitive with underlying pasture, and it is important that the changes in the degree of competition with underlying crops and pastures be monitored throughout the whole tree growing cycle, as proposed by Gomes et al. (2019).

For CON, the decline in animal production in the second cycle could reasonably be attributed to stocking management. Oliveira et al. (2014) observed higher AWG and SR in short swards managed at 27 ± 4.6 cm, whereas in the current study, the sward height of CON ranged from 31 to 46 cm across the seasons. Our results for winter and spring differed only slightly from those reported by Oliveira et al. (2014), the seasons where the authors found no differences for AWG according to the height managed. It seems that CON could be grazed at different heights from SPSs. We conclude that pasture height should not be the sole criterion on which appropriate stocking rate for grazing pastures under trees is assessed. Further studies are warranted to determine a more appropriate criterion for deciding when and how to graze these pastures.

Conclusions

Silvopastoral systems with eucalyptus trees like those studied are unable to support both forage and animal production equivalent to a straight grass pasture by the 8th year after establishment. Further studies are needed to determine appropriate management of the trees to reduce competition for the pasture. One might question the suitability of eucalypts for these silvopastoral systems because of their high levels of competition for water, light and nutrients. Pruning and thinning of the trees have been implemented in an endeavor to reduce competition and these practices have been recommended in the literature (Santos et al. 2016; Lima et al. 2018; Pezzopane et al. 2019, 2020) to reduce radiation interception starting from the 6th to 8th year after establishment. However, eucalyptus trees retain a competitive advantage over pasture even after thinning and tree stands recover rapidly and increase their competition with pasture (Back et al. 2009). Studies to evaluate those interactions between pasture and trees seem warranted.

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Statement of Animal Rights

All procedures were approved by the Ethics and Animal Use Commission of Embrapa Beef Cattle under protocol nº 014/2014.

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Research Paper

Height and mowing of pasture at the end of winter modulate the tillering of Marandu palisadegrass in spring

La altura y el corte de Urochloa brizantha *cv. Marandu al final del invierno afectan su capacidad de rebrote en primavera*

BRUNO HUMBERTO REZENDE CARVALHO[1], LILIAN ELGALISE TECHIO PEREIRA[2], ANDRÉ FISCHER SBRISSIA[3], GABRIEL DE OLIVEIRA ROCHA[1] AND MANOEL EDUARDO ROZALINO SANTOS[1]

[1]*Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil.* famev.ufu.br
[2]*Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brazil.* usp.br/fzea
[3]*Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina, Lages, SC, Brazil.* cav.udesc.br

Abstract

In pastures subjected to stockpiling, the tiller population goes through an intense process of self-thinning, hindering the recruitment of new tillers in the subsequent season. We evaluated different pasture management strategies in late winter in an attempt to modify tiller recruitment during spring. *Urochloa brizantha* cv. Marandu was maintained at 4 different levels (heights) of stockpiled pasture at the end of winter: short (15.1 cm), medium (23.2 cm), tall (31.4 cm) and tall/mown (31.3 cm, mown to 8 cm). In October (early spring), the short and tall/mown pastures had a tiller appearance rate (TAR) and a population stability index (PSI) superior ($P<0.05$) to that of the tall pasture. During the remainder of the growing season, these characteristics (TAR and PSI) were similar for all pastures. Tiller survival rate (TSR) was also highest ($P<0.05$) in short pasture in early spring. TAR values were highest in early spring and these tillers persisted throughout the growing season. When stockpiling Marandu palisadegrass pasture during spring it is important to have it short at the end of winter to ensure early and intense tillering in spring. If pasture is tall at the end of winter mowing at this time before spelling is advantageous.

Keywords: Population stability index, tiller appearance, tiller survival, *Urochloa brizantha* syn. *Brachiaria brizantha*.

Resumen

En sistemas de usos diferidos de un pasto, la población de brotes de plantas sufre un intenso proceso de auto-raleo lo que dificulta la regeneración de nuevos brotes en la temporada siguiente. En un estudio conducido en la Universidade Federal de Uberlândia, Minas Gerais, Brasil, se evaluaron diferentes estrategias de manejo del pasto *Urochloa brizantha* cv. Marandu a finales del invierno con el objeto de favorecer la regeneración de brotes durante la primavera. Para el efecto, a finales del invierno, utilizando pastoreo simulado se generaron parcelas de pasto diferido con cuatro niveles de altura diferentes: los tratamientos Corto (15.1 cm), Medio (23.2 cm), Alto (31.4 cm) y Alto/Cortado (31.3 cm, cortado a 8 cm). En octubre (comienzo de la primavera), los pastos en los tratamientos Corto y Alto/Cortado presentaron una tasa de aparición de brotes y un índice de estabilidad poblacional superior ($P<0.05$) al del pasto en el tratamiento Alto. Durante el resto de la temporada de crecimiento, estas dos características fueron similares para todos los tratamientos. La tasa de supervivencia de brotes también fue más alta ($P<0.05$) en el pasto del tratamiento Corto a principios de la primavera. La tasa de aparición de brotes fue más alta a principios de la primavera y estos brotes persistieron durante toda la temporada de crecimiento. Al diferir el pasto cv. Marandu durante la primavera, es importante manejarlo a corta altura al final del invierno para asegurar un rebrote temprano y abundante en primavera. Por tanto, si el pasto es alto al final del invierno, se sugiere realizar un corte antes de diferirlo.

Palabras clave: Aparición de brotes, índice de estabilidad poblacional, supervivencia de brotes.

Correspondence: M.E.R Santos, Veterinary Medicine School of Federal University of Uberlândia (FAMEV/UFU), Campus Glória, BR-050, km 78, 38410-337, Uberlândia, MG, Brazil.
Email: manoel.rozalino@ufu.br

Introduction

Stockpiling of pasture has been widely adopted in many countries such as Canada, USA and Brazil (Santos et al. 2009; Añez-Osuna et al. 2015; Nave et al. 2016; Silva et al. 2016) as a pasture management strategy in an endeavor to ensure forage is available for fall or winter grazing and to reduce winter feeding costs (Añez-Osuna et al. 2015). When carried out effectively, stockpiling can improve the environmental impact of winter feeding systems (Bakelaar et al. 2017), as well as animal health and welfare (Poore and Drewnoski 2010).

It is important that stockpiled pasture grows rapidly in spring and summer (Santana et al. 2014; Santos et al. 2020), and the response depends on its ability to emit new tillers (Colvill and Marshall 1984), as tiller numbers in pasture are a reflection of the balance between birth and survival rates of tillers (Costa et al. 2016; Pessoa et al. 2016; Duchini et al. 2018).

A lower forage mass in stockpiled pasture at the end of winter may allow increased light incidence at the base of plants in early spring, boosting tillering when environmental conditions become more favorable for plant growth. Santana et al. (2014) evaluated the tillering of *Urochloa decumbens* cv. Basilisk during spring, as affected by the management of previously stockpiled pastures. Stockpiled pastures with shorter heights (10 and 20 cm) at the beginning of the stockpiling period produced more new tillers in spring than those stockpiled at a greater height (30 and 40 cm). The authors attributed this response pattern to lower forage mass at the end of winter in the 10 and 20 cm stockpiled pastures compared with those stockpiled at 30 and 40 cm. Santos et al. (2011) had already found that continuously stocked *Urochloa decumbens* cv. Basilisk maintained at 15 cm average height in winter and 25 cm in spring produced more new tillers than pasture managed at constant height (25 cm) throughout the year. According to these authors, pastures kept short in winter senesce less, reducing the amount of dead forage and the degree of shading at the base of the plants, resulting in a microclimate more favorable to tillering in the spring.

On the other hand, when stockpiled pasture has a high forage mass at the end of winter, tillering in the spring can be reduced or delayed (Santos et al. 2009; Silva et al. 2016). Stockpiled pasture still tall at the end of winter can also contain many dead and old tillers, which remain in the canopy, interspersed with new tillers that appear over the subsequent spring and summer (Souza et al. 2015). In this situation, mowing of stockpiled pasture with a high forage mass in late winter can improve canopy structure in spring (Souza et al. 2015), with positive effects on animal performance, although costs of mowing are an additional expense. In fact, Sousa et al. (2018) found that tall pasture (31.3 cm) of *U. brizantha* cv. Marandu mowed to 8 cm at the end of winter showed a higher percentage of live leaf and a lower percentage of dead stem in the subsequent spring than unmown pasture. Performance of sheep continuously grazing the pastures in spring was 33% higher in the tall/mown pasture than in the unmown pasture.

A possible negative consequence of mowing in tall pasture is the large amount of cut plant material over the plants, which could inhibit tillering, owing to: (i) the likely high C:N ratio of the mowed material, which would decrease the N availability for pasture via immobilization by soil microorganisms (Carneiro et al. 2008); and (ii) greater shading at the base of the pasture, inhibiting tillering. It is worth noting that the effects of mowing at the end of winter on tillering and the stability of the tiller population in spring and summer are still unknown in tropical pastures.

We studied Marandu palisadegrass (*Urochloa brizantha* cv. Marandu) to test the following hypotheses: (i) stockpiled but short pasture at the end of winter produces earlier and more intense tillering in spring than tall pasture; and (ii) mowing of pasture with high forage mass at the end of winter inhibits tillering since the mowed forage shades the growing points at the base of the plant and compromises tiller population stability in spring and summer. Thus, our study aimed to identify management conditions at the end of winter for previously stockpiled Marandu palisadegrass that allow a faster renewal of the tiller population in spring and early summer.

Materials and Methods

Locality and environmental conditions

The experiment was conducted from January 2013 to February 2014, at Capim Branco Experimental Farm, belonging to the Federal University of Uberlândia, in Uberlândia, MG (18º53' S, 48º20' W; 835 masl). According to Köppen´s classification, the climate of the Uberlândia region is Aw-type, i.e. tropical savanna, with mild, dry winters and well-defined dry and rainy seasons (Alvares et al. 2013). Climatic conditions during the experimental period were monitored at a meteorological station 200 m away from the experimental area (Figure 1A). Temperature and monthly precipitation were used to calculate the soil water balance (Thornthwaite and Mather 1955), considering soil stored 50 mm water at field capacity (Figure 1B).

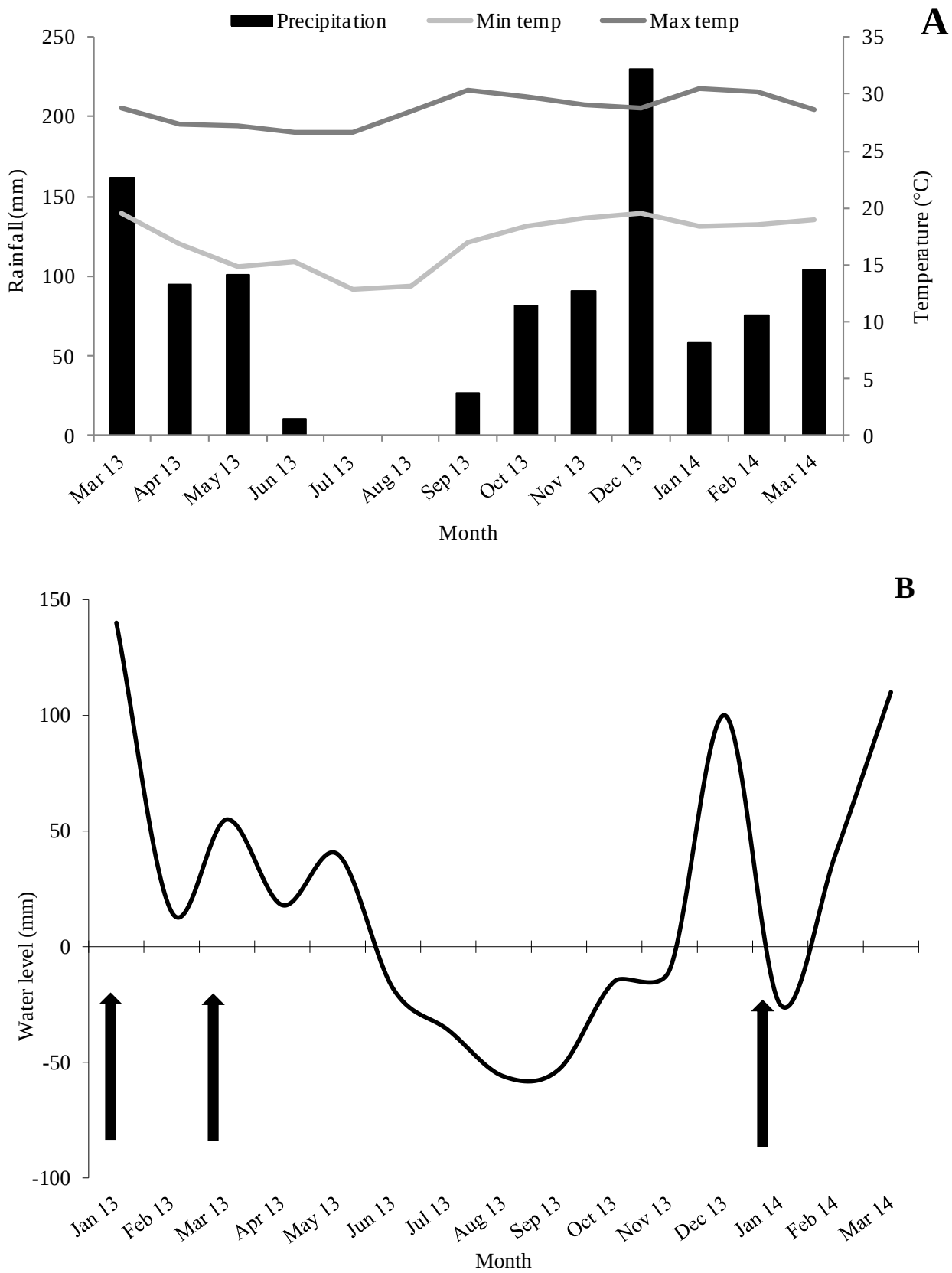

Figure 1. (**A**) Monthly weather data during the experimental period; and (**B**) monthly soil water balance during January 2013 – March 2014. The arrows indicate the months in which fertilizer was applied. Summer: January–March; fall: April–June; winter: July–September; spring: October–December.

The experimental area consisted of a pasture of *Urochloa brizantha* cv. Marandu (Marandu palisadegrass), subdivided into 12 paddocks (experimental units) of 800 m^2 each, in addition to a reserve area, totaling 2 hectares. In January 2013, soil samples were taken from the 0–20 cm layer, and chemical analysis showed the following results: pH (H_2O): 6.1; P: 4.5 mg/dm^3 (Mehlich I); K^+: 139 mg/dm^3; Ca^{2+}: 5.5 $cmol_c/dm^3$; Mg^{2+}: 1.9 $cmol_c/dm^3$; Al^{3+}: 0.0 $cmol_c/dm^3$ (KCl 1 mol/L); effective CEC: 7.3 $cmol_c/dm^3$; pH 7.0; CEC: 10.2 $cmol_c/dm^3$; and base saturation: 72.0%.

History of area use, treatments and experimental design

From January to April 2013, all pastures were continuously stocked with sheep and stocking rates were varied to maintain pastures at 4 average sward heights (15, 25, 35 and 45 cm). Each height was implemented in 3 paddocks. Sward heights were measured weekly from the soil surface to the highest live leaves in the canopy using a graduated rule at 30 random points per paddock and were controlled by adding to or removing from the paddocks sheep with an average body weight of 26 kg.

All pastures were stockpiled, i.e. ungrazed, from 3 April 2013 to 21 June 2013 (79 days). All pastures were then continuously stocked with sheep from 22 June 2013 to 25 September 2013. Initial stocking rate was 4 sheep per paddock, i.e. 2.8 AU (animal units)/ha (1 AU = 450 kg of animal body weight).

At the end of the pasture utilization period on 25 September 2013, sampling revealed that pastures stockpiled at 15, 25 and 35 cm had the following attributes: short (15.1 cm and 4,600 kg DM/ha), medium (23.2 cm and 5,940 kg DM/ha) and tall (31.4 cm and 7,640 kg DM/ha), respectively, compared with the 45 cm stockpiled pasture (31.3 cm and 7,200 kg DM/ha). While we aimed to have this final group of pastures at 45 cm, this was not achieved and the pastures were really similar to the 35 cm stockpiled pasture so we decided to mow the pastures to 8 cm on 27 September 2013 to provide a fourth treatment for comparison and this became known as tall/mown.

Management

From 27 September 2013, the short, medium, tall and tall/mown pastures remained unstocked for 46, 42, 14 and 44 days, respectively, until they reached a target height of 30 cm, when grazing recommenced and continued until 4 February 2014. During this grazing period, all pastures were continuously stocked at variable stocking rates with the aim of maintaining an average height of 25 cm (Silva et al. 2013), using a similar strategy to that imposed before the stockpiling period. Animals used were crossbred Santa Inês × Dorper sheep (mean body weight 30 kg), which had unrestricted access to water and mineral salt.

Based on the results of the soil analysis, application of lime and potassium fertilizer was not necessary. During the rainy season of 2013, 55 kg P and 50 kg N/ha were applied in January, followed by 70 kg N/ha on 15 March 2013 and a further 70 kg N/ha on 12 January 2014.

Measurement of tiller population dynamics

Basal tillering dynamics was evaluated in 3 areas of 0.07 m^2 per paddock, representative of the average pasture condition. The areas were marked with a PVC ring (30 cm in diameter), fixed to the ground with metal clamps. All basal tillers within the ring were counted and marked on 27 September 2013 and subsequently new basal tillers were counted and marked every 30 days with plastic-coated wire of different colors, to identify each tiller generation until 10 February 2014. Tiller appearance rate (TAR, tillers/100 tillers) represented the number of tillers that appeared between 2 evaluations in relation to the total tiller population at the previous evaluation. Tiller survival rate (TSR, tillers/100 tillers) represented the number of tillers that survived between 2 evaluations in relation to the total tiller population at the previous evaluation. The population stability index was calculated by the equation proposed by Bahmani et al. (2003): FP/IP = TSR (1 + TAR), in which FP/IP represents the current or final tiller population (FP) expressed as a percentage of the original or initial tiller population (IP) in a given period of evaluation. Graphs were also generated showing the monthly variation in number of tillers per generation in the pastures.

Measurement of tiller number

In September, November and December 2013, as well as January 2014, total number of tillers in the pastures was recorded, but outside the rings where tillering dynamics was assessed. In this case, 3 counts were performed per paddock at points that represented the average pasture condition. All live basal tillers contained within a 50 × 25 cm rectangle were counted.

Statistical analysis

The analysis of variance of the data was performed in a completely randomized design using the MIXED procedure (mixed models) of the SAS® (Statistical

Analysis System) statistical package, version 9.2. The covariance matrices were chosen using the Akaike Information Criterion (Wolfinger 1993). The effects of pasture condition at the end of winter, months of grazing and their interaction were considered fixed. Different months of the grazing period were considered repeated measures over time. The means of the factors were compared by the Student Newman Keul's test (P<0.05).

Results

Relative tiller appearance and survival rates

All response variables were influenced (P<0.05) by an interaction between pasture condition at the end of winter and month of the year (Table 1). In October, tiller appearance rate (TAR) was highest in the tall/mown pasture and lowest in the medium and tall pastures (P<0.05). For the remaining months, TAR was similar for all pastures (Table 1). TAR declined from October to November-December and then rose in January.

In October, the short pasture had the highest tiller survival rate (TSR), while the tall/mown pasture had the lowest (P<0.05; Table 1). In November and January, TSR was similar in all pastures, while tall and tall/mown pastures had higher TSR than medium pasture in December. With the exception of tall/mown pasture, pastures showed a reduction in TSR in December (P<0.05) followed by an increase in January (Table 1).

Cohort survival diagrams

Based on number of tillers in the Marandu palisadegrass pastures at successive counts (Figure 2), there was a sharp increase in the number of tillers in the months of October and January. Consequently, tillers generated in October dominated the total tiller population. This phenomenon was most pronounced in short and tall/mown pastures. In addition, total tiller population was higher in short and tall/mown pastures than in medium and tall pastures.

Tiller population stability index

The tiller population stability index (PSI) in October was highest in the short and tall/mown pastures and lowest in the tall pasture. In the remaining months, pasture conditions did not influence PSI. For all pastures, the PSI was highest in October, decreasing in November and December and increasing again in January (P<0.05) (Table 2).

Tiller population density

Regardless of the condition of the pasture and the way in which tiller population density was assessed (i.e. inside or outside the tiller dynamics assessment ring), the number of tillers (NT) was lowest in September and highest in January (Table 3). From October to January, NT was generally highest in tall and tall/mown pasture and lowest in tall pasture (Table 3).

Table 1. Rates of appearance and survival of basal tillers in spring and early summer, according to the condition of Marandu palisadegrass pasture at the end of winter and after its use under stockpiling.

Month	Pasture condition in late winter[1]				s.e.m.
	Short	Medium	Tall	Tall/Mown	
	Tiller appearance rate (TAR, tillers/100 tillers)				
October	132.9Ab	90.4Abc	55.5Ac	178.3Aa	26.6
November	14.2Ca	12.8Ca	14.1Ba	11.9Ca	0.6
December	11.7Ca	16.2Ca	14.7Ba	16.0Ca	1.0
January	32.1Ba	46.7Ba	39.8Aa	35.8Ba	3.1
	Tiller survival rate (TSR, tillers/100 tillers)				
October	97.5Aa	89.7Ab	91.4Ab	79.9Bc	0.21
November	89.3Ba	85.0Aa	86.6Aa	86.6Ba	0.03
December	77.7Cab	76.2Bb	80.2Ba	84.8Ba	0.02
January	89.2Ba	91.1Aa	88.6 Aa	95.6 Aa	0.03

[1]Short pasture (15.1 cm and 4,600 kg DM/ha); Medium pasture (23.2 cm and 5,940 kg DM/ha); Tall pasture (31.4 cm and 7,640 kg DM/ha); and Tall/Mown pasture (31.3 cm and 7,200 kg DM/ha, but cut to 8 cm) at end of winter.
For each characteristic, means within columns followed by the same upper-case letter, and within rows followed by the same lower-case letter are not significantly different by the Student Newman Keul's test (P>0.05).

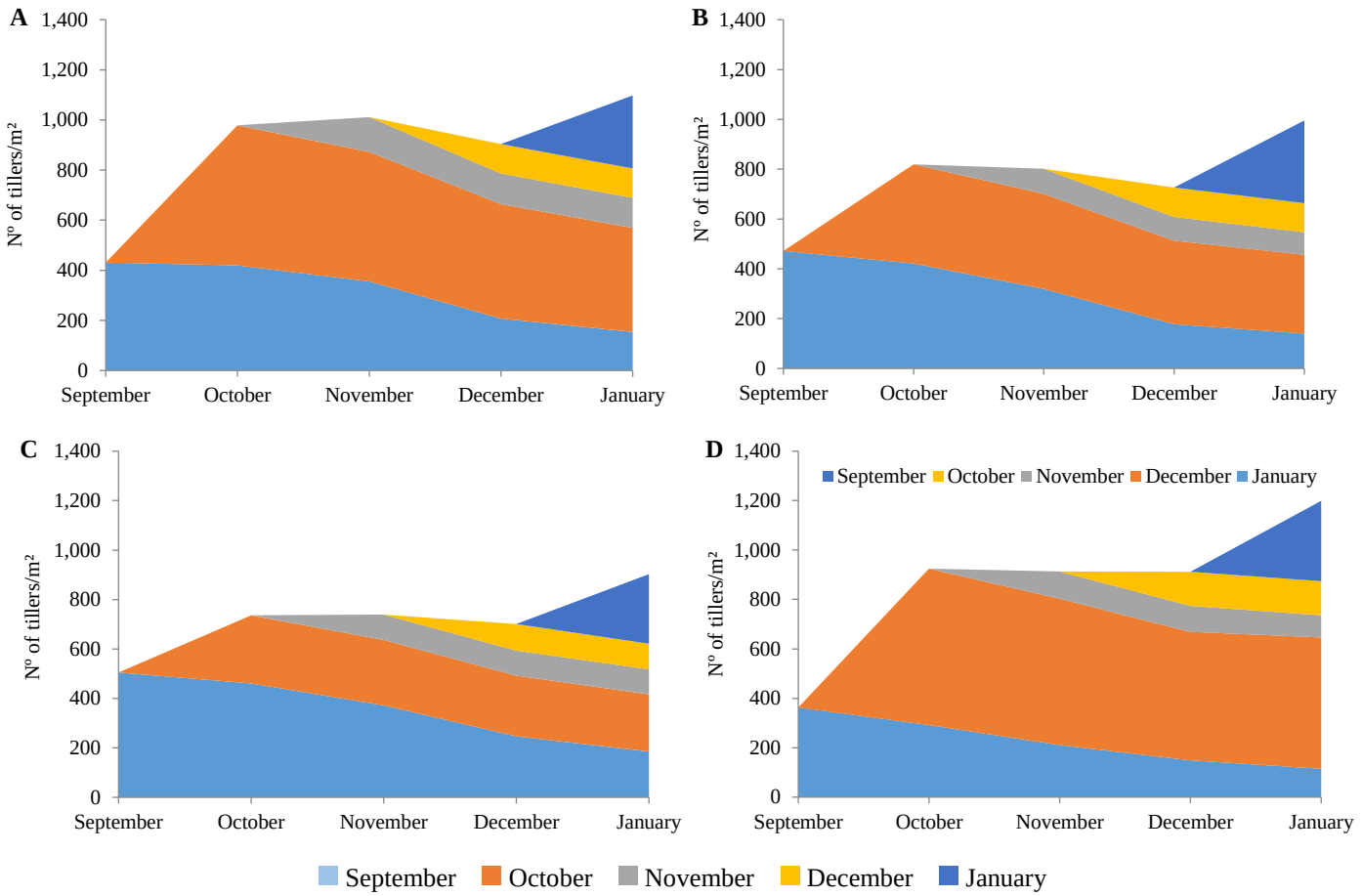

Figure 2. Changes in tiller numbers of Marandu palisadegrass pastures for the cohorts generated each month during the experimental period. **A**: Short pasture (15.1 cm and 4,600 kg DM/ha); **B**: Medium pasture (23.2 cm and 5,940 kg DM/ha); **C**: Tall pasture (31.4 cm and 7,640 kg DM/ha); **D**: Tall/Mown pasture (31.3 cm and 7,200 kg DM/ha, but cut to 8 cm) at end of winter.

Table 2. Basal tiller population index in spring and early summer, according to the condition of Marandu palisadegrass pasture at the end of winter.

Month	Pasture condition in late winter[1]				s.e.m.
	Short	Medium	Tall	Tall/Mown	
October	2.3Aa	1.7Ab	1.4Ac	2.2Aa	0.21
November	1.0BCa	0.9Ca	1.0Ba	1.0Ca	0.03
December	0.9Ca	0.9Ca	0.9Ba	1.0Ca	0.02
January	1.2Ba	1.3Ba	1.2Aa	1.3Ba	0.03

[1]Short pasture (15.1 cm and 4,600 kg DM/ha); Medium pasture (23.2 cm and 5,940 kg DM/ha); Tall pasture (31.4 cm and 7,640 kg DM/ha); and Tall/Mown pasture (31.3 cm and 7,200 kg DM/ha, but cut to 8 cm) at end of winter.
For each characteristic, means within columns followed by the same upper-case letter, and within rows followed by the same lower-case letter are not significantly different by the Student Newman Keul's test (P>0.05).

Table 3. Basal tiller numbers in spring and summer according to the condition of Marandu palisadegrass pasture at the end of winter and after its use under stockpiling.

Month	Pasture condition in late winter[1]				s.e.m.
	Short	Medium	Tall	Tall/Mown	
			Inside the tiller dynamics ring		
September	431Ca	472Ca	505Ca	363Cb	30.6
October	978Ba	819Bb	736Bc	925Ba	54.1
November	1011Ba	802Bb	686Bc	914Ba	70.2
December	904Ba	727BCb	642Bc	912Ba	66.8
January	1,098Aa	996Aa	945Ab	1,199Aa	56.3
			Outside the tiller dynamics ring		
September	511 Ca	469Ca	451Ca	441Ca	15.5
November	724 Ba	475Cb	631Bab	727Ba	59.1
December	712 Ba	751Ba	647Ba	742Ba	23.5
January	956 Aa	884Aa	956Aa	912Aa	17.7

[1]Short pasture (15.1 cm and 4,600 kg DM/ha); Medium pasture (23.2 cm and 5,940 kg DM/ha); Tall pasture (31.4 cm and 7,640 kg DM/ha); and Tall/Mown pasture (31.3 cm and 7,200 kg DM/ha, but cut to 8 cm) at end of winter.
For each characteristic, means within columns followed by the same upper-case letter, and within rows followed by the same lower-case letter are not significantly different by the Student Newman Keul's test (P>0.05).

Discussion

The stability of the tiller population can be assessed by the population stability index (PSI), used for the first time by Bahmani et al. (2003) in perennial ryegrass populations. This index is calculated based on an integrated analysis between survival rates (TSR) and appearance (TAR) of tillers and when its value is equal to 1, the tiller population is in balance and remains stable. Values less than 1 mean that pastures show instability in the tiller population (which may be only transitory) and suggest, in general, that the appearance of new tillers is insufficient to compensate for the deaths of tillers from previous generations. On the other hand, values higher than 1 indicate an increase in the tiller population (Bahmani et al. 2003; Caminha et al. 2010). Based on the values of PSI obtained in this study, which were very close to or greater than one unit (Table 2), it is clear that stability of the tiller population was not compromised in any of the pastures.

In general, and in all pastures, the tiller population remained long-lived in the months following the high tiller appearance in October (Table 1). This fact guaranteed the stability of the plant population throughout spring and until the end of summer (Table 2). As previously observed by several authors and under different grazing methods, such as Caminha et al. (2010) in continuously stocked Marandu palisadegrass, and Pereira et al. (2018) in intermittently stocked Napier elephantgrass (*Cenchrus purpureus*, syn. *Pennisetum purpureum*), the basal tillers of the first generations produced during the beginning of the rainy season are the main contributors to tiller population density and herbage accumulation up to early summer. Similarly, in this experiment, tillers which appeared in October made the greatest contribution to the tiller population up to January (Figure 2).

The higher TAR (Table 1) and PSI (Table 2) in October in short pasture, compared with tall pasture, were expected, since the greater amount of incident light reaching basal gems in low canopies stimulates tillering (Matthew et al. 2000; Sbrissia et al. 2010). Santana et al. (2014) also found an increase in TAR of *U. decumbens* in early spring, following a decrease in sward height at the beginning of the previous stockpiling period. In addition, higher TAR in October than in the other months (Table 1) was promoted by the improvement in climatic conditions (Figure 1), resulting in elevated values of PSI in this month (Table 2). In contrast with the short and tall/mown pastures, the tall pasture produced significantly fewer new tillers in October, probably because of reduced light incidence at the base (Figure 2). The lower PSI value in October (Table 2) in the tall pasture is consistent with its lower tiller population density within the tiller dynamics assessment ring (Table 3).

In October, the highest PSI value observed in short and tall/mown pastures (Table 2) was due to the more intense production of new tillers in these forage canopies, when compared with the medium and tall pastures (Figure 2). This may have compensated for the lower TSR of the tall/mown pasture, causing this pasture to present a high PSI in October (Table 1). The lower TSR of tall/mown pasture in October (Table 1) may have been caused by the elimination of the apical meristem (Matthew et al. 2000) of many tillers during the mowing carried out at the end of September. However, this lower TSR did not compromise tiller population stability of the tall/mown pasture, which

contradicted our hypothesis. It is possible that removal of the apical meristem from tillers during mowing may have reduced apical dominance and stimulated tillering (Santos et al. 2010). In addition, the stock of reserve compounds may have been sufficient to allow high tillering of tall/mown pasture in early spring. It is likely that stockpiled pastures have accumulated a high amount of reserve compounds during stockpiling in autumn, which may have been little used in winter, due to the low growth of the pasture (Silva et al. 2014). These reserve compounds accumulated in autumn and winter may, therefore, be important for the spring tillering of previously stockpiled pastures. However, this hypothesis has yet to be proven.

Considering that the short pasture at the end of the winter was made up of younger tillers, they may have stayed alive longer, which would justify their high TSR in October (Table 1).

The highest TAR in October, compared with the other months (Table 1), would have been a response to the improved climatic conditions (Figure 1), causing PSI values to peak during this month (Table 2). In November and December, the increasing number of tillers in the pastures (Table 3) probably increased the volumetric forage density, which may have intensified the level of shading within the canopies and, thus, decreased the TAR in those months (Table 1). This resulted in a small increase in tiller population density in November and December (Table 3; Figure 2), which promoted a decrease in PSI values in these months (Table 2). In January, nitrogen fertilizer application to the experimental area may have been the main determinant of increases in TAR values (Table 1), since this element is recognized for promoting the activation of dormant gems (Matthew et al. 2000), provided that the leaf area index of the pasture is low. In addition, the soil water deficit that occurred in January (Figure 1) may have been the cause of an unexpected increase in TSR this month, compared with the previous 2 months (Table 1). In this sense, Santos et al. (2011) also observed that, in winter (time of soil water deficit), tillers of *U. decumbens* cv. Basilisk continuously stocked with cattle survived longer than in spring and summer.

The greater tiller survival in times of water deficit may be an ecological strategy by Marandu palisadegrass for nutrient conservation (Santos et al. 2011). This strategy is interesting, since the absorption of nutrients by plants, via mass flow and/or diffusion, is hampered in conditions of water deficit in the soil (Novaes and Smyth 1999). Furthermore, in the months when there is a reduction in the rate of appearance, the tillers survive for a longer time, in order to stabilize the tiller population and, thus, guarantee their persistence in the area under different environmental conditions (Santos et al. 2011). In the present study, a high TSR, combined with the increase in TAR, contributed to the increase in PSI in January (Table 2).

Sbrissia et al. (2010) and Santos et al. (2011) suggest that monthly manipulation of tillers during counting may have an impact on tiller regeneration. In the tall/mown pasture, the possible negative effect on tiller generation of the plant residue deposited on the base of the mown plants could have been minimized or even annulled by the monthly manipulation of tillers within the evaluation rings, and may even have stimulated tillering. However, the numbers of live tillers recorded at points outside the evaluation rings confirmed the data obtained within the rings for tall/mown pasture (Table 3).

The results of our work indicate that the high TAR observed in October was extremely important to maintain the stability of Marandu palisadegrass pastures. Despite this, even in October, TSR values were high (>0.75) and remained high over the following months, while the magnitude of TAR decreased (Table 1).

At the end of the experiment (January), on average, 14.2% of the total tillers were made up of the base generation (first marked generation) of tillers, which probably appeared during the previous autumn, since in winter climatic conditions were restrictive to plant growth. In contrast, 85.8% of all existing tillers had emerged from the end of October. Thus, most tillers present in pastures at the end of summer (late January) can be considered relatively young, less than 90 days old (Paiva et al. 2011). These facts indicate that the Marandu palisadegrass presented a population of young tillers that survived from the end of October until January. The lack of application of nitrogen fertilizer during the spring, added to a single low dose (70 kg N/ha) only in January, would have generated a condition of low N availability and, in effect, contributed to the longevity of the tiller population. This same pattern of response had already been reported by Costa et al. (2016) and Pessoa et al. (2016), who evaluated the tiller dynamics of Marandu palisadegrass under conditions of low nitrogen fertilizer application (50 and 60 kg N/ha during the entire rainy season, respectively) and observed TSR values above 80% from winter to summer. However, despite the relevance of TSR for the persistence of Marandu palisadegrass, it is important that management practices ensure high TAR in early spring (October), a time when this forage plant regains its tiller population density (Costa et al. 2016; Pessoa et al. 2016).

Based on our results, it is also possible to state that the effects of sward height and mowing at the end of winter on tillering occurred only in the 3 months of spring. Stockpiled pastures maintained at lower heights or those maintained at higher heights and then mown at the end of winter, showed the highest TAR and PSI, allowing the maintenance of

higher tiller population density during the spring. During summer all pastures, which were under the same management, showed similar tillering patterns (Table 1) and number of tillers (Table 3).

Our results demonstrate that short or tall/mown pastures have a faster tiller renewal, early in the growing season, compared with medium and tall pastures. In the latter ones, tillers still alive at the end of winter and early spring died off and were replaced by new ones, but with a lag in relation to short or tall/mown pastures. Therefore, the dynamics was the same, with a delay in the taller pastures, caused by the treatments imposed. In other words, pasture tillering patterns responded strongly to variations in availability of climatic growth factors, but these responses were modulated by experimental treatments. It is obvious that mismanagement during the transition from winter to spring may seriously impair the pasture's ability to rebuild tiller population and may compromise production for the entire next pasture growing season.

The importance of tillering dynamics for persistence and high yield of pastures in grazing systems is widely recognized (Colvill and Marshall 1984). The knowledge of seasonal patterns of tiller birth and death has allowed an understanding of how pasture management strategies affect tillering dynamics and has helped to define better grazing practices. This study has shown that the same rationale applies to stockpiled pastures, as both sward height and mowing at the end of winter grazing affect tiller population density and tillering dynamics early in the grazing season, i.e. in spring.

This study has revealed that, when stockpiling *Urochloa brizantha* cv. Marandu for providing winter feed, it is important to keep sward height low at the end of winter or alternatively mow the sward at the end of winter. Both strategies will ensure early and intense tillering in early spring. This strategy will ensure population stability of tillers in the Marandu palisadegrass pasture, as tillers generated in early spring are long-lived and therefore contribute significantly to the total population of tillers in the pasture during the growth season.

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Research Paper

Urochloa brizantha cultivated in aluminum-toxic soil: Changes in plant growth and ultrastructure of root and leaf tissues

Cambios en el crecimiento y en las ultraestructuras de tejidos radiculares y foliares de Urochloa brizantha *cultivado en suelo con niveles tóxicos de aluminio*

LUCAS APARECIDO MANZANI LISBOA[1,2], GUSTAVO HENRIQUE DE OLIVEIRA DIAS[1], HIAGO AUGUSTO AMARAL SACCO[1], JOÃO VITOR RODRIGUES PADOVAN[1], GABRIEL BANOS RODRIGUES[1], KAUÊ BARBAROTTO RIBEIRO[1], GABRIEL GEMINIANO DA SILVA[1], ALAN DOS SANTOS CARDOSO[1], LEANDRO BARRADAS PEREIRA[1] AND PAULO ALEXANDRE MONTEIRO DE FIGUEIREDO[2]

[1]*Fundação Educacional de Andradina, Andradina, SP, Brazil.* fea.br
[2]*Faculdade de Ciências Agrárias e Tecnológicas, Universidade Estadual Paulista, Dracena, SP, Brazil.* dracena.unesp.br

Abstract

Brazilian soils destined for fodder production are infertile and acidic and contain toxic levels of aluminum (Al), which cause a reduction in growth of the root system and aerial plant parts. The main aim of the present pot trial was to determine ultrastructural and developmental changes in root and leaf tissues of *Urochloa brizantha*, when grown in an acid Oxisol containing varying levels of Al. The experimental design was a 3 × 5 factorial arrangement, involving 3 cultivars of *U. brizantha* (Marandu, Paiaguás and Piatã) and 5 concentrations of Al in the soil (0.2, 0.4, 0.8, 1.6 and 3.2 cmol/dm^3), with 4 replications; a total of 60 pots. All cultivars responded negatively to increasing Al concentration in the soil, even in small amounts. Root ultrastructures were damaged even at concentrations of 0.4 cmol Al/dm^3, primarily in the conducting tissues (xylem and phloem) and epidermal cells. Shoot development and leaf tissues were also negatively affected. In general, plant development and ultrastructure of root and leaf tissues in all 3 cultivars of *U. brizantha* were impaired when grown in the presence of Al at doses >0.2 cmol/dm^3 in the soil.

Keywords: Dry matter production, forage, plant morphology, tropical grasses.

Resumen

Los suelos dedicados a la producción de pastos en Brasil se caracterizan en general por baja fertilidad, alta acidez y altas concentraciones de aluminio (Al), lo que conlleva a una reducción del sistema radicular y de las partes aéreas de las plantas. En un experimento de invernadero conducido en Andradina, São Paulo, Brasil, en un Oxisol se evaluaron los efectos de varios niveles de Al en el desarrollo y las ultraestructuras de los tejidos radiculares y foliares de tres cultivares de *Urochloa brizantha* (cvs. Marandu, Paiaguás y Piatã). El diseño experimental fue en un esquema factorial 3 × 5, con los tres cultivares y cinco concentraciones de Al en el suelo: 0.2; 0.4; 0.8; 1.6; y 3.2 cmol Al/dm^3, con cuatro repeticiones, para un total de 60 materas. Los tres cultivares respondieron negativamente al aumentar la concentración de Al en el suelo, incluso en las concentraciones bajas. Las ultraestructuras radiculares sufrieron daños incluso a concentraciones de 0.4 cmol Al, principalmente los tejidos conductores y las células epidérmicas. También el desarrollo de los brotes y los tejidos foliares fueron afectados. En general, el desarrollo de las plantas y las ultraestructuras de tejidos radiculares y foliares se deterioraron cuando los tres cultivares de *U. brizantha* se cultivaron en presencia de Al en el suelo en dosis superiores a 0.2 cmol/dm^3.

Palabras clave: Forraje, hojas, morfología de la planta, producción, raíces.

Correspondence: L.A.M. Lisboa, São Paulo State University (UNESP), College of Technology and Agricultural Sciences, Dracena, São Paulo, Brazil. Email: lucas.lisboa@unesp.br

Introduction

The genus *Urochloa* originated from the African continent, evolving on soils very similar to the infertile soils of the Brazilian Cerrado with a good rainy season and the presence of toxic aluminum (Nunes et al. 1984). *Urochloa* spp. are the most cultivated grass species in Brazil, and display good adaptation and establishment under adverse conditions of soil and climate, producing high yields of dry mass (Ramos et al. 2012; Pezzopane et al. 2015). *Urochloa brizantha*, especially cvv. Marandu, Paiaguás and Piatã, has been popular for more than 30 years. Due to its efficient root system, it easily adapts to acid soils, with fast and steady growth, has good nutritional value and has been claimed to improve physical and chemical attributes of soil degraded by mining (Stumpf et al. 2016a).

Many Brazilian soils are infertile and acidic (Cantú et al. 2016), as well as containing toxic levels of aluminum (Al), which is found as aluminum oxides or aluminosilicates. Al is one of the most limiting abiotic factors affecting plant production in acid soils (Stumpf et al. 2016a; 2016b).

The main symptom of toxicity caused by Al in plants is reduction of the root system and altering of the morphology of its tissues (Duressa et al. 2010; Derré et al. 2013). The injuries to the roots hamper the absorption of water, which influences morphophysiological and biochemical characteristics of the plants, restricting both the development of aerial parts and increase in dry mass, with deformation of shoot growth and chlorosis of leaves (Cantú et al. 2016; Jesus et al. 2016).

The improvement of plant tolerance of Al toxicity in the soil has become a strategy to supply more adapted materials to areas of the Brazilian savannas, where *Urochloa* spp. are the basis of forage improvement programs (Figueiredo et al. 2019). Major research efforts have been made to improve plant tolerance of Al toxicity through selection and breeding. According to Bitencourt et al. (2011), different genotypes within the same species showed differences in their development, and how they behaved when exposed to different doses of Al in solution, mainly in terms of the diameter and length of the main root.

Many researchers have endeavored to characterize the responses of forage plants to presence of Al in the soil, and currently molecular markers are being used to confirm these responses (Worthington et al. 2020). However, it is also necessary to know what changes in morphology of plant organs occur when they are grown in soils containing Al.

The main aim of the present pot study was to determine ultrastructural and developmental changes in root and leaf tissues of grasses, when grown in acid soil containing varying levels of aluminum. Since *Urochloa brizantha* cultivars are so widely grown they were chosen as the test plants.

Materials and Methods

Experimental design

The study was carried out as a pot experiment between February and May 2018, at the Integrated Faculties Stella Maris (FISMA), located in Andradina, São Paulo State, Brazil (20°53' S, 51°22' W; 413 masl). The experimental design was a 3 × 5 factorial arrangement of 3 cultivars of *Urochloa brizantha* (Marandu, Paiaguás and Piatã) and 5 concentrations of aluminum (Al^{3+}) in the soil (0.2, 0.4, 0.8, 1.6 and 3.2 cmol Al/dm^3), with 4 replications, giving a total of 60 pots. Aluminum chloride ($AlCl_3$) in solution was used as the source of aluminum.

Each experimental unit was composed of a single pot with 6 dm^3 capacity filled with sifted soil, fertilized according to Raij et al. (1996). The soil used in the experiment was classified as Vermelho-amarelo distrófico férrico (Santos et al. 2013) and soil pH was maintained at 4.7 to make Al readily available to the plants (Table 1). Five seeds were sown per pot, and 15 days later the most developed 3 plants were selected and others removed. Sixty days after sowing, a standardization cut was performed at 2 cm above soil level. Evaluations were made on the regrowth of the grass after another 30 days, i.e. when aerial plant parts (shoots) were 30 days old and roots 90 days old. By this a maximum expression of the toxic effects of Al was expected.

Plant growth attributes and ultrastructural changes

Shoot attributes measured were number of leaves/pot (NL) and dry mass of the aerial part (DMAP; g/pot). Roots were recovered from soil, washed and dry mass of roots (DMR; g/pot) was determined. Furthermore, fragments of totally expanded leaves and fragments of roots (1 cm in size) were collected.

Table 1. Chemical attributes of the soil used in the experiment.

pH ($CaCl_2$)	SOM (g/dm^3)	P (mg/dm^3)	K	Ca	Mg	H+Al	Al	SB	CEC	BS%	Al%
			($mmol_c/dm^3$)								
4.7	8.0	1.0	0.5	7.0	6.0	20	2.0	13.5	33.5	40	13

SOM = soil organic matter; SB = sum of bases; BS% = base saturation; Al% = saturation of Al; CEC = cation exchange capacity.

The samples were transported to Laboratory of Vegetal Morphophysiology and Forages at College of Agricultural and Technological Sciences – São Paulo State University. The collected material was immersed in FAA 70 (37% formaldehyde, acetic acid and 70% ethanol in the ratio of 1:1:18; V/V). Twenty-four hours later the fragments were washed and stored in 70% ethanol until the date of the analyses, as described by Kraus and Arduin (1997). All fragments of plant tissues were treated using the relevant procedures for dehydration, diaphanization, inclusion and embedding.

By using a Leica microtome that contains steel razors, 8 µm transverse sections were obtained from each embedded fragment. The first undamaged transverse section was chosen for preparation of the histological slides. These sections were fixed with patches (albumin), tinted with safranin with a 1% ratio and set in microscope and glass slides with Entellan® patch (Kraus and Arduin 1997).

All slides were examined with an Olympus optical microscope, model BX 43, with an attached camera in order to photograph the sections. Pictures were used to measure anatomical parameters through the software cellSens Standard, which was calibrated with a microscopic rule, as described by Figueiredo et al. (2013).

By using transverse sections, the following ultrastructural variables were measured: root xylem diameter (RXD; µm); root phloem diameter (RPD; µm); root endoderm thickness (RET; µm); leaf xylem diameter (LXD; µm); leaf phloem diameter (LPD; µm); adaxial epidermal thickness (ADET; µm); and abaxial epidermal thickness (ABET; µm), according to the methodology of Figueiredo et al. (2013). The lower or abaxial epidermal impression of the fragments, collected using cyanoacrylate ester (Segatto et al. 2004), was used to determine density of stomata (SD) and stomatal functionality (SF) according to Castro et al. (2009). Ten measurements were done for all characteristics on each microscope slide. Plots were represented by average values obtained for each characteristic.

Statistical analyses

All variables were submitted to an F test ($P<0.05$); the Tukey test was applied at 5% probability and regression analysis was applied to the Al doses, in which their models were tested for linear, quadratic and cubic relationships (Banzatto and Kronka 2013), by using Assistat 7.7 statistic software (Silva and Azevedo 2016).

Results

Plant growth attributes

Cultivar Paiaguás had the highest average number of leaves (NL) with more dry mass of aerial parts (DMAP) than cv. Marandu ($P<0.05$). On the other hand, dry mass of roots (DMR) for cv. Marandu was greater than those of Paiaguás and Piatã (Table 2).

There was a negative linear relationship between the number of leaves per plant and the concentration of Al in the soil, with the lowest concentration of Al in soil resulting in the greatest number of leaves in all 3 cultivars (Figure 1).

Table 2. Analysis of variance of plant growth characteristics of 3 *Urochloa brizantha* cultivars (cvv. Marandu, Paiaguás and Piatã) grown in soils with different concentrations of aluminum.

	NL (no.)	DMAP (g/pot)	DMR (g/pot)
Forage (F)			
Marandu	62.5b	6.31b	33.5a
Paiaguás	80.6a	8.00a	27.0b
Piatã	64.0b	6.86ab	27.7b
SMD	8.45	1.43	3.34
P value	0.0001	0.0205	0.001
cmol Al/dm^3 (Al)			
0.2	83.3a	9.71a	37.6a
0.4	70.3b	8.33ab	28.8b
0.8	63.0b	6.57bc	28.1b
1.6	69.0b	5.87c	28.1b
3.2	59.6b	4.80c	24.4b
SMD	12.80	2.17	5.06
P value	0.0001	0.0001	0.001
CV (%)	15.9	26.4	14.8
Overall mean	69.0	7.06	29.4
P value F×Al	0.8364	0.6001	0.0239
Analysis of regression variance			
Marandu			
P value	0.0138	0.0001	0.0015
Regression	L*	L**	L**
CV (%)	12.1	16.6	14.7
Paiaguás			
P value	0.0066	0.0037	0.0012
Regression	L**	L**	L**
CV (%)	17.3	34.9	17.9
Piatã			
P value	0.0154	0.0001	0.0005
Regression	L*	L**	L**
CV (%)	15.2	16.6	11.4

NL = number of leaves; DMAP = dry mass of aerial parts; and DMR = dry mass of roots. SMD = significant minimum difference; L = polynomial of 1st degree.

In the same way as for number of leaves, dry mass of aerial parts (shoots) was negatively related to concentration of Al in the soil (Figure 2A). The lower development of aerial parts may be a response to the reduced development of roots, which also decreased as the level of Al in soil increased (Figure 2B).

Large differences in development of roots of all 3 cultivars occurred when Al concentrations in soil increased from 0.2 to 3.2 cmol Al/dm^3 (Figure 3).

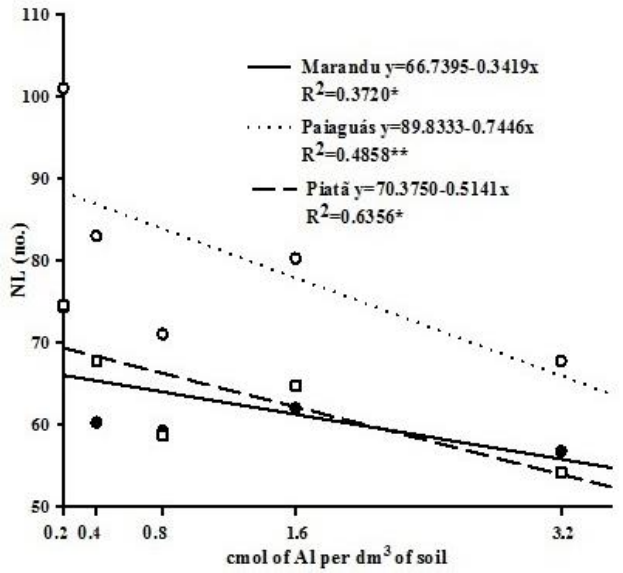

Figure 1. Regression analysis of number of leaves (NL) in 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) in relation to level of aluminum in soil.

Ultrastructural changes in leaf and root tissue

Table 3 presents analyses of variance of the ultrastructural characteristics of leaves and roots of the 3 *U. brizantha* cultivars grown in soils with different concentrations of Al. Stomatal density (SD) on leaves of all 3 cultivars was similar, but stomatal functionality (SF) for cv. Piatã was greater (P = 0.007) than that of cv. Paiaguás. The pattern for roots was different with diameter of root xylem (RXD) for Marandu and Paiaguás being significantly greater than that of Piatã (P=0.0001), while diameter of root phloem (RPD) for Marandu was significantly greater than those of Paiaguás and Piatã (P=0.0001).

Stomatal development on the abaxial surface of leaves of some of the cultivars was impaired as Al concentrations were increased, as shown in Figure 4. Stomatal density (SD) and stomatal functionality (SF) in Marandu declined in a linear fashion as Al concentrations in soil increased, but Al concentration had no impact on these parameters for Paiaguás or on SD for Piatã (Table 3). For Piatã, SF increased initially as Al concentration in soil increased but then declined giving a quadratic response. Peak activity occurred at approximately 1.9 cmol Al/dm^3 in soil.

This negative response to concentration of Al in soil is an important factor in understanding the anatomical responses of plants to varying concentrations of the metal in soil. Figure 5 shows that the morphology of the inner tissues of roots was impaired as Al^{3+} concentration in soil increased from 0.2 to 3.2 cmol/dm^3.

Root xylem diameter (RXD) of Marandu showed a linear negative response to the concentration of Al, while Paiaguás and Piatã showed quadratic responses, reaching peaks between 0.8 and 2.1 cmol/dm^3 (Figure 5A).

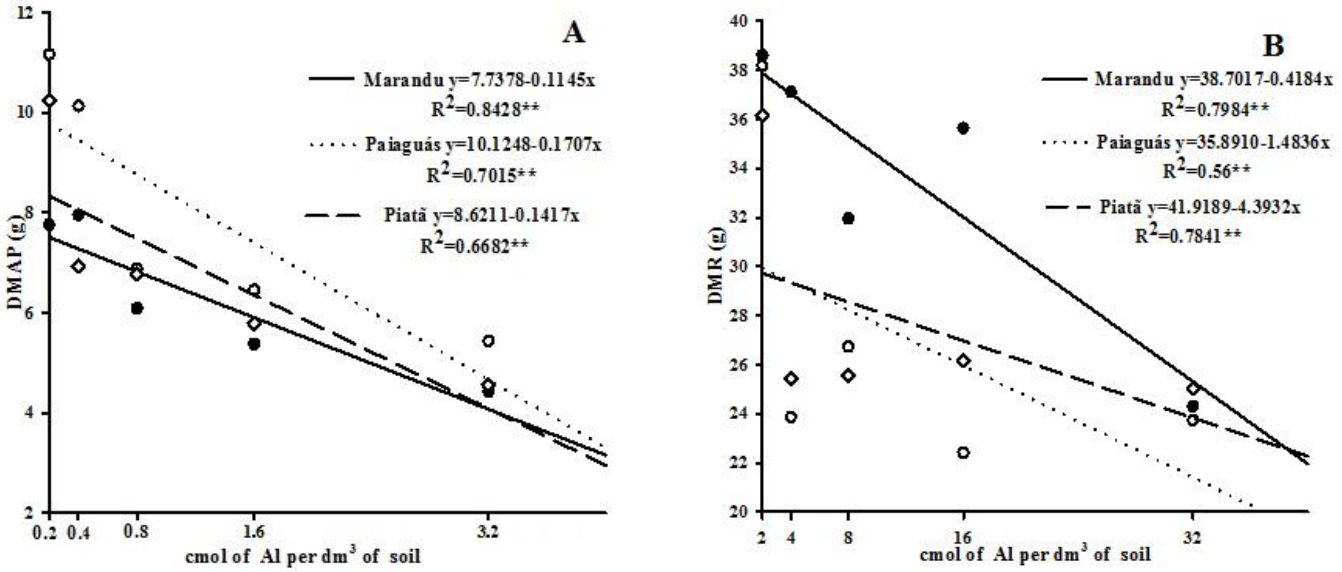

Figure 2. Regression analysis of: **A** – dry mass of aerial parts (DMAP); and **B** – dry mass of roots (DMR) of 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) in relation to level of aluminum in soil.

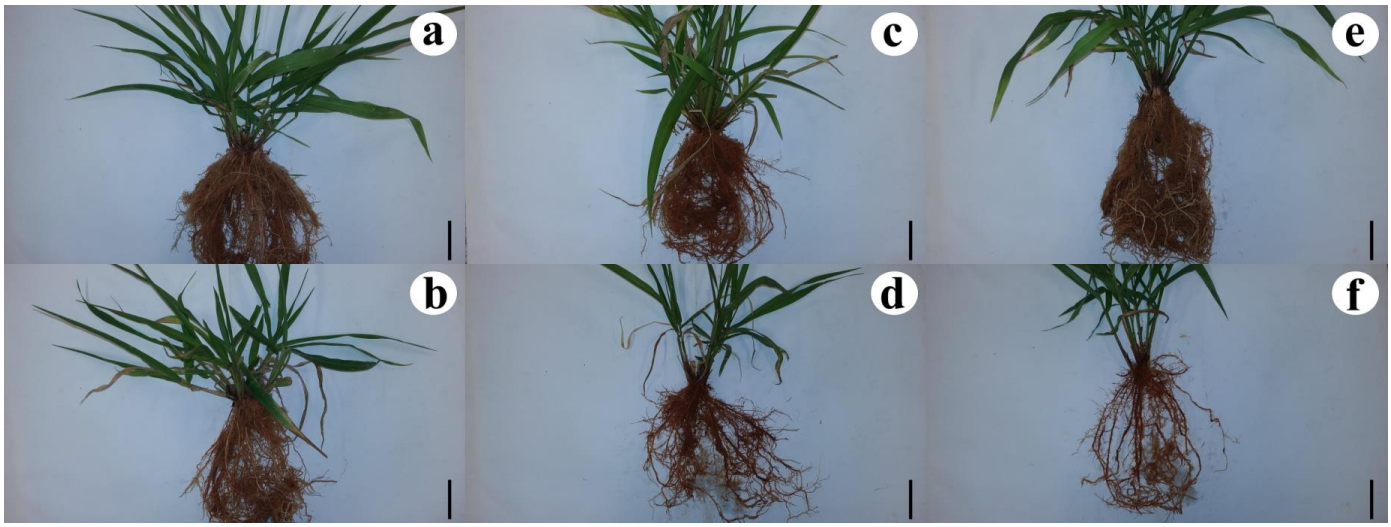

Figure 3. *Urochloa brizantha* plants grown in soils with different concentrations of aluminum: **a** – cv. Marandu in soil with 0.2 cmol Al/dm^3; **b** – cv. Marandu in soil with 3.2 cmol Al/dm^3; **c** – cv. Paiaguás in soil with 0.2 cmol Al/dm^3; **d** – cv. Paiaguás in soil with 3.2 cmol Al/dm^3; **e** – cv. Piatã in soil with 0.2 cmol Al/dm^3; and **f** – cv. Piatã in soil with 3.2 cmol Al/dm^3. Bars = 5.0 cm.

Table 3. Analysis of variance of 5 ultrastructural characteristics of 3 *Urochloa brizantha* cultivars (cvv. Marandu, Paiaguás and Piatã) grown in soils with different concentrations of aluminum.

	SD (no./mm^2)	SF	RXD (µm)	RPD (µm)	RET (µm)
Forage (F)					
Marandu	104.0a	2.82ab	46.7a	10.16a	17.2a
Paiaguás	114.9a	2.52b	42.6a	7.61b	16.7a
Piatã	104.6a	3.06a	36.6b	7.37b	16.3a
SMD	15.09	0.39	4.81	1.20	2.45
P value	0.1555	0.0070	0.0001	0.0001	0.6532
cmol Al/dm^3 (Al)					
0.2	112.3a	2.97a	41.4ab	8.35ab	16.83b
0.4	111.4a	2.97a	42.1ab	9.64a	19.80ab
0.8	108.3a	2.89a	44.1a	9.09ab	21.25a
1.6	107.0a	3.00a	46.3a	7.55b	16.12b
3.2	100.1a	2.16b	35.9b	7.37b	9.62c
SMD	22.85	0.59	7.28	1.82	3.71
P value	0.5750	0.0007	0.0035	0.0026	0.0001
CV (%)	18.21	18.19	14.92	18.70	19.06
Overall mean	107.8	2.80	42.0	8.38	16.73
P value F×Al	0.3563	0.5295	0.1863	0.0001	0.5518
Analysis of regression variance					
Marandu					
P value	0.065	0.0112	0.0059	0.0218	0.0002
Regression	L**	L*	L**	L*	Q**
CV (%)	10.6	18.2	11.1	15.9	16.5
Paiaguás					
P value	0.5968	0.1729	0.0216	0.0002	0.0054
Regression	ns	ns	Q*	Q**	Q**
CV (%)	22.9	20.1	17.3	10.6	23.9
Piatã					
P value	0.7292	0.0121	0.0214	0.0212	0.0001
Regression	ns	Q*	Q*	L*	Q**
CV (%)	16.6	15.9	15.5	27.6	18.1

SD = stomatal density; SF = stomatal functionality (= ratio polar diameter/equatorial diameter); RXD = root xylem diameter; RPD = root phloem diameter; and RET = root endoderm thickness. SMD = significant minimum difference; L = polynomial of 1st degree; and Q = polynomial of 2nd degree.

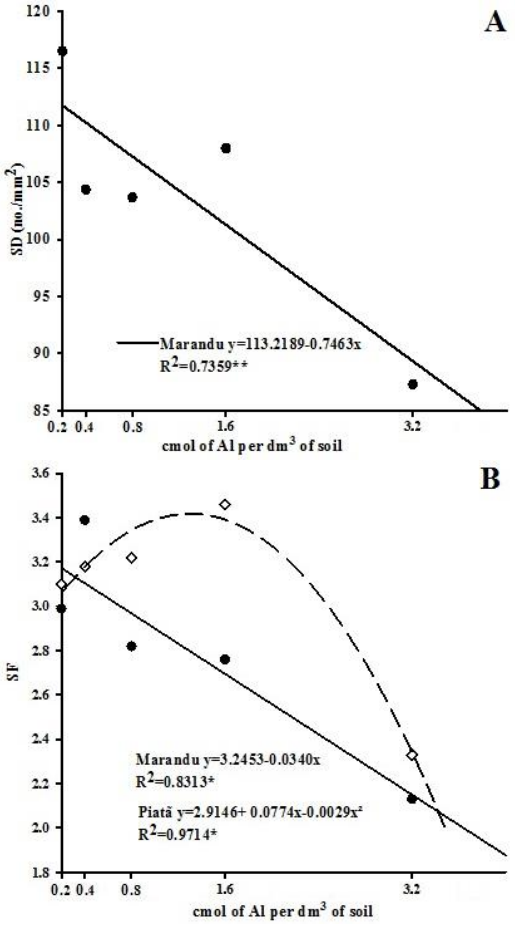

Figure 4. Effects of aluminum concentration in soil on: **A** – stomatal density (SD); and **B** – stomatal functionality (SF) in leaves of *Urochloa brizantha* cvv. Marandu and Piatã.

For RPD, Marandu and Piatã displayed linear negative effects, while Paiaguás presented a quadratic response, with the greatest effect at 2.3 cmol/dm^3 of Al in soil (Figure 5B). For RET, all cultivars showed quadratic responses, as shown in Figure 5C, in which Marandu peaked at 0.1 cmol Al/dm^3 in soil, while Paiaguás and Piatã peaked at 0.9 and 1.3 cmol Al/dm^3, respectively.

Marked injury to roots of the *U. brizantha* cultivars due to the presence of Al in the soil is depicted in Figure 6 with red arrows indicating the injuries to the endodermis, as the plant was subjected to 3.2 cmol Al/dm^3 in soil.

A statistical difference was observed between the cultivars for leaf xylem diameter (LXD), where Paiaguás showed higher values, and for leaf phloem diameter (LPD), where the values of Piatã were lower. For abaxial epidermal thickness (ABET) there was no statistical difference between cultivars. However, Piatã showed higher adaxial epidermal thickness (ADET) than both Paiaguás and Marandu. A negative response to increasing Al concentration in soil was also found in the morphology of some internal tissues of leaves (Table 4).

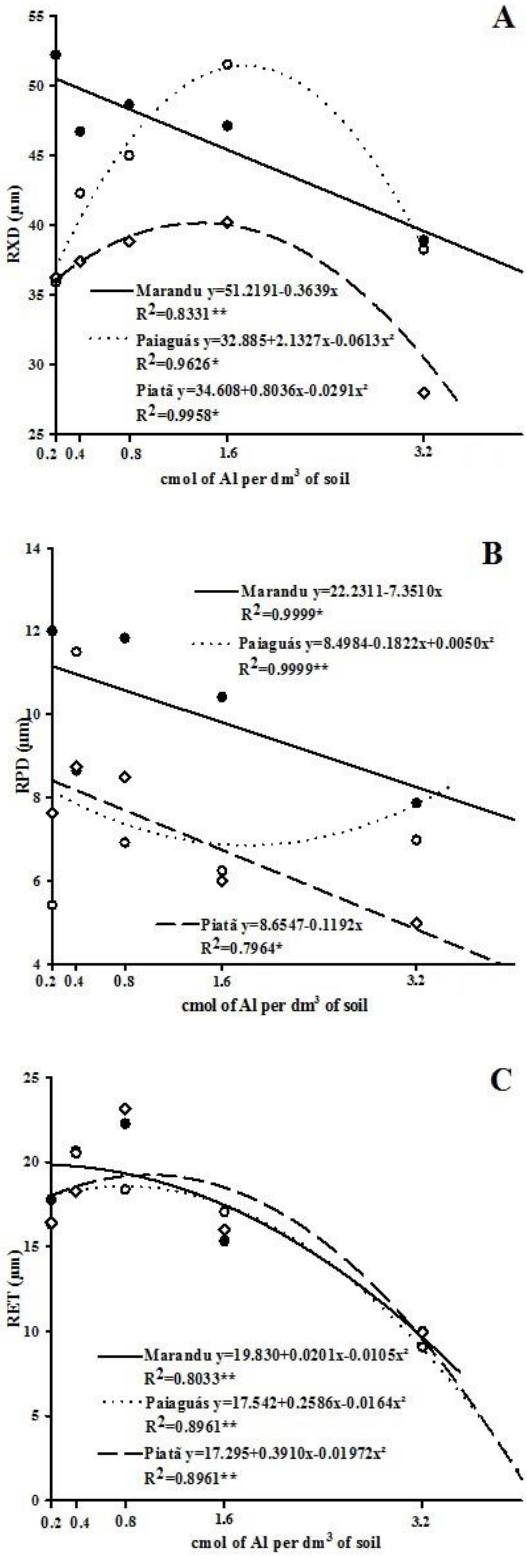

Figure 5. **A** – Root xylem diameter (RXD); **B** – root phloem diameter (RPD); and **C** – root endodermis thickness (RET) of 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) grown in soil with different concentrations of aluminum.

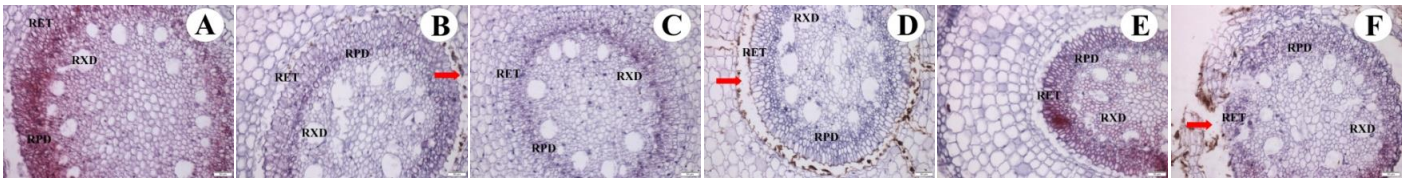

Figure 6. Root ultrastructural changes observed in 3 *Urochloa brizantha* cultivars grown in soil with contrasting levels of aluminum. **A** – cv. Marandu in soil with 0.2 cmol Al/dm^3; **B** – cv. Marandu in soil with 3.2 cmol Al/dm^3; **C** – cv. Paiaguás in soil with 0.2 cmol Al/dm^3; **D** – cv. Paiaguás in soil with 3.2 cmol Al/dm^3; **E** – cv. Piatã in soil with 0.2 cmol Al/dm^3; and **F** – cv. Piatã in soil with 3.2 cmol Al/dm^3. Zoom: 400×. The red arrows indicate the injuries to the root endodermis. RXD = root xylem diameter; RPD = root phloem diameter; and RET = root endoderm thickness.

Table 4. Analysis of variance of 4 ultrastructural characteristics of leaves of 3 *Urochloa brizantha* cultivars (cvv. Marandu, Paiaguás and Piatã) grown in soil with different concentrations of aluminum.

	LXD (µm)	LPD (µm)	ADET (µm)	ABET (µm)
Forage (F)				
Marandu	25.5a	3.98ab	7.26b	7.12a
Paiaguás	22.0b	4.00a	6.87b	6.45a
Piatã	22.3ab	3.48b	9.10a	6.80a
SMD	3.47	0.51	1.47	1.12
P value	0.0345	0.0310	0.0014	0.3624
cmol Al/dm^3 (Al)				
0.2	27.0a	4.39a	9.39a	7.46a
0.4	20.5b	3.82ab	8.93ab	7.54a
0.8	24.1ab	3.61ab	7.00bc	7.31a
1.6	24.0ab	3.88ab	8.47ab	6.56ab
3.2	21.06b	3.40b	4.92c	5.10b
SMD	5.36	0.78	2.22	1.70
P value	0.0068	0.0133	0.0001	0.0008
CV (%)	19.44	17.73	24.72	21.58
Overall mean	23.33	3.82	7.74	6.79
P value F×Al	0.0926	0.0807	0.0734	0.1987
Analysis of regression variance				
Marandu				
P value	0.7987	0.0011	0.1776	0.0012
Regression	ns	L**	ns	L**
CV (%)	14.9	15.5	27.4	16.8
Paiaguás				
P value				
Regression	ns	ns	L*	L*
CV (%)	24.5	22.3	20.5	22.6
Piatã				
P value	0.0015	0.6044	0.0008	0.0331
Regression	L**	ns	L**	L*
CV (%)	19.0	13.5	24.4	25.7

LXD = leaf xylem diameter; LPD = leaf phloem diameter; ADET = adaxial epidermal thickness; and ABET = abaxial epidermal thickness. SMD = significant minimum difference; and L = polynomial of 1st degree.

Leaf xylem diameter (LXD) of Piatã showed a linear negative response to increasing concentrations of Al in soil (Figure 7A), while leaf phloem diameter (LPD) of Marandu showed a linear negative response (Figure 7B). In a similar way, leaves of Paiaguás and Piatã showed linear negative responses in ADET to increasing concentrations of Al (Figure 7C). For ABET, all 3 cultivars showed linear negative responses as Al concentrations increased (Figure 7D).

Ultrastructural changes in inner tissues of leaves of the cultivars were observed as Al concentration in soil increased (Figure 8).

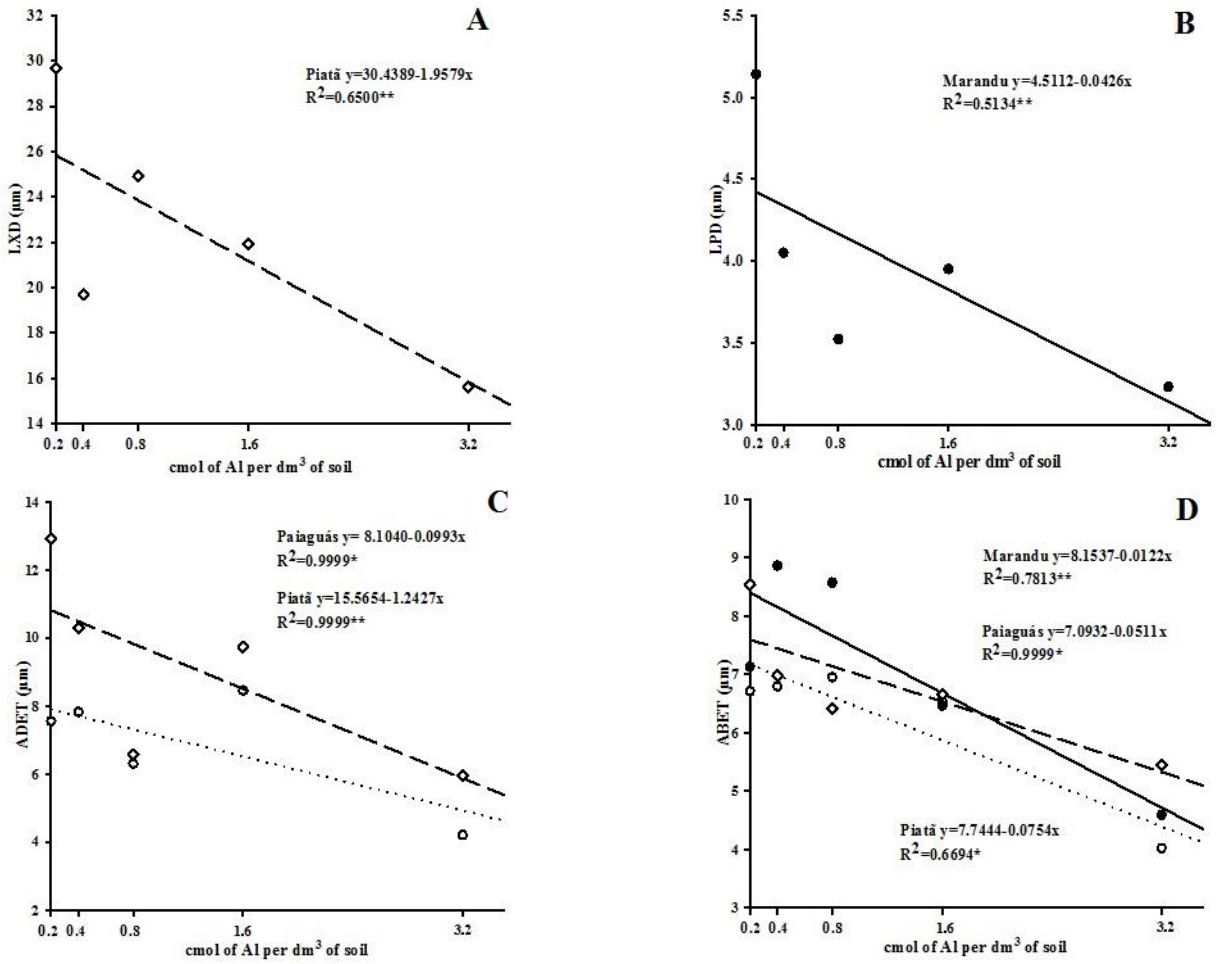

Figure 7. Leaf xylem diameter (LXD), leaf phloem diameter (LPD), adaxial epidermal thickness (ADET) and abaxial epidermal thickness (ABET) of 3 *Urochloa brizantha* cultivars (cvv. Marandu, Paiaguás and Piatã) in soil with different concentrations of aluminum.

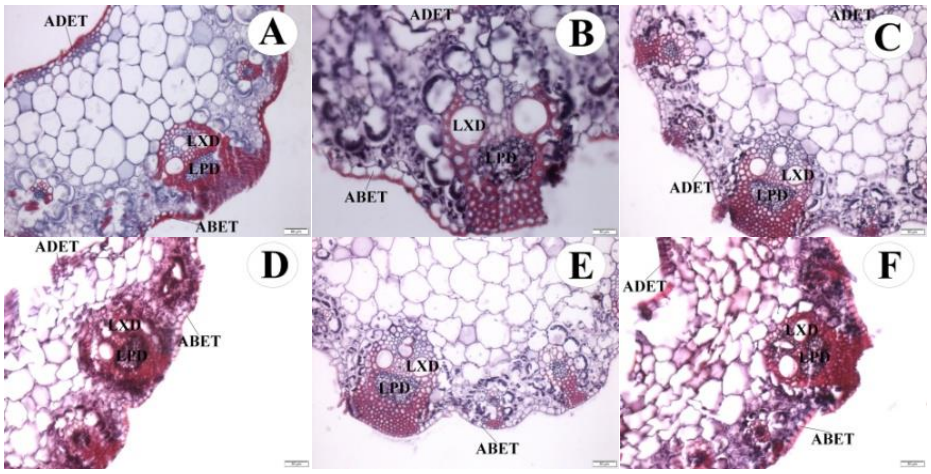

Figure 8. Ultrastructural changes in leaf tissues of 3 *Urochloa brizantha* cultivars grown in soil with contrasting concentrations of aluminum. **A** – cv. Marandu in soil with 0.2 cmol Al/dm^3; **B** – cv. Marandu in soil with 3.2 cmol Al/dm^3; **C** – cv. Paiaguás in soil with 0.2 cmol Al/dm^3; **D** – cv. Paiaguás in soil with 3.2 cmol Al/dm^3; **E** – cv. Piatã in soil with 0.2 cmol Al/dm^3; and **F** – cv. Piatã in soil with 3.2 cmol Al/dm^3. Zoom: 400×. LXD = leaf xylem diameter; LPD = leaf phloem diameter; ADET = adaxial epidermal thickness; and ABET = abaxial epidermal thickness.

Discussion

This study has provided clear indications of the ultra-structural and developmental changes in plants of some *U. brizantha* cultivars when grown in soils with varying concentrations of Al. The decrease in number of leaves of the grasses as concentration of Al increased could reduce net photosynthesis, thereby lowering accumulation of dry mass in the aerial parts and roots (Figure 2B). Al becomes harmful to plant growth as concentration in soils increases and as its availability is increased in acid soils, i.e. with lower pH in soil solution (Cai et al. 2011).

It is worth mentioning that the cultivars presented different responses to cultivation in acid soils in presence of Al. It is for such soils in the Brazilian savanna region that cv. Marandu was launched in 1985, followed by cv. Xaraés in 2003. Following further genetic improvement, new cultivars like Piatã and Paiaguás have become alternatives to Xaraés and Marandu, as they present a greater accumulation of leaves as reported by Valle et al. (2007). Our findings are in agreement with that earlier work and suggest these new cultivars are a useful alternative for cattle producers on acid soils.

The first negative response to the presence of Al in soil is atrophy of the root system owing to inhibition of cell division in the root cap or to small injuries in this area (Čiamporová 2002; Guo et al. 2014; Wang et al. 2016; Xu et al. 2016). Enzyme activity can be reduced as a response to stress to which the plant is submitted in the presence of Al (Kumari et al. 2008; Duressa et al. 2011).

This reduced development of the root system results in lower absorption of nutrients, which will impair the growth of aerial parts of the plant (Figures 2B and 3). In all situations, plants that display poor development of the root system also display problems in their above-ground structure (Reis et al. 2017; Lisboa et al. 2019).

The impairment of stomatal density and functionality of the grasses with increase in Al concentration in soil could be a result of low availability of nutrients supplied to leaves from the root system. Vegetation may react by activating ALMT (aluminum-activated malate transporter) found in plasma membrane or on the tonoplast of plant cells (Palmer et al. 2016). This response can be impaired in the presence of calcium ions, lightless conditions and even abscisic acid action (Sasaki et al. 2010; Araújo et al. 2011).

Marked changes were detected in conducting tissues of the roots (xylem and phloem), as Figures 5A and 5B show, which may depress cell formation in the aerial parts of the plant (Figure 8). Al is stored in pericycle and can lead to formation of xylem. As the concentration of Al increases in acidic soils, deposition occurs within the plants which starts to interfere with the transport of sap within the xylem and phloem vessels. The displacement of Al inside these conducting vessels occurs in the form of Al-citrate, when Al associates with citric acid, since the metal is found in the root cortex reaching a high internal concentration in the vessels, and producing lesions in these root tissues (Klug et al. 2011; Ma and Hiradate 2011) as Figures 6B, 6D and 6F show.

As the A1-citrate is translocated to leaf cells, the plant may show an anti-oxidative physiological response, possibly even chelating the metal, fixing it as oxalate, which is inactive and may be stored within cells (Souza et al. 2018). This process may produce a hardness in the inner tissues, impairing the development of the axial or radial cells of leaf tissues.

At low Al concentrations in soil, an increase in thickness of root endodermis occurred, peaking between 0.9 and 1.3 cmol Al/dm^3, but this was followed by an acute fall in epidermal thickness as concentrations of Al in soil increased. This morphological response is critical for the root system, as root epidermis acts as a barrier to protect the root vessels. While low concentrations of Al had a positive effect, higher concentrations had a marked negative effect, which could interfere with the volume of cytoplasm within the cell (Poschenrieder et al. 2008; Duressa et al. 2011; Ma and Hiradate 2011).

Conclusions

All 3 *U. brizantha* cultivars studied responded negatively to increasing Al concentration in the soil, in amounts >0.2 cmol/dm^3, through impairment of plant development and ultrastructure of root and leaf tissues. Both shoot development and leaf tissue production were reduced. However cv. Paiaguás produced more leaves and more above-ground biomass than cv. Marandu at all Al concentrations. Further studies in the field are warranted to determine if these findings can be reproduced on a larger scale.

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(Note of the editors: All hyperlinks were verified 13 January 2020.)

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Research Paper

Chemical composition, fermentation profile, microbial population and dry matter recovery of silages from mixtures of palisade grass and forage peanut

Composición química, perfil de fermentación, población microbiana y recuperación de materia seca en ensilajes de Urochloa brizantha *y* Arachis pintoi

FRANÇOISE MARA GOMES[1], KARINA GUIMARÃES RIBEIRO[2], IGOR ALEXANDRE DE SOUZA[1], JANAINA DE LIMA SILVA[1], MARIELE CRISTINA NASCIMENTO AGARUSSI[2], VANESSA PAULA DA SILVA[2], THIAGO CARVALHO DA SILVA[2] AND ODILON GOMES PEREIRA[2]

[1]*Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brazil.* ufvjm.edu.br
[2]*Universidade Federal de Viçosa, Viçosa, MG, Brazil.* www.ufv.br

Abstract

The study evaluated chemical composition, fermentation profile, microbial population and dry matter recovery of silages made from mixtures of palisade grass (*Urochloa brizantha* cv. Marandu) and forage peanut (*Arachis pintoi* cv. Belmonte). The experiment was conducted and analyzed in a complete randomized factorial design using 5 levels of each forage (0, 25, 50, 75 and 100% on a fresh matter basis), with and without microbial inoculant and 3 replications. The crude protein concentration increased linearly (P<0.05) and fiber concentration decreased linearly (P<0.05) as forage peanut level in silage increased. There was a positive quadratic effect (without inoculant) and positive linear effect (with inoculant) on lactic acid concentration (P<0.05) and a positive quadratic effect (P<0.05) on lactic acid bacteria population with increasing forage peanut levels in silage. The main effects of the addition of forage peanut to palisade grass at ensiling were improvement in the chemical composition and fermentation profile of the grass silage. We recommend adding 25–75% forage peanut to palisade grass prior to ensiling to improve the quality of the resulting silage but there is little merit in adding microbial inoculant to the forage at ensiling. Feeding studies with animals would verify potential benefits in production from inclusion of legume with grass at ensiling, while studies with addition of energy sources at ensiling would determine any further benefits to be achieved in silage quality.

Keywords: Ammonia nitrogen, *Arachis pintoi*, effluent, microbial inoculant, organic acids, pH, *Urochloa brizantha*.

Resumen

En la Universidade Federal de Viçosa, Minas Gerais, Brasil, se evaluaron la composición química, el perfil de fermentación, la población microbiana y la recuperación de materia seca en ensilajes de diferentes mezclas de *Urochloa brizantha* cv. Marandu y *Arachis pintoi* cv. Belmonte (maní forrajero). El diseño experimental fue factorial (5 × 2) completamente al azar, utilizando cinco niveles de cada especie (0, 25, 50, 75 y 100% con base en materia fresca), con y sin inoculante microbiano, y tres replicaciones por tratamiento. La concentración de proteína cruda aumentó linealmente (P<0.05), mientras que la concentración de fibra disminuyó linealmente (P<0.05) con niveles crecientes de maní forrajero. Los niveles crecientes de maní forrajero presentaron un efecto cuadrático positivo (sin inoculante) y lineal positivo (con inoculante) en la concentración de ácido láctico (P<0.05), y cuadrático positivo (P<0.05) en la población de bacterias acido-lácticas. Los principales efectos de la adición de maní forrajero a la gramínea al momento

Correspondence: Karina Guimarães Ribeiro, Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, CEP 36570-900, MG, Brazil. Email: karinaribeiro@ufv.br

de ensilar fueron el mejoramiento en la composición química y el perfil de fermentación del ensilaje, mientras que la adición de inoculante microbiano mostró pocos beneficios. Los mejores resultados se obtuvieron con pasto *U. brizantha* cv. Marandu en mezcla con 25–75% de maní forrajero, sin aplicación de inoculante. Se requieren estudios complementarios de alimentación con ganado para confirmar los beneficios potenciales de esta mezcla, igualmente estudios para incrementar la calidad de las mezclas mediante la adición de fuentes de energía al momento de ensilar.

Palabras clave: Ácidos orgánicos, efluente, inoculante microbiano, nitrógeno amoniacal, pH.

Introduction

In tropical regions, pasture areas of the genus *Urochloa*, including *Urochloa brizantha*, with potential for silage production of reasonable quality have been established. According to Dawo et al. (2007), the production of tropical grass silage intercropped with legumes may be a strategy to increase dry matter yields and nutritive value of diets for ruminants. Recently, Silva et al. (2018) found that palisade grass (*Urochloa brizantha* cv. Xaraés) and stylo (*Stylosanthes capitata* mixture with *S. macrocephala* cv. Campo Grande) mixed silages had good nutritive value and fermentation profile. In addition, the introduction of legumes into production systems has several benefits, such as increasing nutritive value, voluntary intake and performance of livestock, as well as contributing to lower greenhouse gas emissions (Lüscher et al. 2014).

Arachis pintoi cv. Belmonte originated from a non-seeding accession collected in the area of Belmonte, Bahia, Brazil and was the first *A. pintoi* cultivar released for vegetative propagation (Paganella and Valls 2002). The possibility of ensiling forage peanut and obtaining appropriate chemical composition and fermentative characteristics was investigated by WingChing-Jones and Rojas-Bourrillón (2006), who suggested ensiling it as a means of incorporating an ingredient with moderate protein concentration in the total ration at low cost.

Kung Jr et al. (2003) recommended the use of microbial inoculants to reduce losses when ensiling tropical grasses, since homolactic bacteria compete with the existing microflora of epiphytic microorganisms, thus increasing fermentation efficiency. This view was supported by Muck (2010), who reported that inoculants based on homolactic bacteria have been the predominant additives for use when ensiling, with beneficial effects on both fermentation and storage efficiency.

The objective of this study was to evaluate the chemical composition, fermentation profile, microbial population and dry matter recovery of silages made from mixtures of palisade grass and forage peanut with or without microbial inoculation.

Materials and Methods

Silage material and treatments

The trial was performed at the Animal Science Department of the Federal University of Viçosa (Universidade Federal de Viçosa - UFV), Minas Gerais, Brazil (20º45' S, 42º51' W; 657 masl), where mean annual rainfall is 1,341 mm, of which 86% occurs between October and March.

Palisade grass (*Urochloa brizantha* cv. Marandu) forage was harvested at 60 days of regrowth and forage peanut (*Arachis pintoi* cv. Belmonte) at the beginning of flowering, at 5 cm from ground level, using a steel blade brush cutter (STIHL®). The palisade grass and forage peanut presented dry matter yields of 7.2 t/ha and 2.6 t/ha, respectively.

Silage making

After harvesting, the forages were chopped separately into particles of approximately 2 cm using a stationary forage harvester, before being weighed and mixed in the following proportions of palisade grass and forage peanut, respectively: 100:0, 75:25, 50:50, 25:75 and 0:100 by weight (fresh matter). Each mixture was then halved and one half was inoculated with microbial inoculant, while the other remained un-inoculated. The inoculant was Sil-All® 4x4 water soluble (Alltech, Paraná, Brazil) and contained: *Lactobacillus plantarum*, *Pediococcus acidi-lactici*, *L. salivarius* ssp. *salivarius* and *Enterococcus faecium*; enzymes (xylanase, amylase, cellulase and hemicellulolytic enzyme); silicone dioxide; and saccharose. The inoculant was added at the recommended rate of 5 g/t fresh forage, diluted in deionized water and applied using a 2-L hand sprayer by spraying uniformly onto the forage that was constantly hand-mixed. Untreated material received a volume of water equal to the amount of inoculant.

After the inoculant was applied, the forage mixtures were ensiled in 20-L plastic silos equipped with snap-on lids fitted with a Bunsen valve that enabled gas release

only from fermentation. At the bottom of each silo, 4 kg of sand was placed inside a cotton bag to capture the effluent. Compression of forage was performed to give a mean density of 580 kg/m^3 (fresh matter). There were 3 replications of each treatment giving 30 silos, which were weighed at the beginning of the experiment, and stored in a covered area at 25 ± 1 ºC for 60 days. After 60 days, each silo was weighed and evaluated for effluent loss and recovery of dry matter, according to techniques described by Jobim et al. (2007).

Chemical and microbial analyses

Buffering capacity (BC) of silages was determined as described by Playne and McDonald (1966). Concentrations of water-soluble carbohydrate (WSC) in forage and of residual water-soluble carbohydrate (RWSC) in silage were determined according to the technique described by Silva and Queiroz (2002). The fermentation coefficient (FC) of the forage was calculated according to the following equation proposed by Weissbach and Honig (1996) and cited by Oude-Elferink et al. (2000): FC = DM + 8 × (WSC/BC),

where:

DM is dry matter (g/kg);

WSC is water-soluble carbohydrate (g/kg); and

BC is buffering capacity (meq HCl/100 g DM).

To determine chemical composition, fresh forage and silage samples were dried in an oven at 55 °C until constant weight, and then ground in a Wiley mill with a 1-mm sieve. These samples were used to determine the concentrations of: DM (AOAC 2005; method number 930.15); crude protein (CP; from total N) according to the Kjeldahl method (AOAC 2005; method number 976.05); acid detergent-insoluble nitrogen (ADIN) according to Licitra et al. (1996); acid detergent fiber (ADF) and lignin according to AOAC (2005; method number 973.18); and ash- and protein-free neutral detergent fiber (NDFap) according to Licitra et al. (1996) and Mertens (2002).

To conduct the microbial counts, 25 g of fresh forage was transferred into a sterile container with 225 mL of sterile solution (Ringers Solution®) to obtain a dilution of 10^{-1} and was then homogenized for 4 min in an industrial blender. Serial dilutions were prepared with MRS (Man, Rogosa and Sharp) agar (*Lactobacillus* MRS Broth®, Difco Laboratories, Detroit, MI, USA) to determine lactic acid bacteria (LAB) numbers, after incubation at 37 ºC for 48 hours, and to determine enterobacteria numbers, after incubation at 37 °C for 24 hours in VRB (Violet Red Bile) agar (Difco Laboratories, Detroit, MI, USA) using the pour-plate technique. Mold and yeast numbers were determined using 3M™ Petrifilm™, after incubation at 25 °C for 3 and 5 days for yeast and mold, respectively. The mold and yeast colony-forming units (cfu) were enumerated separately, according to their macromorphological features, using values between 30 and 300 cfu for counting, and the results obtained were transformed into log x in order to achieve a normal distribution. Duplicate samples were assessed for each species.

Chemical composition, buffering capacity (BC), fermentative capacity (FC) and microbial population of fresh palisade grass, forage peanut and their mixtures prior to inoculation, are shown in Table 1.

Table 1. Chemical and microbial composition of fresh *Urochloa brizantha* cv. Marandu (palisade grass) and *Arachis pintoi* cv. Belmonte (forage peanut) prior to inoculation and ensiling.

Parameter	Palisade grass	Forage peanut
Dry matter (g/kg)	262	210
Crude protein (g/kg DM)	51.4	179
NDF (g/kg DM)	775	472
NDFap (g/kg DM)	729	375
ADF (g/kg DM)	455	338
ADIN (g/kg DM)	100	134
Lignin (g/kg DM)	26.8	58.5
Lignin:ADF ratio	0.06	0.17
WSC (g/kg DM)	15.2	49.8
BC (meq HCl/100 g DM)	4.25	7.14
Fermentative capacity	22.7	35.6
LAB (log cfu/g FM)	5.90	6.63
Enterobacteria (log cfu/g FM)	6.49	8.01
Molds + yeasts (log cfu/g FM)	5.76	6.85

NDF = neutral detergent fiber; NDFap = ash- and protein-free neutral detergent fiber; ADF = acid detergent fiber; ADIN = acid detergent-insoluble nitrogen; WSC = water-soluble carbohydrate; BC = buffering capacity; LAB = lactic acid bacteria; FM = fresh matter; log = denary logarithm of the numbers; cfu = colony-forming unit.

To determine pH, 25 g of silage from each silo was homogenized in 225 mL of distilled water in an industrial blender for 1 min and pH was immediately measured with a pH meter. For determination of NH_3-N concentration (expressed as % of total nitrogen, TN), the extract was filtered through filter paper and the filtrate was used according to Bolsen et al. (1992).

To determine organic acids in the silages, 25 g of silage was homogenized with 225 mL distilled water in an industrial blender for 1 min. The aqueous extracts were filtered, acidified with 20% metaphosphoric acid solution and centrifuged for 15 min, according to Kung Jr (1996). Analysis of organic acids was performed using high-performance liquid chromatography (HPLC) of Shimadzu-BIORAD mark, SPD-10 model, C18 column, reverse phase at a wavelength of 210 nm.

Statistical analyses

The experiment was analyzed as a complete randomized factorial (5 × 2) design using increasing fresh matter levels of *A. pintoi* cv. Belmonte (0, 25, 50, 75 and 100%) in silages of *U. brizantha* cv. Marandu, with and without microbial inoculant, and 3 replicates per treatment. The results were subjected to analysis of variance, with the means of the quantitative factors subjected to regression analysis, selecting equations with a coefficient of determination >0.5, and the means of the qualitative factors were compared using the F-test with 5% probability for a type I error using the statistical program SAEG 9.1 (UFV 2007)

Results

Chemical composition of silages

The concentrations of DM, CP, ADIN, ADF, lignin and RWSC were affected (P<0.01) by increasing levels of forage peanut (Table 2). The concentrations of DM and ADF decreased linearly, while CP, ADIN and lignin increased linearly with increasing levels of forage peanut in silage (Table 3). NDFap concentration was affected by an interaction between forage peanut level and microbial inoculant (P<0.05) (Table 2), decreasing linearly with increasing forage peanut level, with and without microbial inoculant (Table 3). The RWSC concentration was affected (P<0.01) by increasing level of forage peanut (Table 2), but no statistical model was adjusted to the RWSC data since mean concentration was only 14 g/kg DM (Table 3).

Treatment with microbial inoculant affected ADIN (P<0.01), NDFap (P<0.01), lignin (P<0.01) and RWSC (P<0.05) concentrations (Table 2).

Fermentation profile, microbial population and effluent loss

There was a significant interaction between forage peanut level and microbial inoculant with respect to pH (P<0.05) and organic acids (P<0.01) (Table 4). The pH of silage increased linearly as forage peanut level increased (P<0.05), in both un-inoculated and inoculated silages (Table 3). While NH_3-N percentage was affected (P<0.05) by increasing level of forage peanut (Table 4), no statistical model adjusted to the NH_3-N data, which showed a mean of 93.5 g/kg total N (Table 3).

Table 2. Chemical composition of silages made from *Urochloa brizantha* cv. Marandu (palisade grass) and *Arachis pintoi* cv. Belmonte (forage peanut) and their mixtures without and with microbial inoculant.

	Forage peanut level (%)						Significance			CV (%)
	0	25	50	75	100	Mean[1]	L	MI	L×MI	
					Dry matter (g/kg)					
C	265	258	243	226	207	240	**	NS	NS	0.9
I	269	261	243	226	208	241				
					Crude protein (g/kg DM)					
C	54.7	79.2	106	134	164	108	**	NS	NS	4.0
I	59.3	82.1	102	131	162	107				
					ADIN (g/kg DM)					
C	75.1	95.3	106	121	125	104a	**	**	NS	5.1
I	69.7	82.8	99.1	106	123	96.2b				
					NDFap (g/kg DM)					
C	706	633	543	462	388	546a	**	**	**	3.1
I	584	509	441	483	396	488b				
					ADF (g/kg DM)					
C	455	434	409	384	359	408	**	NS	NS	2.1
I	449	432	416	388	356	408				
					Lignin (g/kg DM)					
C	31.1	38.5	47.6	54.6	62.1	44.8a	**	**	NS	7.1
I	24.2	33.1	46.2	47.6	53.0	40.8b				
					RWSC (g/kg DM)					
C	12.5	18.8	8.8	34.2	11.9	17.2a	**	*	NS	51.7
I	3.5	21.6	2.6	16.3	9.7	10.7b				

NDFap = neutral detergent fiber corrected for ash and protein; AIDN = acid detergent-insoluble nitrogen; ADF = acid detergent fiber; RWSC = residual water-soluble carbohydrate; C = Control (un-inoculated); I = inoculated; L = forage peanut level; MI = microbial inoculant; L × MI = interaction between forage peanut level and microbial inoculant. [1]Means within the same column and parameter followed by different letters differ significantly at P<0.05.

Table 3. Regression equations for silages made from *Urochloa brizantha* cv. Marandu (palisade grass), *Arachis pintoi* cv. Belmonte (forage peanut) and their mixtures without and with microbial inoculant.

Variable	Regression equation	r^2/R^2
Dry matter (g/kg)	$Y = 271.033 – 0.610133X$	0.98
Crude protein (g/kg DM)	$Y = 54.1667 + 1.0608X$	0.99
NDFap[1] (g/kg DM)	$Y = 707.655 – 3.2232X$	0.98
NDFap[2] (g/kg DM)	$Y = 563.126 – 1.50958X$	0.66
ADF (g/kg DM)	$Y = 454.187 – 0.929467X$	0.97
ADIN (g/kg total N)	$Y = 74.7867 + 0.510133X$	0.96
Lignin (g/kg DM)	$Y = 28.7533 + 0.3008X$	0.95
RWSC (g/kg DM)	$\overline{X} = 14$	
pH[1]	$Y = 4.14067 + 0.00608X$	0.96
pH[2]	$Y = 4.30333 + 0.00421333X$	0.97
NH_3/TN (%NH_3 of total N)	$\overline{X} = 93.5$	
Lactic acid[1] (g/kg DM)	$Y = 19.3696 + 0.237867X – 0.00170056X^2$	0.81
Lactic acid[2] (g/kg DM)	$Y = 10.3608 + 0.140792X$	0.91
Acetic acid[1](g/kg DM)	$Y = 10.6319 + 0.10101X$	0.87
Acetic acid[2] (g/kg DM)	$Y = 15.3107 - 0.108578X + 0.00156997X^2$	0.58
Propionic acid[1] (g/kg DM)	$\overline{X} = 4.8$	
Propionic acid[2] (g/kg DM)	$Y = 4.00847 + 0.0349003X$	0.91
Butyric acid (g/kg DM)	$\overline{X} = 0.8$	
Lactic acid bacteria (log cfu/g FM)	$Y = 7.63156 + 0.00725874X – 0.0000936216X^2$	0.50
Molds and yeasts[1] (log cfu/g FM)	$Y = 2.85373 + 0.0113453X$	0.80
Molds and yeasts[2] (log cfu/g FM)	$Y = 2.81131 + 0.0228047X – 0.000154406X^2$	0.86
DM recovery[1] (%)	$\overline{X} = 86.5$	
DM recovery[2] (%)	$\overline{X} = 85.6$	

NDFap = neutral detergent fiber corrected for ash and protein; ADF = acid detergent fiber; ADIN = acid detergent-insoluble nitrogen; RWSC = residual water-soluble carbohydrate; NH_3/TN = ammonia N as a percentage of total nitrogen; X = 0, 25, 50, 75 and 100% forage peanut in the mixture with palisade grass. [1]Control (un-inoculated); [2]Inoculated.

Table 4. Fermentation parameters of silages made from *Urochloa brizantha* cv. Marandu (palisade grass), *Arachis pintoi* cv. Belmonte (forage peanut) and their mixtures without and with microbial inoculant.

	Forage peanut level (%)						Significance			CV (%)
	0	25	50	75	100	Mean[1]	L	MI	L×MI	
					pH					
C	4.14	4.29	4.45	4.61	4.74	4.44b	**	**	*	0.9
I	4.29	4.41	4.52	4.63	4.71	4.51a				
					NH_3/Total N (%)					
C	73.0	101	83.7	162	92.3	102	*	NS	NS	29.6
I	90.7	76.1	104	90.4	61.2	84.5				
					Lactic acid (g/kg DM)					
C	19.7	23.2	27.9	27.5	26.1	24.9a	**	**	**	7.1
I	11.2	12.7	17.9	19.9	25.2	17.4b				
					Acetic acid (g/kg DM)					
C	11.6	12.2	14.4	20.0	20.3	15.7	**	NS	**	4.9
I	13.8	16.8	13.0	13.9	21.3	15.8				
					Propionic acid (g/kg DM)					
C	4.50	5.20	4.80	4.80	4.80	4.82b	**	**	**	6.4
I	4.20	4.70	5.70	6.40	7.70	5.74a				
					Butyric acid (g/kg DM)					
C	0.80	0.70	0.90	0.70	0.90	0.80	**	NS	**	3.7
I	0.70	0.80	0.80	0.70	0.80	0.76				

C = Control (un-inoculated); I = inoculated; L = forage peanut level; MI = microbial inoculant; L × MI = interaction between forage peanut level and microbial inoculant. NH_3/Total N = ammonia nitrogen as a proportion of Total N. [1]Means within the same column and parameter followed by different letters differ significantly at $P<0.05$.

In the absence of microbial inoculant, concentration of lactic acid showed a positive quadratic relationship (P<0.01; Table 3) with increasing forage peanut level in silage, and estimated maximum value was 27.7 g/kg DM with 75% forage peanut (fresh matter); in the presence of microbial inoculant, lactic acid concentration increased linearly with increasing forage peanut level (P<0.01; Table 3). In contrast, a linear increase (P<0.01; Table 3) in acetic acid concentration was observed in the absence of microbial inoculant, while with microbial inoculant, there was a negative quadratic effect (P<0.01; Table 3), with an estimated maximum value of 13.4 g/kg DM with approximately 35% forage peanut (fresh matter). In the absence of microbial inoculant, an average concentration of 4.8 g propionic acid/kg DM was recorded, while with microbial inoculant, propionic acid concentration increased linearly with increasing forage peanut level (P<0.01; Table 3). While butyric acid concentration was affected (P<0.01) by increasing levels of forage peanut, no statistical model adjusted to the butyric acid data which had a mean of 0.8 g/kg DM (Table 3).

Population of LAB was affected by increasing levels of forage peanut (P<0.05; Table 5). There was a quadratic relationship between forage peanut level and LAB population (P<0.05; Table 3) with a calculated maximum value of 7.77 log cfu/g FM with 38.2% forage peanut (fresh matter). Microbial inoculant had no effect (P>0.05) on the LAB population (mean 7.64 log cfu/g FM).

Populations of molds + yeasts were affected by an interaction between level of forage peanut and microbial inoculant (P<0.01; Table 5). There was a linear increase (P<0.01; Table 3) in mold + yeast populations in silages without microbial inoculant, ranging from 2.85 to 2.87 log cfu/g FM with increasing forage peanut level. However, in inoculated silages, there was a quadratic effect (P<0.01; Table 3), with an estimated maximum value of 3.65 log cfu/g FM with 73.8% forage peanut (fresh matter). No enterobacteria were detected in the silages.

Effluent losses were similar (mean 4.56 kg/t FM; P>0.05) for all silages and the total DM recovery was affected by a significant interaction between level of forage peanut and microbial inoculant (P<0.01; Table 5). However, no statistical model was adjusted to the total DM recovery data where means were 86.5% (un-inoculated) and 85.6% (inoculated) (Table 3).

Table 5. Microbial population, effluent losses and dry matter recovery of silages made from *Urochloa brizantha* cv. Marandu (palisade grass), *Arachis pintoi* cv. Belmonte (forage peanut) and their mixtures without and with microbial inoculant.

	Forage peanut level (%)						Significance			CV (%)
	0	25	50	75	100	Mean	L	MI	L×MI	
						LAB (log cfu/g FM)				
C	7.57	7.67	7.76	7.82	7.39	7.64	*	NS	NS	2.2
I	7.71	7.84	7.70	7.55	7.39	7.64				
						Molds + yeasts (log cfu/g FM)				
C	2.83	3.23	3.27	3.82	3.95	3.42	**	NS	*	5.5
I	2.82	3.26	3.60	3.62	3.56	3.37				
						Effluent losses (kg/t FM)				
C	3.91	3.94	6.36	4.14	3.62	4.39	NS	NS	NS	30.9
I	4.69	5.48	4.01	3.40	6.10	4.73				
						DM recovery (%)				
C	83.9	88.9	87.9	84.4	87.6	86.5	**	*	**	1.1
I	86.6	87.3	86.0	82.9	85.1	85.6				

LAB = lactic acid bacteria; FM = fresh matter; log = denary logarithm of the numbers; cfu = colony-forming unit; C = Control (un-inoculated); I = inoculated; L = forage peanut level; MI = microbial inoculant; L × MI = interaction between forage peanut level and microbial inoculant.

Discussion

Estimated dry matter (DM) concentrations in ensiled material ranged from 262 to 210 g/kg DM, with concentrations declining with increasing levels of forage peanut. However, this difference was not enough to affect the effluent loss. Although McDonald et al. (1991) recommended 30 g DM/kg fresh forage as the minimum value in forage at ensiling to minimize loss of effluent, effluent losses in our study can be considered low for a perennial tropical grass and legume. Despite the reduction in DM concentration of silage with the addition of forage peanut, the average value of 262 g/kg for palisade grass before ensiling meets the minimum value of 260 g/kg recommended by Haigh (1999) for good-quality silage production. In contrast, the DM concentration observed in forage peanut (210 g/kg FM) was lower than this recommendation. WingChing-Jones and Rojas-Bourrillón (2006) also recorded a low DM concentration (200 g/kg FM) for 2 cultivars of forage peanut harvested at 12 weeks of regrowth. Wilting forage before ensiling would have overcome this issue but all silages in our study produced limited amounts of effluent, even without wilting.

According to Mahanna (1993), concentration of desirable water-soluble carbohydrates (WSC) in forages before ensiling should fall in the range of 40–60 g/kg, if DM concentration in forage is <350 g/kg FM and good fermentation is expected. In our study, the WSC concentration in palisade grass (15.2 g/kg DM) was much lower than that for forage peanut (49.8 g/kg DM) before ensiling. The WSC concentration in forage is critical for the production of good quality silage because it is the main source of nutrients for the growth of microorganisms that produce lactic acid. However, tropical species usually have a low concentration of WSC because higher temperatures increase metabolic activity and the synthesis of structural compounds, causing decrease in WSC (Van Soest 1994). Other authors have also found low WSC concentrations in *U. brizantha* cv. Marandu, e.g. Bernardes et al. (2005) (11 g/kg DM) and Arroquy et al. (2014) (21.4 g/kg DM).

The differing concentrations of WSC in the 2 forages were reflected in the differing fermentation coefficient (FC), which was lower in palisade grass (22.7) than in forage peanut (35.6). According to Oude-Elferink et al. (2000), forages with insufficient fermentable substrate or low DM concentration have a FC <35. The ensiling potential of 10 tropical forage legumes and one tropical forage grass was evaluated by Heinritz et al. (2012) and FC ranged from 30 to 68 for legumes, while for *Brachiaria* (now: *Urochloa*) grass hybrid cv. Mulato II FC was 52. In our study, FC for cv. Marandu was much lower than that reported for Mulato II, possibly due to lower WSC and DM concentrations. Addition of energy sources, e.g. sucrose or molasses, at ensiling is practised in some situations to increase fermentable energy supply for bacteria (Heinritz et al. 2012; Bureenok et al. 2013; Rosa et al. 2018).

It is important to highlight that the chemical composition of palisade grass silage was improved by the inclusion of forage peanut due to the higher CP concentration in the legume. Including 25–75% forage peanut with grass at ensiling increased CP concentration in the resulting silage by 41–133%. Qu et al. (2013) also reported that CP% in silage made from intercropped corn and lablab bean [*Lablab purpureus* (L.) Sweet] was greater and fiber concentration was lower than those of corn monoculture.

The lower NDFap and ADF concentrations observed with increasing proportion of forage peanut in the silage are due to lower concentrations of these cell wall constituents in the legume compared with grass. In contrast, lignin concentration and lignin:ADF ratio increased with increasing proportion of forage peanut in the ensiled material, which could contribute to a reduction in silage digestibility (Van Soest 1994). While we did not determine digestibility in the present study, Cardoso et al. (2018) found higher in vitro DM degradability for forage peanut than for *Urochloa decumbens* cv. Basilisk.

A fact related to fermentation profile of the silages was the increase in pH values with increasing levels of forage peanut, probably due to the higher buffering capacity of legumes (McDonald et al. 1991; Kung Jr et al. 2018), as found by Heinritz et al. (2012), who registered an average pH >5.0 for tropical legume silages.

The ammonia nitrogen (NH_3-N) concentrations obtained in silages in this study were within the recommended ranges for good quality silage. According to Kung Jr et al. (2018), plant and microbial proteolytic processes lead to changes in nitrogenous compounds in silages, and the fermentation results in an increase in NH_3-N (usually less than 100–150 g/kg total N). Furthermore, higher than normal levels of soluble N and NH_3-N in wet legume silages are usually a result of proteolytic activity from clostridia. However, we did not verify this in our study.

According to Kung Jr et al. (2018), typical concentrations of lactic acid in commonly fed silages range from 20 to 40 g/kg DM. While acetic acid usually ranges from 10 to 30 g/kg DM, propionic acid is usually undetectable or at very low concentrations (<10 g/kg DM) in good silages and butyric acid should not be detectable in well-fermented silages. In our study, the estimated lactic acid concentrations in the various silages were around 20 g/kg DM (except in inoculated palisade grass, when, based on the respective equation in Table 3, there was up to 50% forage peanut), while acetic acid concentrations were between 10 and 20 g/kg DM, propionic acid concentration was below 10 g/kg DM, and butyric acid concentration was 0.8 g/kg DM.

The ratio of lactic acid to acetic acid is also commonly used as a qualitative indicator of fermentation and this ratio should be 2.5:1 to 3.0:1 in good quality silage (Kung Jr et al. 2018). However, according to these authors, silages with very high lactic acid:acetic acid ratios may sometimes be more aerobically unstable than those with normal ratios because low concentrations of acetic acid may not be sufficient to inhibit lactate assimilating yeasts. In contrast, lactic acid:acetic acid ratios below 1.0 are usually an indication of abnormal fermentations. In our study, the ratio of lactic acid to acetic acid was below 2.5, indicating that the silage would not be considered good quality silage; however, the values were above 1.0, indicating that there was not an abnormal fermentation, except for inoculated palisade grass (lactic:acetic ratio = 0.67).

The LAB population found in palisade grass and forage peanut before ensiling was higher than that reported by Muck (1996) (5.0 log cfu/g FM), under temperate climate conditions, as adequate for the occurrence of good fermentation in silage. In our silages, LAB population exceeded 7.0 log cfu/g FM.

While analyses indicate that DM recovery in the silages was affected by an interaction between increasing level of forage peanut and inoculant, losses were quite inconsistent and failed to follow a definite pattern, with overall means for DM recovery of 86.5 and 85.6%, for un-inoculated and inoculated silages, respectively. This finding is in agreement with the results reported by Cezário et al. (2015) that addition of microbial inoculant did not improve DM recovery (mean of 85%) in palisade grass silages. The authors attributed this to variation in the population of epiphytic bacteria and fungi pre-existing in the forage that could interact with the microbial inoculant. According to Muck (2010), the inefficiency of many commercial inoculants in wet tropical grass silages may result from the inclusion of inappropriate species of lactic acid bacteria or species unable to effectively compete with epiphytic flora when applied at low doses.

Conclusions

The addition of forage peanut to palisade grass during the ensiling process improved the chemical composition and fermentation profile of resulting silage over that of pure grass silage, while adding microbial inoculant at ensiling produced no significant benefit to the resulting silage. We recommend adding 25–75% forage peanut to palisade grass (FM basis) prior to ensiling to produce better quality silage than that from pure grass, with higher levels of legume having the potential to support higher production levels in animals because of increased CP concentration. Feeding studies with animals should be conducted to determine production benefits to be obtained from the mixed silage. Since the mixed silages studied are still not of good quality, further studies to enhance quality by adding energy sources at ensiling seem warranted.

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Research Paper

Dry matter yields and quality parameters of ten Napier grass (*Cenchrus purpureus*) genotypes at three locations in western Oromia, Ethiopia

Producción de materia seca y calidad nutritiva de diez genotipos de Cenchrus purpureus *en tres localidades en Oromia occidental, Etiopía*

ABUYE TULU, MEKONNEN DIRIBSA AND WORKU TEMESGEN

Oromia Agricultural Research Institute, Bako Agricultural Research Center, Bako, Western Ethiopia. www.iqqo.org

Abstract

Ten Napier grass genotypes (accessions) were assessed across 3 locations, Bako, Boneya Boshe and Gute, for forage dry matter (DM) yield, crude protein (CP) concentration, leaf:stem ratio, nutrient composition and digestibility characteristics during 2016 and 2017. The genotypes were evaluated in a randomized complete block design with 3 replications. Mean DM yield was higher for accession ILRI 16804 across all locations followed by ILRI 16801 and ILRI 16800. Leaf:stem ratio, CP concentration and CP and digestible organic matter (OM) yields also varied significantly among genotypes with the highest values obtained for accession ILRI 16804 across all locations, followed by ILRI 16800 and ILRI 16801. Yields of DM, CP and digestible OM and leaf:stem ratio were higher at Boneya Boshe and Gute than at Bako and higher during 2017 than during 2016. The consistently superior performance of ILRI 16804, ILRI 16801 and ILRI 16800 in both years across the 3 sites suggests that these genotypes should be studied further on farms and in differing environments before being recommended for general cultivation in this area. Examining performance with more frequent harvests and feeding studies with livestock would confirm the benefits to be obtained from planting these new accessions.

Keywords: Digestibility, diversity, quality traits, tropical grass.

Resumen

Durante 2016 y 2017 se evaluaron diez genotipos (accesiones) del pasto Napier (*Cenchrus purpureus*) en las localidades Bako, Boneya Boshe y Gute, en el occidente de la región Oromia, Etiopía, por rendimiento de materia seca (MS), relación hoja:tallo, concentración de proteína cruda (PC), digestibilidad y composición nutricional. Se empleó un diseño experimental de bloques completos al azar con tres repeticiones. El rendimiento promedio de MS fue mayor para la accesión ILRI 16804 en todas las localidades, seguido por ILRI 16801 e ILRI 16800. La relación hoja:tallo, la concentración de PC y los rendimientos de PC y materia orgánica (MO) digestible, igualmente variaron significativamente entre genotipos; los valores más altos de estas características se obtuvieron para la accesión ILRI 16804 en todas las localidades, seguido por ILRI 16800 e ILRI 16801. Los rendimientos de MS, PC y MO digestible y la relación hoja:tallo fueron más altos en Boneya Boshe y Gute que en Bako y en 2017 que en 2016. El desempeño consistentemente superior de las accesiones ILRI 16804, ILRI 16801 e ILRI 16800 en ambos años y en los tres sitios sugiere que estos genotipos deben ser evaluados a nivel de productor y en diferentes ambientes, antes de ser recomendados para cultivo en esta región. Además se recomiendan estudios con cosechas más frecuentes y mediciones de producción animal.

Palabras clave: Digestibilidad, diversidad, gramíneas tropicales, pasto Napier, valor nutritivo.

Correspondence: A. Tulu, Oromia Agricultural Research Institute, Bako Agricultural Research Center, PO Box 03, Bako, Western Ethiopia. Email: armdilla@gmail.com

Introduction

A major problem facing livestock producers in tropical countries is how to provide adequate nutrition for their animals (Muhammad 2016). In Ethiopia, like other tropical countries, poor nutrition is a major constraint to livestock production in small-holder crop-livestock farming, especially during the dry season, when pastures and cereal residues are both limited in quantity and of low nutritional value (Tolera et al. 2000). Rangeland pastures (54.6%) and crop residues (31.4%) are the main feed resources for the greater part of the country, but they fail to meet nutritional requirements of livestock (McDonald et al. 2002; CSA 2017). To improve livestock production, a sustainable solution to seasonal deficiencies in feed availability and quality is required.

Napier grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*) shows great potential to alleviate the problem because it is adaptable, vigorous and drought-tolerant and can produce high dry matter yields (Alemayehu 2002). It is a tall perennial grass, which is well adapted to elevations from sea level up to 2,000 m. It is reported to be tolerant of drought and will grow in areas where the rainfall range is from 200 to 4,000 mm (FAO 2016). It is also palatable and can be fed fresh as cut-and-carry forage and as hay or silage or directly grazed in the field (Alemayehu 2002; Getnet 2003). According to Boonman (1993), forage yields of 85.4 t DM/ha without fertilizer and a record of 130 t DM/ha with 1,320 kg N/ha have been recorded. However, forage yields vary significantly depending on variety, season, location, soil fertility and management practices (Ogoshi et al. 2010; Rengsirikul et al. 2011). Testing of Napier grass genotypes for both qualitative and quantitative attributes under diverse environmental conditions in order to select superior genotypes for particular environments seems warranted.

The Napier grass variety, accession ILRI 16792, which was introduced in 1998 from International Livestock Research Institute (ILRI), is well adapted to the area and the most commonly grown local variety. However, it is now considered to have limited capacity to produce high forage yields. Therefore, the current study was undertaken to identify Napier grass genotypes superior to the local variety in terms of forage yield, nutritive value and digestibility at 3 locations in western Oromia, Ethiopia.

Materials and Methods

Locations

The experiment was conducted at 3 locations (Bako Agricultural Research Center, Bonaya Boshe and Gute subsites), located in the western part of Oromia regional state, Ethiopia, which represent the subhumid mid-altitude maize-growing area of western Oromia. Specific locations were: Bako (9°06' N, 37°09' E; 1,650 masl; 2,285 mm mean annual rainfall); Bonaya Boshe (9°54' N, 37°00' E; 1,645 masl; 1,295 mm); and Gute (9°01' N, 36°40' E; 1,880 masl; 1,586 mm) (Figure 1). Monthly rainfall means and maximum and minimum temperatures at the 3 sites are shown in Figures 2a, 2b and 2c. The soil type at Bako location is classified as a Nitosol with 2.5% organic carbon, 10 ppm available P, 0.22% total N and pH (H_2O) 5.18, while that at Bonaya Boshe is a clay loam with 1.86% organic carbon, 12 ppm available P, 0.16% total N and pH (H_2O) 4.6; soil at Gute has 60% silt, 35% sand and 5% clay with 1.98% organic carbon, 6.2 ppm available P, 0.17% total N and pH (H_2O) 4.43. Farming systems in the study area are mixed crop-livestock systems, in which production of maize (*Zea mays*), teff (*Eragrostis tef*), noug (*Guizotia abyssinica*), sorghum (*Sorghum* spp.), hot pepper (*Capsicum annuum*) and sugar cane (*Saccharum officinarum*) are the major crops, and cattle, small ruminants and poultry are the most important livestock species.

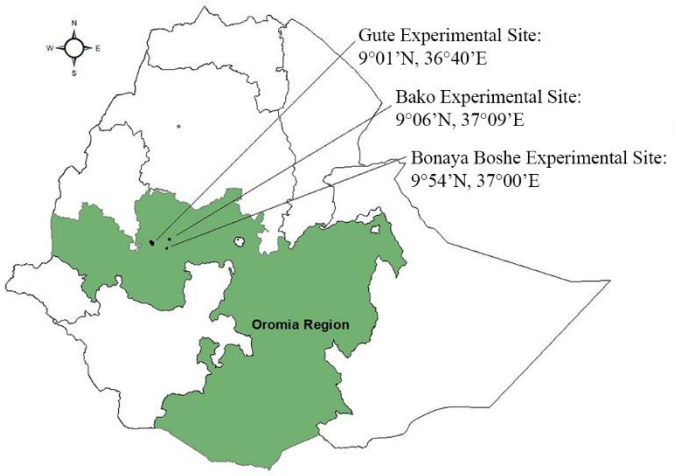

Figure 1. Map of the study area.

Experimental land preparation and planting

Thirty 6 m^2 plots (2 × 3 m) were established on 1 June 2016 at each location. Cuttings of 10 Napier grass genotypes with 3 nodes were planted into a well-prepared seedbed, 2 nodes deep, at an angle of about 45°, with a row spacing of 60 cm and 50 cm between cuttings within rows. Fertilizer was applied according to the recommendation of Tessema et al. (2003), i.e. diammonium phosphate (DAP) at 100 kg/ha at planting, plus urea at 50 kg/ha close to the root slips a month after planting when the Napier grass was well established. In the second year, experimental plots were top-dressed with 50 kg urea/ha, of which one-third was applied immediately after cutting and the remaining two-thirds 2 weeks later.

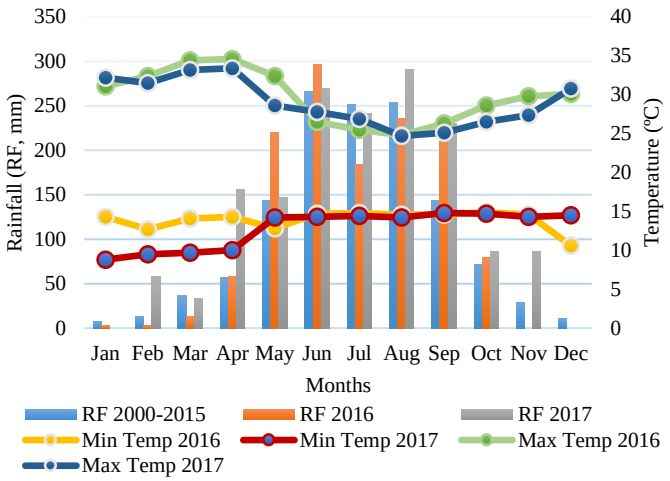

Figure 2a. Mean monthly rainfall and minimum and maximum temperatures at Bako.

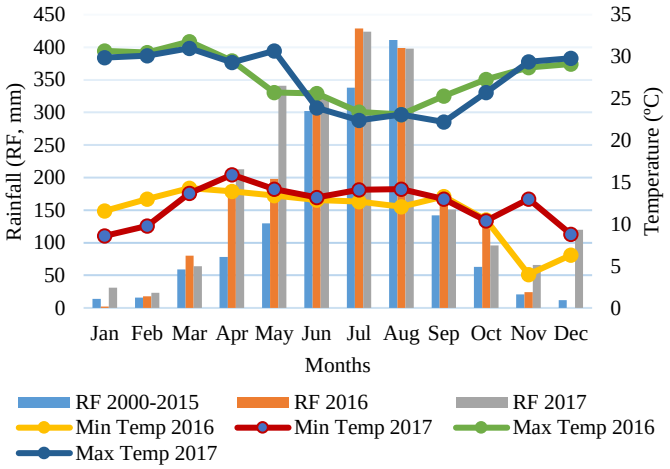

Figure 2b. Mean monthly rainfall and minimum and maximum temperatures at Gute.

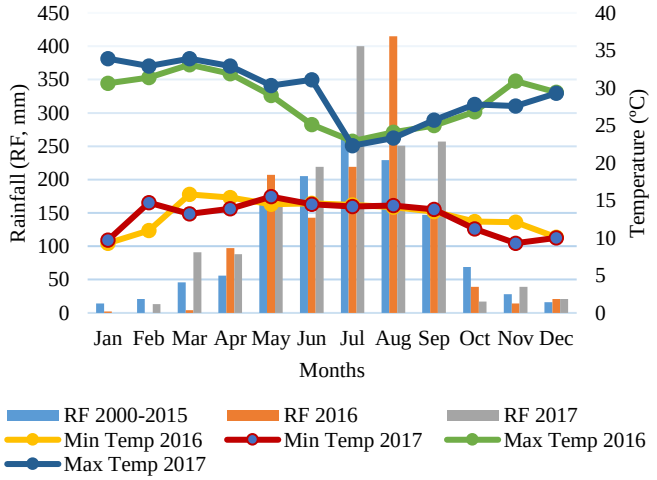

Figure 2c. Mean monthly rainfall and minimum and maximum temperatures at Bonaya Boshe.

Experimental design and treatments

The design was a complete randomized block design with 10 genotypes, 3 locations and 3 replications, giving a total of 30 observations per location. The genotypes tested were: accessions ILRI 14389, ILRI 15473, ILRI 16785, ILRI 16787, ILRI 16798, ILRI 16800, ILRI 16801, ILRI 16804, ILRI 16840 and ILRI 16792. Accession ILRI 16792, the local variety, was included as the standard/check variety for comparison (Control).

Source of planting materials

The 10 genotypes evaluated were selected from a previous screening and preliminary variety trial conducted at Bako Agricultural Research Center on the basis of their yield performance and adaptation to the subhumid climatic conditions of Bako. Cuttings for planting were obtained from International Livestock Centre for Africa (ILCA), now International Livestock Research Institute (ILRI).

Forage yield and calculated yield measurements

Harvesting of forage for data collection was done only once during the first year, while during the second year harvesting was done twice and yields from the 2 harvests combined to provide the total dry matter (DM) yield. Harvesting was done when Napier grass reached about 1.5 m tall, which is the recommended height for harvesting at the Bako Agricultural Research Center. For herbage yield measurements, forage in the middle row of each plot was harvested manually with a sickle at 20 cm above ground level. The fresh forage was weighed with a suspended field balance just after mowing. Then subsamples of 300 g were taken from each replication of each treatment at each location and oven-dried at 65 °C for 72 hours until constant weight to determine forage DM yields. After measuring the fresh forage mass, 5 plants were selected at random from each plot, sorted into leaf and stem and oven-dried to constant weight at 65 °C for 72 hours for estimating leaf:stem ratio on a DM basis. Yields of crude protein (CP) and digestible forage were estimated as a product of total dry forage yield and CP% and in vitro organic matter digestibility, respectively (Schroeder 2013).

Chemical composition and in vitro digestibility analyses

For herbage chemical composition analysis, chopped herbage from the 3 replications was pooled, thoroughly mixed and 1 representative subsample was taken for each accession at each site. The samples were dried in an oven at 65 °C for 72 h and ground to pass through a 1 mm sieve. Then, DM, nitrogen (N) and ash concentrations were

determined according to AOAC (1990), and organic matter (OM) percentage was calculated by deducting the ash concentration from 100. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations were determined using the procedures of Van Soest et al. (1991). In vitro organic matter digestibility (IVOMD) was determined using the Tilley and Terry (1963) method. Metabolizable energy (ME) values were estimated from IVOMD using the equation of Saha et al. (2010): ME (MJ/kg DM) = 0.15 × IVOMD.

Data analyses

Statistical analyses were done using analysis of variance (ANOVA) following the General Linear Model (GLM) procedure of SAS, Version 9.3 (SAS 2007), and significantly different means were separated using the least significant difference (LSD) test at P<0.05. For forage DM, CP and digestible OM yield measurements and leaf:stem ratio, genotypes, year, location and their interactions were considered as independent variables in the model indicated as:

$$Yijkl = \mu + Gi + Ej + Yk + (Gi \times Ej \times Yk) + Bl(j) + eijkl,$$

where:
Yijkl = response variable;
μ = overall mean;
Gi = genotypic effect;
Ej = environmental effect;
Yk = year effect;
Gi×Ej×Yk = interaction effect of genotype, environment and year;
Bl(j) = block effect; and
eijkl is the random error.

For quality traits, since only a composite sample per treatment was taken from each location, location was considered as a replicate and hence the data were subjected to the following model:

$$Yij = \mu + Gi + Ej + eij,$$

where:
Yij refers to the response of forage quality traits;
μ = overall mean;
Gi = effect of genotypes I;
Ej = environmental effect (replicate); and
eij is the random error.

Results

Dry matter, crude protein and digestible organic matter yields and leaf:stem ratio

The results from analysis of variance for DM yield, crude protein (CP) yield and digestible OM yield, and leaf:stem ratio of the 10 Napier grass genotypes (accessions) over the 3 sites are shown in Table 1. All yields were significantly (P<0.01) affected by genotype, location and year with significant interactions, so data for individual sites and treatments are presented.

DM yield varied significantly (P<0.001) and ranged among genotypes from 19.2 to 35.2 t DM/ha at Bako, 31.5 to 50.7 t DM/ha at Bonaya Boshe and 30.5 to 50.0 t DM/ha at Gute (Table 1). The highest mean DM yield over the 3 locations was recorded for ILRI 16804 (45.3 t DM/ha) followed by ILRI 16801 (39.5 t DM/ha) and ILRI 16800 (38.3 t DM/ha). Overall yields at Bonaya Boshe and Gute were higher than at Bako. Yields of CP and OM also

Table 1. Cumulative yields of dry matter, crude protein and digestible organic matter (DOM) and leaf:stem ratios of 10 Napier grass accessions at 3 locations over 2 years (2016 and 2017) in western Oromia, Ethiopia.

Accession	Dry matter yield (t/ha)			Crude protein yield (t/ha)			DOM yield (t/ha)			Leaf:stem ratio		
	Bako	Bonaya	Gute	Bako	Bonaya	Gute	Bako	Bonaya	Gute	Bako	Bonaya	Gute
ILRI 14389	25.6c[1]	40.4c	41.8b	1.76c	3.07c	2.97b	13.6d	20.9c	20.3cd	1.73cd	1.67d	1.81c
ILRI 15743	21.3e	33.8de	36.9c	1.28e	1.99e	2.25c	11.6ef	16.0e	19.2def	1.70cd	1.88bcd	1.86c
ILRI 16785	21.3e	31.5e	36.8c	1.22e	1.86ef	2.25c	9.1g	14.0f	16.9fgh	1.75cd	1.85cd	1.97bc
ILRI 16787	21.4e	32.2de	35.8cd	1.54d	2.22d	2.39c	11.3ef	17.6de	20.1cde	1.88bc	1.86bcd	1.76c
ILRI 16798	19.2f	33.0de	30.5e	1.23e	1.92ef	1.86e	10.8f	16.8de	14.6h	1.76bcd	1.92bc	1.79c
ILRI 16800	28.9b	43.1bc	42.8b	2.08b	2.89c	3.13b	16.3c	21.3c	21.8c	1.98ab	2.09b	2.18ab
ILRI 16801	30.1b	44.6b	43.7b	2.17b	3.57b	3.10b	18.0b	25.9b	26.6b	1.88bc	2.04bc	2.20ab
ILRI 16804	35.2a	50.7a	50.0a	2.78a	4.10a	4.50a	21.5a	30.9a	29.9a	2.17a	2.33a	2.36a
ILRI 16840	22.6de	34.3d	34.3cd	1.22e	2.02de	2.20cd	10.8f	17.1de	16.6gh	1.74cd	1.92bc	1.75c
Control[2]	23.7d	33.5de	33.8d	1.25e	1.71f	1.97de	12.3e	18.0d	17.8efg	1.56d	1.81cd	1.72c
Overall mean	25.0	37.7	38.6	1.65	2.54	2.66	13.5	19.8	20.4	1.82	1.94	1.94
$LSD_{0.05}$	1.54	1.96	2.15	0.11	0.12	0.14	0.87	1.02	1.99	0.22	0.24	0.29
P-level	***	***	***	***	***	***	***	***	***	**	**	***
CV (%)	5.3	4.4	4.8	5.5	4.3	4.6	5.5	4.4	8.4	10.6	10.5	13.0

[1]Means within columns followed by different letters differ significantly (P<0.05). [2]ILRI 16792.

varied significantly (P<0.001) among the tested genotypes as well as between locations (Table 1). CP yields ranged from 1.22 to 2.78 t/ha at Bako, 1.71 to 4.1 t/ha at Bonaya Boshe and 1.86 to 4.5 t/ha at Gute, with the highest values recorded for ILRI 16804 at all locations followed by ILRI 16801 and ILRI 16800. Similarly, digestible OM yield also ranged among genotypes from 9.1 to 21.5 t/ha at Bako, 14.0 to 30.9 t/ha at Bonaya Boshe and 14.6 to 29.9 t/ha at Gute, with the highest values again recorded for ILRI 16804 at all locations followed by ILRI 16801 and ILRI 16800.

As shown in Table 1, the value for leaf:stem ratio ranged among genotypes from 1.56 to 2.17 at Bako (mean 1.82), 1.67 to 2.33 at Bonaya Boshe (mean 1.94) and 1.72 to 2.36 at Gute site (mean 1.94) with the highest value recorded for genotype ILRI 16804 followed by ILRI 16800 and ILRI 16801 across locations (Table 2).

Significant variation in DM (P<0.001), CP (P<0.001) and digestible OM yields (P<0.001) and leaf:stem ratios (P<0.001) were observed among the tested genotypes across locations (Table 2). DM yield ranged from 22.3 to 38.1 t/ha in 2016 and 32.8 to 52.4 t/ha in 2017 with the highest value recorded for genotype ILRI 16804 in both years followed by ILRI 16801 and ILRI 16800. CP yield ranged from 1.35 to 3.19 t/ha in 2016 and 1.91 to 4.39 t/ha in 2017, whereas digestible OM yield ranged from 10.9 to 23.1 t/ha in 2016 and 15.8 to 31.8 t/ha in 2017. Values for leaf:stem ratio ranged among the tested genotypes from 1.57 to 2.27 (mean 1.78) in 2016 and 1.79 to 2.31 (mean 2.01) in 2017. As for DM yield in both years, accession ILRI 16804 gave the highest CP and digestible OM yields plus leaf:stem ratio, followed by accession ILRI 16801 and ILRI 16800. Overall mean DM, CP and digestible OM yields and leaf:stem ratio were higher in 2017 than in 2016 (P<0.0001).

Nutrient composition

Mean nutrient composition of the 10 Napier grass genotypes across the 3 sites is shown in Table 3. Concentrations of CP, OM and ash differed between genotypes (P<0.001). The highest CP concentration (83.3 g/kg DM) occurred in ILRI 16804, followed by ILRI 16801, ILRI 16800, ILRI 16787 and ILRI 14389 (mean 71.6 g/kg DM), while the lowest was recorded for ILRI 16792 (Control), ILRI 16840, ILRI 16798, ILRI 16785 and ILRI 15743 (mean 58.6 g/kg DM). Ash and OM concentrations in the 10 genotypes ranged from 65.7 to 94.3 g/kg DM and 906 to 934 g/kg DM, respectively. Differences in fiber and lignin concentrations were generally non-existent.

In vitro digestibility

Table 4 shows the mean in vitro organic matter digestibility (IVOMD) and metabolizable energy (ME) values for the 10 Napier grass genotypes over the 3 locations. Both quality traits showed marked variation (P<0.001) among genotypes. The highest IVOMD values occurred in accessions ILRI 16804 and 16801 (mean 60.1%) and the lowest in ILRI 16785 (44.3%). ME values were highest in ILRI 16804 and 16801 (mean 9.0 MJ/kg DM).

Table 2. Dry matter (DM), crude protein (CP) and digestible organic matter (DOM) yields and leaf:stem ratios of 10 Napier grass accessions in 2 years across 3 locations in western Oromia, Ethiopia.

Accession	DM yield (t/ha)		CP yield (t/ha)		DOM yield (t/ha)		Leaf:stem ratio	
	2016	2017	2016	2017	2016	2017	2016	2017
ILRI 14389	30.1c[1]	41.9c	2.18c	3.03d	14.6d	21.9d	1.57c	1.91c
ILRI 15743	25.2d	36.2d	1.51de	2.17f	12.8e	18.4f	1.69c	1.93c
ILRI 16785	24.4de	35.4d	1.45ef	2.10f	10.9g	15.8h	1.73c	1.99bc
ILRI 16787	22.9ef	36.6d	1.58d	2.52e	12.6ef	20.2e	1.68c	1.98bc
ILRI 16798	22.3f	32.8e	1.35f	1.99g	11.4fg	16.7g	1.73c	1.92c
ILRI 16800	31.8b	44.7b	2.24c	3.16c	16.4c	23.1c	1.93b	2.24a
ILRI 16801	32.8b	46.1b	2.46b	3.43b	19.5b	27.5b	1.94b	2.14ab
ILRI 16804	38.1a	52.4a	3.19a	4.39a	23.1a	31.8a	2.27a	2.31a
ILRI 16840	25.4d	35.4d	1.51de	2.11f	12.4ef	17.3g	1.68c	1.92c
Control[2]	25.5d	35.1d	1.38f	1.91g	13.5de	18.6f	1.59c	1.79c
Mean	27.9	39.7	1.9	2.7	14.7	21.1	1.78	2.01
$LSD_{0.05}$	1.52	1.56	0.1	0.1	1.35	0.84	0.17	0.20
P-level	***	***	***	***	***	***	***	***
CV (%)	5. 8	4.2	5.7	4.1	9.7	4.2	10.4	10.8

[1]Means within columns followed by different letters differ significantly (P<0.05). [2]ILRI 16792.

Table 3. Chemical composition of 10 Napier grass accessions across 3 locations in western Oromia, Ethiopia.

Accession	Parameter (g/kg DM)					
	Ash	CP	OM	NDF	ADF	ADL
ILRI 14389	80.0bc[1]	72.0b	920bc	640	398b	75.8
ILRI 15743	94.3a	60.0cd	906d	647	437a	73.0
ILRI 16785	87.0ab	59.0cd	913cd	656	432a	78.5
ILRI 16787	79.3c	69.3b	921b	639	436a	77.7
ILRI 16798	87.7a	61.0c	912d	646	443a	75.8
ILRI 16800	68.6d	70.7b	931a	644	432a	79.0
ILRI 16801	77.4c	74.3b	923b	635	433a	75.0
ILRI 16804	65.7d	83.3a	934a	631	432a	73.3
ILRI 16840	78.3c	59.0cd	922b	655	432a	78.5
Control[2]	78.0c	54.0d	922b	655	433a	80.4
Overall mean	79.6	66.3	920	645	431	7.67
$LSD_{0.05}$	0.75	0.64	0.75	2.49	1.54	0.8
CV (%)	5.5	5.6	0.48	2.3	2.1	6.1
P-level	***	***	***	NS	**	NS

[1]Means within columns followed by different letters differ significantly (P<0.05). [2]ILRI 16792. CP = crude protein; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin.

Table 4. In vitro organic matter digestibility (IVOMD) and metabolizable energy (ME) concentration of 10 Napier grass accessions across 3 locations in western Oromia, Ethiopia.

Accession	IVOMD (% DM)	ME (MJ/kg DM)
ILRI 14389	52.5b[1]	7.9b
ILRI 15743	51.2bc	7.7bc
ILRI 16785	44.3d	6.7d
ILRI 16787	54.5b	8.2b
ILRI 16798	51.5bc	7.7bc
ILRI 16800	52.1bc	7.8bc
ILRI 16801	59.6a	8.9a
ILRI 16804	60.7a	9.1a
ILRI 16840	48.6c	7.3c
Control[2]	52.7b	7.9b
Overall mean	52.8	7.9
$LSD_{0.05}$	3.87	0.58
CV (%)	4.3	4.3
P-level	***	***

[1]Means within columns followed by different letters differ significantly (P<0.05). [2]ILRI 16792.

Discussion

The present study has provided useful information to assess the relative performance in terms of both quality and quantity of the 10 Napier grass genotypes under the different environmental conditions tested. While there was significant variation in biomass yield, CP concentration and yield, leaf:stem ratio and in vitro digestibility among genotypes over the 3 locations, ranking of genotypes remained remarkably consistent across the 3 locations. Results obtained in a single environment would give a good indication of how a particular genotype would perform relative to another but absolute yields etc. at a given site could be misleading if applied to a different environment. Significant differences in DM yield have been observed previously among 20 oat varieties and 14 grass pea lines tested across various locations in Ethiopia (Fekede 2004) and Iran (Ahmadi et al. 2012), respectively. In the current study, environmental effects were highlighted by the marked differences in overall yields of DM and CP at Bonaya Boshe and Gute compared with Bako (approximately 50% greater). In a similar fashion, mean DM yields recorded for the genotypes tested, particularly for ILRI 16804, ILRI 16801 and ILRI 16800, were higher than the values reported by Tessema (2005), who studied 10 Napier grass accessions (labeled as ILRI 14983, ILRI 14984, ILRI 15743, ILRI 16834, ILRI 16835, ILRI 16786, ILRI 16791, ILRI 16798, ILRI 16836 and local check) at Adet Agricultural Research Center, Ethiopia. As reported by Boonman (1997), agro-meteorological variables such as rainfall, soil fertility, air temperature and wind have major impacts on crop growth and development.

In evaluating forage crops, it is well accepted that DM yield should not be considered as the sole parameter for evaluating a species. Quality of the forage produced must be considered along with DM yields to give nutrient yields, as the optimal time to utilize a forage is always a compromise between DM yield and CP concentration plus digestibility. Including CP% and DM digestibility with DM yield in determining the overall value of a forage provides an overall picture of the nutritional value of a crop. In the current study, despite variation between sites, there was surprising consistency across the 3 different locations in terms of the genotypes which performed at

the highest level. Not only did ILRI 16804 produce the highest DM yields at all sites, but also it had the highest CP% and equal highest in vitro digestibility. ILRI 16804, ILRI 16801 and ILRI 16800 consistently outperformed the local Control variety (accession ILRI 16792), indicating that local farmers could improve production by changing the genotype they are growing.

The higher leaf:stem ratio recorded for accessions ILRI 16804, ILRI 16800 and ILRI 16801 reinforced their superiority over other genotypes in terms of DM yield, given that leaf is generally of higher nutritive value than stem (Islam et al. 2003). The overall mean leaf:stem ratio recorded across locations in the current study (1.82–1.94) was lower than the 1.7–3.1 reported by Nyambati et al. (2010). This difference might be attributed to environmental variation where the studies were carried out as well as genetic variability among the tested genotypes and stage at which they were harvested.

The higher yields of DM, CP and digestible OM, and higher leaf:stem ratio observed during the second production year might be attributed predominantly to the perennial nature of Napier grass, which produces more tillers and higher vegetative growth as the pasture develops following planting. Fekede et al. (2005) and Tessema and Alemayehu (2010) reported that tiller production by Napier grass increases with time after planting and density of vegetative growth increases as the pasture consolidates due to the perennial nature of the grass. In addition to stand development, the higher and better distributed rainfall received during the second production year across the 3 locations could be expected to produce growth superior to that recorded in the establishment year.

Forage quality is highly variable among and within forage types with forage species, variety and stage of maturity at harvest all contributing to this variation (NRC 2000). CP concentrations obtained (54–83.3 g/kg DM) in the current study span the minimum level of CP required for effective rumen function reported in the literature. Van Soest (1982) reported that, at CP concentrations less than 70 g CP/kg DM, rumen function suffers and feed intake in ruminants can be depressed. While most genotypes studied either failed to reach this level or barely did so, ruminants fed on this forage as cut-and-carry would require protein supplements, particularly so lactating animals. However, stage of maturity at which the forage is harvested plus soil fertility have a marked influence on CP%. Harvesting more frequently would provide forage with higher CP%. Only ILRI 16804 in this study produced forage with CP% considered acceptable for providing a diet above a maintenance level (Norton 1982). Even though NDF concentrations did not vary significantly among genotypes, the range (631–656 g/kg DM) was slightly below the average value of 662 g/kg DM reported for tropical grasses (Barton et al. 1976). The overall mean of ADF recorded in the current study was 431 g/kg DM which is comparable with the findings reported by Keba et al. (2013) who studied the nutritive value of common grass species in the semi-arid rangelands of Borana, southern Ethiopia.

Digestibility of a forage is important because of its influence on energy and protein extracted by animals and feed intake in ruminant production systems. The IVOMD values (44.3–60.7%) obtained in the current study fall within the general range reported for tropical grasses of 50–60% (Owen and Jayasuriya 1989). With the exception of genotypes ILRI 16785 (44.3%) and ILRI 16840 (48.6%), which had relatively low digestibility percentages, all Napier genotypes, especially ILRI 16804 (60.7%) and ILRI 16801 (59.6%), produced forage which would be quite acceptable for ruminant nutrition. The ME values obtained in this study (6.7–9.1 MJ/kg DM) agree with the values reported for other tropical grasses, e.g. 7.1–9.4 MJ/kg DM by Krishnamoorthy et al. (1995) and 5.76–9.12 MJ/kg DM by Nogueira Filho et al. (2000).

The variation in performance of the different genotypes in the 3 locations indicates the potential for selecting superior genotypes in terms of both yield and quality for use in environments suitable to those involved. The consistent high performance of ILRI 16804, ILRI 16800 and ILRI 16801 across the 3 sites relative to the Control accession ILRI 16792 suggests that further studies be carried out with these genotypes in the area as well as similar environments. These studies should examine more frequent harvesting of the forage in an endeavor to improve the quality of forage without significant yield depression. Demonstrations on farms should encourage farmers to replace the existing variety with these apparently superior lines. These future studies should focus not only on performance of the grass but also the performance of ruminants fed on the forage to determine if the apparent better quality of the forage produced by these new genotypes is reflected in superior animal performance.

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Research Paper

Fermentation quality and aerobic stability of Napier grass ensiled with citric acid residue and lactic acid bacteria

Calidad fermentativa y estabilidad aeróbica del pasto Napier ensilado con residuo de ácido cítrico y bacterias ácido-lácticas

XUXIONG TAO[1], CHONGWEN JI[1], SIFAN CHEN[1], JIE ZHAO[1], SIRAN WANG[1], JUNFENG LI[1], FUXIN SUN[2] AND TAO SHAO[1]

[1]*College of Agro-grassland Science, Nanjing Agricultural University, Nanjing, China.* cyxy.njau.edu.cn
[2]*Jiangsu Guoxin Union Energy Co. Ltd, Yixing, China.*

Abstract

This study was conducted to investigate the effects of adding citric acid residue (CAR) with or without lactic acid bacteria (LAB) to Napier grass (*Cenchrus purpureus*; syn. *Pennisetum purpureum*) cv. Sumu No. 2 at ensiling on the fermentation quality and aerobic stability of the resulting silage. Treatments included: Control (Napier grass forage without additives); and Napier grass inoculated with lactic acid bacteria (*Lactobacillus plantarum* and *L. buchneri*) at 1×10^6 cfu/g fresh weight (FW) forage (LAB) or 36 g citric acid residue/kg FW forage (CAR) or a mixture of CAR and LAB (CL). Forty-five days after ensiling the silages were tested for chemical and microbial composition and an aerobic stability test was conducted. The addition of CAR with or without LAB increased the DM and lactic acid concentrations in silage and decreased pH plus acetic acid, ammonia nitrogen (NH_3-N), neutral detergent fiber and cellulose concentrations relative to Control. The pH in LAB silage was lower than in Control, while lactic acid concentration was higher. During the first 2 days of aerobic exposure, all additives increased the water-soluble carbohydrate (WSC) and lactic acid concentrations and decreased pH plus NH_3-N and acetic acid concentrations. Moreover, CL silages had the highest WSC and the lowest NH_3-N and acetic acid concentrations during aerobic exposure. However, all additives failed to improve the aerobic stability of the silage. While CAR with or without LAB inoculant improved the fermentation quality of silage made from Napier grass, more studies are warranted to identify additives which can improve aerobic stability of the silage after opening.

Keywords: Aerobic deterioration, antibacterial effect, *Cenchrus purpureus*, ensilage, silage additives.

Resumen

En Nanjing, provincia de Jiangsu, China, se investigaron los efectos de la adición de residuo de ácido cítrico con o sin bacterias ácido-lácticas, al ensilar pasto Napier (*Cenchrus purpureus*; sin. *Pennisetum purpureum*) cv. Sumu No. 2, en la calidad fermentativa y estabilidad aeróbica del ensilaje resultante. Los tratamientos incluyeron: Testigo (pasto sin aditivos); y pasto inoculado con bacterias ácido-lácticas (*Lactobacillus plantarum* y *L. buchneri*) a una concentración de 1×10^6 ufc/g de peso fresco del forraje (LAB) o 36 g de residuo de ácido cítrico/kg de forraje (CAR) o una mezcla de CAR y LAB (CL). Cuarenta y cinco días después del ensilado, se analizaron la composición química y microbiana de los ensilajes y se realizó una prueba de estabilidad aeróbica. En relación con el Testigo, la adición de CAR con o sin LAB aumentó las concentraciones de materia seca y ácido láctico en el ensilaje y disminuyó el pH, las concentraciones de ácido acético, nitrógeno amoniacal (NH_3-N), fibra detergente neutro y celulosa. El pH en el ensilaje LAB fue menor que en el Testigo, mientras que la concentración de ácido láctico fue mayor. Durante los primeros dos días de exposición aeróbica, todos los aditivos aumentaron las concentraciones de carbohidratos solubles en agua (WSC) y ácido láctico, y disminuyeron el pH y las concentraciones de NH_3-N y ácido acético. Además, los ensilajes CL presentaron las concentraciones más altas de WSC y las concentraciones más bajas de NH_3-N y ácido acético durante la exposición aeróbica. Sin embargo, los aditivos no mejoraron la estabilidad aeróbica del

Correspondence: Tao Shao, Institute of Ensiling and Processing of Grass, College of Agro-grassland Science, Nanjing Agricultural University, Weigang 1, Nanjing 210095, China.
Email: taoshaolan@163.com

ensilaje del pasto Napier. Si bien el CAR con o sin inoculante LAB mejoró la calidad fermentativa del ensilaje, se necesitan más estudios para identificar aditivos que mejoren la estabilidad aeróbica después de la apertura del silo.

Palabras clave: Aditivos de ensilaje, *Cenchrus purpureus*, deterioro aeróbico, efecto antibacteriano, ensilado.

Introduction

Napier grass [*Cenchrus purpureus* (Schumach.) Morrone; syn. *Pennisetum purpureum* Schumach.] is a fast-growing forage widely cultivated in tropical areas due to its high potential dry matter (DM) yield (Bureenok et al. 2012). The forage is an important crop for biofuel and animal feed production and is routinely stored by ensiling for feeding ruminants year-round. However, high quality silage of Napier grass is difficult to produce because the coarse and stemmy structure of the forage leads to poor compaction during silage preparation (Desta et al. 2016). The presence of excess air in forage mass at ensiling encourages the growth of undesirable microorganisms during the initial stages of ensiling, inducing abundant loss of nutrients. Thus, various biological and chemical additives have been developed to improve the fermentation quality of Napier grass (Ferreira et al. 2013; Desta et al. 2016; Khota et al. 2018).

Organic acids, e.g. formic, acetic and propionic, are common additives at ensiling that cause a rapid reduction in pH and suppression of undesirable bacteria, thereby improving silage quality (Muck et al. 2018; Wilkinson and Rinne 2018). However, the use of organic acids increases the cost of silage making. Citric acid residue (CAR) is the main by-product of citric acid production, which contains some citric acid, crude protein and other nutrients. Citric acid is widely used in food preparation owing to its safety and antibacterial properties (Bou et al. 2017). With the rapid growth in demand for citric acid in the food industry, the amount of CAR generated from citric acid production has increased in recent years (Zhang et al. 2014). A large amount of this CAR is merely discarded, which is a wasted resource and might cause environmental pollution. Previous studies reported that citric acid improved the fermentation quality of alfalfa silage, limiting proteolysis and improving poly-unsaturated fatty acid composition during ensiling (Ke et al. 2017; 2018). In addition, feeding trails confirmed that citric acid increased feed digestion and utilization in the diet of steers (Wang et al. 2009). While CAR might have similar effects on the fermentation quality of silage, literature on the incorporation of CAR during silage making is scarce, and further investigation is needed.

Lactic acid bacteria (LAB) are also commonly applied during silage making, based on ensuring the presence of enough efficient LAB during ensiling to enhance lactic fermentation (Moselhy et al. 2015). Citric acid can be utilized by some LAB strains, which might promote the growth of these LAB (McDonald et al. 1991). Therefore, inoculating CAR-treated silage with LAB might have a synergistic effect on fermentation quality. The objective of this study was to investigate the effects of adding CAR with or without LAB inoculant at ensiling on fermentation quality and aerobic stability of Napier grass silage.

Materials and Methods

Silage preparation

Napier grass cv. Sumu No. 2 was cultivated in an experimental field of Nanjing Agricultural University, located in Nanjing, Jiangsu, China (32°03′ N, 118°88′ E; 20 masl). The grass was harvested at the heading stage (approximately 2.5 m tall) and chopped into lengths of about 2–3 cm with a forage cutter (93ZT-300, Xingrong Co. Ltd, Guangzhou, China). LAB inoculant and CAR were used as additives in the experiment. The LAB inoculant was a mixture of *Lactobacillus plantarum* MTD-1 (Ecosyl Products Ltd, Stokesley, UK) and *Lactobacillus buchneri* 40788 (Lallemand Animal Nutrition, Milwaukee, WI, USA) at a ratio of 1:1. The chemical composition of CAR (Jiangsu Guoxin Union Energy Co. Ltd, Yixing, China) is shown in Table 1. The chopped Napier grass was treated in various ways to form the different treatments: (1) Napier grass without additives (Control); (2) Napier grass with LAB inoculant (LAB); (3) Napier grass with 36 g CAR/kg fresh weight (CAR); and (4) Napier grass with 36 g CAR/kg fresh weight + LAB inoculant (CL). The LAB inoculant was dissolved in sterile distilled water and sprayed on each replicate of LAB and CL treatments (5 mL/kg fresh weight) to give an equivalent of 1×10^6 colony-forming units (cfu)/g fresh weight before thorough mixing with the chopped forage. The CAR was added manually to chopped forage for each replicate of CAR and CL treatments and mixed thoroughly. The same amount of sterile distilled water was applied to the Control and CAR treatments. Approximately 3.2 kg treated forage was packed into 5 L laboratory silos (polyethylene bottles with a diameter of 17.3 cm and a height of 26.5 cm; Lantian Biological Experimental Instrument Co. Ltd, Jiangsu, China). The silos were stored at ambient temperature (17–22 °C) after being sealed with screw tops and plastic tape. Five silos for each treatment were opened after 45 days of ensiling for subsequent analyses.

Table 1. Chemical composition of citric acid residue.

Parameter	Value
pH	2.50
Concentrations (% FW)	
Dry matter	44.0
Citric acid	6.00
Crude protein	11.5
Crude fiber	24.3
Water soluble carbohydrate	ND
Ether extract	1.20
Crude ash	0.80

FW - fresh weight; ND - not detected.

Aerobic stability

Two kg of silage was placed loosely in 5 L plastic buckets (5 replicates for each treatment) without sealing to monitor aerobic stability. Each bucket was covered with a layer of cheesecloth to avoid contamination but allow air flow. Thermocouple wires were placed at the center of the silage mass and connected to a data logger (SMOWO MDL-1048A, Tianhe Automation Instrument Co. Ltd, Shanghai, China) that measured the temperature every 30 min for 6 days. When the temperature of the silage increased by 2 °C above ambient temperature (17–22 °C), the silage was considered to be undergoing aerobic deterioration. Subsamples of the air-exposed silages (100 g) were removed from each plastic bucket after 2, 4 and 6 days to quantify the levels and rates of change of chemical and microbial compositions.

Chemical and microbial analyses

At silo opening, a cold-water extract was prepared by blending a 60 g sample of silage with 120 mL distilled water and stored in a refrigerator at 4 °C for 24 h. The extracts were then filtered through 2 layers of cheesecloth and Whatman filter paper (11 µm pore size, Xinhua Co., Hangzhou, China) and the pH of the filtrate was measured immediately with a pH meter (HANNA pH 211, Hanna Instruments Italia Srl, Villafranca Padovana, Italy). The filtrate was stored at -20 °C for subsequent determination of ammonia nitrogen (NH_3-N) and organic acids. After thawing, the filtrate was centrifuged for 10 min at 4 °C (10,000 × G) and filtered through a microfilter (0.22 µm) for determination of organic acids and ethanol, which was carried out using Agilent 1260 HPLC system (Agilent Technologies Inc., Waldbronn, Germany) equipped with a refractive index detector (column: Carbomix® H-NP5, Sepax Technologies Inc., Newark, DE, USA; eluent: 2.5 mmol/L H_2SO_4, 0.5 mL/min; temperature: 55 °C). The NH_3-N was determined by the phenol-hypochlorite reaction (Broderick and Kang 1980), while buffering capacity was determined according to the method described by Playne and McDonald (1966).

The dry matter (DM) concentrations of fresh material and silages were determined by drying samples in a forced-draft oven to a constant weight at 60 °C for 72 h, and then ground through a 1 mm screen in a laboratory knife mill (FW100, Taisite Instrument Co. Ltd, Tianjin, China). The ground samples were analyzed for: water-soluble carbohydrates (WSC) by colorimetry after reaction with anthrone reagent (Arthur Thomas 1977); total nitrogen (TN) by a Kjeldahl nitrogen analyzer (Kjeltec 8200, FOSS, Hillerød, Denmark); and neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Van Soest et al. (1991), using the ANKOM filter bag technique with an ANKOM 200i fiber analyzer (ANKOM Technologies Inc., Fairport, NY, USA). Crude protein (CP) was calculated as TN% × 6.25. Hemicellulose concentration was calculated as NDF minus ADF, and cellulose concentration as ADF minus ADL.

Approximately 10 g of fresh material or silage was serially diluted 10-fold with sterilized saline solution (0.85% sodium chloride). The LAB were enumerated on deMan, Rogosa and Sharp agar medium after incubation in an anaerobic incubator at 37 °C for 2 days. Yeasts and molds were enumerated on potato dextrose agar with 0.25% chloramphenicol (Sincere Biotech Co. Ltd, Shanghai, China) after incubation at 30 °C for 3 days.

Statistical analyses

The microbial data were converted to log10. Since the experiment had a completely randomized design, all data were analyzed using the General Linear Model (GLM) procedure of SPSS 22 software. Effects of additives on fermentation characteristics and microbial composition during ensiling were subjected to one-way ANOVA with the model: $Y_{ij} = u + T_i + E_{ij}$, where: u is general mean; T_i is the fixed effect of treatment; and E_{ij} is experimental error. The aerobic stability parameters, including pH, plus WSC, NH_3-N, lactic and acetic acid concentrations and the counts of yeasts and molds, were analyzed via repeated measures ANOVA in a GLM with additives, days of air exposure and their interaction in the model. Means of different treatments were compared for significance by Duncan's multiple range test and significance was declared at $P<0.05$

Results

As shown in Table 2, the DM concentration of fresh Napier grass was 249 g/kg FW, while chemical composition was (g/kg DM): CP 64.2; WSC 58.5; NDF 674; and ADF 416. Buffering capacity was 49.7 meq/kg DM and numbers of LAB and yeasts plus molds were 5.08 and 4.38 log cfu/g FW, respectively.

Table 2. Chemical and microbial composition of fresh Napier grass at ensiling.

Parameter	Concentration
Dry matter (g/kg FW)	249
Crude protein (g/kg DM)	64.2
Water soluble carbohydrate (g/kg DM)	58.5
Neutral detergent fiber (g/kg DM)	674
Acid detergent fiber (g/kg DM)	416
Buffering capacity (meq/kg DM)	49.7
Lactic acid bacteria (log cfu/g FW)	5.08
Yeasts and molds (log cfu/g FW)	4.38

FW - fresh weight; DM - dry matter; cfu - colony-forming units. Data presented represent means of 5 replicates.

At 45 days after ensiling, all silages containing additives had lower pH and counts of yeasts and molds but higher lactic acid concentrations than the Control (Table 3). Addition of CAR significantly increased (P<0.05) DM concentration and decreased (P<0.05) acetic acid, propionic acid, NH_3-N, NDF and cellulose concentrations compared with Control. Concentrations of WSC in silage containing only LAB were lower than those of the Control silage but the difference just failed to reach significance (P = 0.054). Adding LAB at ensiling increased the numbers of LAB in silage (P = 0.049) compared with the Control, while CAR silage was intermediate. Adding LAB plus CAR at ensiling significantly decreased (P<0.05) hemicellulose concentration compared with the Control.

The effects of treatments on aerobic stability of Napier grass silages are shown in Figures 1 and 2. All silages remained stable for more than 60 h after being exposed to the atmosphere. However, none of the additives was able to improve the aerobic stability. The additive treatments, days of aerobic exposure and their interactions had significant (P<0.05) effects on pH, concentrations of WSC, NH_3-N, lactic acid and acetic acid, plus counts of yeasts and molds during exposure to air. All silages showed an increase in pH and decreases in lactic acid and acetic acid concentrations with the progression of exposure to air. This effect was most pronounced between Days 2 and 4 of aerobic exposure. Associated with these changes was a marked increase in NH_3-N concentrations, except in the silage containing both LAB and CAR. The WSC concentrations remained basically stable in silages containing LAB but declined in both CAR and Control, with the greatest decline in Control (P<0.05). Counts of yeasts and molds increased rapidly in all silages during the first 4 days of exposure but then plateaued.

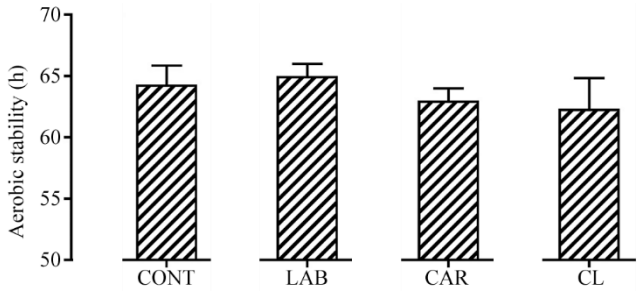

Figure 1. Effect of adding lactic acid bacteria and citric acid residue at ensiling on aerobic stability of Napier grass silages. CONT - Control; LAB - 1 × 10^6 log cfu/g lactic acid bacteria (*Lactobacillus plantarum* + *L. buchneri*) added at ensiling; CAR - 36 g/kg citric acid residue added at ensiling; CL - CAR + LAB. There was no significant difference between treatments.

Table 3. Final composition of Napier grass silage 45 days after ensiling.

Parameter	CONT	LAB	CAR	CL	s.e.m.	P value
DM (g/kg FW)	232b	241ab	253a	260a	3.99	0.033
pH	4.70a	3.88b	3.78b	3.51b	0.149	<0.001
Lactic acid (g/kg DM)	17.2b	30.9a	28.9a	35.4a	2.36	0.011
Acetic acid (g/kg DM)	19.3a	16.0a	9.75b	6.22b	1.71	0.025
Propionic acid (g/kg DM)	0.42a	0.61a	0.07b	0.17b	0.069	0.001
Butyric acid (g/kg DM)	0.41	0.26	0.35	0.15	0.046	0.195
Ethanol (g/kg DM)	26.1	15.9	21.5	20.5	1.57	0.142
WSC (g/kg DM)	8.76	6.41	9.54	10.1	0.553	0.054
NH_3-N (g/kg TN)	66.6a	62.6a	23.4c	31.6b	5.77	<0.001
LAB (log cfu/g FW)	7.55b	8.40a	7.97ab	8.35a	0.132	0.049
Yeasts and molds (log cfu/g FW)	5.47a	4.06c	4.76b	4.45bc	0.178	0.006
NDF (g/kg DM)	685a	674a	625b	619b	9.39	<0.001
ADF (g/kg DM)	428	418	391	398	6.10	0.084
ADL (g/kg DM)	52.3	51.4	46,8	59.7	1.57	0.974
Hemicellulose (g/kg DM)	257a	256a	233ab	221b	5.72	0.018
Cellulose (g/kg DM)	376a	366ab	344bc	338c	5.47	0.023

DM - dry matter; FW - fresh weight; WSC - water soluble carbohydrate; LAB - lactic acid bacteria; NDF - neutral detergent fiber; ADF - acid detergent fiber; ADL - acid detergent lignin; CONT - Control; LAB - 1 × 10^6 log cfu/g lactic acid bacteria (*Lactobacillus plantarum* + *L. buchneri*) added at ensiling; CAR - 36 g/kg citric acid residue added at ensiling; CL - CAR + LAB. Data presented represent means of 5 replicates. Values in the same row without common letters are significantly different (P<0.05).

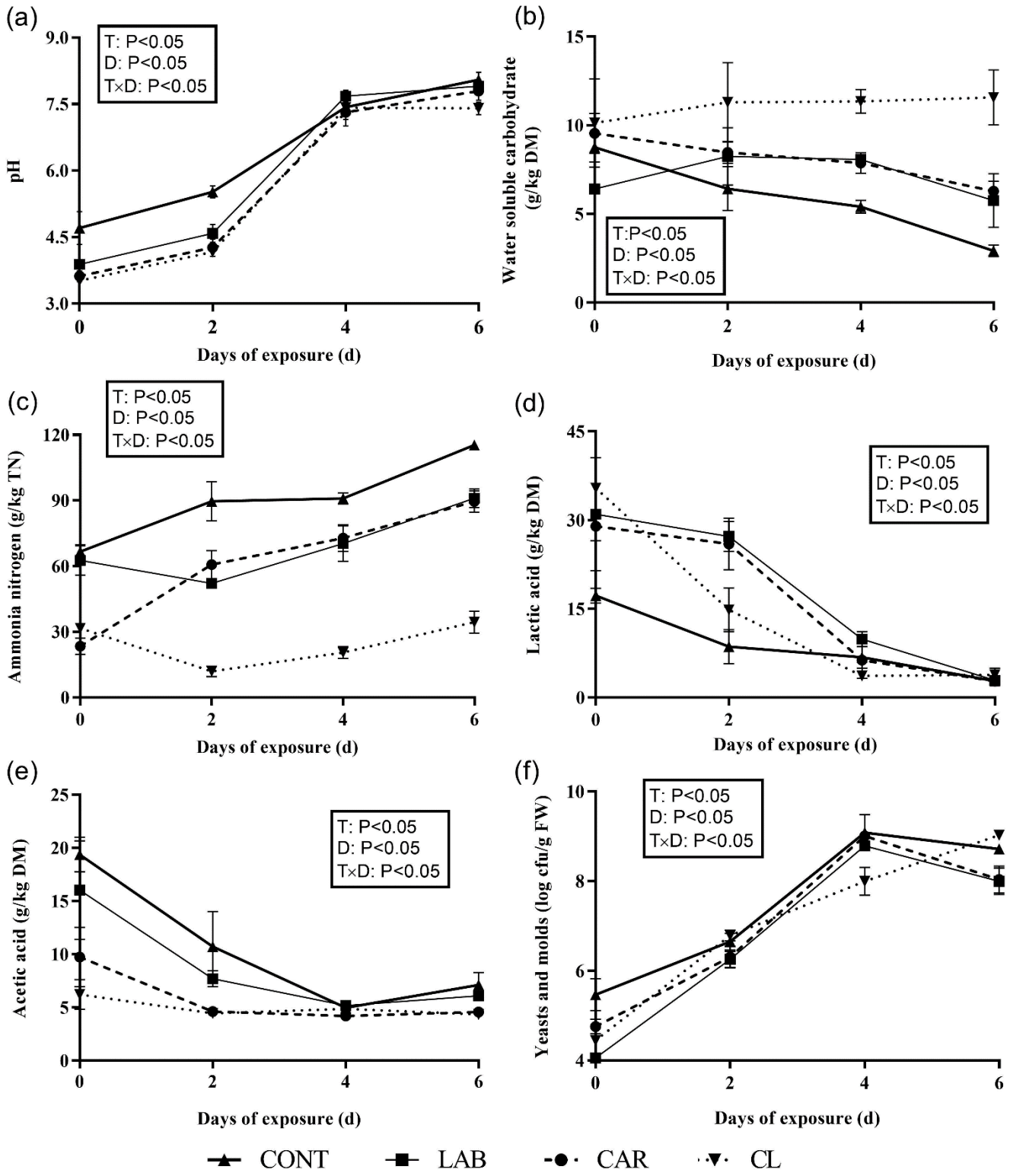

Figure 2. Changes in: (a) pH; (b) water soluble carbohydrate; (c) ammonia nitrogen (NH_3-N); (d) lactic acid; (e) acetic acid; and (f) yeasts and molds in Napier grass silage during aerobic exposure. FW - fresh weight; DM - dry matter; NH_3-N - ammonia nitrogen; TN - total nitrogen; cfu - colony-forming units; CONT - Control; LAB - 1×10^6 log cfu/g lactic acid bacteria (*Lactobacillus plantarum* + *L. buchneri*) added at ensiling; CAR - 36 g/kg citric acid residue added at ensiling; CL - CAR + LAB. Data presented represent means of 5 replicates. T - effect of treatment; D - effect of length of air exposure; T × D - interaction between treatment and length of exposure ($P<0.05$).

Discussion

Fermentation quality

As a tropical forage, Napier grass has a low DM and high fiber concentration (at certain stages of harvesting and rainfall conditions), which is beneficial for the growth of undesirable bacteria like clostridia during ensiling (Khota et al. 2018). Achieving low pH during the initial stage of ensiling could effectively inhibit the activity of undesirable bacteria and decrease the loss of nutrients (McDonald et al. 1991). In addition, wilting to reduce the moisture concentration in forage prior to ensiling is an option to inhibit the activity of undesirable bacteria in silage. In this study, all additives had a positive effect on the fermentation quality of Napier grass, as indicated by the lower pH and higher lactic acid concentrations. In detail, CAR-treated silages had lower pH plus acetic acid and NH_3-N concentrations and higher DM and lactic acid concentrations than silages without CAR, indicating that CAR had a positive effect in improving fermentation quality of Napier grass. In agreement with our results, Ke et al. (2017) found that treating alfalfa with 0.1% or 0.5% citric acid (0.22% in this study) at ensiling improved fermentation quality and limited proteolysis, which might be related to the antibacterial properties of citric acid and the direct acidification it produces (Bou et al. 2017). The antibacterial activity of citric acid during ensiling was confirmed by Lv et al. (2020), who found that application of citric acid to *Amomum villosum* at ensiling decreased the number of undesirable bacteria, such as *Enterobacter*, *Escherichia*, *Shigella* and *Pantoea*. In addition to containing citric acid, CAR is rich in crude fiber, crude protein and other nutrients that could provide additional substrates and thereby increase DM% in CAR-treated silages. Furthermore, by limiting the activity of undesirable bacteria, adding CAR at ensiling reduced substrate losses in these silages, as evidenced by the higher DM concentration in silages containing CAR.

Compared with the Control silage, the higher lactic acid and lower acetic acid concentrations, observed in CAR-treated silages, indicate that adding CAR to forage at ensiling favors lactic fermentation. Besides, more substrates would have been converted to lactic acid by LAB, because CAR inhibited the activity of undesirable bacteria. The results of Ke et al. (2018) showed that treating alfalfa with citric acid with or without LAB at ensiling increased lactic acid concentration in the resulting silage compared with that of the Control. Inoculation with LAB also increased the lactic acid concentration due to the predominant population of LAB during the initial stages of ensiling. However, a combination of CAR and LAB had no synergistic effects on lactic acid production, which is probably because the low pH caused by CAR limited the effects of LAB inoculant. Compared with the Control silages, ethanol concentrations in silages with additives were relatively low. This would probably be due to the lower yeast counts in these silages. High concentrations of ethanol in silages are often associated with high populations of yeast (Kung et al. 2018), and ethanol is considered to be the main fermentation product of yeasts.

During ensiling, substantial amounts of forage proteins are degraded into peptides and amino acids, the latter being further deaminated to NH_3-N and decarboxylated to amines (Rooke and Hatfield 2003; Scherer et al. 2019). In general, plant enzymes play a major role in proteolysis, and low pH can inhibit their activity (Ding et al. 2013). In the present study, lower NH_3-N concentrations in CAR and CL silages were mainly attributed to the lower pH caused by the acidification produced by CAR and the accumulation of lactic acid. It is consistent with the results of Lv et al. (2020), that a decrease of NH_3-N concentration was observed in silage treated with citric acid.

To improve fiber digestibility in ruminant diets, interest in improving degradation of structural carbohydrates (especially lignin and ADF, which cannot be fermented by the ruminal microflora) of silage has increased in recent years (Lynch et al. 2014). Acidolysis, enzymatic action and microbial activity are considered the primary factors influencing degradation of structural carbohydrates (Zhao et al. 2018). In the present experiment, CAR-treated silages showed lower NDF, cellulose and hemicellulose concentrations than the Control. It is possible that CAR and organic acids added during ensiling have a hydrolyzing effect on the structural carbohydrates. A previous study reported that treating Napier grass with formic acid at ensiling reduced structural carbohydrates and increased WSC concentrations in silage relative to silage without additives (Desta et al. 2016). Thus, the relatively high WSC concentrations in CAR-treated silages might be due to the degradation of structural carbohydrates.

Aerobic stability

When silos are opened for feeding, the silage is exposed to air. Under the aerobic conditions, undesirable bacteria, which remain dormant in the absence of air, multiply, resulting in a deterioration of the silage (McDonald et al. 1991). This deterioration is usually manifested as a rise in temperature. In this study, none of the additives was able to delay the aerobic deterioration of Napier grass silage (Figure 1).

During the first 4 days of aerobic exposure, the rapid increase in pH and counts of yeasts and molds plus the sharp decrease in lactic and acetic acid concentrations, observed in all silages, indicated that all silages underwent aerobic deterioration during this period. However, silages treated with additives (especially CAR combined with LAB) had lower pH plus NH_3-N and acetic acid concentrations and

higher WSC and lactic acid concentrations than Control during the first 2 days of exposure to conditions, which suggested that these additives can preserve stability of silage for the first 2 days of exposure to air. These results provide a valuable guide for use when opening silos. Generally, yeasts are considered to play the main role in aerobic deterioration (Pahlow et al. 2003). The lactic acid and WSC, which remain in silages when opened, are potential sources of readily available substrates for the growth of yeasts, when the silages are exposed to air (Wilkinson and Davies 2013). Application of CAR at ensiling of the forage failed to improve aerobic stability of the resulting silage, which could be related to the relatively high WSC concentration in the CAR-treated silages. In the study of Adesogan and Salawu (2002), addition of formic acid at ensiling increased residual WSC concentration and improved aerobic stability in pea-wheat bi-crop silages. The reason for the different findings may relate to the different forage species involved. Ke et al. (2017) reported that adding citric acid to alfalfa at ensiling promoted the growth of yeasts in the resulting silage, which might explain the comparable aerobic stability times displayed by the CAR-treated and Control silages. Hetero-fermentative LAB like *Lactobacillus buchneri* are commonly added to forage at ensiling to improve aerobic stability of silage, although inoculation of forage with LAB had no positive effects on aerobic stability of the resulting silage in this study. This is possibly related to the lower acetic acid concentrations in the silage. The LAB inoculant might have specificity for different forage species. It is interesting that aerobic stability of all silages in the study exceeded 60 hours, even though growth of yeasts and molds occurred rapidly in all silages during the first 2 days of aerobic exposure. An explanation for this phenomenon could be that high moisture levels in ensiled material required more energy for heating (Tomaz et al. 2018). The low DM concentrations (maximum 26%) in all silages are likely to have delayed the rise of temperature.

Conclusions

The results of this study have demonstrated the benefits of using CAR as an additive when ensiling Napier grass. While addition of CAR alone or in combination with LAB improved the fermentation quality of the silage, no positive effects on aerobic stability of the silage were observed. Further investigations of the effects of CAR combined with other inhibitors of aerobic deterioration in silage making seem warranted, as the search for additives to improve aerobic stability of silages continues.

Competing interests

The authors have declared that no competing interests exist.

Acknowledgments

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(Note of the editors: All hyperlinks were verified 10 January 2020.)

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Research Paper

Selection for resistance to fungal diseases and other desirable traits in kikuyu grass (*Cenchrus clandestinus*)

Selección por resistencia a enfermedades fungosas y otras características deseables en germoplasma del pasto kikuyo (Cenchrus clandestinus) *en Australia*

WILLIAM J. FULKERSON[1], NATHAN R. JENNINGS[2], MARK CALLOW[1], KAREN J. HARPER[3], PERCY T.W. WONG[4] AND PETER M. MARTIN[4]

[1]*Research and Development Office, Norco Co-operative Ltd, Lismore, NSW, Australia.* norco.com.au
[2]*North Coast Local Land Services, Lismore, NSW, Australia.* northcoast.lls.nsw.gov.au
[3]*School of Agriculture and Food Sciences, The University of Queensland, Lawes, QLD, Australia.* gatton.uq.edu.au
[4]*Plant Breeding Institute, University of Sydney, Cobbitty, NSW, Australia.* sydney.edu.au

Abstract

While kikuyu (*Cenchrus clandestinus*) is an important grass for dairy and beef production in the subtropical region of Australia and the world, the most common cultivar, Whittet, is seriously affected by the fungal diseases, kikuyu yellows (*Verrucalvus flavofaciens*) and black spot (*Bipolaris* spp.). Thus resistance to these diseases is a priority in selecting a better kikuyu cultivar, along with higher herbage quality and yield and better winter growth. A study was conducted to identify suitable candidates from kikuyu ecotypes collected along the east coast of Australia plus lines obtained by subjecting Whittet to a mutagenic agent. Initial glasshouse studies identified 19 lines that were resistant to the KY1A strain of kikuyu yellows and 4 of these, with forage quality and yield superior to Whittet, were further evaluated in the field at 2 sites using Whittet as the control. At Site 1, line 12A demonstrated a much higher level of resistance to kikuyu yellows than Whittet, with 85% of plants resisting infection compared with only 15% of Whittet plants. At Site 2, the numbers of 12A and Whittet plants infected were similar. Further tests, using kikuyu yellows inoculum collected from 11 sites along the east coast of Australia, found that only 15% of 12A plants became infected compared with 61% of Whittet plants. Thus, kikuyu line 12A was resistant to most, but not all, strains of the kikuyu yellows pathogen. Annual yield of 12A (19,008 kg DM/ha) was 24% higher than that of Whittet and 12% higher than Acacia, but the difference was significant only for Whittet. During summer, 12A produced 10,212 kg DM/ha (24% higher than Whittet), was more active in early spring, had slightly higher dry organic matter digestibility (66.7 vs. 64.0%) and was resistant to black spot infection.

Keywords: Black spot (*Bipolaris* spp.), cow preference, forage quality, kikuyu yellows (*Verrucalvus flavofaciens*), tropical pasture.

Resumen

El pasto kikuyo (*Cenchrus clandestinus*) es una especie ampliamente utilizada para la producción de ganado de leche y carne en la región subtropical de Australia. El cultivar (cv.) Whittet es el más utilizado; no obstante es seriamente afectado por dos enfermedades fungosas, el amarillamiento de kikuyo, causado por *Verrucalvus flavofaciens*, y la mancha negra (*Bipolaris* spp.). Por tanto, la resistencia a estas enfermedades es una prioridad en el desarrollo de mejores cultivares de esta gramínea, conjuntamente con una mayor producción y calidad del pasto y mejor crecimiento en época de invierno. En New South Wales, Australia, se realizó un estudio para seleccionar líneas superiores entre ecotipos de kikuyo recolectados a lo largo de la costa este de Australia, y germoplasma que se obtuvo después de someter plantas

Correspondence: W.J. Fulkerson, PO Box 486, Lismore, NSW 2480, Australia. Email: billf@norco.com.au

del cv. Whittet a un agente mutagénico. En estudios de invernadero iniciales se identificaron 19 líneas resistentes a la cepa KY1A del amarillamiento de kikuyo. Cuatro de ellas, que presentaron calidad forrajera y rendimiento superiores a cv. Whittet, se evaluaron a nivel de campo en dos sitios, usando cv. Whittet como testigo. En el Sitio 1, la línea 12A demostró un nivel más alto de resistencia al amarillamiento de kikuyo que el cv. Whittet, con 85% de las plantas resistentes a la infección, en comparación con solo 15% de las plantas del cv. Whittet. En el Sitio 2, el número de plantas infectadas de la línea 12A y cv. Whittet fue similar. En pruebas adicionales, utilizando inóculo del amarillamiento de kikuyo recolectado en 11 sitios a lo largo de la costa este de Australia, solo 15% de las plantas de la línea 12A quedaron infectadas en comparación con 61% de las plantas del cv. Whittet. Por tanto, la línea 12A fue resistente a la mayoría, pero no a todas, de las cepas del patógeno. El rendimiento anual de la línea 12A (19,008 kg MS/ha) fue 24% mayor que el del cv. Whittet y 12% mayor que el de otro cultivar comercial en Australia, cv. Acacia, pero la diferencia solo fue significativa para cv. Whittet. Durante el verano, la línea 12A produjo 10,212 kg de MS/ha (24% más que cv. Whittet), creció mejor a principios de la primavera, presentó una digestibilidad de la materia orgánica ligeramente más alta (66.7 vs. 64.0%) y fue resistente a la infección por mancha negra.

Palabras clave: Amarillamiento de kikuyo (*Verrucalvus flavofaciens*), calidad forrajera, gramínea tropical, mancha negra (*Bipolaris* spp.), palatabilidad relativa.

Introduction

Kikuyu grass (*Cenchrus clandestinus*) is a C4 summer grass, native to northeastern parts of Africa, predominantly in the Aberdare Mountain region of Kenya, which is inhabited by the Kikuyu tribe; hence the name, kikuyu. It is found at elevations between 1,950 and 2,700 m in the tropics, creating a niche subtropical climate with maximum temperature of 16–24 °C and a minimum of 2–8 °C (Morrison 1969), which led Pearson et al. (1985) to conclude that kikuyu had an optimal temperature range for growth of 8–21 °C. However, kikuyu is very adaptable, having become naturalized in areas with far more extreme temperatures, such as South Africa and Australia, where it grows successfully from the tropical highlands of the Atherton Tableland (North Queensland) to East Gippsland, Victoria (temperate climate) and the irrigated pastures of southwest Western Australia (Mediterranean climate).

'Common' kikuyu grass, introduced into Australia in 1919, was traditionally established by cuttings until Wilson (1968) identified a seeding line of kikuyu grass at Grafton Research Station in northern NSW. This line was primarily selected for its high level of free seeding and more vigorous growth than Common kikuyu grass, particularly in winter, and was registered as cultivar Whittet in 1970.

Three more lines were subsequently selected and registered:

1. 'Breakwell', selected for its ability to set seed but similar to Common kikuyu grass in other aspects, was registered in 1971.
2. 'Crofts' is claimed to have greater cold tolerance and to be slightly higher yielding than Whittet and was released in 1983.
3. 'Noonan' was selected for resistance to kikuyu yellows (*Verrucalvus flavofaciens*) (Wong and Wilson 1983) and was registered in 1983 but is now used mainly as a turf grass.
4. 'Acacia' selected from the Acacia Plateau at high elevation in the subtropical region was registered in 2013.

A survey in 1994 (Anonymous 1994) revealed that kikuyu grass was the basic forage for 30% of dairy pastures in NSW and it was estimated that 70% of milk production in summer came from kikuyu pastures. However, since then, the spread of the fungal diseases, kikuyu yellows and, to a lesser extent, black spot (*Bipolaris* spp.), inter alia, have reduced its contribution. When Mears (1970) reviewed kikuyu grass in 1970, the adverse impact of kikuyu yellows on kikuyu pastures was already evident. He concluded that "improvement (of kikuyu grass) should be directed towards disease resistance (e.g. kikuyu yellows), possibly higher digestibility and cold tolerance rather than vigour or free seeding". The dairy industry still considers these traits are top priority for selecting a better kikuyu cultivar.

Resistance/tolerance to fungal diseases

Wong (1975) found that an undescribed fungus, subsequently described as a new species, *Verrucalvus flavofaciens*, by Dick et al. (1984), caused kikuyu yellows. It is spread by water-borne zoospores which infect the roots, resulting in death of the grass in patches. No commercial fungicides are currently available to control kikuyu yellows effectively.

With black spot disease, the tips of the more mature leaves of infected kikuyu plants often turn yellow and numerous dark brown to black spots appear on the leaf

blades and sheaths, especially during periods of high humidity and rainfall. Normally, black spot does not kill the plant, but growth is slightly reduced, and more importantly, the leaves become chlorotic and unpalatable to cattle.

Tolerance of low temperatures

In the subtropical dairy region of NSW, kikuyu pastures are typically over-sown with short-rotation ryegrass in autumn to provide winter feed, when kikuyu growth is low (Fulkerson et al. 2010). This over-sowing is costly and results can be variable, dependent on weather. Finding a kikuyu genotype more tolerant of winter cold would eliminate the need to sow ryegrass or shorten the period when it was needed.

Improved forage quality

Selection for improved forage quality, specifically digestibility, is also a common dairy industry request, as a means of achieving higher milk production. While ryegrass can produce 20 L milk/cow/day with good management on-farm, Reeves (1998) found cows grazing kikuyu during mid-lactation produce about 15 L milk/day, i.e. at a time when virtually all milk is coming from feed and not body reserves.

Based on these factors, the present study aimed to identify a kikuyu line with one or more of the following desirable traits: resistance to fungal diseases, improved herbage quality, greater tolerance of winter cold and higher yield than cv. Whittet, in that order of priority..

Materials and Methods

There were 4 stages in the evaluation of available genetic material of kikuyu as outlined below:

Stage 1 - Yield, forage quality and resistance to kikuyu yellows of 115 kikuyu lines, as single plants, were evaluated.

Stage 2 - Kikuyu plants identified as resistant to kikuyu yellows in Stage 1 were tested in the field, using Whittet as the control.

Stage 3 - The kikuyu line, 12A, identified as resistant to kikuyu yellows in Stage 2, and the commercial kikuyu cultivar, Whittet, were further tested for resistance to kikuyu yellows by using inoculum from kikuyu yellows-infected plants, collected over a wide geographical region.

Stage 4 - The 2 commercial cultivars, Whittet and Acacia, plus line 12A were evaluated against 6 lines found to be highest in other desirable traits (yield and forage quality) during Stage 1.

Stage 1 – Initial screening

Material tested. The study commenced in March 2015 with the evaluation of 115 kikuyu lines, selected from 1,600 lines held at the Animal Science Precinct, University of Queensland from a previous study on the genetic variation in kikuyu grass (Lowe et al. 2010) with those lines originating from 3 different sources:

- Wollongbar collection (DAN063) - These plants originated from Whittet seed subjected to mutagenic agents by Dr D. Luckett, plant breeder, Department of Primary Industries, Agricultural Research Institute, Wagga Wagga, NSW in 1996 and initially evaluated at Wollongbar Agricultural Institute, NSW.
- Kevin Lowe collection - Kikuyu ecotypes collected from throughout Queensland.
- Peter Martin collection - Kikuyu ecotypes collected from throughout NSW.

Forage yield and quality. The plants were tested for the following:

- Dry matter yield of lines as individual potted plants at the recommended stage of regrowth (4.5 new leaves/ tiller).
- Ash-free neutral detergent fiber (NDFom) and indigestible NDFom (iNDFom) (Harper and McNeil 2015) at the School of Agriculture and Food Sciences, University of Queensland, Gatton Campus, Queensland. The samples were analyzed for iNDFom using long-term (10 days) in vitro fermentation. This procedure is based on a fermentation procedure described by Goering and Van Soest (1970), adapted for use with Ankom filter bags using ANKOM Daisy incubators. Neutral detergent fiber concentration was determined using the procedure described by Goering and Van Soest (1970) and adapted for an Ankom fiber analyzer.
- Dry organic matter digestibility (DOMD) by the rumen fluid fermentation method (Tilley and Terry 1963) at the NATA-accredited feed testing laboratory, Department of Primary Industries, Charles Sturt University, Wagga Wagga, NSW.

The mean, standard deviation (SD) and range of the results for the 115 kikuyu lines were: NDFom 62.4% (±3.8) (range 54.3–71.9%); and iNDFom 20.7% (±4.6) (9.2–29.9%).

Resistance to kikuyu yellows infection. The sensitivity of the kikuyu plants to kikuyu yellows was tested in a glasshouse at the Plant Breeding Institute, University of Sydney, Cobbitty, NSW, using kikuyu yellows-infected kikuyu leaves as described by Wong (2011). Where there was any doubt that the yellowing symptoms were due to

the disease, yellow leaves were collected at random to re-isolate the pathogen and to serve as a check on the method. This method was also used to confirm kikuyu yellows infection in the regional samples in Stage 3. Nineteen kikuyu lines showed resistance to the KY1A strain of kikuyu yellows, one of the 3 strains previously identified by Dr P. Wong and believed to be the most prevalent.

Selected lines. Four of these kikuyu lines (Table 1), with forage quality (NDFom, iNDFom and DOMD) at least as good as Whittet, were chosen for further evaluation against Whittet, as the control, in Stage 2.

Table 1. Neutral detergent fiber (NDFom), indigestible NDFom (iNDFom), dry organic matter digestibility (DOMD) (all as % DM) and yield (g DM/plant), sampled in March 2015 from individual plants of 4 kikuyu lines and Whittet as control.

Kikuyu line/cultivar	NDFom (%)	iNDFom (%)	DOMD (%)	Yield (g DM/plant)
12A	62.0	16.4	69	10.7
11C	59.5	17.0	69	8.6
Whittet	61.9	19.7	67	8.3
15A	57.4	15.7	70	10.7
25D	61.2	14.3	68	9.3

The origins of these 5 lines were as follows:

- Lines 15A, 11C and 12A were unidentified plants, which originated from the combined Queensland and Wollongbar collections.
- Line 25D was the only identifiable plant and had shown some resistance to kikuyu yellows in the initial field evaluation at Wollongbar (Dairy Australia-funded project, DAN063).

Stage 2 - Field evaluation of kikuyu lines for resistance to kikuyu yellows

Propagation and establishment. Cultivar Whittet plus the 4 kikuyu lines selected for the field evaluation were propagated vegetatively from the original 'mother' plants by means of rooted stolons. These were planted out on 20 December 2015 in 1 m^2 plots, forming a grid of 5 lines of kikuyu grass with 5 plants/line, replicated 4 times at 2 locations (Site 1 – Kyogle: 28° S, 153° E; 76 masl; and Site 2 – Lismore: 29° S, 153° E; 12 masl). This grid structure facilitated weeding and helped to identify lines and prevent incursion between lines through stolon growth. The soils were clay loams on a flood-plain aspect.

The climate at both sites is subtropical with Site 1 having lower rainfall and humidity but higher summer temperature than Site 2. From planting to the completion of the study on 30 May 2016, Site 1 received 323 mm of rain and Site 2 had 406 mm.

At planting, the areas where the plots were located, showed obvious signs of current kikuyu yellows infestation (stunted growth, yellowing of the leaves, short stolon internodes and little root development, making the plants easy to pull from the soil). The soils at both sites were highly fertile with high original soil nitrate levels (>80 mg/kg). In order to stress the plants, in an effort to maximize active kikuyu yellows infestation, supplementary irrigation was applied only for the first 4 weeks after planting to enable the plants to establish effectively and no N fertilizer was applied.

Confirmation of kikuyu yellows status. On 16 March 2016, stolon and leaf samples were taken from one 'yellow' plant of each kikuyu line in each replicate as well as from the 'green' 12A plants. All samples were tested for kikuyu yellows infection, to confirm or reject the visual symptoms, using the following methods:

- Plating out of leaf pieces on water agar plates after surface sterilization (P. Wong, unpublished data); and
- Floating leaf pieces of kikuyu in soil extract after surface sterilization (Wong 1975).

Stage 3 - Resistance to disease inoculum from the north coast of NSW and SE Queensland

In order to determine the geographical extent of the resistance of line 12A to kikuyu yellows relative to Whittet, kikuyu leaves from plants, which were obviously affected by kikuyu yellows, were collected from 11 dairy pastures located along the north coast of NSW and SE Queensland and used as a source of inoculum (Wong 2011). In this experiment, 3 replicates of each inoculum were tested against 12A and Whittet. The plants were kept in a glasshouse at 30:25 °C (day:night) temperature for about 3 months from mid-January. Kikuyu yellows infection usually became obvious after 5–7 weeks. One tiller or leaf from each plant, suspected to be infected, was tested to confirm kikuyu yellows, as outlined previously.

Stage 4 - Field evaluation of kikuyu lines for yield, forage quality, stolon vigor, black spot susceptibility and cow preference

This plot experiment was undertaken at the same location as used in Stage 2, i.e. Site 2 (Lismore). The climatic data during Stage 4 are shown in Figure 1.

Six kikuyu lines selected in Stage 1 for high quality (DOMD, NDFom and iNDFom) or yield, as individual plants were selected for evaluation in this stage (Table 2).

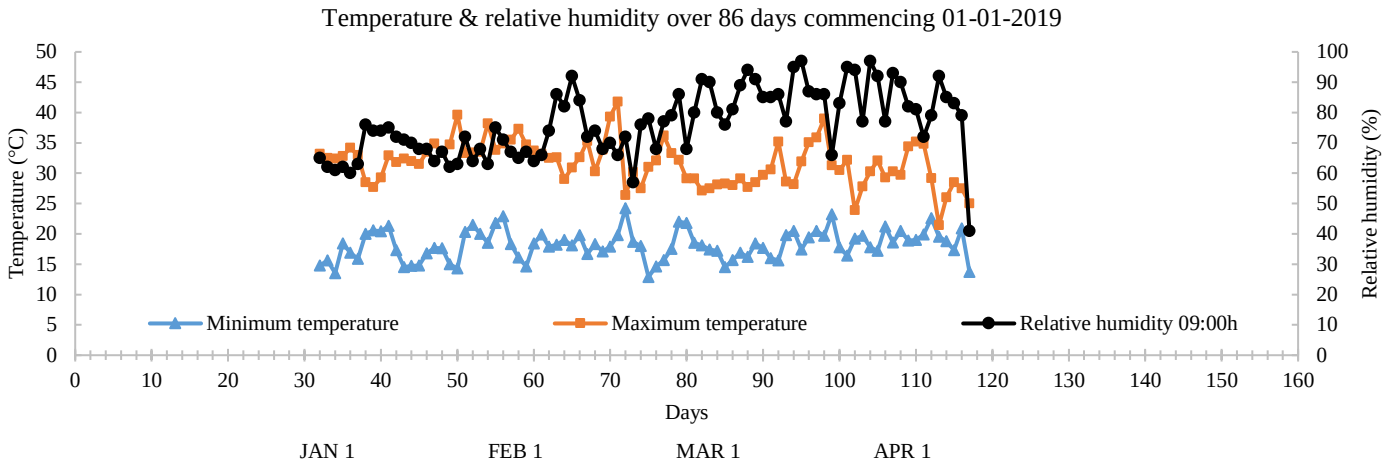

Figure 1. The minimum and maximum ambient temperatures (°C) and relative humidity (%) at 09:00 h during the experiment.

Table 2. Kikuyu lines selected in Stage 1 for high quality (DOMD, NDFom and iNDFom) or yield, as individual plants, evaluated in Stage 4 in plots.

Kikuyu line	NDFom (% DM)	iNDFom (% DM)	DOMD (% DM)	Yield (g DM/plant)
15A	57.4	15.7	70	10.7
18A	52.6	14.2	71	5.7
7A	55.9	18.4	65	11.6
14B	56.1	13.6	68	8.8
2A	57.3	9.2	68	7.7
3A	59.4	9.2	64	8.4

The commercial cultivars, Whittet and Acacia, and kikuyu line 12A, which was shown to be resistant to kikuyu yellows in Stages 2 and 3, were also included in the experiment (see Table 1 for quality and yield data).

Parameters measured were: seasonal yield, forage quality, stolon vigor, level of black spot infestation, winter growth and cow preference, in 2 × 1 m field plots, randomly allocated within 3 replicates. The evaluation continued for 11 months (24 November 2016–24 October 2017). The plants were vegetatively propagated from the original 'mother' plants in August 2016 and transplanted into the plots on 6 October. Recording of yield and other traits commenced with the first recorded defoliation on 8 December 2016.

Site preparation. Lime and a complete fertilizer (Rubisca plus at 350 kg/ha containing N:P:K:S at 12:5:14:9.7 mg/kg plus trace elements) were applied to the total plot area before planting to ensure that soil pH and soil nutrients [Colwell P - 110; exchangeable K - 227; S - 30 in mg/kg; pH ($CaCl_2$) - 5.1] were not limiting kikuyu growth. Urea was then applied after each defoliation at a rate equivalent to a per-month recommendation of 100 kg/ha.

Methodology and chemical analysis. The plots were cut to 6 cm stubble height with a Massport hand push mower each time the plants had regrown 4–4.5 new leaves/tiller (after approximately 14 days in 'summer' extending out to 35 days or more in 'winter'), i.e. the stage when herbage quality and yield are optimized (Reeves 1998). The 'green' plot yield and weight of a 'green' subsample were recorded before the green subsample was dried at 65 °C for 48 h in a forced-draft oven.

At the defoliations on 27 February, 24 April and 2 December 2017, herbage samples were taken for analyses of DOMD, NDFom and iNDFom (for details see Stage 1) and the number of stolons protruding from each plot was recorded as an indication of stolon vigor.

The presence of the fungal infection, black spot, was scored as: 0 (no infection), 1 (slight infection - 4–6 yellow leaf tips/plot), 2 (moderate - more than half the leaf tips yellow) and 3 (severe - nearly all leaf tips yellow), prior to each defoliation from 4 December 2016 to 9 May 2017.

A cow preference test (Horadagoda et al. 2009) was performed 14 days after the final harvest of the experiment on 16 December 2017 (when 4 new leaves/tiller had regrown and at a time when there was no black spot infection). The test involved 2 observers recording which plots were being grazed every 10 seconds for 40 minutes by 3 Jersey milking cows, accustomed to grazing kikuyu pastures.

Results

Stage 2 - Field evaluation of kikuyu lines for resistance to kikuyu yellows

The status of the kikuyu plants at Site 1 (Kyogle) on 20 April (130 days after planting) and at Site 2 (Lismore) on 31 May (171 days after planting) is shown in Table 3.

Observations on plots at Lismore were continued until May as a number of plants were seen to be recovering in April, whereas most plants that were infected with kikuyu yellows at Kyogle had died by 20 April.

The photos below (Figure 2) show Replicate 2 at Site 1, 38 days after planting and at 130 days after planting, illustrating the impact of kikuyu yellows on the kikuyu lines.

Table 3 shows the very marked differences at Site 1 between line 12A and the other 4 kikuyu lines which had all, apart from Whittet, shown resistance to the kikuyu yellows strain KY1A in the initial glasshouse tests in Stage 1. At Site 2, the proportion of plants infected with kikuyu yellows was substantially lower than at Site 1 and all lines recorded similar levels of infection, but a high percentage of infected plants recovered in late autumn. In addition, the onset of infection was later, which suggests the strain of kikuyu yellows at Site 1 was different from that at Site 2.

At Site 1, kikuyu yellows was isolated from 19 leaf/stolon samples that displayed symptoms of kikuyu yellows infection, confirming the presence of the disease, while 2 plants of 12A, identified as 'green' or symptomless, were confirmed as free from kikuyu yellows infection. At Site 2, 15 of the 18 'yellow' leaf/stolon samples were confirmed as infected and 2 leaves/stolons identified as 'green' were also infected with kikuyu yellows.

Stage 3 - Resistance to disease inoculum from the north coast of NSW and SE Queensland

In this test, only 1 or 2 plants of the 3 became infected, probably because some of the leaves originally collected and showing obvious symptoms of kikuyu yellows may not have had viable inoculum at the time of sampling. Therefore, it was not possible to say definitively at which sites 12A would be resistant to kikuyu yellows. However, the results support the proposition that 12A is far more resistant to kikuyu yellows than Whittet with only 5 plants (of 33 treated) becoming infected and 2 recovering, compared with 20 Whittet plants becoming infected, of which 6 recovered.

Stage 4 - Field evaluation of kikuyu lines for forage quality and other desirable traits

The forage quality of the 7 kikuyu lines and the 2 commercial cultivars used in this stage are shown in Table 4.

In 'summer', DOMD of kikuyu line 12A was 2.7% units higher than that of Whittet but the differences were not significant. The DOMD of line 18A was significantly higher than that of any other line except 3A. There were no significant differences in DOMD for the samples taken in 'autumn' and 'winter-spring'.

Table 3. The kikuyu yellows status of kikuyu plants at Kyogle (Site 1) on 20 April and Lismore (Site 2) on 31 May. Values indicate no. of plants in the respective categories.

Kikuyu line/cultivar	Kyogle				Lismore[1]			
	Green	Yellow	Dead	Recovered	Green	Yellow	Dead	Recovered
12A	17	2	0	1	3	0	7	10
11C	3	3	12	2	6	4	5	5
Whittet	3	0	17	0	6	0	8	6
15A	1	3	15	1	5	1	10	4
25D	6	1	12	1	8	0	2	6

[1]There were only 16 plants available for line 25D at Lismore.

Figure 2. The status of kikuyu lines in Replicate 2 at Site 1 at 38 days after planting (left) and 130 days after planting (right). Lines (from left to right) are: 12A (all green), 11C (2 dead, 2 yellow, 1 green), Whittet (all dead), 15A (4 dead, 1 yellow) and 25D (3 dead, 1 yellow).

Table 4. Dry organic matter digestibility, ash-free neutral detergent fiber and indigestible NDF (% DM) for kikuyu samples taken on the dates shown.

Kikuyu line/cultivar	27/02/2017 'Summer'	24/04/2017 'Autumn'	2/12/2017 'Winter-spring'
	Dry organic matter digestibility (DOMD)		
12A	66.7bcd[1]	63.1abcd	65.9
Whittet	64.0de	62.9bcd	65.7
Acacia	66.9bc	64.0abc	66.0
15A	62.6e	59.6d	NA[2]
18A	69.7a	64.7ab	66.7
7A	65.1cde	61.7cd	NA
14B	66.8bc	66.5a	NA
2A	64.0e	61.7cd	66.0
3A	68.7ab	65.7ab	67.0
Significance	P = 0.001	P = 0.022	NS
	Ash-free neutral detergent fiber (NDFom)		
12A	61.0b	65.0	57.3
Whittet	62.2ab	65.0	57.0
Acacia	65.0a	66.7	57.3
15A	61.0b	NA	NA
18A	59.0b	65.0	55.9
7A	60.3b	NA	NA
14B	60.1b	NA	NA
2A	59.3b	65.0	56.7
3A	62.0b	65.7	58.8
Significance	P = 0.028	NS	NS
	Indigestible NDFom (iNDFom)		
12A	20.0abc	10.5bc	14.9
Whittet	22.4a	10.0c	15.0
Acacia	18.8bcd	12.7a	14.7
15A	21.2ab	NA	NA
18A	16.7d	11.7ab	13.6
7A	20.4abc	NA	NA
14B	19.2bcd	NA	NA
2A	21.1ab	10.7bc	14.6
3A	18.4cd	10.0c	15.6
Significance	P = 0.008	P = 0.015	NS

[1]Within columns and parameters means without a common letter are significantly different at the levels indicated.
[2]Analysis of kikuyu lines 15A (low quality and yield) and 7A and 14B (both highly affected by black spot fungus) were discontinued after the first analysis in summer owing to the issues indicated.

The NDFom of Acacia was significantly higher than that of all other kikuyu lines except Whittet in 'summer', while NDFom values of all kikuyu lines in 'autumn' and 'winter-spring' were similar.

The iNDFom in 'summer' was highest for Whittet and lowest for 18A and the difference was significant but, by contrast, the iNDFom of Acacia in 'autumn' was significantly higher than that of any other line, except 18A. There was no difference in iNDFom between lines in 'winter-spring'.

Dry matter yield. Forage yields for the 11-month period from 8 December 2016 to 24 October 2017 are shown in Table 5. Clearly, 12A was higher-yielding than both Whittet and Acacia during the main 'summer' growth period but not different from lines 2A, 7A, 18A and 14B. Line 12A also produced higher yields than Whittet in the 'winter-spring' period but the difference was not significant (P>0.05). Total yield for 12A was significantly greater than that for Whittet but not for Acacia nor lines 2A, 7A and 18A.

Table 5. Dry matter yields during 'summer', 'autumn' and 'winter-spring' and total yield for the 7 kikuyu lines (including 12A) and the 2 commercial cultivars, Whittet and Acacia.

Kikuyu line/cultivar	Dry matter yield (kg DM/ha)			
	24/11 to 29/3 'Summer'	30/3 to 9/5 'Autumn'	10/5 to 24/10 'Winter/ spring'	Total
12A	10,212a[1]	1,396	7,480	19,088a
Whittet	8,226bc	1,245	5,963	15,434c
Acacia	8,258bc	1,460	7,283	17,001abc
15A	8,343bc	1,114	5,934	15,390c
18A	9,126ab	1,355	6,473	16,954abc
7A	9,397ab	1,500	7,040	17,937abc
14B	8,841abc	1,382	6,169	16,391bc
2A	9,481ab	1,585	8,260	19,327a
3A	7,553c	1,352	6,456	15,360c
	P = 0.024	NS	P = 0.1	P = 0.05

[1]Within columns means without a common letter are significantly different at the levels indicated.

Other desirable traits. The incidence of black spot and indications of stolon vigor for 12A, Whittet and Acacia were recorded during the 'summer' and 'autumn' periods from 22 December 2016 to 9 May 2017, while the cow preference test was recorded in early December 2017, when there were no symptoms of black spot infection and none was expected owing to the low relative humidity at that time (Table 6).

Black spot infestation on 12A was zero and on Acacia was low but the mean score for Whittet was 1.6, being between moderate and severe at each defoliation over the 'summer' growth period and this difference was significant.

Whittet showed the highest stolon vigor, being more than 70% greater than 12A and nearly 3 times as great as Acacia but these differences were not significant (P>0.05). While line 12A was preferred by cattle over both Whittet and Acacia, differences were again not significant, but did indicate that 12A was not less palatable than the cultivars at this stage of growth.

The germination rate of line 12A and the cultivars, Whittet and Acacia, were all over 80% and seedling vigor was similar.

Table 6. Mean score for incidence of black spot fungal infection during the 'summer-autumn' growth period, stolon vigor and cow preference.

Kikuyu line/ cultivar	Black spot[1] (score)	Stolon vigor[2] (No./harvest)	Cow preference[3] (recordings)
12A	0	11.7	17
Whittet	1.6	20.1	11
Acacia	0.1	6.8	5
Significance	P<0.009	NS	NS

[1]0 = no infection; 1 = slight infection, 4–6 leaves/plot were yellow; 2 = moderate, more than half the leaf tips were yellow; and 3 = severe, nearly all leaf tips were yellow; recorded during the experimental period.
[2]Mean number of stolons protruding outside the plots at a defoliation; recorded during the experimental period.
[3]Recorded grazings in 40 minutes at 10-second intervals; at the end of the experiment.

Discussion

In this study, a line of kikuyu grass, 12A, was identified as being more resistant to the fungal diseases kikuyu yellows and black spot and higher yielding than the cultivars Whittet and Acacia and similar to the two commercial cultivars for stolon vigor, herbage quality and cow preference. The most important attribute, where 12A was superior to the other lines, was resistance to the fungal disease, kikuyu yellows, in most, but not all cases. The resistance to kikuyu yellows differed markedly between the 2 evaluation sites. At Site 1, the resistance was outstanding, with only 15% of 12A plants infected with kikuyu yellows, whereas over 85% of plants in the other lines, including Whittet, were infected and all infected plants died. This was despite the fact that all lines, except Whittet, were found to be resistant to the KY1A strain of kikuyu yellows, when individual plants were tested under glasshouse conditions in Stage 1 of the study.

In contrast, at Site 2, line 12A showed no advantage over the other kikuyu lines under test in terms of resistance to kikuyu yellows with 85% of plants infected. However, 49% of all plants infected with kikuyu yellows in summer recovered in the autumn, compared with only 8% at Site 1. This was despite the fact that the minimum temperature remained above 15 °C longer than normal and well into April (Table 7), a temperature conducive to kikuyu yellows activity. In addition, the onset of visual symptoms of kikuyu yellows infection was later at Site 2 than at Site 1. This difference between sites was presumably due to strain differences of the pathogen at each site with the strain at Site 1 being more pathogenic.

The regional study also supports the contention that line 12A has greater resistance to kikuyu yellows than Whittet with only 16% of 12A plants becoming infected compared with 64% for Whittet and nearly all inoculum that infected 12A plants came from 2 properties on the lower north coast of NSW. In addition to displaying resistance to kikuyu yellows, 12A also had significantly higher 'summer' and total yields than Whittet plus earlier onset of growth in early spring with dry matter yields of 12A and Acacia being twice that of Whittet at the first spring defoliation. The results also indicate that forage quality, stolon vigor and cow preference for 12A are at least as good as those for the 2 commercially-available kikuyu cultivars.

Table 7. The long-term mean minimum ambient temperature for Site 2 (Lismore) from January to April and the actual minimum temperature in 2016 (data for Site 1 at Kyogle were not available).

Month	Average Min. temp. (°C)	2016 Min. temp. (°C)
January	18.7	17.4
February	18.6	18.8
March	17.0	17.7
April	14.1	16.6

The complete absence of black spot infection in 12A, compared with a very high level of infection in Whittet and other lines tested in Stage 4, is also very important. Unlike kikuyu yellows, black spot does not kill the kikuyu plant and scarcely affects its growth, as only the tips of the mature leaves are affected, but palatability of the infected herbage for grazing cattle is very low. Thus, dairy farmers consider that black spot is just as important a problem as kikuyu yellows.

Interestingly, the DOMD for samples of 12A were not significantly different from those of Whittet in any season. In this study, DOMD was assessed by the Tilley and Terry method (Tilley and Terry 1963), which is considered to most accurately reflect the availability of nutrients to the ruminant animal. One can be confident that forage from 12A was not inferior to that of Whittet.

We used 4.5 new leaves/tiller as the optimal time to defoliate, as digestibility is considered to be at a maximum at this point, after which the oldest leaves sequentially begin to senesce and the proportion of stolon:leaf growth increases (Reeves et al. 1996). In this environment, 4.5 leaves is usually reached at about 2-weeks regrowth in 'summer' and 3–4-weeks regrowth in late 'spring and early 'autumn' and is almost entirely dependent on ambient temperature.

While the DM yield of 12A over the main 'summer' period was greater than those of both Whittet and Acacia plus 15A and 3A, total DM yield of 12A over the 11 months was greater than those of Whittet, 15A, 3A and 14B only. The observation that leaves of Whittet were yellow during 'winter', while 12A and Acacia remained green, was in keeping with the higher yields of 12A and Acacia at the first defoliation after 'winter' on 6 October.

This was not unexpected for Acacia, as it was selected from the Acacia plateau at a higher elevation and in a colder climate. This 'winter' activity is a highly desirable trait for a dairy pasture and more particularly for beef farms, where over-sowing of kikuyu with ryegrass in autumn, to provide winter feed, is not practiced often, primarily due to cost.

Conclusions

This study has identified a kikuyu line, 12A, which appears superior to the commercial cultivars Whittet and Acacia, in terms of resistance to fungal infection, i.e. kikuyu yellows, in most but not all situations, and *Bipolaris* spp., both of which have been responsible for a major decline in the area of kikuyu pastures grown in the subtropical dairy region of Australia and a hesitancy by farmers to re-establish kikuyu grass with the existing cultivars. This successful selection of 12A has been achieved along with an increase in forage yield over that of the most common commercial cultivar, Whittet, and, most importantly, without a decline in forage quality and cow preference. The study has also given indications of the genetic diversity in kikuyu grass for the traits tested. Evaluation of this line under grazing to assess the benefits of improved disease resistance and yields in terms of increased livestock production would provide further evidence for recommending widespread plantings.

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Research Paper

Effects of a nitrogen-based supplement on intake, live weight and body energy reserves in breeding *Bos indicus* cross cows

Efecto de una suplementación basada en nitrógeno en el consumo de forraje, la ganancia de peso vivo y las reservas energéticas de vacas Bos indicus × B. taurus

ROB M. DIXON[1] AND ROBERT J. MAYER[2]

[1]*Queensland Alliance for Agriculture and Food Innovation (QAAFI), Centre for Animal Science, The University of Queensland, Rockhampton, QLD, Australia.* qaafi.uq.edu.au
[2]*Queensland Department of Agriculture and Fisheries, Maroochy Research Facility, Nambour, QLD, Australia.* daf.qld.gov.au

Abstract

Breeding cows grazing seasonally dry rangelands usually lose substantial live weight (LW) during the dry season, when in late pregnancy. An experiment investigated the effects of feeding a N-based supplement to cows in late pregnancy on voluntary intake, total live weight (T-LW), body condition score (CS) and estimated body net energy content (Body-NE), as well as carry-over effects during lactation. In Phase A for 139 days from mid-pregnancy, mature *Bos indicus* cross breeders [initially 438 kg T-LW and 5.7 CS units (9-point scale)] were fed in pens on low quality tropical grass hay alone (Control) or with a N supplement (Supplemented). Most (17/22) of the cows calved during this interval. Voluntary hay intake averaged 6.74 g DM/kg T-LW/d in Control cows, and was increased by 35% (P<0.001) when supplement was fed. As a result, feeding supplement reduced loss in conceptus-free live weight (CF-LW) by 30% (from 1.11 kg/d to 0.78 kg/d; P<0.001) and in Body-NE by 20% (from 26.6 to 21.2 MJ NE/d; P = 0.007). Control cows mobilized 24% of maternal LW and 32% of body energy when fed low quality hay during late pregnancy, and these losses were substantially reduced when a N-based supplement was fed. During Phase B, when the lactating cows with their calves grazed a high quality rainy season grass-*Stylosanthes* pasture, the previously supplemented cows produced more milk (P = 0.065) and their calves grew faster (P = 0.077) in early lactation than Control cows. In addition, during early lactation Control cows exhibited compensatory LW gain relative to the Supplemented cows (0.80 vs. 0.43 kg/d, respectively; P<0.001) and there was no discernable weight difference between the groups by 205 days of lactation. In conclusion the losses in LW and body energy reserves by late pregnant cows fed low quality tropical grass hay were substantially reduced by a N supplement, but the differences were not maintained when the cows subsequently grazed high quality pasture.

Keywords: Growth rates, reproduction, supplementation, tropical rangelands.

Resumen

En pastizales nativos estacionalmente secos, las vacas de cría generalmente pierden peso en forma sustancial durante la estación seca cuando se encuentran al final de la gestación. En un experimento realizado en el norte de Australia se estudiaron los efectos de un suplemento basado en nitrógeno en el consumo voluntario, el cambio de peso vivo (PV) total, la condición corporal de los animales y el contenido energético corporal al final de la gestación de las vacas, así como los efectos posteriores durante la lactancia. En la Fase A del experimento se alimentaron vacas *Bos indicus* × *B. taurus* adultas durante 139 días a partir de la mitad de la gestación [438 kg de PV inicial y 5.7 unidades de condición corporal en una escala de 9 puntos)] en confinamiento con solo heno de baja calidad (testigo) de una gramínea nativa (*Heteropogon contortus*) o heno más un

Correspondence: R.M. Dixon, Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, PO Box 6014, Rockhampton, QLD 4702, Australia.
Email: r.dixon2@uq.edu.au

suplemento nitrogenado. Diecisiete de 22 vacas parieron durante este intervalo. El consumo voluntario de heno fue de 6.74 g MS/kg PV por día en las vacas testigo e incrementó en 35% (P<0.001) cuando se suministró el suplemento. Como resultado, el suplemento redujo las pérdidas de PV sin el concepto ('conceptus-free live weight') en 30% (de 1.11 para 0.78 kg/día; P<0.001) y las del contenido energético corporal en 20% (de 26.6 para 21.2 MJ/día; P = 0.007). Las vacas testigo movilizaron 24% del PV materno y 32% de la energía corporal cuando fueron alimentadas con heno de baja calidad durante la última etapa de la gestación; estas pérdidas se redujeron sustancialmente cuando fueron suplementadas. Durante la Fase B, cuando en la temporada de lluvias las vacas lactantes estuvieron con sus crías en una pastura de alta calidad (gramínea nativa en activo crecimiento asociada con la leguminosa *Stylosanthes*), las vacas anteriormente suplementadas produjeron más leche (P = 0.065) y sus terneros crecieron más rápido (P = 0.077) en la lactancia temprana que las vacas testigo. Las vacas testigo presentaron una ganancia de peso compensatorio superior al de las vacas suplementadas (0.80 vs. 0.43 kg/d; P<0.001); a los 205 días de lactancia no se registró diferencia de PV significativa entre los grupos de animales. En conclusión, las pérdidas de PV y reservas de energía corporal de vacas en gestación avanzada y alimentadas con heno de baja calidad, se redujeron sustancialmente cuando se les suministró un suplemento nitrogenado. Sin embargo, las diferencias no fueron iguales cuando posteriormente las vacas tuvieron acceso a una pastura de alta calidad.

Palabras clave: Gramíneas tropicales, reproducción, suplementación, tasa de crecimiento.

Introduction

In breeding beef cows grazing rangelands in the seasonally dry tropics the intake of nutrients typically varies widely between seasons during the annual cycle. The metabolizable energy (ME) and protein concentrations and voluntary intakes of pastures, and therefore liveweight (LW) gains, are typically high during the early to mid-rainy season but decrease markedly with declining pasture quality in the late rainy season and the dry season (Winks 1984; Entwistle and McCool 1991; Dixon et al. 2011a; Holroyd and McGowan 2014). Breeding herds that are 'control mated' are usually managed to calve during the late dry season or early rainy season, when high quality rainy season pastures are expected to be available to meet the high nutritional demands for lactation and calf growth. Year-round-mated herds tend to have a similar calving pattern due to most conceptions occurring during the rainy season. The importance of managing the nutrition, mating and weaning of breeding herds so that cows have at least moderate body reserves at parturition is well recognized. Numerous studies have reported general relationships between cow LW or body condition score (CS) and reconception of lactating cows and cow mortality in seasonally dry tropical environments, especially in southern Africa (Richardson et al. 1975; Buck et al. 1976) and northern Australia (Goddard et al. 1980; Doogan et al. 1991; Holroyd and Fordyce 2001; Mayer et al. 2012; Holroyd and McGowan 2014). However, maintaining LW and CS in cows grazing low quality senesced tropical pastures is often difficult in environments with infertile soils and short pasture growing seasons, and especially during years when the seasonal rainfall break is late and dry season conditions are prolonged. In such environments and seasonal conditions, breeding cows often do not obtain sufficient intakes of nutrients to meet their requirements for pregnancy and lactation and also to maintain body reserves of live weight and condition.

During the mid- to late dry season senesced tropical grass pastures are usually deficient in N for grazing cattle. Numerous pen experiments have reported that supplementation of growing cattle fed low quality hays with non-protein N (NPN), usually provided as urea, substantially increased voluntary intake and reduced LW loss but had little effect on dry matter (DM) digestibility (Ernst et al. 1975; Lindsay et al. 1984; Hunter and Siebert 1985; Hennessy and Williamson 1990; Kennedy et al. 1992). Experiments with growing cattle grazing tropical grass pastures during extended dry seasons have often reported benefits from NPN supplementation with reduced liveweight loss and occasionally increased liveweight gain (Winks et al. 1979; Foster and Blight 1984; Coates and Dixon 2008; Dixon and Coates 2010). In addition NPN supplements can substantially reduce LW loss of late-pregnant cows fed low quality C4 grass hay in pens (Lindsay et al. 1982). However, experiments with breeding cows grazing native speargrass pastures in northern Australia have reported large variation between years in the responses to NPN supplements. The responses of breeding cows to NPN supplements expressed as changes in LW and CS have ranged from no effect in years with benign dry season conditions (e.g. when there were early seasonal breaks and some winter rainfall) through to reductions in liveweight up to 35 kg and losses in CS (on a 9-point scale) of c. 1 unit during harsh dry seasons (Holroyd et al. 1977, 1983, 1988; Dixon 1998; Dixon et al. 2011a). Commensurate with higher LW and CS at the end of the dry season, reconception rates of cows have been increased by up to 15 percentage units during the following rainy season (Holroyd et al. 1979, 1988; Dixon 1998; Dixon et al. 2011a). In these and other experiments (Ford 1976; Taylor et al. 1982), cow mortality,

or the proportion of cows considered at risk of dying, has been reduced by NPN supplements.

In the low-input management systems typical of extensive beef cattle production in seasonally dry tropical rangelands, including in northern Australia, NPN supplementation is widely practiced as it provides one of the few economically viable management options to improve nutrient intake during the dry season and to reduce losses of LW and CS and mortality of breeding cows. An essential aspect of applying quantitative cattle nutrition for management of breeding cows in seasonally dry environments is to improve quantitative understanding of nutrient intakes during the annual cycle, maintenance requirements, the utilization of nutrients for tissue growth (e.g. as conceptus growth, milk production and cow body reserves) and the mobilization of body tissues when intakes of nutrients are insufficient (Hogan 1996; Dixon et al. 2007). The nutritional requirements of breeding cows are generally well established (e.g. CSIRO 2007) and the intakes of nutrients of grazing cattle, including in herds on-farm, can be estimated with a variety of diagnostic tools such as on-ground and remote-sensing pasture evaluation, and fecal near infrared spectroscopy. However, there is limited information on the circumstances and extent to which *Bos indicus* cross breeding cows mobilize body energy reserves of fat and muscle, and the extent to which such mobilization can be reduced by provision of NPN supplements in the dry season, when cows are usually also pregnant (Hogan 1996). Improved understanding of the latter aspects of cow nutrition is essential for better application of quantitative nutrition in these production systems.

The present experiment investigated the responses in voluntary intake, LW and estimated mobilization of maternal body energy reserves to a N-based supplement in late-pregnant *Bos indicus* cross cows fed a diet of low quality tropical grass hay in pens. It also investigated the carry-over effects of providing this N supplement on cow and calf growth during lactation. While studies on supplementation of breeding cows under grazing conditions have been conducted previously, this study under closely controlled conditions allowed us to examine in detail and partition the severe losses in LW and body energy reserves that occur in pregnant cows in seasonally dry tropical environments during harsh dry seasons, and the recovery when high quality pasture is available.

Materials and Methods

General

A group of 25 *Bos indicus* × *Bos taurus* (Droughtmaster type with c. 5/8 *Bos indicus* - 3/8 *Bos taurus*) cows, 5–6 years of age and initially 3–6 months pregnant, were used in a study conducted at the Swans Lagoon Research Station (20°4' S, 147°15' E) situated about 100 km south-southeast of Townsville in northern Queensland, Australia. Mean T-LW (including weight of conceptus) on 5 July 1996 was 438 ± (SD) 36 kg (range 391–513 kg) and CS was 5.7 ± 0.53 (range 4.5–6.5). All experimental procedures were carried out according to the Code of Practice for the Care and Use of Animals for Scientific Purposes and with the approval of the relevant Animal Ethics Committee of the Queensland Department of Primary Industries operating when the experiment was conducted.

Phase A – cows held in pens

On 5 July 1996 the cows were placed in individual pens and fed hay alone for 11 days to allow adaptation to pens and to identify any animals with unsatisfactory behavior. On 16 July cows were allocated by stratified randomization based on stage of pregnancy and CS to 4 groups, which were allocated at random to 1 of 2 dietary treatments. These treatments comprised a low quality tropical native grass hay fed alone (Control), and hay plus a N-based supplement (Supplemented). Both the hay and supplements were offered ad libitum for 139 days (16 July–3 December 1996). The hay consisted of native pasture grasses, predominantly black speargrass (*Heteropogon contortus*), harvested from mature pasture during the mid-dry season. The supplement comprised a loose mix containing (g/kg) 267 cottonseed meal, 225 urea, 167 dried molasses, 150 salt, 117 monodicalcium phosphate and 75 ammonium sulfate.

For 99 days of the 139 days of Phase A, the 4 groups of cows were group-fed in 4 pens, but for 2 interim periods (M1, 2–26 August; M2, 2–18 October) were fed in individual pens. During group-feeding, hay was fed as large round bales in hay feeders and the groups were moved at weekly intervals through the 4 pens to counteract any differences individual pens might have on cow intake or performance. During individual-feeding, chopped hay was offered in feed troughs 3 times each week at a level 10–30% above previous average intake and refusals were collected. DM intake was measured during the last 7 days of each individual-feeding period (M1 and M2). The supplement was offered in separate troughs and adequate trough space was provided in group pens for all cows to access the supplement. Feces were collected for analysis from fresh dung pats as well as per rectum during the last 7 days of periods M1 and M2. The calving period extended from 17 October 1996 to 8 January 1997 with a mean calving date of 13 November. While most (17/22) cows calved during Phase A when the cows were in the pens, some (5/22) calved during Phase B when cows grazed pasture, as described below. Unfasted LW and CS of cows estimated on a 9-point scale (NRC 1996) were recorded at 1–3 week intervals.

Phase B – cows and calves grazing grass-legume pasture

The seasonal rainfall break occurred on 23 November 1996 (54 mm rain) and subsequent falls of rain maintained pasture growth (December 31 mm, January 117 mm, February 306 mm, March 247 mm, April 0 mm, May 66 mm, June 32 mm and July 0 mm). On 3 December 1996 at the commencement of Phase B, all cows and calves were removed from the pens to graze rainy season native grass-*Stylosanthes* spp. pastures on a rotational basis as a single herd through a series of 6 similar 40 ha paddocks. The herd was moved every 1–2 weeks to a new paddock until 27 July 1997, when the study terminated. Every 1–3 weeks cows and calves were weighed without fasting, and CS of cows was assessed. On 7 occasions milk production by cows was measured using a weigh-suckle-weigh procedure (Neville 1962) with calves removed from cows for 8 hours before weighings commenced.

Laboratory analyses, calculations and statistical analyses

DM concentration of hay and supplement offered and refused, and of feces, was determined by oven drying (70 ºC). Concentrations of organic matter, total N and minerals in the hay and supplement were analyzed as described by Dixon et al. (2011a). Digestibility of the hay was estimated from near infrared reflectance spectroscopy of feces (F.NIRS) and application of calibration equations appropriate to the pasture system (Dixon and Coates 2009; Coates and Dixon 2011). Conceptus-free live weight (CF-LW) of cows was calculated from the measured cow LW and the day of pregnancy calculated from the actual calving date (O'Rourke et al. 1991). To distinguish between total LW (including conceptus) and the CF-LW the former is abbreviated as T-LW for measurements during pregnancy, while LW is used post-partum when there was no conceptus to be considered. Body net energy content of the cows (Body-NE) was calculated from LW and CS (CSIRO 2007), assuming a standard reference weight of 550 kg. The rates of change in cow T-LW and CF-LW from 5 July 1996 until parturition, and cow and calf LW gains from parturition to Day 90 and from Day 90 to 205 of lactation of individual cows, were calculated by linear regression of LW with time. Milk production was calculated for the same intervals.

Intake of pasture by cows when grazing was estimated as described by CSIRO (2007) using the QuikIntake V5 spreadsheet (S.R. McLennan and D.P. Poppi, unpublished software) along with measurements of mean cow LW, LW gain and milk production plus calf live weight and liveweight gain during the interval. The diet was estimated to contain 8.8 MJ metabolizable energy (ME)/kg DM; this was the 4-year average ME concentration measured in subsequent years with F.NIRS during the 3 months after the seasonal break with grass-*Stylosanthes* pastures in the same trial area (Dixon 2005).

Statistical analyses were conducted separately for the pregnancy phase (5 July in mid-pregnancy to parturition) and the lactation phase (Days 1–90 and Days 91–205 post-partum) by ANOVA using GENSTAT (release 16.1 9VSN International Ltd, Hemel Hemstead, UK). Individual cows were considered as experimental units. The coefficients of the linear regressions of cow LW, CF-LW and calf LW with time for Days 1–90 and 91–205 of lactation were adopted as the best estimates of LW change during these intervals, while the LWs predicted from these regressions were adopted as the best estimates of LW at Days 90 and 205 after parturition. Initial LW, CF-LW and CS of cows on 5 July 1996, calculated day of conception and gender of the calf were examined as potential covariates in the analyses of response variables, and were included when there was >0.9 probability that the covariate did affect the response.

Results

Phase A – cows held in pens

One cow in the Control group aborted and 2 cows in the Supplemented group were withdrawn from the experiment due to low body condition so that during pregnancy data from 22 cows (12 Controls and 10 Supplemented) were analyzed. In addition, during the individual feeding period M2 in October, 1 cow had extremely low intakes of hay and data for this cow for this period were excluded. Measurements for 2 cows, whose calves died neonatally, were included in the data set during pregnancy but not during lactation. Most (17/22) of the cows calved during Phase A (Table 1).

Table 1. The number of cows calving in fortnightly intervals during the final 8 weeks of Phase A to 3 December 1996, and during the first 6 weeks of Phase B.

Phase	Time relative to end of Phase A	Treatment	
		Control	Supplemented
A	6–8 weeks before	1	0
A	4–6 weeks before	3	5[1]
A	2–4 weeks before	3	3
A	0–2 weeks before	2	0
B	0–2 weeks after	2	1[1]
B	2–4 weeks after	1	0
B	4–6 weeks after	0	1
Total		12	10

[1]One calf in each of these subgroups died neonatally, so data were available for 10 cows and 8 cow-calf pairs in the Supplemented treatment during pregnancy and lactation, respectively.

The hay offered contained 6.9 g total N/kg DM, 0.6 g phosphorus/kg DM and 2.8 g calcium/kg DM. In vivo DM digestibility and metabolizable energy (ME) concentration of the diets measured by near infrared reflectance spectroscopy of feces were 528 g/kg DM and 7.4 MJ ME/kg DM, respectively. The hay thus contained c. 5.8 g crude protein/MJ ME. The supplement contained 130 g total N/kg DM, 87% of which was non-protein N. Fecal N concentration averaged 14 g N/kg DM.

Supplemented cows consumed 0.38 and 0.24 kg supplement DM/day (0.95 and 0.59 g supplement DM/kg T-LW/d) during periods M1 and M2, respectively, which provided 49 and 31 g N/d, respectively. These compare with mean intake of supplement during the 139 days of Phase A of 0.21 ± 0.066 kg/d or 0.53 ± 0.16 g DM/kg T-LW/d, which provided 27 g N/d. Intakes of supplement during M1 and M2 were 81 and 14% greater, respectively, than average intake when the cows were fed as groups. Mean voluntary intakes of hay by the Control group were similar during periods M1 and M2 (6.36 and 7.13 g DM/kg T-LW/d, respectively) (Table 2). During periods M1 and M2 the supplement increased hay intakes in Supplemented cows to 9.15 and 9.03 g DM/kg T-LW/d, i.e. by 44 and 27%, respectively (P<0.001 and P = 0.022). Total intakes of the diets were

Table 2. Changes in total live weight (T-LW), conceptus-free live weight (CF-LW), condition score (CS) and body net energy content (Body-NE) of cows from mid-pregnancy through to 3–5 days after calving plus hay and supplement intakes. Cows were held in pens and fed either hay alone or hay with a urea-based supplement (Control and Supplemented, respectively). Intakes of hay and supplement were measured during periods M1 and M2 when cows were fed in individual pens. Means were adjusted for the covariates where these were significant at P<0.10.

Parameter		Supplementation treatment		s.e.	Covariate[1]	Probability
		Control	Supplemented			
Number of cows		12	10			
Mean date of parturition		15 Nov	11 Nov	-	-	-
T-LW	Initial (kg)	446	438	-	-	-
	Final (kg)	331	356	5.9	2,5	0.008
	Change (kg/d)[2]	-0.87	-0.68	0.051	5	0.014
CF-LW	Initial (kg)	436	427	-	-	-
	Final[3] (kg)	331	356	5.9	2,5	0.008
	Change (kg/d)[2]	-1.11	-0.78	0.044	5	<0.001
CS	Initial	5.6	5.8	-	-	-
	Final	3.4	4.2	0.11	3,5	0.011
	Change	-2.3	-1.6	0.20	5	0.015
Body-NE	Initial (GJ)	10.88	10.77	-	-	-
	Final (GJ)	7.38	8.22	0.17	2,5	0.002
	Change (GJ)	-3.50	-2.55	0.172	2,5	0.005
	Change (MJ/d)	-26.6	-21.2	1.33	2,5	0.007
Voluntary feed intake – Period M1						
	n	12	10	-	-	-
	Hay (kg DM/d)	2.53	3.78	0.165	2	<0.001
	Supplement (kg DM/d)	0	0.38	-	-	-
	Total (kg DM/d)	2.53	4.16	0.171	2	<0.001
	Hay (g DM/kg T-LW/d)	6.36	9.15	0.397	-	<0.001
	Supplement (g DM/kg LW/d)	0	0.95	-	-	-
	Total (g DM/kg T-LW/d)	6.36	10.10	0.409	-	<0.001
Voluntary feed intake – Period M2						
	n	11	10	-	-	-
	Hay (kg DM/d)[4]	2.66	3.49	0.245	2	0.021
	Supplement (kg DM/d)	0	0.24	-	-	-
	Total (kg DM/d)	2.66	3.73	0.252	2	0.002
	Hay (g DM/kg T-LW/d)[4]	7.13	9.03	0.557	2,5	0.022
	Supplement (g DM/kg LW/d)	0	0.59	-	-	-
	Total (g DM/kg T-LW/d)	7.13	9.62	0.609	2	0.004

[1]Covariates: 1, initial LW; 2, initial CF-LW; 3, initial CS; 4, initial Body-NE; 5, day of conception.
[2]Changes in T-LW and CF-LW were calculated by regression.
[3]T-LW and CF-LW after parturition were identical.
[4]Results for intakes by one cow during M2 were not included.

increased to 10.10 and 9.62 g DM/kg T-LW/d, i.e. by 59 and 35% in periods M1 and M2, respectively. When intakes of hay and total DM during M1 and M2 were examined in a repeated measures analysis across time, it was revealed that the supplement increased (P<0.001) intakes of both hay and total DM but neither the main effect of time nor the time × supplement interaction was significant (P>0.10). Initial CF-LW of cows on 5 July and day of pregnancy were significant (P<0.001) covariates affecting intakes of hay and total DM.

From 5 July 1996, when the cows were in mid-pregnancy, until shortly after parturition Supplemented cows lost less (P<0.05 to P<0.001) T-LW (-0.68 vs. -0.87 kg/d), CF-LW (-0.78 vs. -1.11 kg/d), CS (-1.6 vs. -2.3 units) and Body-NE (-21.2 vs. -26.6 MJ ME/d) than Control cows (Table 2). Losses for Control and Supplemented cows represented 26 and 19% of T-LW, 24 and 17% of CF-LW and 32 and 24% of Body-NE, respectively.

Phase B – cows and calves grazing grass-legume pasture

Data from 2 cows in the Supplemented treatment with neonatal deaths of calves were excluded, so data during

Table 3. Changes in live weight (LW), condition score (CS) and body net energy content (Body-NE) of cows and LW of calves post-parturition. LW gains of the cows and calves were calculated by regression of LW with time, while changes in CS and Body-NE were calculated by difference. Means were adjusted for the covariates where these were significant at P<0.10.

Parameter	Treatment during late pregnancy		s.e.	Covariate[1]	Probability
	Control	Supplemented			
Cows					
Number of cows	12	8[2]	-	-	-
Cow LW					
Post-calving (kg)	326	358	6.2	2,5	<0.001
Day 90 – lactation (kg)	387	391	7.0	2,5	0.703
Change (Days 1–90) (kg/d)	0.80	0.43	0.062	5	<0.001
Day 205 – lactation (kg)	450	422	12.4	-	0.108
Change (Days 90–205) (kg/d)	0.33	0.27	0.031	5	0.184
Change (Days 1–205) (kg/d)	0.55	0.42	0.035	5,6	0.005
Cow CS					
Post-calving	3.3	4.2	0.17	5,3	<0.001
Day 90 – lactation	4.4	4.8	0.15	5,3	0.125
Change (Days 1–90)	1.1	0.5	0.15	-	0.014
Day 205 – lactation	4.8	5.1	0.19	3	0.217
Change (Days 90–205)	0.4	0.4	0.15	5	0.898
Change (Days 1–205)	1.5	0.9	0.19	5	0.045
Cow Body-NE					
Post-calving (GJ)	7.24	8.32	0.152	2,5	<0.001
Day 90 – lactation (GJ)	9.01	9.26	0.177	2,5	0.310
Change (Days 1–90) (MJ/d)	19.2	10.9	1.86	-	0.004
Day 205 – lactation (GJ)	10.52	10.24	0.310	2	0.513
Change (Days 90–205) (MJ/d)	12.6	9.3	2.45	5	0.324
Change (Days 1–205) (MJ/d)	15.6	9.9	1.74	5	0.027
Milk production (Days 1–90) (kg/d)	4.5	5.4	0.32	5	0.065
Milk production (Days 90–205) (kg/d)	4.6	4.8	0.21	-	0.525
Calves					
Number of calves	12	8	-	-	-
Mean date of birth	15 Nov	11 Nov	-	-	-
Birth weight (kg)	24.3	23.9	1.28	-	0.824
Calf LW (kg)					
Day 90	90.9	99.8	3.47	5	0.077
Day 205	190	194	5.6	-	0.545
Calf LW gain (kg/d)					
Days 1–90	0.77	0.85	0.036	5	0.111
Days 90–205	0.81	0.80	0.027	-	0.852

[1]Covariates: 1, initial LW; 2, initial conceptus-free live weight; 3, initial CS; 4, initial body energy content; 5, day of conception; 6, gender of calf.

[2]Twenty-two cows calved but 2 calves in the Supplemented treatment died neonatally so observations were made on only 8 cows & calves.

lactation were available for 12 and 8 cow-calf pairs in the Control and Supplemented treatments, respectively (Table 1). Shortly after calving Supplemented cows were 10% heavier (358 vs. 326 kg) and had 15% higher Body-NE (8.32 vs. 7.24 GJ) (both P<0.001) than Control cows (Table 3). During the first 90 days post-partum, when high quality rainy season pasture was available for most of the time for most cows, Control cows exhibited compensatory growth relative to previously Supplemented cows, gaining LW (0.80 vs. 0.43 kg/d, P<0.001) and Body-NE (19.2 vs. 10.9 MJ/d, P<0.004) more rapidly than Supplemented cows. By Day 90 post-partum there were no discernable differences (P>0.05) in LW or Body-NE between the 2 groups of cows. From Day 90 to Day 205 post-partum, Control cows continued to gain LW (0.33 kg/d) and Body NE (12.6 MJ/d), but these rates of change were not significantly affected (P>0.05) by supplementation during late pregnancy. Birth weight of calves (mean 24.1 kg) was similar for Control and Supplemented groups (Table 3). Cows supplemented during pregnancy tended (P = 0.065) to produce more milk during the first 90 days of lactation than Control cows and their calves tended (P = 0.077) to be heavier (8.9 kg) at 90 days post-partum (Table 3).

Discussion

The present experiment demonstrated that N supplementation of late-pregnant mature *Bos indicus* cross cows fed low quality tropical grass hay in pens for about 4½ months substantially reduced LW loss during this period. Ingestion of on average 27 g supplementary N/d, most as urea, would have increased crude protein (CP) concentration in the total diet to 10–11% and the dietary CP:ME ratio from c. 6 g CP/MJ ME to c. 13–16 g CP/MJ ME. Thus the rumen-degradable N available for microbial growth would have been deficient in the Control diet and adequate in the Supplemented diet (Hogan 1996; CSIRO 2007). If rumen ammonia concentrations in ruminants fed C4 grasses are lower than for temperate C3 grasses as reported by Hogan et al. (1989), the availability of rumen degradable N in the Control cows may have been much lower than indicated by the 6 g CP/MJ ME and there may have been a severe deficiency of N as a substrate for rumen microbial growth. For the Supplemented cows, although the average amount of supplementary rumen-degradable N ingested by the cows during the entire 139 days of Phase A (27 g N/day) was lower than during the M1 or M2 periods, this lower intake of supplementary N would still have provided a high dietary CP:ME ratio. Thus it is unlikely that the supply of rumen-degradable N as ammonia would have limited voluntary intake of hay in the Supplemented cows.

The very low voluntary intakes of hay by Control cows in the present experiment, and the large increases in hay intake (44 and 27% during periods M1 and M2, respectively) in the cows fed the N supplement, were comparable with the intakes and increases in intakes by late-pregnant cows also fed mature native pasture hay reported by Lindsay et al. (1982) and in other experiments summarized by Hogan (1996). In addition, the large increases in hay intake by Supplemented cows were in accord with increases reported in growing cattle fed comparable low quality tropical grass hays in pens and fed NPN supplements (Ernst et al. 1975; Lindsay et al. 1984; Hennessy and Williamson 1990; Kennedy et al. 1992; Hogan 1996), and hence were not unexpected. The reduced T-LW and CF-LW losses in the pregnant cows in the present experiment were also consistent with similar reduced losses in pregnant cows grazing dry season speargrass native pasture and ingesting a diet with c. 6 g CP/MJ ME reported by Dixon et al. (2011a), and with the effects of NPN supplements on cow LW discussed below.

Numerous grazing experiments have measured the changes in LWs of herds of breeding cows through annual cycles in seasonally dry tropical environments and calving over 3–4 months, including with and without NPN supplementation. However unfortunately the reports of such experiments have not included the data needed to calculate rates of loss of CF-LW during late pregnancy. Usually reports have provided the overall mean LWs of treatment groups at infrequent intervals (e.g. June and October in the dry season and the following April in the late rainy season), but not changes in LW or maternal LW of sub-groups of cows within treatment groups that calved at various times, including before and after the seasonal rainfall break. Firstly, these measurements underestimate the losses in maternal LW (CF-LW) since the true losses are masked by the increasing weight of the conceptus that typically weighs c. 54–66 kg in *Bos indicus* cows at parturition (O'Rourke et al. 1991). Since it is the cow's own tissues that provide the body reserves that impact on survival and subsequent fertility, both the T-LW and CF-LW in late pregnancy are important for quantitative nutrition. Secondly, reported LW changes of treatment groups are usually confounded with the time of the seasonal rainfall break. This is important because cows are generally losing LW before the seasonal break and gaining LW after the break. An example of the consequences of these difficulties can be observed in the data from a 3-year experiment reported by Holroyd et al. (1988) in cows grazing native pasture. During 2 harsh dry seasons cows were reported to have lost 20 and 27 kg LW during July–October, but cows were mated in the previous January and the conceptus would have grown by

about 20 kg during July–October. Thus the actual loss in maternal tissues of the cows (CF-LW) would have been c. 40–47 kg. Furthermore, the graphical representation of the results indicated that from July to the following December–January cows lost c. 80–95 kg LW during 2 harsh dry seasons, LW losses comparable with those observed from July to parturition in the present experiment. More generally, where the calving season extends over several months as generally occurs, it is usually not possible from the data reported in the literature to calculate the changes in maternal body weight of the sub-groups of cows calving during intervals before and after the seasonal break. This knowledge is essential for understanding nutrient balances and applying quantitative nutrition. Estimates of changes in maternal body weight (e.g. estimated as CF-LW in the present experiment) are needed to estimate changes in body energy of cows. If a substantial proportion of cows calve before the seasonal break when cows are losing LW, and the remainder after the seasonal break when cows are gaining LW, the mean LW of the herd at any time provides a poor estimate of the true losses in maternal LW during the late dry season, and will seriously underestimate weight loss in cows calving earlier. It is often these sub-groups of cows in the herd that are most at risk from undernutrition. It is not possible to calculate changes in body energy reserves of various sub-groups of cows in published reports of breeding herds to compare with the results of the present experiment.

A number of studies (e.g. Lamberth 1969; Forbes 1970, 1996; Penzhorn and Meintjies 1972) have reported that voluntary intake of lower quality forage diets by cattle generally decreases during the last 6–8 weeks of pregnancy and suggested that this was due to the physical limitations of abdominal capacity and possibly also hormonal changes (Forbes 1984). However, there was no such decline in intake in the present experiment. This observation was in accord with reports of Hunter and Siebert (1986), who also observed no change in intake of a forage diet in *Bos indicus* cross heifers during late pregnancy. The absence of any decrease in intake in the present experiment may have been associated with the very low intakes of hay (<10 g DM/kg LW/d) and, based on low calf birth weights, a low volume of the conceptus. Both factors would presumably have reduced any effects of lower abdominal capacity in late pregnancy on intake. Most importantly the increase in intake of low quality hay and the reduction in liveweight loss (0.2–0.3 kg/d) due to N supplementation in these late-pregnant cows were comparable with responses previously reported in growing cattle (Winks 1984; Dixon 2011) and in similar genotype cows in pens (Lindsay et al. 1982) or grazing senesced tropical dry season pastures (Holroyd et al. 1988; Dixon 1998; Dixon et al. 2011a).

In the present experiment, although supplementation had substantial effects on cow live weight and body reserves immediately after parturition, the effects on calf growth were small. Calf birth weight was not affected by N supplementation, although birth weight (mean 24 kg) was considerably lower than the 30–34 kg reported in a series of experiments with cows of very similar genotype and grazing native pastures (Holroyd et al. 1979; 1983; 1988). Although milk production and calf growth tended to be higher during the first 3 months of lactation in Supplemented cows, there were no discernable differences in calf weights at 205 days of age, representative of weaning weight. Milk production and calf growth rates, and the small response to provision of N-based supplements during the dry season, are in accord with previous experiments with cows of similar genotype at the same experimental site (Holroyd et al. 1979; 1983; 1988). The observation that Control cows provided sufficient milk for calves to grow at 0.77 kg/d, while gaining 0.80 kg/d themselves, during the first 90 days post-partum indicated that the cows must have achieved very high nutrient intakes when grazing the grass-*Stylosanthes* pasture during Phase B. Our calculations suggested that Control cows were ingesting 42 g DM/kg LW/d and 133 MJ ME/d, while the previously Supplemented treatment cows consumed 35 g DM/kg LW/d and 116 MJ ME/d. In the other experiment used to estimate dietary ME concentration the pastures were continuously grazed, while in the present study the pastures were rotationally grazed and at a low stocking rate. Hence the ME concentration in the diet of cows when grazing in the present experiment may well have been underestimated, and feed intake overestimated, by the calculations. Regardless, by 90 days post-partum the Control cows had recovered 88% of the LW difference between the 2 treatment groups immediately after parturition.

The magnitude of this compensatory growth of Control cows during early lactation appears unusual for breeding cows in the seasonally dry tropics and was likely associated with the high quality of the grass-*Stylosanthes* pasture available to the cows. The adequate and well-distributed rainfall combined with temperatures and light excellent for growth of such pastures, and the regular movement to paddocks of fresh pasture, would have provided unusually high quality pasture compared with that usually available to cows in early lactation. The high compensatory growth observed in the present experiment was in accord with the large and rapid compensatory growth of *Bos taurus* beef cows during lactation following feed restriction during pregnancy in temperate pasture production systems,

presumably of high nutritional quality (Nicol and Kitessa 1997). In more typical tropical rangeland circumstances, where cows are grazing native grass pastures containing little or no legume, diet quality and voluntary intake during the rainy season are likely to be appreciably lower than in the present experiment. As a result lesser compensatory growth in cows with low live weight and body condition at the seasonal break would be expected. This hypothesis is supported by observations in other experiments at Swans Lagoon Research Station (North Queensland), where the extent of compensatory growth in similar cows during the rainy season and the first 3 months post-partum, relative to heavier cows in better condition was only c. 50% (Dixon et al. 2011a; 2011b) and much lower than the 88% compensation observed in the present study. The rapid LW gain of Control cows in the present experiment suggests that in general the primary limitation to the recovery of live weight by low CS mature cows in early lactation is from their inability to ingest sufficient nutrients from available pasture rather than from any carryover effects of severe undernutrition when pregnant during the dry season.

In general, benefits from dry season N supplementation are associated with higher LW and body reserves of breeding cows in the late dry season and near parturition. This provides the cow with a buffer of energy reserves to alleviate the consequences of a delayed seasonal break and/or a failed rainy season, as cows calve in higher body condition and the risk of cow mortalities is reduced. It is generally neither possible nor acceptable to handle or transport cows in late pregnancy or early lactation and the usual alternative management option for reducing cow mortalities is high-cost supplementation with molasses-urea mixtures or other concentrate supplements. The present study demonstrated the importance of cow body reserves in the dry season to sustain cows through annual cycles in harsh seasonally dry tropical environments with extended dry seasons. From the mid-dry season in July through to immediately after calving in the late dry season (December), Control cows mobilized 105 kg (24%) of their conceptus-free live weight and an estimated 3.5 GJ (32%) of their net energy in body reserves. The latter mobilization of body net energy was equivalent to 26.6 MJ/day. Despite these high losses over 4½ months of poor nutrition and during the equivalent of harsh dry season conditions, the cows survived until rains came, and good rainy season pasture enabled them to lactate satisfactorily and produce weaners of 190 kg at 205 days of age. The reasonable weights and body condition (446 kg and 5.6 CS) of the cows in the mid-dry season in July were no doubt important to allow such extensive tissue mobilization. Nevertheless comparable nutritional conditions are likely to apply in some regions of the seasonally dry tropics where herd weaning rates are regularly <55% and many cows conceive, calve and lactate only every second year as a consequence of lactation stress combined with inadequate nutrition, resulting in extended anestrous periods after calving. In such environments cow survival and production are possible only because many cows replenish body reserves during alternate rainy seasons when not pregnant or lactating.

Conclusions

This study provided detailed information on the effects of N supplementation on performance of late-pregnant cows ingesting low quality N-deficient forage. It demonstrated in a closely controlled situation the potential of N-based supplements to substantially reduce loss of live weight and body reserves and thus delay the adverse effects of severe undernutrition in reproducing cows. In commercial herds and circumstances this liveweight advantage due to supplementation of cows in the late dry season would be expected to result in fewer mortalities in breeding cows. When high quality pasture was available in early lactation, which allowed rapid recovery of cow body reserves as well as calf growth, there were negligible carry-over benefits of supplementation on cow live weight by weaning. However, the carry-over benefits of supplementation are likely to be greater in environments where lower pasture quality and availability prevent a rapid recovery of cow live weight as was observed in the present experiment.

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Research Paper

Comparison of forage production and nutritive value of 10 *Grona* spp. accessions in Danzhou, Hainan, China

Producción y valor nutritivo de 10 accesiones de Grona *spp. en Danzhou, Hainan, China*

LINLING YAN[1], RONGSHU DONG[1], WENQIANG WANG[1], SABINE DOUXCHAMPS[2], MARY ATIENO[2], GUODAO LIU[1] AND YIMING LIU[1]

[1]*Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences (CATAS), Ministry of Agriculture & Rural Affairs, Danzhou, Hainan, PR China.* catas.cn
[2]*Tropical Forages Program, CIAT-Asia, Hanoi, Vietnam.* ciat.cgiar.org

Abstract

The demand for high-quality forages is increasing in tropical regions, and could be filled with legume species of the genus *Grona*, which have good nutritive value. In this study, a comparison of the forage production and nutritive value of 10 accessions of *Grona* spp. was carried out in the field at Danzhou, Hainan from 2016 to 2018. Yield, plant height, survival rate, leaf:stem ratio and concentrations of crude protein, crude fiber, crude fat (ether extract), nitrogen free extract, crude ash, calcium and phosphorus were measured. Results showed that *Grona strigillosa* (syn. *Desmodium strigillosum*) cv. Reyan No. 27 and *G. heterocarpa* subsp. *ovalifolia* (syn. *Desmodium ovalifolium*) cv. Maquenque displayed the best performance, owing to their 261.3% and 235.6% higher dry matter yields, respectively, compared with the Control germplasm, *G. heterocarpa* subsp. *ovalifolia* cv. Reyan No. 16 in 2018. Cultivar Maquenque had a higher survival rate than the Control ($P<0.05$). Regarding nutritive value, cv. Reyan No. 27 exhibited higher crude fat and crude fiber but lower Ca concentrations than the Control ($P<0.05$). Based on PCA ranking, we concluded that cvv. Maquenque and Reyan No. 27 could be used as suitable candidate materials for livestock production in tropical regions of China. Further studies on their tannin concentrations and their acceptability by animals are needed before practical recommendations can be made.

Keywords: Comprehensive evaluation, *Desmodium*, forage legumes, PCA, tropical pastures.

Resumen

La demanda de forrajes de alta calidad está aumentando en las regiones tropicales y podría ser satisfecha con leguminosas del género *Grona* que se caracterizan por un buen valor nutritivo. En un estudio de campo, llevado a cabo en Danzhou, Hainan, entre 2016 y 2018, fueron comparadas la producción de forraje y el valor nutritivo de 10 accesiones de *Grona* spp. Para el efecto se determinaron el rendimiento, la altura de planta, la tasa de supervivencia, la relación hoja:tallo y las concentraciones de proteína cruda, fibra cruda, grasa bruta, extracto libre de nitrógeno, ceniza, calcio y fósforo en tejido. Según los resultados, *Grona strigillosa* (sin. *Desmodium strigillosum*) cv. Reyan No. 27 y *G. heterocarpa* subsp. *ovalifolia* (sin. *Desmodium ovalifolium*) cv. Maquenque mostraron el mejor desempeño, debido a sus rendimientos de materia seca más altos (261.3% y 235.6%, respectivamente), en comparación con el germoplasma testigo (*G. heterocarpa* subsp. *ovalifolia* cv. Reyan No. 16) determinados en 2018. La tasa de supervivencia del cv. Maquenque fue más alta que la del testigo ($P<0.05$). En cuanto al valor nutritivo, el cv. Reyan No. 27 presentó concentraciones de

Correspondence: Guodao Liu and Yiming Liu, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences (CATAS)/Key Laboratory of Crop Gene Resources and Germplasm Enhancement in Southern China, Ministry of Agriculture & Rural Affairs, Danzhou, Hainan 571737, PR China.
Email: liuguodao2008@163.com and lymsjtu@foxmail.com

grasa bruta y fibra cruda más altas pero una concentración de Ca más baja que las del testigo (P<0.05). Con base en la clasificación por PCA, los cvs. Maquenque y Reyan No. 27 son buenos candidatos para contribuir a la producción animal en la región tropical de China. No obstante, se requieren estudios sobre las concentraciones de taninos en estos materiales y su aceptabilidad por los animales antes de poder hacer recomendaciones prácticas.

Palabras clave: *Desmodium*, evaluación compuesta, leguminosas forrajeras, pastos tropicales, PCA.

Introduction

The demand for high-quality animal products in developing countries is increasing year by year with the improvement of living standards and consumption changes (Lee 2018). However, the development of livestock production is usually limited by insufficient high quality forage supply. For example, in the tropical region of Hainan province of China, the main forages are king grass [*Cenchrus purpureus* × *C. americanus* (syn. *Pennisetum purpureum* × *P. glaucum*)] and *Stylosanthes* spp., which are not adapted to all edaphic conditions and production systems, resulting in livestock production below its potential (Zi et al. 2018). Therefore, it is crucial to invest in developing and utilizing new forage resources with high production and nutritive value in tropical regions (Kambashi et al. 2014).

Tropical forage legumes have the potential to contribute significantly to sustainable intensification of livestock production (Schultze-Kraft et al. 2018). Based on our prior evaluation of nutritional concentrations (Chen et al. 2010; Liu et al. 2014), an important and well known legume genus is *Desmodium*, from which the genus *Grona* was recently separated, based on morphological, palynological and molecular data (Ohashi and Ohashi 2018). *Grona* comprises currently 21 species and subspecies recognized by GRIN, the taxonomic database of the USDA Genetic Resources Information Network (npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch), among them some species well known by the tropical forages plant research community, such as *G. barbata*, *G. heterocarpa* subsp. *ovalifolia*, *G. heterophylla*, *G. strigillosa* and *G. triflora*.

The Chinese Academy of Tropical Agricultural Sciences (CATAS) introduced more than 800 *Desmodium* and *Grona* accessions to China from the International Center for Tropical Agriculture (CIAT). Evaluations of *Grona heterocarpa* subsp. *ovalifolia* (Prain) H. Ohashi & K. Ohashi [syn. *Desmodium ovalifolium* (Prain) Wall. ex Merr.] accession CIAT 350 and *G. strigillosa* (Schindl.) H. Ohashi & K. Ohashi (syn. *Desmodium strigillosum* Schindl.) accession CIAT 13158 resulted in the release of cultivars Reyan No. 16 and Reyan No. 27, respectively, with good performance in Hainan. *G. heterocarpa* subsp. *ovalifolia* is a creeping stoloniferous herb or subshrub, with height ranging from 0.1 to 0.5 (occasionally 1.0) m, which grows well in tropical and subtropical climates, is adapted to infertile acid soils and drought-tolerant (Cook et al. 2020). *G. strigillosa* has been less researched so far. It is a semi-erect subshrub growing up to about 0.5 m, morphologically similar to *G. heterocarpa* subsp. *heterocarpa* (Figure 1). The species has shown promise on acid, low-fertility soils in Colombia and southwestern Nigeria (Thomas and Schultze-Kraft 1990; Larbi et al. 2000).

Figure 1. *Grona strigillosa* at CATAS, Hainan, China.

Previous studies have shown that *Grona* spp. present average tannin concentrations at a relatively moderate level of about 2.09% (Li et al. 2013), but reduction of methanol-extractable condensed tannin concentration in leaves of *G. heterocarpa* subsp. *ovalifolia* (syn. *D. ovalifolium*) by the addition of polyethylene glycol resulted in increased intake and nitrogen retention by sheep (Carulla et al. 2001). Plants of *Grona* spp. can be used as protein supplements with roughage for ruminants, increasing the value of feed and reducing production costs.

Production and nutritive value of tropical forage legumes can vary widely in different areas with different climatic conditions and soil types. *G. heterocarpa* subsp. *ovalifolia* (syn. *D. ovalifolium*) performed well on a low-phosphorus, acid soil in southern Ethiopia, producing dry matter (DM) yields of 2,977 kg/ha and 2,652 kg/ha in 1989 and 1990, respectively (Larbi et al. 1995). Based on forage production and quality, *G. strigillosa* (CIAT 13155), along with *Centrosema arenarium* (CIAT 5236), were considered the most promising species for the development of silvopastoral systems in the west African forest-savanna transition zone and in similar tropical environments (Larbi et al. 2000).

However, little information is available about the relative performance of various *Grona* spp. accessions in tropical China, both in terms of forage production and nutritive value. To meet the demand for high-quality animal products in China and improve livestock production, it is essential to increase the quantity and quality of forage available, which can be achieved through selecting and breeding the accessions with the highest production and nutritive value. Therefore, the objective of this study was to examine the performance of 9 accessions of *G. heterocarpa* subsp. *ovalifolia* (syn. *D. ovalifolium*) and 1 accession of *G. strigillosa* in terms of yield and nutritive value, in the tropical region of China, over 3 years.

Materials and Methods

Site characteristics

The experimental site was located east of Nada, Danzhou, PR China in the province of Hainan (19°30′ N, 109°30′ E; 149 masl). It has a tropical monsoon climate characterized by hot and rainy summers (May–October), and cold, dry winters and springs (November–April). Mean annual rainfall (2010–2018) is 2,153 mm. Mean monthly maximum and minimum temperatures range from 22 to 35 °C and from 14 to 25 °C, respectively. The soil type is a latosol formed on granite, with characteristics of the top 40 cm as follows: pH 4.5–5.5; alkaline hydrolyzable nitrogen 85–100 mg/kg; organic matter 10–12 g/kg; available P 8.5–12.5 mg/kg detected by the hydrochloric acid-ammonium fluoride method (LY/T1233-1999/5); and available K 50–65 mg/kg.

Experimental materials

Ten *Grona* spp. accessions, including cvv. Reyan No. 16, Reyan No. 27 and Maquenque, were used in this experiment, all of which were obtained from CIAT (Table 1).

Experimental design and management

A single factor randomized block design was used with 3 replications, for a total plot number of 30 (10 accessions × 3 replications). Plot size was 26 m^2 (6.5 × 4 m), and plant spacing was 0.5 × 0.5 m. Guard rows were set up around the experimental site. Seedlings were propagated in a greenhouse by planting seeds on 4 January 2016 and were transplanted into the plots on 25 April 2016. No fertilizer was applied during the experiment, and weeds were removed manually every 2 weeks.

Measurements

The first harvest was carried out 3 months after transplanting in July 2016, leaving a stubble height of 20 cm, and forage yields from the whole plots were recorded subsequently every 4 months until 2018. Fresh material was weighed immediately in the field and random samples of about 1,000 g were collected from each plot and oven-dried at 70 °C until constant weight for determining DM yields.

Before each harvest, 10 plants were selected at random from each plot and height was measured, using a rule to measure from the ground to the highest leaves of the plant. After the final harvest in 2018, all surviving plants were counted, and survival rate was calculated as the ratio of the number of plants remaining and the number transplanted into the plots in April 2016 expressed as a percentage.

Leaf:stem ratio was determined during the first harvest each year, i.e. 25 July 2016, 27 February 2017 and 25 February 2018, respectively. Five to 10 plants (total fresh weight about 1,000 g) were collected at random from each plot and separated into leaf and stem before oven-drying at 70 °C for calculation of leaf:stem ratio.

Samples for determining nutrient composition were collected before the first harvest in 2017 and crude protein (CP), crude fat (ether extract, EE), crude fiber (CF), nitrogen free extract (NFE), crude ash (ash), calcium (Ca) and phosphorus (P) concentrations were determined following the protocols of Owens et al. (2010).

Data analysis

All data were subjected to analysis of variance (SAS 8.1; SAS Institute Inc., Cary, NC). Treatment means were separated using the least significant difference test at P=0.05.

Comprehensive evaluation of production and nutritive value using PCA

Comprehensive evaluation of the 10 accessions was performed on the data collected using Principal Component Analysis (PCA), and ranking values for each accession were calculated with the formula:

Ranking value $=\sum_{i=1}^{n} x_i PC_i$,

where: x is the contribution of PC_i; and n is the number of PCs considered for the ranking (in this case the first 4) (Liu et al. 2015).

Table 1. Details of 10 *Grona* spp. accessions used in this study.

Accession No.	Species	Origin
CIAT 13083	*Grona heterocarpa* subsp. *ovalifolia*	Ubon Ratchathani, Thailand
CIAT 13305	*Grona heterocarpa* subsp. *ovalifolia*	Terengganu, Malaysia
CIAT 13108	*Grona heterocarpa* subsp. *ovalifolia*	Negeri Sembilan, Malaysia
CIAT 13114	*Grona heterocarpa* subsp. *ovalifolia*	Terengganu, Malaysia
CIAT 13120	*Grona heterocarpa* subsp. *ovalifolia*	Yala, Thailand
CIAT 13087	*Grona heterocarpa* subsp. *ovalifolia*	Prachuap Khiri Khan, Thailand
CIAT 3788	*Grona heterocarpa* subsp. *ovalifolia*	Narathiwat, Thailand
cv. Reyan No. 27 (CIAT 13158)	*Grona strigillosa*	Surin, Thailand
cv. Maquenque (CIAT 13651)	*Grona heterocarpa* subsp. *ovalifolia*	Trat, Thailand
Control cv. Reyan No. 16 (CIAT 350)	*Grona heterocarpa* subsp. *ovalifolia*	Southeast Asia (commercial cover crop cultivar)

Results

Dry matter yield

Only CIAT 3788 and CIAT 13120 had significantly higher DM yields (mean 12,180 kg/ha) than Control *G. heterocarpa* subsp. *ovalifolia* cv. Reyan No. 16 (9,730 kg/ha) in 2016 but neither exceeded the DM yield of *G. strigillosa* cv. Reyan No. 27 (11,688 kg/ha; Table 2). In 2017, only *G. strigillosa* cv. Reyan No. 27 had significantly higher DM yield than the Control cv. Reyan No. 16 (13,438 vs. 9,890 kg/ha). In 2018, both *G. strigillosa* cv. Reyan No. 27 and cv. Maquenque had significantly higher DM yields than Control cv. Reyan No. 16 (9,933 and 8,956 vs. 3,801 kg/ha, respectively), representing increases of 161 and 136%, respectively (Table 2). Over 3 years, *G. strigillosa* cv. Reyan No. 27, CIAT 3788, CIAT 13120 and cv. Maquenque had higher DM yields than Control cv. Reyan 16, while CIAT 13083 and CIAT 13108 had the lowest biomass production.

Plant height

Height of the 10 accessions ranged between 17.0 and 63.1 cm, with *G. strigillosa* Reyan No. 27 being tallest, while CIAT 13083 and CIAT 13108 were the shortest ($P<0.05$) (Table 3).

Survival rate

Survival rates varied markedly from 13 to 93% with cv. Maquenque having a significantly higher survival rate than the Control cv. Reyan No. 16, while CIAT 13083 had a significantly lower survival rate than all other accessions ($P<0.05$).

Leaf:stem ratio

While leaf:stem ratio varied from 0.71:1 (*G. strigillosa* cv. Reyan No. 27) to 0.98:1 (CIAT 13108 and 13120), there were no significant differences among accessions for this parameter ($P>0.05$) (Table 3).

Nutrient composition

Significant differences were observed in CF, EE, ash, NFE, Ca and P concentrations among the 10 accessions (Table 4). However, the most important component, i.e. crude protein %, did not differ between accessions, with an average of 12.7%. Only *G. strigillosa* cv. Reyan No. 27 had a significantly higher EE (2.81%) than the Control. CIAT 13305, CIAT 13108, CIAT 13087 and *G. strigillosa* cv. Reyan No. 27 had significantly higher CF (mean 35.8%) than the Control (34.6%). Ca concentration varied from 0.74 to 1.57% with no accession containing a higher level than Control ($P>0.05$). Similarly, P concentration varied from 0.19 to 0.29% in the various accessions with no accession containing a higher level than the Control ($P>0.05$).

Comprehensive evaluation

The first 4 components of the PCA (PC1, PC2, PC3 and PC4) explained 87.6% of the variance between the 10 *Grona* spp. accessions (Table 5). HT, EE and P were driving PC1, with loadings >0.4; NFE and DM were driving PC2, with loadings >0.3; ash and CP were driving PC3, with loadings >0.3; and for PC4, ash and Ca were the key parameters, with loadings >0.4.

The resulting ranking order for the 10 accessions was: cv. Maquenque > cv. Reyan No. 27 > CIAT 3788 > cv. Reyan No. 16 > CIAT 13305 > CIAT 13087 > CIAT 13108 > CIAT 13114 > CIAT 13120 > CIAT 13083 (Table 6).

Table 2. Dry matter yields of 10 *Grona* spp. accessions from 2016 to 2018.

Accession No.	Dry matter yield (kg/ha)			
	2016	2017	2018	Total
CIAT 13083	4,215e[1]	3,467d	568e	8,250
CIAT 13305	9,406cd	9,832bc	2,703cde	21,942
CIAT 13108	5,354e	3,243d	1,192 de	9,789
CIAT 13114	10,281abc	8,762bc	3,976 bc	23,020
CIAT 13120	12,102a	10,328b	4,279bc	26,708
CIAT 13087	11,877ab	7,117c	3,442cd	22,436
CIAT 3788	12,257a	10,442b	6,013b	28,713
cv. Reyan No. 27	11,688ab	13,438a	9,933a	35,060
cv. Maquenque	7,460d	10,189bc	8,956a	26,605
Control cv. Reyan No. 16	9,730bc	9,890bc	3,801bc	23,421
Mean	9,437	8,671	4,486	

[1]Within columns, means with different letters are significantly different at P<0.05.

Table 3. Height and leaf:stem ratio of 10 *Grona* spp. accessions and survival rate in 2018.

Accession No.	Height (cm)	Survival rate (%)	Leaf:stem ratio
CIAT 13083	17.0c[1]	13.1d	0.84:1a
CIAT 13305	26.2bc	57.4bc	0.86:1a
CIAT 13108	18.1bc	53.7bc	0.98:1a
CIAT 13114	24.9bc	41.7bc	0.93:1a
CIAT 13120	27.8bc	31.4c	0.98:1a
CIAT 13087	27.2bc	54.5bc	0.75:1a
CIAT 3788	34.9bc	78.2ab	0.94:1a
cv. Reyan 27	63.1a	65.1bc	0.71:1a
cv. Maquenque	35.4b	93.3a	0.85:1a
Control cv. Reyan 16	25.8bc	64.4bc	0.86:1a
Mean	30.0	55.3	0.87:1

[1]Within columns, means with different letters are significantly different at P<0.05.

Table 4. Nutritive value of 4-month-old forage from 10 *Grona* spp. accessions in 2017.

Accession No.	Crude protein (%)	Ether extract (crude fat) (%)	Crude fiber (%)	Crude ash (%)	Nitrogen free extract (%)	Ca (%)	P (%)
CIAT 13083	12.8a[1]	1.68bc	33.1c	5.49ab	37.5bc	0.78c	0.20e
CIAT 13305	12.8a	1.92abc	35.8a	4.68d	35.7efg	0.84c	0.25bc
CIAT 13108	13.0a	1.77bc	35.5a	5.38abc	35.1fg	1.15ab	0.19e
CIAT 13114	12.1a	1.15c	33.3c	5.04cd	38.5ab	1.57a	0.20e
CIAT 13120	13.0a	2.42ab	33.6c	5.69a	36.2def	0.97ab	0.29a
CIAT 13087	12.6a	1.84bc	35.9a	5.4abc	34.5gh	0.91ab	0.22d
CIAT 3788	12.6a	1.79bc	31.6d	5.19bc	39.2a	0.74c	0.27ab
cv. Reyan No. 27	12.9a	2.81a	36.1a	5.08bcd	33.8h	0.78c	0.27ab
cv. Maquenque	12.6a	2.47ab	31.2d	5.74a	38.4ab	1.1ab	0.24cd
cv. Reyan No. 16	12.8a	1.65bc	34.6b	5.08bcd	36.7cd	1.08ab	0.27ab
Mean	12.7	1.95	34.1	5.28	36.6	0.99	0.24

[1]Within columns, means with different letters are significantly different at P<0.05.

Table 5. Principal components eigenvalues, proportion of variance explained and loadings for each variable.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	3.749	2.423	1.725	0.859	0.597	0.424	0.157	0.067	0
Proportion of variance	0.37	0.24	0.17	0.09	0.06	0.04	0.02	0.01	0
Cumulative proportion	0.37	0.61	0.79	0.88	0.94	0.98	0.99	1	1
Dry matter	0.381	0.342	-0.248	-0.041	0.317	0.063	-0.089	-0.197	0.355
Height	0.428	0.157	-0.169	0.246	0.060	-0.528	0.262	0.576	-0.025
Survival rate	0.224	0.364	-0.114	0.281	-0.762	0.345	-0.153	0.041	0.008
Crude protein %	0.279	-0.370	0.369	-0.134	-0.130	0.388	0.556	0.247	0.149
Ether extract %	0.452	-0.012	0.253	0.294	0.074	-0.110	0.235	-0.681	-0.050
Crude fiber %	0.136	-0.511	-0.396	0.119	0.018	0.128	-0.215	0.028	0.608
Ash %	-0.035	0.053	0.665	0.446	0.188	0.012	-0.445	0.234	0.253
Nitrogen free extract %	-0.247	0.505	0.178	-0.307	-0.040	-0.053	0.305	-0.011	0.587
Calcium %	-0.311	0.173	-0.253	0.549	0.374	0.464	0.366	0.071	-0.085
Phosphorus %	0.403	0.199	0.044	-0.382	0.340	0.449	-0.254	0.205	-0.250

Table 6. Coordinates of the accessions on the first 4 components (PC1, PC2, PC3 and PC4) and resulting ranking values for 10 *Grona* spp. accessions.

Accession No.	PC1	PC2	PC3	PC4	Ranking value	Ranking order
CIAT 13083	10.73	6.35	-3.07	1.73	15.74	10
CIAT 13305	27.92	23.79	-13.14	16.78	55.35	5
CIAT 13108	21.07	18.77	-9.27	14.59	45.17	7
CIAT 13114	21.63	20.58	-9.74	11.36	43.83	8
CIAT 13120	22.79	16.13	-8.77	10.05	40.21	9
CIAT 13087	26.84	21.42	-12.17	17.07	53.17	6
CIAT 3788	35.04	36.92	-14.57	23.33	80.72	3
cv. Reyan No. 27	47.77	32.42	-21.07	28.88	88.00	2
cv. Maquenque	38.83	42.27	-15.86	28.56	93.80	1
cv. Reyan No. 16	28.74	27.51	-13.09	18.43	61.59	4

Discussion

Forage quality is determined mainly by DM yield and nutrient composition (Kambashi et al. 2014). An important reason why different forage materials show great variations in these parameters is genetic diversity. Previous studies show that *Grona* spp. have a rich genetic diversity in terms of morphology and molecular markers. Fan et al. (2010) used 10 morphological parameters to cluster 23 *Grona* spp. and *Desmodium* spp. accessions into 4 groups, while Liu et al. (2014) divided 37 accessions of *Grona* spp. and *Desmodium* spp. into 6 groups using 16 morphological indicators. Similarly, Luo et al. (2016) used 8 EST-SSR to analyze the genetic diversity and phylogenetic relationship of 16 *Grona* spp. and *Desmodium* spp. accessions, dividing them into 5 groups according to cluster analysis, when the similarity coefficient was 0.73. Wang et al. (2017) used the amplified fragment length polymorphism (AFLP) molecular marker technique to analyze the genetic diversity and phylogenetic relationships of 46 *Grona* spp. and *Desmodium* spp. accessions from 5 local species and 2 introduced species, and a NTSYS cluster analysis divided them into 6 categories. The rich phenotypic diversity among different accessions of *Grona* is obviously a reflection of their genetic differences. Our study showed that yields and nutritional composition differed significantly between the 10 accessions tested, which suggests that there are genetic differences between the accessions.

Plant height had a marked impact on DM yields of the 10 *Grona* spp. accessions. The DM yield decrease from 2016 to 2018 may be a reflection of declining survival rates as some plants from all accessions died during the study. CIAT 13083 had the lowest DM yield in 2018 (568 kg/ha) and had the lowest survival rate (13%). In contrast cv. Maquenque had an excellent survival rate of 93% and equal highest DM yield in 2018 (8,956 kg/ha). The differences between accessions in terms of survival rates were possibly a function of the regular 4-monthly harvest times and accessions with higher survival rates may have higher tolerance of regular harvesting (Mukangango et al. 2020).

The fact that *G. strigillosa* cv. Reyan No. 27 was the tallest accession at 63.1 cm reflects the growth form of this species (semi-erect subshrub vs. the prostrate growth habit of *G. heterocarpa* subsp. *ovalifolia*). The somewhat shrubby habit of *G. strigillosa* was also reflected in the lowest leaf:stem ratio, although differences were not significant.

Interestingly CIAT 13108 had a leaf:stem ratio of 0.98:1, but CP% of forage was not different from that of the other accessions. Despite differences in leaf:stem ratio from 0.70:1 to 0.98:1, no significant differences were detected and no differences in CP concentration.

Crude protein, crude fat and crude fiber are usually used as important indicators of the nutritive value of forages (Lauriault and Kirksey 2004). In this study, average CP, EE and CF concentrations were 12.6, 2.47 and 31.2% for cv. Maquenque and 12.9, 2.81 and 36.1% for cv. Reyan No. 27, respectively. When compared with average CP and ADF concentrations (10.5 and 39.7%, respectively) of *Stylosanthes guianensis* reported by Li et al. (2014), the *Grona* spp. accessions tested have a relatively good nutritive value, especially with respect to CP concentration. Our results also showed that *G. strigillosa* cv. Reyan No. 27 had significantly lower Ca concentration than Control *G. heterocarpa* subsp. *ovalifolia* cv. Reyan No. 16.

Conclusions

Based on this comprehensive evaluation of 10 *Grona* spp. accessions, it appears that *G. heterocarpa* subsp. *ovalifolia* cv. Maquenque and *G. strigillosa* cv. Reyan No. 27 were the most promising accessions in this environment, displaying good DM yields, high CP concentration and good survival rates under regular harvesting. They are possible candidate materials for improving livestock production in tropical regions of China, but further evaluation, e.g. feeding experiments, should be conducted to test acceptability to animals and performance of livestock, especially as forage from *Grona* spp. can contain high levels of tannins.

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Research Paper

Varietal differences in yield and nutritional quality of alfalfa (*Medicago sativa*) accessions during 20 months after planting in Ethiopia

Diferencias varietales en producción y calidad nutritiva de accesiones de alfalfa (Medicago sativa) *en Etiopía*

TESSEMA TESFAYE ATUMO[1] AND CHRISTOPHER STEPHEN JONES[2]

[1]*Arba Minch Agricultural Research Center (AMARC), Arba Minch, Ethiopia*. www.sari.gov.et
[2]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia*. www.ilri.org

Abstract

Feed supply in terms of quality and quantity plays an important role in livestock production and productivity. Here we report on varietal differences in yield and nutritional quality among 9 alfalfa accessions over 7 harvests following planting in Ethiopia. Experimental design was a randomized complete block with 3 replications at Chano Mille, Southern Ethiopia on a sandy loam soil where mean annual rainfall is 544 mm. Days to harvesting, plant height, dry matter yield, seed yield and the concentrations of the nutritional quality parameters crude protein (CP), neutral detergent fiber, acid detergent fiber, acid detergent lignin, hemicellulose and cellulose plus in vitro dry matter digestibility (IVDMD) and relative feed value (RFV) were assessed to rank the accessions. There were significant (P<0.001) differences between accessions and harvests in plant height, dry matter yield and seed yield. Accession ILRI_7323A performed best in all agro-morphological aspects. All accessions, except 1, produced forage with CP in excess of 30% and IVDMD greater than 80% with RFV greater than 150 at 50% flowering, indicating the high quality of forage produced. Further studies to assess the longevity of stands of the various accessions seem warranted along with studies in higher rainfall environments or under irrigation.

Keywords: Crude protein, digestibility, fiber, forage legume, quality, yield.

Resumen

La disponibilidad de forraje en términos de calidad y cantidad tiene un papel importante en la producción y productividad del ganado. En un experimento conducido en un suelo franco arenoso en Chano Mille, al sur de Etiopía, se determinaron las diferencias varietales en rendimiento y calidad nutritiva entre nueve accesiones de alfalfa en siete cosechas después de la siembra. La precipitación media anual en la zona es de 544 mm. Los tratamientos fueron dispuestos en un diseño de bloques completos al azar con tres repeticiones. Se evaluaron los siguientes parámetros: días hasta la cosecha, altura de planta, rendimiento de materia seca (MS) y las concentraciones de proteína cruda (PC), fibra detergente neutra, fibra detergente ácida, lignina detergente ácida, hemicelulosa y celulosa, y la digestibilidad in vitro de la materia seca (DIVMS). Para clasificar las accesiones, se utilizó el valor relativo del forraje (VRF). La altura de planta y el rendimiento de MS y de semilla presentaron diferencias significativas (P<0.001) entre accesiones y cosechas. La accesión ILRI_7323A mostró el mejor comportamiento en todos los aspectos agromorfológicos. Cuando las plantas presentaban 50% de floración, todos los materiales, con excepción de una accesión, produjeron forraje con una concentración de PC superior al 30%, DIVMS superior al 80% y un VRF superior a 150, lo que indica la alta calidad del forraje. Se discute la necesidad de estudios complementarios para evaluar la longevidad de las accesiones y su comportamiento en ambientes de mayor precipitación o bajo riego.

Palabras clave: Digestibilidad, fibra, leguminosa forrajera, proteína cruda, valor nutritivo.

Correspondence: T.T. Atumo, Arba Minch Agricultural Research Center (AMARC), PO Box 2228, Arba Minch, Ethiopia.
Email: tessema4@gmail.com

Introduction

Livestock production is highly dependent on the production and availability of quality feed and forage resources (Thornton 2010). According to the Central Statistical Agency (CSA 2018), total livestock population in Ethiopia is estimated to be 193.23 million units (excluding beehives), with cattle making up 60.39 million, sheep 31.3 million, goats 32.74 million, horses 2.01 million, donkeys 8.85 million, mules 0.46 million, camels 1.42 million and poultry 56.06 million. The livestock sector produces 15–17% of Ethiopia's GDP, 35–40% of agricultural GDP and 37–87% of household incomes (GebreMariam et al. 2013). The major impediment to improved production of the Ethiopian livestock sector is considered to be insufficient feed, in terms of both quality and quantity (Tesfay et al. 2016). To help combat this situation and reduce the nutritional constraints to livestock production, the use of adapted, high-yielding, drought-tolerant improved forages of high quality is recommended (Derseh et al. 2016; Bashe et al. 2018).

Alfalfa (*Medicago sativa*) is a high-yielding, perennial (2–3 years with highest economical yield) forage legume that is well suited to hay, silage or pasture production and is known as the "Queen of Forages" for its excellent quality, especially in terms of high crude protein concentration. This species can tolerate frequent harvesting (as frequent as every 35–40 days) by storing energy in the crown to support re-growth after cutting (Undersander et al. 2011). It adds nitrogen to the soil via symbiotic nitrogen fixation in bacterial nodules on the roots, and can also withstand long periods of water deficit by halting vegetative growth and accessing water from deep in the soil through its deep root system (Annicchiarico and Pecetti 2010). These properties make alfalfa one of the most widely grown forage crops in the world.

The project reported here focused on the adaptability, forage yield, seed yield and nutritional quality of 9 alfalfa accessions, selected in consultation with the forage genebank manager of the International Livestock Research Institute (ILRI), based on previous experience and consideration of which accessions may be most suitable to perform well in the selected environment, and planted in Arba Minch Agricultural Research Center, Southern Ethiopia.

Materials and Methods

Varietal evaluation of yield and nutritional quality of 9 accessions of alfalfa (*Medicago sativa*) was conducted between September 2016 and February 2018 at the Arba Minch Agricultural Research Center, Chano Mille substation (6°06' N, 37°35' E; 1,206 masl), where mean annual rainfall is 544 mm. Weather data including mean monthly rainfall and maximum and minimum temperatures during the course of the trial are presented in Figure 1.

Laboratory analysis of a composite (0–30 cm) soil sample collected from the experimental site (Chano Mille) revealed that soil texture is a sandy loam, with pH 6.2, available phosphorus 14.5 g/kg, total nitrogen 0.29%, organic carbon 1.19%, organic matter 1.63% and potassium 1.12 cmolc/kg. The pH of the experimental soil is within the range for productive soils (FAO 2000) and the soil is considered medium in available P, medium to high in N fertility class (Tadesse et al. 1991) and in the medium range for organic carbon (Herrera 1995), which makes it satisfactory for producing good growth and yields of alfalfa.

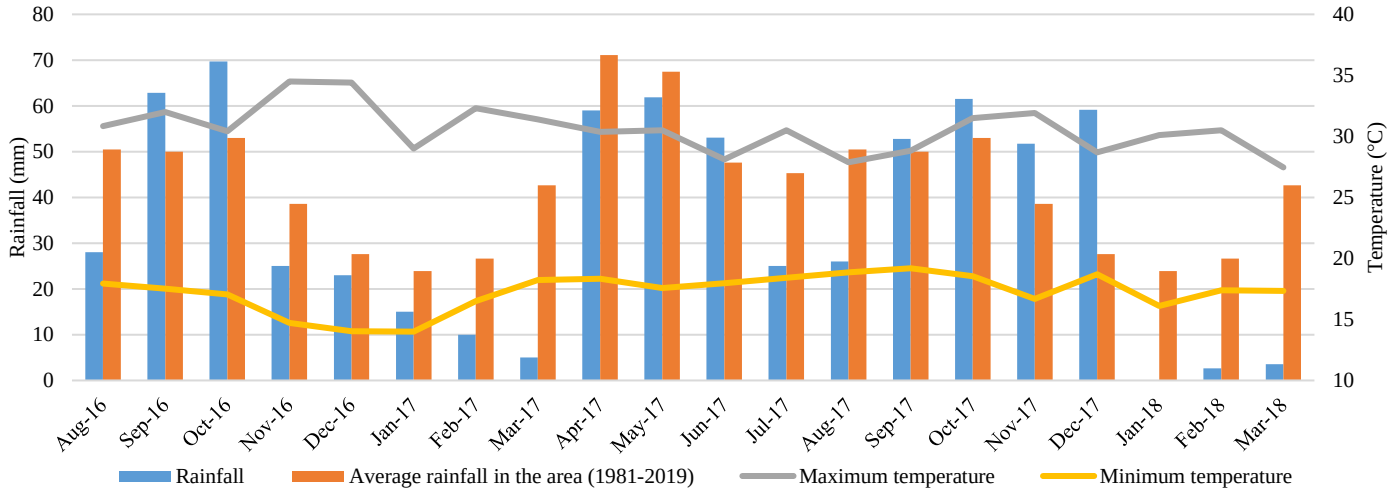

Figure 1. Rainfall and temperature data for Arba Minch area, Gamo Gofa Zone, Ethiopia during the study.

The experiment involved 9 alfalfa accessions (Table 1) with 3 replications and was laid out in a randomized complete block design. Seed was sown in plots (3 × 2 m), consisting of 9 rows of 3 m with 20 cm between rows, using a seed drill on 6 October 2016 during the main cropping season. All recommended field management practices and packages, e.g. weeding, fertilizer application [100 kg/ha NPS (19% N:38% P_2O_5:7% S) fertilizer as basal], were performed uniformly over all plots for the duration of the trial. K application was not recommended by the Ethiopia Soil Information Service (EthioSIS) for this location, so none was applied.

Table 1. Alfalfa accessions used in the study.

Accession	Cultivar name	DOI
ILRI_14176A	WL 514	10.18730/FSNAR
ILRI_15585A	WL 516	10.18730/FTTW5
ILRI_5680A	Moopa	10.18730/G5AZY
ILRI_5681A	wild	10.18730/G5B0Z
ILRI_7323A	wild	10.18730/G6C2G
ILRI_7369A	Kohli	10.18730/G6D7G
ILRI_9234A	Siriver	10.18730/G7R6P
ILRI_9237A	Sheffield	10.18730/G7R9S
ILRI_9239A	Trifecta	10.18730/G7RBV

Seven consecutive harvests were conducted with the criterion for harvesting being when 50% of plants were flowering. The central 5 rows were harvested at 7 cm above ground level with a hand sickle and data collected included: plant height; dry matter yield; and nutritional quality parameters. Briefly, for plant height measurement a total of 10 plants from each plot were randomly selected and measured from ground to the top of the plant just prior to forage harvesting. Following harvesting, green forage was gathered and weighed with a spring balance to determine the fresh matter yield (FMY) per plot. A 500 gram sample from each plot was then placed in a pre-weighed cloth bag and oven dried at 105 °C for 12 h to a constant weight. Dry matter yield (DMY) was calculated using the formula DMY (t/ha) = DM% × FMY/ha. Dried samples were then preserved for subsequent analysis of nutritional quality parameters. Of the remaining 4 rows the outer 2 rows were discarded as border rows, while the other 2 rows were used to obtain seed yields. The border rows were harvested with the seed rows after the forage rows were harvested to minimize the impact of any border effect on the seed rows. Plants from these rows were cut to 20 cm above ground level, taking care to avoid loss of pods and seed, and plants were threshed to obtain seed. The seed was dried in the shade to a constant weight to calculate seed yield.

Forage nutritional quality, in terms of ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations, was assessed in the laboratory according to National Forage Testing Association procedures (Undersander et al. 1993). Relative feed value (RFV) was calculated using the formula: RFV = DDM (% of DM) × DMI (% of BW)/1.29 (Saha et al. 2010), where DDM is the digestible dry matter derived from ADF [DDM = 88.9 – (0.779 × ADF)] and DMI is dry matter intake as a % of body weight (BW) derived from NDF (DMI = 120/NDF). Hemicellulose and cellulose concentrations of the accessions were calculated by the formulae: neutral detergent fiber (NDF) – acid detergent fiber (ADF); and ADF – acid detergent lignin (ADL), respectively.

The data generated were statistically analyzed using the analysis of variance procedure and least significance difference, at the 5% probability level, of Genstat statistical sofware (Version 16, VSN International Ltd, UK) and correlation analysis was undertaken using the SAS (2004) software package

Results

The first harvest of alfalfa accessions was conducted 104 days after planting (18 January 2017), with subsequent harvests a further 140, 48, 44, 33, 44 and 92 days (on 7 June, 25 July, 7 September, 10 October and 23 November 2017 and 23 February 2018, respectively) after the previous harvest. This wide range in harvest cycles reflected the plants' ability to re-grow given the available soil moisture and temperature in this dryland system (Figure 1), which represents what happens in the fields of smallholder farmers in the region. The overall means for plant height and DM yield of the 9 alfalfa accessions for the 7 harvests are shown in Tables 2 and 3, respectively.

Plant height varied significantly ($P<0.001$) between accessions, being greatest for Accession ILRI_7323A (70.0 cm) followed by ILRI_9239A (66.8 cm), while the shortest accession was ILRI_14176A (57.4 cm) (Table 2). Mean DM yields also varied significantly ($P<0.05$) among the accessions, with the highest and most consistent yield being recorded for accession ILRI_7323A (4.99 t DM/ha) followed by ILRI_5680A (4.73 t DM/ha), while the lowest yield was recorded for ILRI_14176A (3.86 t DM/ha) (Table 3). Mean DM yields at different harvests varied significantly ($P<0.05$), which was not surprising given the different lengths of inter-harvest intervals plus variation in rainfall and temperature for the different periods. Highest DM yields were recorded in September, October and June and the lowest in July (Table 3). Seed yields (SY) of alfalfa accessions over the course of the experiment (Table 4) varied significantly ($P<0.001$) among accessions. Pooled mean values showed that the highest SY was recorded for genotype ILRI_7323A (107.7 kg/ha), while the lowest was for ILRI_9237A (32.9 kg/ha).

Table 2. Mean herbage plant height (cm) of 9 alfalfa accessions across 7 harvests following planting in October 2016 in Ethiopia.

Accession	Jan 17	Jun 17	Jul 17	Sep 17	Oct 17	Nov 17	Feb 18	Mean
ILRI_14176A	54.8	68.5	36.0	56.3	68.3	58.3	59.6	57.4d
ILRI_15585A	57.5	76.0	39.1	68.0	69.3	63.3	67.9	63.0bc
ILRI_5680A	59.3	76.7	43.5	59.5	76.5	63.1	71.5	64.3bc
ILRI_5681A	52.7	69.2	45.2	65.6	79.7	61.2	57.2	61.5cd
ILRI_7323A	63.3	81.7	55.3	73.7	83.5	67.1	65.5	70.0a
ILRI_7369A	55.4	65.8	40.8	65.0	60.3	52.7	65.8	58.0d
ILRI_9234A	57.5	69.7	37.5	60.8	75.7	59.4	70.3	61.6cd
ILRI_9237A	58.1	72.5	32.7	61.2	71.7	64.6	69.8	61.5cd
ILRI_9239A	63.7	78.7	43.9	61.7	82.5	59.3	77.9	66.8ab
Mean	58.1d	73.2a	41.6e	63.5c	74.2a	61.0cd	67.3b	62.7

CV (%) = 11.1. $LSD_{0.05}$: accessions = 4.24, harvests = 3.74. Means within columns and rows with a common letter are not significantly different (P>0.05).

Table 3. Mean dry matter yields (t/ha) of 9 alfalfa accessions across 7 harvests following planting in October 2016 in Ethiopia.

Accession	Jan 17	Jun 17	Jul 17	Sep 17	Oct 17	Nov 17	Feb 18	Total yield	Mean
ILRI_14176A	2.68	4.52	2.23	4.61	4.51	3.16	5.29	27.0	3.86d
ILRI_15585A	3.46	4.99	2.43	5.31	4.47	2.91	5.19	28.8	4.11cd
ILRI_5680A	3.66	5.74	3.29	5.58	6.02	3.76	5.05	33.1	4.73ab
ILRI_5681A	3.08	5.36	3.06	5.63	5.58	3.47	5.48	31.7	4.52abc
ILRI_7323A	3.51	4.84	3.97	6.26	6.80	4.25	5.29	34.9	4.99a
ILRI_7369A	3.39	4.97	3.17	4.56	4.71	3.76	5.29	29.9	4.27bcd
ILRI_9234A	3.42	4.67	2.57	4.76	4.64	3.52	5.78	29.4	4.19cd
ILRI_9237A	3.34	6.35	2.70	4.85	4.71	3.52	5.68	31.2	4.45bc
ILRI_9239A	4.06	4.92	3.01	4.95	5.02	3.64	5.15	30.8	4.39bc
Mean	3.40b	5.15a	2.94c	5.17a	5.16a	3.55b	5.35a	30.7	4.39

CV (%) = 17.6. $LSD_{(0.05)}$: accessions = 0.47, harvests = 0.42. Means within a column and row with a common letter are not significantly different (P>0.05).

Table 4. Mean seed yield (SY, kg/ha) and crude protein yield (CPY, t/ha) of 9 alfalfa accessions across 7 harvests following planting in October 2016 in Ethiopia.

Accession	SY (kg/ha)	CPY (t/ha)
ILRI_14176A	44.2de	1.68bcd
ILRI_15585A	86.1b	1.77bcd
ILRI_5680A	40.8ef	1.25e
ILRI_5681A	45.3de	1.53d
ILRI_7323A	107.7a	1.84abc
ILRI_7369A	58.9c	1.63cd
ILRI_9234A	52.1cd	2.06a
ILRI_9237A	32.9f	1.69bcd
ILRI_9239A	43.6de	1.91ab
Mean	56.9	1.70
$LSD_{0.05}$	9.94	0.27
CV (%)	10.2	9.0

Mean values within columns with a common letter are not significantly different (P>0.05).

Mean values for forage quality traits for the 9 alfalfa accessions across the 7 harvests in terms of ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations plus in vitro dry matter digestibility (IVDMD) and relative feed value (RFV) are presented in Table 5. There was significant (P<0.05) variation among accessions in terms of CP concentration, IVDMD and RFV. All accessions except ILRI_5680A recorded CP concentration overall in excess of 30% and all accessions recorded IVDMD in excess of 80%. Crude protein yields over the total period ranged from 1.25 (ILRI 5680A) to 2.06 (ILRI_9234A) t/ha (Table 4). RFV varied from 160 to 184. No significant variation (P>0.05) was detected in concentrations of ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose and cellulose.

Table 5. Mean chemical composition, in vitro dry matter digestibility (IVDMD) and relative feed value (RFV) of 9 alfalfa accessions across 7 harvests following planting in October 2016 in Ethiopia.

Accession	Forage quality trait (% DM)								
	Ash	CP	NDF	ADF	ADL	Hemicel-lulose	Cellulose	IVDMD	RFV
ILRI_14176A	12.6	31.7bcd	38.6	21.7	5.7	16.8	16.0	82.2bc	179ab
ILRI_15585A	12.0	32.6bcd	38.7	20.6	5.9	18.1	14.8	83.8ab	177b
ILRI_5680A	13.2	27.4e	37.7	24.2	5.8	13.6	18.3	82.9bc	173b
ILRI_5681A	13.5	30.2d	40.8	24.6	6.1	16.1	18.6	81.3bc	160c
ILRI_7323A	12.9	33.2abc	37.5	22.4	5.3	15.1	17.1	82.1bc	178ab
ILRI_7369A	12.5	31.2cd	37.2	23.4	6.1	13.8	17.3	80.5c	177ab
ILRI_9234A	12.2	35.1a	38.5	21.2	5.5	17.3	15.7	87.0a	177b
ILRI_9237A	12.7	31.8bcd	36.6	21.7	5.1	14.8	16.6	83.0bc	184a
ILRI_9239A	12.4	33.8ab	37.3	21.8	5.6	15.4	16.3	83.3bc	180ab
LSD	NS	2.51	NS	NS	NS	NS	NS	3.31	6.98
CV (%)	5.1	4.5	10.0	8.6	13.6	15.0	9.1	2.3	2.3

Means within columns with a common letter are not significantly different (P>0.05).

Discussion

This study has shown that all alfalfa accessions grew satisfactorily during the period, producing acceptable yields of very high quality forage. Rainfall seemed to have a greater influence on growth rate of the various accessions than genetic differences, and timing of consecutive harvests appeared to be primarily dependent on weather conditions during the growth period. When sufficient moisture was present, i.e. during and immediately after the onset of the rainy season, plants grew more rapidly and flowered more quickly, as can be seen by the 30–45 day inter-harvest intervals for the 3rd, 4th, 5th and 6th harvests in July, September, October and November 2017 (Figure 1). However, when weather conditions were less favorable for alfalfa growth, it took more than 90 days to reach the harvesting stage, i.e. 50% flowering. The long periods between harvests, from October to January and January to June in the establishment year and November to June in the second year, were associated with dry conditions in February and March. From these results we can infer that optimum yields throughout the year would be achievable only by applying irrigation water during periods of low rainfall.

The variations in growth and yield performance of different alfalfa accessions would be due to genetic differences between them and their response to environmental conditions experienced during the growing season, especially soil moisture. This finding concurs with those of previous reports, which show that environmental conditions play a significant role in the variation in dry matter yield among alfalfa cultivars (Veronesi et al. 2010). It has also been reported that cutting frequency has a significant effect on forage yield and yield components in alfalfa (Tabacco et al. 2002; Borreani et al. 2006; Testa et al. 2011; Atıs et al. 2019) and that the crop harvesting cycle has a significant effect on other parameters including stand height (Testa et al. 2011). Our harvest intervals were determined by flowering patterns, with 50% flowering being the criterion for harvesting. Since time of year has a significant impact on incidence of flowering, this factor also affected intervals between harvests, adding to the marked variation in this parameter throughout the year.

While the range of overall means for plant height of the various accessions across all harvests was 57.4 cm (ILRI_14176A) to 70.0 cm (ILRI_7323A), there was also significant (P<0.05) variation in mean plant height of all accessions across harvests with stands at the 2nd (73.2 cm) and 5th (74.2 cm) harvests significantly taller than at all other harvests. The fact that the accessions took 140 days to reach this height before the second harvest and only 33 days to do so before the fifth harvest highlights the influence that time of year and soil moisture have on plant growth. Plant height is strongly associated with the total biomass yield of the crop (Tilly et al. 2013) and previous research has shown that plant height of alfalfa varies between cultivars (Djaman et al. 2020) as well as with crop management (Ullah et al. 2009) and soil fertility (Massaliyev et al. 2015). In our study, time of year and environmental conditions had a greater influence on plant height than the particular accession. Mean DM yields varied significantly (P<0.05) among accessions and ranged from 3.86 t DM/ha for ILRI_14176A to 4.99 t DM/ha for ILRI_7323A. This was to be expected as previous studies have reported significant differences in DM yields among alfalfa cultivars, with yields of 2.0–3.3 t DM/ha (Yüksel et

al. 2016), and 2.6–3.6 t DM/ha (mean 3.2; Altinok and Karakaya 2002) under rain-fed conditions.

Seed yield improvement is critical for the commercial development of alfalfa varieties (Huyghe et al. 2001). Variation in seed production of alfalfa is a product of the genetic potential of the crop and environmental factors such as insect pollinator attractants (Sreedhara et al. 2012). In this experiment ILRI_7323A, which performed best across the full range of agro-morphological traits, proved to have the highest relative seed yield, whereas ILRI_9237A was the lowest seed-yielding accession (Table 4). Seed yields presented here, while similar to those reported by some researchers (Al-Kahtani et al. 2017), were significantly lower than those reported by others, although they were consistent in that genotypic variation affected seed yield over cutting periods (Falcinelli 1999; Bolaños-Aguilar et al. 2000; Huyghe et al. 2001).

Although some of the forage quality parameters, such as ash, NDF, ADF and cellulose concentrations, were not significantly different among the alfalfa accessions in the present experiment, significant variation among accessions was detected in this study for CP concentration, IVDMD and RFV. These results concur with the findings for alfalfa quality assessment by other researchers (Milić et al. 2011). Forages with a RFV of greater than 100 are generally considered to be of high quality (Saha et al. 2010; Schroeder 2013) and all cultivars in our study had values above 150. In addition, according to the alfalfa quality rating of Orloff and Marble (1997), premium quality alfalfa forage contains 29% or less ADF. The results from our experiment demonstrate that, according to these criteria, all 9 accessions tested in our study would be considered to produce very high quality forage. In fact, since all accessions had CP concentration in excess of 30% at 50% flowering and IVDMD in excess of 80%, the forage could be considered of exceptionally high quality. The CP values are considered quite high but broadly similar to those reported by other scholars, e.g. Yolcu et al. (2008), who reported values of 24–32% for 12 cultivars and Awad and Bakri (2009), who reported values of 20–27% for 3 cultivars.

Conclusion and Recommendations

In this study, the 9 alfalfa accessions achieved very satisfactory yields (27–35 t DM/ha) of very high quality forage (>80% IVDMD, >30% CP) during the 20 months following planting in a 544 mm rainfall environment. The effects of harvest and accession were significant for plant height and DM yield, which indicated genotype differences under a single management system. The superior performance of accession ILRI_7323A in terms of growth, seed yield and overall forage quality demonstrated its potential value for production in the region. Further studies are warranted to determine longevity of the stands and the improvement in yields in higher rainfall environments or when irrigated.

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Research Paper

Evaluation of sainfoin accessions exposed to powdery mildew disease at four locations in Iran

Evaluación de accesiones de esparceta expuestas al oídio (mildiú polvoroso) en cuatro localidades de Irán

MOHAMMAD ALI ALIZADEH[1], ALI ASHRAF JAFARI[2], KARAM SEPAHVAND[3], SAIED DAVAZDAHEMAMI[4], MOHAMMAD RAHIM MOEINI[5], FARID NORMAND MOAIED[6] AND BITA NASERI[7]

[1]*Natural Resources of Gene Bank Group, Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran.* en.rifr-ac.ir
[2]*Rangelands Research Division, Research Institute of Forests and Rangelands, AREEO, Tehran, Iran.* en.rifr-ac.ir
[3]*Department of Natural Resources, Lorestan Agricultural & Natural Resources Research & Education Center, AREEO, Lorestan, Iran.* areeo.ac.ir
[4]*Isfahan Agricultural & Natural Resources Research & Education Center, AREEO, Isfahan, Iran.* areeo.ac.ir
[5]*Plant Protection Research Department, Zanjan Agricultural & Natural Resources Research & Education Center, AREEO, Zanjan, Iran.* areeo.ac.ir
[6]*Tabriz Agricultural & Natural Resources Research & Education Center, AREEO, Tabriz, Iran.* areeo.ac.ir
[7]*Plant Protection Research Department, Kermanshah Agricultural & Natural Resources Research & Education Center, AREEO, Kermanshah, Iran.* areeo.ac.ir

Abstract

In order to evaluate resistance of sainfoin (*Onobrychis viciifolia*) to powdery mildew, seeds of 19 accessions were collected from different parts of Iran and sown at 4 locations, i.e. Kheirabad, Khoramabad, Semirom and Tabriz, in 2014. Accessions were evaluated for powdery mildew severity index (DSI), forage dry matter yield (DM), dry matter digestibility (DMD) and crude protein (CP) and water soluble carbohydrate (WSC) concentrations over 4 years. Based on Duncan's test, accessions 15353 and 3001 showed disease severity index lower than 25% and were nominated as resistant to powdery mildew. Accessions Oshnavieh and Polycross were considered semi-resistant due to their DSI ranging from 25 to 50%. Other accessions were considered susceptible because their DSI was higher than 50%. The resistant accessions (15353 and 3001) with average yields of 3,341 and 3,304 kg DM/ha were ranked as having high DM production, in addition to displaying high DMD plus high CP and WSC concentrations. Severity of powdery mildew infection was linked negatively with all 3 quality traits, i.e. DMD and CP and WSC concentrations. According to Eberhart/Russell regression results, stability of accessions 3001 and 15353 for DSI and DM yield was confirmed across 4 locations. We recommend the use of accessions 3001 and 15353 in future breeding programs to increase resistance to powdery mildew, while at least maintaining yield and quality attributes. Evaluation of other sources of sainfoin germplasm should continue to identify further resistant accessions.

Keywords: Forage; nutritive value, *Onobrychis*; productivity, susceptibility, tropical legumes.

Resumen

En un experimento multilocacional conducido durante 4 años en 4 localidades de Irán (Kheirabad, Khoramabad, Semirom y Tabriz), se evaluaron 19 accesiones de esparceta (*Onobrychis viciifolia*), una leguminosa forrajera, recolectadas en diferentes regiones de Irán. Las evaluaciones incluyeron: susceptibilidad al oídio (*Leveillula taurica/*

Correspondence: Bita Naseri, Plant Protection Research Department, Kermanshah Agricultural & Natural Resources Research & Education Center, AREEO, Kermanshah, Iran.
E-mail: bitanaseri@yahoo.com

Oidiopsis sp.); rendimiento de materia seca (MS); digestibilidad de la MS; y concentraciones de proteína cruda (PC) y carbohidratos solubles. Las accesiones 15353 y 3001 presentaron un índice de severidad (IS) de la enfermedad inferior a 25% y fueron consideradas resistentes al oídio. Las accesiones Oshnavieh y Polycross presentaron resistencia media (IS entre 25 y 50%). Las demás accesiones presentaron un IS >50% y se calificaron como susceptibles. Las accesiones resistentes (15353 y 3001) presentaron altos rendimientos promedio de MS (3,041 y 3,304 kg MS/ha, respectivamente) además de alta digestibilidad y altas concentraciones de PC y carbohidratos solubles. La severidad de la infección por el oídio presentó relaciones negativas con la digestibilidad y las concentraciones de PC y carbohidratos solubles. El análisis de estabilidad de Eberhart/Russell confirmó la estabilidad de las accesiones 3001 y 15353 para la resistencia al IS y el rendimiento de MS a través de las localidades. Por tanto, se sugiere usar estas accesiones en futuros programas de mejoramiento de esparceta. La evaluación de otras fuentes de germoplasma de esta especie debe continuar para identificar adicionales accesiones resistentes.

Palabras clave: Forrajes, leguminosas subtropicales, *Onobrychis*, productividad, susceptibilidad, valor nutritivo.

Introduction

Common sainfoin, *Onobrychis viciifolia* Scop. (syn. *O. sativa* Lam.), is a most important perennial forage legume, which is highly regarded by farmers due to high levels of palatability and nutrient concentrations (Delgado et al. 2008). Sainfoin was introduced to agriculture as a drought- and salinity-tolerant plant, can produce yields comparable with those of alfalfa and due to its deep roots is well adapted to dry and desert ecosystems (Soares et al. 2000). It contains condensed tannins which lower bloat incidence in grazing animals and improve protein digestion in the intestines (Rumball and Claydon 2005). Sainfoin, also known as holy clover, is often intercropped with forage grasses to improve soil fertility and pasture quality via nitrogen (N) fixation (Lu et al. 2000). The ability of this forage crop to fix N can reduce applications of chemical fertilizers (Greub et al. 1984). The honey obtained from nectar of sainfoin flowers is very bright and sweet, with a distinct flavor, and displays concentrated crystallization at any temperature with yields in the range of 20–51 kg/ha (Pérez-Arquillue et al. 1995). Sainfoin is also tolerant of *Hypera postica* (Gyllenhal) (Curculionidae), a common pest of alfalfa crops (Allen and Allen 1981).

The *Onobrychis* genus comprises 69 species (both annual and perennial) in Iran (Mabberley 1997). The species are concentrated in the Zagros mountains of Iran (60 species) and Turkey (52 species) (Çelik et al. 2011), and these areas appear to be the major origins of genetic diversity in sainfoin populations (Mohajer et al. 2013). The plant is commonly grown in both irrigated and dry lands of Iran, including Charmohal Bakhtiary, Lorestan, Fars, Kerman, Kordestan, Kermanshah, Zanjan and Mazandaran provinces (Hidarian and Mollaei 2001) and is capable of acceptable growth in cropping systems, which are inappropriate for clover and alfalfa cultivation.

However, more widespread cultivation of sainfoin in the main growing regions of Iran is limited by the incidence of powdery mildew, which reduces forage yield during the second harvest (Majidi 2010). Severe infections of powdery mildew occur in Azarbayjan, Charmohal Bakhtiary, Esfahan, Fars, Kerman, Kermanshah, Kordestan, Lorestan, Mazandaran and Zanjan provinces (Bamdadian 1991; Behdad 1996). Naseri and Alizadeh (2017) reported a number of climatic indicators of development of powdery mildew infections in Zanjan province. This disease can appear at the end of the growing season and causes noticeable yield losses at the last cutting. The causal agent of this disease is a fungus known as *Leveillula taurica*, with *Oidiopsis* sp. as the asexual form. This pathogen also infects other plant species such as alfalfa, sunflower, safflower and hemp. The aerial plant parts severely infected by this pathogen develop spots, become dehydrated and are shed. Depending on the region, the disease appears in August–September in Iran (Sharifnabi and Banihashemi 1990; Ershad 1995; Naseri and Marefat 2008). Alizadeh and Jafari (2014) evaluated the susceptibility of 56 accessions of sainfoin to powdery mildew in Alborz province during 2010–2012. Their results showed that 4 accessions (Polycross, Oshnavieh, 3001 and 15353) were more resistant than the remaining accessions and were considered desirable parents for breeding superior sainfoin cultivars.

The objectives of this study were: (i) to test the resistance to powdery mildew disease of these promising accessions across 4 geographical regions of Iran in comparison with a set of 15 inbred accessions collected in different parts of the country; and (ii) to identify accessions with superior dry matter yield and forage quality.

Materials and Methods

The study was conducted at 4 locations (Khoramabad, Kheirabad, Semirom and Tabriz) in Iran as described in Table 1. These 4 locations (Figure 1) were included in the current research to cover a range of agro-ecological conditions for powdery mildew development and sainfoin productivity. In this experiment, reactions of tolerant and semi-tolerant accessions in comparison with susceptible accessions were evaluated during 4 growing seasons under irrigated conditions using a randomized complete block design with 3 replications. The seeds of 19 accessions (Table 2) were collected from plants grown in the earlier research project. For each accession, seeds were sown in 4 drilled rows (2 m long and 0.25 m apart) in sward conditions in April 2014. Irrigation was applied according to the plant requirements. Weeds were controlled mechanically and fertilizers (75 kg N/ha as ammonium nitrate and 150 kg P/ha as superphosphate) were applied based on scientific advice and recommendations. In the establishment year (2014), plots were cut once and no data were collected.

Table 1. Ecological and geographical characteristics of the 4 test locations in Iran.

Location	Province	Climate	Elevation (masl)	Temperature (°C)			Lat (N)	Long (E)	Annual rainfall (mm)	Soil type
				Maximum	Minimum	Mean				
Koramabad	Lorestan	Semi-arid	1,148	47.8	-1	22.9	33°47'	48°36'	406	Loamy clay
Tabriz	Azarbayjan	Cold, semi-arid	1,359	39	-22.5	14.0	38°08'	46°27'	322	Sandy loam
Semirom	Esfahan	Cold, arid	1,612	47.8	-1	22.5	32°65'	51°28'	300	Clay
Kheirabad	Zanjan	Cold, arid	1,763	18.4	-29	10.7	36°41'	48°29'	267	Loamy clay

Figure 1. Map of Iran showing the 4 study locations in their respective provinces. Source: Encyclopædia Britannica.

Table 2. Identification and origin of 19 sainfoin (*Onobrychis viciifolia*) accessions studied at 4 locations in Iran.

Gene bank code	Collection area	
	Province	District
334	Alborz	Karaj
1601	Gorgan	Gorgan
2399	Tehran	Tehran
2759	Hamadan	Hamadan
3062	North Khorasan	North Khorasan
3800	Semnan	Garmsar
4083	Esfahan	Semirom
8199	Tehran	Tehran
8799	Kermanshah	Kermanshah
9147	Alborz	Karaj
9262	Alborz	Karaj
9263	Alborz	Karaj
12542	Unknown	Unknown
15364	Alborz	Karaj
19402	Hamadan	Hamadan
3001	Alborz	Karaj
15353	Alborz	Karaj
Oshnavieh	Urumia	Oshnavieh
Plc (Polycross)	Alborz	Karaj

In the second year, assessments of powdery mildew were made under natural infections in which all accessions (tolerant and susceptible) were cultivated adjacent to each other. Thus, disease severity was evaluated when plants were exposed to airborne contamination from natural infections under environmental conditions specific to the 4 study regions. A disease severity index (DSI) was applied to each accession according to the percentage of aerial parts covered with fungal mycelium. This assessment was performed a few days before the second and third harvests, when powdery mildew infections on plants were rated according to a 0–4 scale (Horsfall and Cowling 1978), with a score of 0–2 (resistant) allocated when fungal mycelium covered 0–25% of aerial parts, a score of >2–3 (semi-resistant) when mycelium covered 25–50% of aerial parts and >3–4 (susceptible) when mycelium covered 51–100% of aerial parts as an indicator of plant susceptibility to powdery mildew.

At the 50% flowering stage plants were harvested by hand and forage produced by each plot was weighed immediately. Three harvests were performed at each site, i.e. early May (first harvest), early June (second harvest) and early September (third harvest). A 300 g representative subsample of fresh forage from each plot was collected, placed in a bag and transferred to the laboratory, where it was oven-dried at 75 °C for 48 hours and then weighed to determine DM percentage. DM yields were calculated for each experimental plot. In addition, subsamples were milled and dry matter digestibility (DMD) plus crude protein (CP) and water-soluble carbohydrate (WSC) concentrations were determined in the laboratory of Research Institute of Forests and Rangeland, Tehran, Iran, based on the method of Jafari et al. (2003).

Statistical analysis

To simplify interpretation of statistical analysis of quality-severity-yield data obtained from 19 sainfoin accessions examined over 4 growing seasons at 4 different geographical locations, low variable data over years were pooled and analyzed to estimate the extent of variability among genotypes and locations. Thus, due to the lack of significant effects of year, annual DM yields, disease severity ratings and quality traits (DMD, WSC and CP) were averaged over the study years to be used for combined analysis over 4 locations. Mean comparisons were conducted based on Duncan's method. Although several stability parameters have been proposed, Eberhart and Russell (1966) considered a stable genotype should have a slope (b value) equal to unity and deviation from regression (S^2_d) equal to zero. Stable genotypes would be those having mean yield higher than the average yield of all genotypes under test. This method has been used widely for evaluating yield stability in both annual and perennial plants. Based on the Eberhart/Russell stability regression model, regression coefficient values (bi) and deviation from regression (S^2_{di}) were calculated for each of the 19 genotypes. Stable genotypes with high mean yields were identified if the regression coefficient equated to one (bi = 1) and deviation from regression equated to zero (S^2_{di} = 0; Eberhart and Russell 1966). The stability tests were performed for both DM yield and disease severity index (DSI) using AGROBASE (Agronomix Software Inc., Winnipeg, Canada). Minitab 16 was used to illustrate relationships among genotypes and environments.

Results

Considering the lack of significant effects of study year, the combined analysis of variance across 4 environments showed significant differences between locations (except for DMD; P>0.01), accessions and G × E interactions for all traits (P<0.01; Table 3). Mean values for DM yield, CP and WSC concentrations, DMD and DSI at each location and averages over 4 locations for sainfoin accessions are presented in Tables 4–8.

While some accessions performed consistently in terms of DM yield across all locations, others performed

Table 3. Combined analysis of variance of DM yield (DMY), disease severity index (DSI), dry matter digestibility (DMD) and crude protein (CP) and water soluble carbohydrate (WSC) concentrations for sainfoin accessions (G) over 4 locations (E) in Iran.

Source	df	Mean Square				
		DMY	DSI	DMD	CP	WSC
E	3	565484**	9819.49**	128.25	355.47*	11.50**
Rep (E)	8	20838	345.29	93.28	73.24	6.42
G	18	16073**	1794.09**	20.81**	5.03**	2.92**
G × E	54	17776**	471.34**	11.32**	2.77**	1.44**
Error	144	8787	40.67	8.10	0.89	0.60
CV (%)		2.92	15.52	3.87	4.39	4.18

well at some locations but relatively poorly at others. Accession 8199 was a consistent performer producing higher DM yield at Esfahan (3,390 kg/ha) and Khoramabad (5,653 kg DM/ha) than most other accessions, and had equal highest mean yield over all locations (3,897 kg DM/ha), along with accession 9263 (3,614 kg DM/ha) (Table 4). While accession 9263 performed very well at 3 locations (mean 4,253 kg DM/ha), DM yield at Esfahan was only half that of the highest yield (1,700 vs. 3,390 kg DM/ha) at that location. All accessions grew well in at least one location but often grew poorly at others, e.g. Polycross at Tabriz (4,470 kg DM/ha) and Koramabad (2,600 kg DM/ha) and accession 19402 at Koramabad (5,554 kg DM/ha) and Zanjan (1,681 kg DM/ha).

Crude protein concentrations at Tabriz were consistently lower for all accessions than at the remaining locations (Table 5) and there was much less variation between accessions in terms of CP% than there was for DM yield. Overall CP means for individual accessions across the 4 locations ranged from 18.1 to 20.3%. As for CP%, dry matter digestibility of accessions at Tabriz was consistently lower than at the other 3 locations (Table 6). While accessions 2759, 3001 and 19402 had consistently high DMD values at all locations, almost all accessions had overall mean DMD greater than 68%. As for CP% and DMD, WSC concentrations at Tabriz were much lower than at the other 3 locations (Table 7). While significant differences between accessions for WSC concentration were observed, overall mean values for different accessions varied from 17.3 to 19.0%, with only 4 accessions below 18.0%.

Table 4. Mean forage dry matter yield (kg DM/ha) for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	2,603f	4,080d	3,881c	1,601e	3,041ef
1601	Gorgan	2,499g	2,865e	3,084d	3,048b	2,874g
2399	Tehran	2,661ef	4,305c	3,443cd	2,207d	3,154e
2759	Hamadan	1,826k	4,298c	4,444a	1,310f	2,969f
3062	North Khorasan	2,676ef	3,831d	3,728c	2,227d	3,115e
3800	Garmsar	2,980b	4,355c	3,286d	2,748c	3,342dc
4083	Semirom	2,371h	2,945e	3,701c	1,789e	2,701h
8199	Tehran	3,390a	5,653a	3,983c	2,563c	3,897a
8799	Kermanshah	2,766d	2,864e	3,683c	2,184d	2,874g
9147	Karaj	2,849c	4,619b	3,595cd	2,328d	3,347dc
9262	Karaj	2,192i	4,537bc	3,482cd	2,222d	3,108e
9263	Karaj	1,700l	4,847b	4,571a	3,340a	3,614ab
12542	Unknown	1,383m	4,772b	3,606cd	1,876e	2,909fg
15364	Karaj	2,253i	4,779b	3,572cd	3,057b	3,415b
19402	Hamadan	1,962j	5,554a	4,261b	1,681e	3,364c
3001	Karaj	2,447hg	5,425a	3,736c	1,611e	3,304d
15353	Karaj	2,474g	4,532bc	4,122b	2,238d	3,341dc
Oshnavieh	Oshnavieh	2,890c	4,264c	3,826c	2,503c	3,370c
Plc	Karaj	2,697ed	2,600e	4,470a	2,272d	3,009f
Mean		2,454	4,270	3,814	2,253	3,198

Values within columns followed by the same letter are not significantly different (P>0.05).

Table 5. Mean crude protein concentration (%) for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	20.1b	19.1b	17.2a	22.0a	19.6c
1601	Gorgan	18.6c	17.2c	15.6b	21.0ab	18.1e
2399	Tehran	19.6bc	19.0b	16.4ab	22.1a	19.3d
2759	Hamadan	22.7a	19.9b	16.0b	20.6b	19.8c
3062	North Khorasan	22.6a	20.2b	15.6b	21.4a	19.9b
3800	Garmsar	20.8b	18.9b	15.6b	22.2a	19.4cd
4083	Semirom	20.8b	20.8ab	16.3ab	22.8a	20.1a
8199	Tehran	23.8a	19.7b	14.7b	21.1ab	19.8bc
8799	Kermanshah	18.6c	18.1bc	15.3b	20.8b	18.2e
9147	Karaj	19.3bc	19.2b	15.4b	22.0a	19.0e
9262	Karaj	21.5ab	19.3b	16.5ab	21.4a	19.7c
9263	Karaj	19.6bc	18.5b	15.1b	20.8b	18.5e
12542	Unknown	21.4ab	20.7ab	15.3b	21.3ab	19.7c
15364	Karaj	22.3a	19.9b	17.2a	21.7a	20.3a
19402	Hamadan	22.1a	21.4a	15.8b	20.5b	19.9b
3001	Karaj	21.3ab	20.0b	17.8a	21.4a	20.1a
15353	Karaj	22.9a	19.7b	17.1a	20.2b	20.0b
Oshnavieh	Oshnavieh	21.6ab	20.1b	15.9b	22.4a	20.0b
Plc	Karaj	20.5b	19.6b	14.9b	22.4a	19.4d
Mean		21.1	19.5	16.0	21.48	19.5

Values within columns followed by the same letter are not significantly different (P>0.05).

Table 6. Mean dry matter digestibility (%) for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	69.3c	72.1b	62.0c	74.1ab	69.4bc
1601	Gorgan	67.3cd	71.4b	66.1a	72.2ab	69.3bc
2399	Tehran	66.8d	74.3ab	61.8c	74.1ab	69.3bc
2759	Hamadan	75.1a	75.9a	64.7b	75.4a	72.0a
3062	North Khorasan	66.3d	73.9ab	57.2d	70.8b	67.1d
3800	Garmsar	68.2cd	74.0ab	61.6c	74.6ab	69.6bc
4083	Semirom	68.4cd	74.0ab	62.7bc	74.7ab	70.0bc
8199	Tehran	69.8c	72.5b	64.0b	73.7ab	70.0b
8799	Kermanshah	69.2c	70.0b	67.7a	74.0ab	70.2b
9147	Karaj	66.2d	72.6b	61.5c	72.3ab	68.1c
9262	Karaj	67.3cd	73.8ab	68.7a	73.3ab	70.8b
9263	Karaj	66.9d	73.4ab	6119c	73.6ab	68.7c
12542	Unknown	71.4c	69.8b	61.6c	71.7b	68.6c
15364	Karaj	70.3c	72.7b	60.4c	71.6b	68.8 c
19402	Hamadan	73.2b	75.5ab	64.7b	73.2ab	71.6a
3001	Karaj	71.0c	73.6ab	68.6a	76.3a	72.4a
15353	Karaj	70.5c	73.1b	61.7c	75.1a	70.1b
Oshnavieh	Oshnavieh	70.7c	74.0ab	61.3c	73.4ab	69.9bc
Plc	Karaj	71.8c	74.7ab	67.1a	75.1a	72.2a
Mean		69.5	73.2	63.4	73.6	69.9

Values within columns followed by the same letter are not significantly different (P>0.05).

Table 7. Mean water soluble carbohydrate concentration (%) for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	18.7ab	18.5c	16.3ab	18.6ab	18.0d
1601	Gorgan	19.2ab	19.3b	15.9b	17.8b	18.0d
2399	Tehran	18.6ab	19.5b	16.2ab	18.1ab	18.1d
2759	Hamadan	21.2a	19.6b	16.2ab	18.8ab	19.0a
3062	North Khorasan	17.5b	18.3c	15.6b	17.9b	17.3e
3800	Garmsar	19.5ab	19.8b	16.5ab	18.9ab	18.7b
4083	Semirom	20.2ab	19.2b	16.6ab	18.9ab	18.7b
8199	Tehran	17.9b	19.2b	17.0ab	18.5ab	18.1cd
8799	Kermanshah	18.9ab	18.1c	15.1b	18.7ab	17.7de
9147	Karaj	18.8ab	19.1b	15.9b	18.1ab	18.0d
9262	Karaj	19.6ab	20.3a	15.5b	18.4ab	18.4c
9263	Karaj	19.5ab	18.5c	16.7ab	18.4ab	18.3c
12542	Unknown	19.9ab	18.1c	16.9ab	18.7ab	18.3c
15364	Karaj	18.1b	19.1b	15.5b	17.7b	17.6e
19402	Hamadan	21.7a	19.1b	15.7b	19.2 a	18.9a
3001	Karaj	19.9ab	19.6b	17.2a	18.6ab	18.8a
15353	Karaj	18.8ab	19.2b	17.6a	18.6ab	18.5bc
Oshnavieh	Oshnavieh	20.6ab	19.2b	17.4a	18.6ab	19.0a
Plc	Karaj	21.5a	19.3b	15.9b	18.9ab	18.9ab
Mean		19.5	19.1	16.3	18.5	18.3

Values within columns followed by the same letter are not significantly different (P>0.05).

Overall mean disease severity index (DSI) values for the 4 locations were much higher at Zanjan (56.1) and Tabriz (48.0) than at Esfahan (31.6) and Koramabad (28.6), indicating a greater incidence of powdery mildew at the former 2 locations (Table 8). However, at all locations accession 3001 consistently displayed the greatest resistance to powdery mildew infection (DSI 5.0–22.3). Over all locations this accession displayed a mean DSI of 12.9, compared with an overall mean for all accessions of 41.1. While accessions 15353, 2759, 9263 and Polycross performed well at Esfahan and Koramabad, they were severely infected at Tabriz and Zanjan.

Correlation analysis

The results of correlation analysis demonstrated that the only associations between DSI and other parameters were a positive correlation (P<0.01) between DM yield and DSI at Esfahan (Table 9) and negative correlations between DSI and both CP% and WSC% at Tabriz and overall, as well as between DSI and DMD overall. There was a significant negative correlation (P<0.05) between DM yield and WSC concentration at Esfahan and Zanjan locations. The strongest relationships from the data were strong positive correlations between DMD and WSC concentration for 3 of the 4 locations and overall (Table 9).

Stability analysis

Results of Eberhart/Russell regression response indices (b), deviation from regression (S^2_d) and mean DM yield and DSI for 19 sainfoin accessions over 4 locations are presented in Table 10. Plots of the relationships between regression coefficients (bi) and mean DM yield for 19 accessions (Figure 2) showed that accessions 8199, 9263, 15353, 15364, 9147 and Oshnavieh had DM yields higher than the average value and b values were near unity, indicating their DM yield was stable across environments. Accessions 19402 and 3001 with bi >1 coupled with high DM yield had above-average stability for high performing environments, Khoramabad and Tabriz. A higher deviation from regression indicated sensitivity to environmental changes for DM yield (Table 10).

Similarly, the regression coefficients plotted against the test accessions for DSI are presented in Figure 3. For DSI, accessions 15353, 3001 and 9262 with b values near unity were considered stable over the 4 locations. Oshnavieh in Khoramabad and accessions 2759, 9263 and Plc (Polycross) in Esfahan with a greater resistance to powdery mildew were stable only for those locations. In general, accessions 15353, 9262 and 3001, located close to the regression line, appeared to be more stable in terms of higher production and lower disease severity index across locations.

Table 8. Mean disease severity index for 19 sainfoin accessions at 4 locations in Iran.

Accession	Origin	Location				
		Esfahan	Koramabad	Tabriz	Zanjan	Mean
334	Karaj	50.0c	37.2c	46.9b	62.1b	49.1cd
1601	Gorgan	50.0	30.7c	55.8a	76.1a	53.2bc
2399	Tehran	35.0de	51.2a	55.6a	80.9a	55.7b
2759	Hamadan	10.0g	2.7e	50.8b	50.4c	28.5h
3062	North Khorasan	25.0e	27.5c	57.5a	50.7c	40.2f
3800	Garmsar	70.0a	49.9a	48.1b	79.4a	61.8a
4083	Semirom	20.0f	44.9ab	58.3a	66.5b	47.4d
8199	Tehran	63.3b	41.1b	39.7c	63.4b	51.9c
8799	Kermanshah	56.6bc	42.6b	59.7a	56.8c	54.0b
9147	Karaj	51.6c	13.0e	54.7a	62.3b	45.4e
9262	Karaj	28.3e	18.5d	48.1b	45.6d	35.1g
9263	Karaj	6.6g	26.0c	32.8d	67.0b	33.1g
12542	Unknown	11.6g	40.2b	51.1b	62.2b	41.3f
15364	Karaj	26.6e	45.5ab	39.7c	46.6d	39.6f
19402	Hamadan	30.0e	26.8c	49.8b	60.9b	41.9f
3001	Karaj	5.0g	6.7e	17.6e	22.4e	12.9h
15353	Karaj	6.6g	11.4e	46.4b	28.6e	23.3h
Oshnavieh	Oshnavieh	48.3d	8.0e	45.3b	29.5e	32.8g
Plc	Karaj	5.9g	20.5d	54.2a	54.2c	33.7g
Mean		31.6	28.6	48.0	56.1	41.1

Values within columns followed by the same letter are not significantly different (P>0.05).

Table 9. Correlations between traits for 19 sainfoin accessions at 4 locations in Iran.

Trait	Location	DMY	DSI	CP	WSC
DSI	Esfahan	0.68**			
	Koramabad	-0.16			
	Tabriz	-0.22			
	Zanjan	0.25			
	Mean	-0.12			
CP	Esfahan	-0.02	-0.24		
	Koramabad	0.35	-0.15		
	Tabriz	-0.19	-0.42*		
	Zanjan	0.03	0.19		
	Mean	0.17	-0.43*		
WSC	Esfahan	-0.42*	-0.36	-0.01	
	Koramabad	0.16	-0.27	-0.01	
	Tabriz	0.19	-0.49*	0.18	
	Zanjan	-0.52*	-0.04	-0.03	
	Mean	-0.01	-0.40*	0.30	
DMD	Esfahan	-0.33	-0.36	0.48*	0.65**
	Koramabad	0.16	-0.37	0.40*	0.60**
	Tabriz	0.02	-0.16	0.07	-0.20
	Zanjan	-0.39	-0.24	0.02	0.61**
	Mean	-0.10	-0.44*	0.20	0.75**

CP = crude protein concentration; DMD = dry matter digestibility; DMY = dry matter yield: DSI = disease severity index; WSC = water soluble carbohydrate concentration.

Table 10. Regression response indices (b), deviation from regression (S^2_d) and mean DM yield and Disease Severity Index determined for 19 sainfoin accessions across 4 locations in Iran.

Accession	Origin	DM yield (kg/ha)			Disease severity index (DSI)		
		Mean	b	S^2_d	Mean	b	S^2_d
334	Karaj	3,041	1.12**	174085	49.1	0.62**	55.1
1601	Gorgan	2,874	0.08**	97248	53.2	1.30**	83.2
2399	Tehran	3,154	0.90**	71732	55.7	1.21**	155.2
2759	Hamadan	2,969	1.60**	204442	28.5	1.90**	62.0
3062	North Khorasan	3,115	0.78**	36073	40.2	1.12**	72.8
3800	Garmsar	3,342	0.63**	160196	61.8	0.46**	294.5
4083	Semirom	2,702	0.67**	336430	47.4	1.26**	204.3
8199	Tehran	3,897	1.20**	417212	51.9	0.22**	246.9
8799	Kermanshah	2,874	0.41**	319531	54.1	0.38**	46.8
9147	Karaj	3,348	0.96**	117272	45.4	1.28**	302.2
9262	Karaj	3,108	1.10**	92653	35.1	1.00**	36.1
9263	Karaj	3,614	1.21**	914955	33.1	1.63**	255.1
12542	Unknown	2,909	1.53**	214034	41.3	1.31**	261.1
15364	Karaj	3,415	0.93**	399054	39.6	0.30**	98.3
19402	Hamadan	3,365	1.86**	52218	41.9	1.23ns	-3.8
3001	Karaj	3,305	1.61**	307139	12.9	0.63ns	-0.9
15353	Karaj	3,341	1.15*	2325	23.3	1.05**	199.1
Oshnavieh	Oshnavieh	3,371	0.81**	12757	32.8	0.38**	470.4
Plc	Karaj	3,010	0.47**	144876	33.7	1.69**	146.3

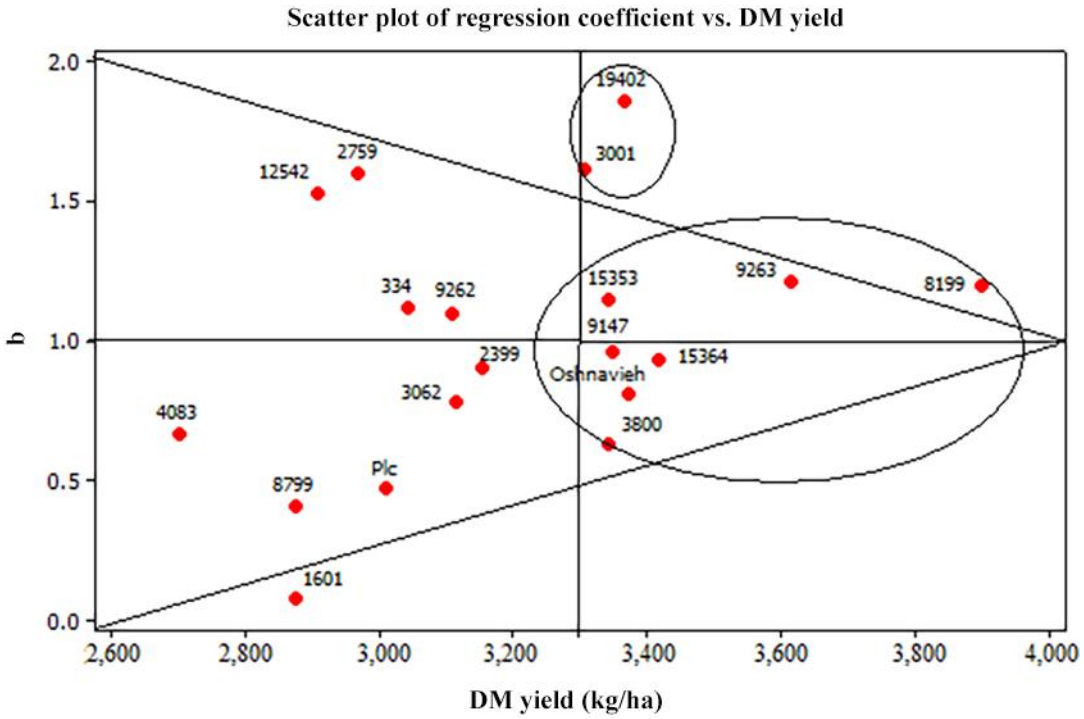

Figure 2. Regression coefficient b plotted against DM yield for 19 sainfoin accessions in Iran.

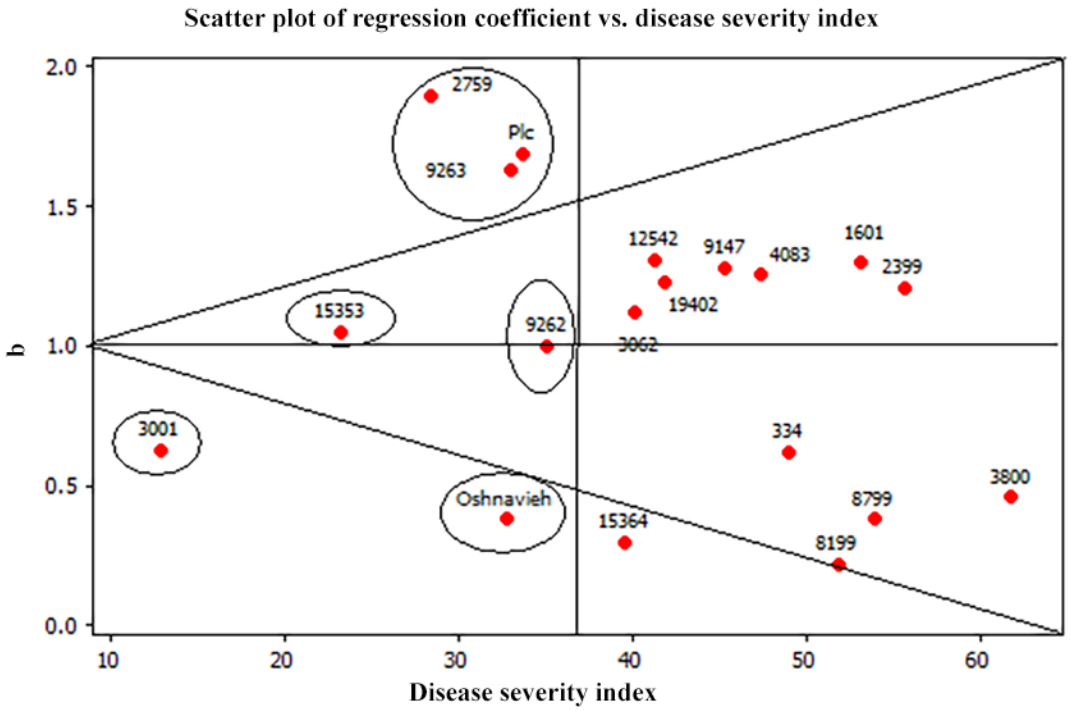

Figure 3. Regression coefficient b plotted against disease severity index for 19 sainfoin accessions in Iran.

Discussion

In order to improve sainfoin as bioactive forage, improvements in resistance to powdery mildew, yield and quality traits are required. As an initial step, quantifying the variability in existing sainfoin germplasm would allow the identification of superior genotypes with disease resistance and yield stability across various environments. According to earlier evidence, Iran is recognized as one of the most important sources of genetic diversity in populations of sainfoin worldwide (Mohajer et al. 2013). From Iranian germplasm the present research has identified 2 promising sainfoin accessions not only resistant to powdery mildew, but also with good forage quality and high DM yield, which was stable across a range of different environments and growing seasons. Since the arid and warm climate in Iran provides a high genetic diversity in populations of *Leveillula* powdery mildew (Khodaparast et al. 2012), testing under natural conditions in Iran provided an ideal environment for comparing accessions. It would seem reasonable to conclude that differences in climatic conditions across the diverse geographical areas would result in genetically diversified populations of the pathogen providing an ideal challenge to accessions. Moreover, this variability in environmental conditions across different Iranian regions resulted in significant differences in powdery mildew severity, DM yield and quality traits among the same accessions grown at 4 study locations. Although Naseri and Alizadeh (2017) reported the association of climate with sainfoin powdery mildew resistance and yield in Zanjan, the interaction of environmental parameters from different regions with the disease, resistance, quality and yield of this valuable forage crop merits further investigation in future.

Our findings demonstrated low disease severity index (<25%) in accessions 15353 and 3001 across 4 different environments, identifying them as powdery mildew-resistant sainfoin genotypes. Furthermore, accessions 9262 and Oshnavieh were considered as semi-resistant or semi-susceptible to powdery mildew. While various levels of sainfoin resistance to powdery mildew based on data from a single location or year have been reported previously (Alizadeh et al. 2014; Alizadeh and Jafari 2014), to the best of our knowledge this is the first report of stability of disease resistance in 2 sainfoin genotypes over a 4-year study conducted across different geographical areas. This information is a valuable first step in global breeding programs to increase powdery mildew resistance in sainfoin populations.

It has been reported that forage WSC provides efficient energy needed for plant growth following reduction in photosynthesis, rejuvenation after leaf loss and recovery after drought and freezing stresses (Humphreys and Eagles 1988; Humphreys 1989). According to Wilkins and Lovatt (1989), it is desirable to maintain CP

concentration of forage above 12%, to ensure rumen microflora have adequate nitrogen for both milk and meat production by livestock. According to our findings, there were significant negative correlations between DSI and forage quality traits tested, indicating that lower powdery mildew levels on accessions corresponded with higher DMD plus CP and WSC concentrations. There are a number of earlier Iranian reports on the linkage of sainfoin powdery mildew severity with forage quality (Mohajer et al. 2013; Alizadeh et al. 2013); however, none of them explored the stability of these relationships over diverse environments and seasons. Moreover, the present research identified a positive correlation between DMD and WSC and CP concentrations in the accessions of sainfoin tested. This result was in agreement with an earlier report of Alizadeh et al. (2013). Therefore, one of the important benefits of reducing powdery mildew infections via breeding of resistant lines may be maintenance of sufficient CP, DMD and WSC to improve sainfoin revival and produce high quality forage. It seems that accessions 3001 and 15353 are potential candidates for both commercial production and use in breeding programs. Further testing of genetic material to identify other accessions for use in breeding programs seems warranted.

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(Note of the editors: All hyperlinks were verified 15 December 2020.)

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Research Paper

Salinity tolerance of *Avena sativa* fodder genotypes

Tolerancia de genotipos de avena forrajera a la salinidad

AJOY KUMAR ROY[1], DEVENDRA RAM MALAVIYA[1], ANJALI ANAND[2], RANG NATH CHOUBEY[1], MIRZA JAYNUL BAIG[3], KULDEEP DWIVEDI[4] AND PANKAJ KAUSHAL[5]

[1]*ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India.* igfri.res.in
[2]*ICAR-Indian Agricultural Research Institute, New Delhi, India.* iari.res.in
[3]*ICAR-National Rice Research Institute, Cuttack, India.* icar-nrri.in
[4]*Amity University, Gwalior, India.* amity.edu/gwalior
[5]*ICAR-National Institute of Biotic Stress Management, Raipur, India.* nibsm.res.in

Abstract

Oats (*Avena sativa* L.) is an important winter season fodder cultivated in many parts of the world. India faces huge shortages of green forage and possesses large salt-affected areas, so identification of salt-tolerant material offers scope for breeding of cultivars for increasing production from salt-affected soils. Forty-eight genotypes of oats comprised of cultivars, germplasm accessions and advanced breeding lines were evaluated with the aim of identifying salt-tolerant genotypes for use on saline soils and/or in programs to breed more salt-tolerant cultivars. Screening was carried out at different growth stages in both pot and field studies. Germination and seedling vigor at different levels of salinity in terms of electrical conductivity (EC), i.e. EC4, EC8, EC12 and EC16, were assessed. Field-level salinity tolerance was assessed in pits where soils had EC ranging from 3.3 to 3.6 dS/m and pH 9.6. Sand culture experiments were carried out on 2 genotypes at different levels of NaCl solution as well as saline soil scrap solution so as to simulate a real field situation. Na, K, Ca and proline concentrations were estimated to understand the mechanism of salinity tolerance of the crop. The study resulted in identification of some suitable genotypes with acceptable levels of salt tolerance, which can be used in developing productive cultivars for saline soils.

Keywords: Diversity, germination, growth, oats, salt stress.

Resumen

En varias partes del mundo la avena (*Avena sativa* L.) es un forraje importante cultivado para la temporada de invierno. India enfrenta una alta escasez de forraje verde y posee grandes áreas afectadas por salinidad, por lo que la identificación de materiales tolerantes a la salinidad ofrece un amplio margen para el mejoramiento de cultivares adaptados con el fin de aumentar la producción en este tipo de suelos. En Jhansi, India, se evaluaron 48 genotipos forrajeros de avena incluyendo cultivares, accesiones de germoplasma y líneas de mejoramiento avanzadas con el objetivo de identificar aquellos tolerantes a la salinidad para uso posterior en suelos salinos y/o en programas de mejoramiento. El estudio se llevó a cabo en diferentes etapas de crecimiento de las plantas, tanto a nivel de invernadero como de campo. Se evaluaron la germinación y el vigor de plántulas a diferentes niveles de salinidad en términos de conductividad eléctrica (CE): CE4, CE8, CE12 y CE16. La tolerancia a la salinidad a nivel de campo se evaluó en fosas llenadas con suelo salino que tenían una CE que variaba entre 3.3 y 3.6 dS/m y un pH de 9.6. Además se realizaron experimentos en arena con dos genotipos a diferentes concentraciones de NaCl, así como en solución de la costra de suelo salino para simular una situación real. También se determinaron las concentraciones de Na, K, Ca y prolina en las plantas para entender el mecanismo de tolerancia a la salinidad. El estudio permitió la identificación de algunos genotipos con niveles aceptables de tolerancia a la salinidad los cuales pueden ser utilizados en el desarrollo de cultivares productivos para suelos salinos.

Palabras clave: *Avena sativa*, crecimiento, diversidad, germinación, estrés por salinidad.

Correspondence: Devendra Ram Malaviya, ICAR-Indian Grassland and Fodder Research Institute, Jhansi –284003, India.
Email: drmalaviya47@rediffmail.com

Introduction

Soil salinity is one of the most important abiotic stresses and is a limiting factor for plant production worldwide (Zhu 2001) with Gao et al. (2016) estimating that more than 6% of the world's total land area is affected by salinity. Saline-sodic soils in India occupy approximately 7% of total land area (1 billion ha) and 20% of the irrigated arable land in arid and semi-arid regions, and this area is increasing (Agarwal et al. 2013). Soil salinity reduces crop growth and its performance, divided into primary and secondary effects. Primary salt effects include metabolic disturbances plus inhibition of growth and development. Secondary salt effects include nutrient deficiency and osmotic dehydration. Soil reclamation and water desalination practices have been implemented in an endeavor to overcome salinity effects but these strategies are very expensive. Developing salt-tolerant lines of plant species appears a feasible option for achieving higher biomass production and yield from salt-affected soils.

Considering the shortage of fodder for livestock in India, it is imperative to develop suitable technologies for increasing fodder production and productivity (Roy et al. 2019a; 2019b) and increasing yields in saline areas would make a valuable contribution. The area under cultivation to produce forage crops has remained constant for the last few decades and there is little prospect of any increase in the area of arable land devoted to forage cultivation. Development of technology to identify suitable genotypes for growing in problem soils, e.g. salt-affected soils, could reduce the forage deficit.

In spite of the fact that forages include much wild and weedy germplasm, which offers better potential for reclamation of saline soils, owing to higher tolerance of biotic and abiotic stresses, there have been limited studies on salt tolerance of forage crops (Maas and Hoffman 1977; Galluzzi et al. 2014; Malaviya et al. 2015; Roy et al. 2019a). Further, efforts to evaluate crops have been restricted to studies of a few selected forages in certain areas. The wide range of genetic variability in many forage genera has not been evaluated in defined conditions.

Oats (*Avena sativa* L.), belonging to the Poaceae family, is grown throughout the world, including Russia, Canada, Poland, Finland, Australia, United States, Spain, United Kingdom, Sweden, Germany and India, for both grain and forage production. The total area cultivated with oats in India is about 0.5 million ha annually. Cultivated oats is an allohexaploid (2n = 6x = 42) derived from 3 ancestral diploid *Avena* genomes (A, C and D). Size of the hexaploid oat genome was found to be 1C = 11.7 pg, which corresponds to 11,443 Mbp (1 pg = 978 Mbp) (Bennett and Leitch 1995). Oat-based food is considered healthy because of the high dietary fiber content of oat groats, particularly beta-glucan (Martínez-Villaluenga and Peñas 2017). It is a popular winter cereal fodder crop grown in north-western and central India and is now planted in the eastern and southern regions. It produces good yields of palatable and nutritious forage.

The genus *Avena* is known for its tolerance of high alkalinity (Holden 1969; Loskutov and Rines 2011) and oats is tolerant of high pH conditions and quite tolerant of salt stress (Zhao et al. 2007; NSW-DPI 2017; Bai et al. 2018). Bhagmal et al. (2009) reported that oats possessed high tolerance of salinity and suggested it could be used for reclamation of saline soils. Salt-tolerance is a complex, multigenic trait and is often a composite response of the integrated biological system. The first step in developing salt-tolerant cultivars of this forage crop would be screening of the wide range of available diverse germplasm lines.

In the present study, oats genotypes, comprised of a few advanced breeding lines, germplasm from Nordic Gene Bank and some existing cultivars, were evaluated under saline conditions and various growth parameters were monitored to identify salt-tolerant lines. Impacts on performance from germination through survival and growth and nutrient concentrations in forage were examined.

Materials and Methods

A series of experiments were conducted in the laboratory and experimental farm of ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India. Forty-eight accessions of oats were used in the study; the list along with their status is presented in Table 1.

In vitro screening for germination and seedling vigor

The 48 genotypes mentioned in Table 1 were evaluated for tolerance of 4 salinity levels, viz. EC4, EC8, EC12 and EC16. One-hundred seeds of each genotype were placed on sterilized filter paper in petri dishes. For Control sets, the filter papers were soaked with distilled water (DW), while for saline treatments, soaking was done with saline water with electrical conductivity (EC) of EC4, EC8, EC12 and EC16 (dS/m or mmho/cm, where 1 dS/m = 1 mmho/cm). Treatment solutions were prepared by dissolving different quantities of NaCl in distilled water so as to get the desired EC level. Data on germination were recorded on Day 7 after soaking by recording the total number of germinated seeds with radicle and plumule growth. Radicle and plumule length were recorded on 3 seedlings in each set on Day15 as a measure of seedling growth.

Table 1. Oats genotypes evaluated for salt tolerance.

SN	Genotype	Details
1	NGB2114	Germplasm
2	NGB2117	Germplasm
3	NGB2118-1	Germplasm
4	NGB2120-1	Germplasm
5	NGB2718	Germplasm
6	NGB4417	Germplasm
7	NGB4467	Germplasm
8	NGB4470	Germplasm
9	NGB4474-1	Germplasm
10	NGB4732	Germplasm
11	NGB4757	Germplasm
12	NGB4758	Germplasm
13	NGB4870	Germplasm
14	NGB4871	Germplasm
15	NGB4872	Germplasm
16	NGB4887	Germplasm
17	NGB6189	Germplasm
18	NGB6368	Germplasm
19	NGB6370	Germplasm
20	NGB6374	Germplasm
21	NGB6963	Germplasm
22	NGB6968	Germplasm
23	NGB6995	Germplasm
24	NGB6997	Germplasm
25	NGB7002	Germplasm
26	NGB7003	Germplasm
27	NGB7007	Germplasm
28	NGB7013	Germplasm
29	NGB7021	Germplasm
30	NGB7026	Germplasm
31	NGB7244	Germplasm
32	NGB7245	Germplasm
33	NGB7247	Germplasm
34	NGB7252	Germplasm
35	NGB7253	Germplasm
36	NGB7259	Germplasm
37	NGB7279	Germplasm
38	JHO2000-3	Advanced breeding line
39	JHO2000-5	Advanced breeding line
40	JHO2001-2	Advanced breeding line
41	JHO2001-4	Advanced breeding line
42	OS6x851-1	Advanced breeding line
43	OS-7x320	Advanced breeding line
44	UPO94xIGO-220	Advanced breeding line
45	JHO822	Cultivar
46	JHO851	Cultivar
47	JHO99-1	Cultivar
48	JHO99-2	Cultivar

Salinity intensity index (SII) was calculated as SII = 1-X_{SS}/X_{NS}, where X_{SS} and X_{NS} are the means for all accessions in salinity-stressed (SS) and non-stressed (NS) environments as per Fisher and Maurer (1978). Salt susceptibility index (SSI) was calculated as SSI = (1-Y_{SS}/Y_{NS})/SII, where Y_{SS} and Y_{NS} are the mean values for a given accession in stressed and non-stressed environments following Bayuelo-Jiménez et al. (2002). Based on SSI values, the genotypes were grouped as susceptible, tolerant and highly tolerant with lower SSI values being an indication of higher tolerance. Standard deviation, Student's t test, 2-factor analysis of variance and regression analyses were performed using MS Excel program.

Sand culture experiment

Seeds of 2 genotypes, JHO2000-5 and OS6x851-1, were sown in pots filled with sand, which had been thoroughly sterilized and washed with distilled water, in 3 replications with 5 treatments including a Control. Salt stress was created by an aqueous solution of NaCl in one treatment. Secondly, in order to simulate the natural salt stress condition, the upper crust of the salt-affected soil from natural condition was scraped, when salts came out on the crust with receding soil moisture, and was then dissolved in distilled water, termed here as saline soil scrap (SSS) solution. Pots were irrigated with one of the following solutions to give the various treatments: (i) distilled water mixed with nutrient solution (Control); (ii) 0.5% NaCl + nutrient solution; (iii) 0.75% NaCl + nutrient solution; (iv) saline soil scrap solution (SSS1) + nutrient solution (EC8 dS/m); and (v) saline soil scrap solution (SSS2) + nutrient solution (EC10.4 dS/m). Twenty-five oat seeds were sown in each pot. The pots were irrigated every day with 500 ml nutrient solution as described by Shannon and Noble (1995) supplemented with salts as described above. After every 5 days the sand in each pot was irrigated with running plain water for 5 minutes to prevent salt build-up. Germination percentage was recorded on Day 10 after sowing and plant survival/ mortality was recorded on Day 25 after sowing. Plant height, leaf length and leaf width were recorded on 3 plants from each pot on Days 30, 45 and 70 after sowing. Three elements (Na, K and Ca) were estimated in plant samples from the various treatments using flame photometry following Jeffery et al. (1989); for this, plants were uprooted, washed in water and blotted dry followed by oven-drying at 80 ºC for 4 days.

Evaluation for field level salinity tolerance

The experiment comprised 44 genotypes, of which 41 genotypes were as per Table 1, except NGB2118-1, NGB4467, NGB4822, NGB4887, NGB7007, NGB7244

and OS-7x320, which were not included due to paucity of seeds. Additionally 3 genotypes, NGB6975, NGB7249 and JHO2001-3, were included. For field-level salinity tolerance, 90–100 seeds of each genotype were sown in rows 50 cm apart in pits in an unreplicated trial due to limited availability of saline pits. The pits were 1 m deep and made of bricks and were filled with natural saline sodic soil collected from nearby villages. Initial EC of the soil was very high, so some salt, which appeared as deposits on the soil surface after irrigation, was removed and the soil homogenized. Final EC of soil in the pits ranged from 3.3 to 3.6 dS/m and the pH was 9.6. Number of germinated seeds and survival of seedlings were recorded 38 days after sowing. Height and leaf dimensions of plants were measured on 3 plants/genotype at 38 days after sowing. Furthermore, proline concentration was estimated for 10 random genotypes and the Control by extraction in 3% sulfosalicylic acid and subsequent colorimetric method of Bates et al. (1973). Proline gets accumulated in plants in response to a variety of abiotic stresses and plays a significant role in stress tolerance, including salt stress (Ashraf and Harris 2004; Ashraf and Foolad 2007; Verbruggen and Hermans 2008).

Results

In vitro screening for germination and seedling vigor

Most genotypes evaluated for germination under saline conditions showed very good germination even at EC16 (Table 2). Mean germination percentages were 92.7, 90.3, 88.8 and 84.2% at EC4, EC8, EC12 and EC16, respectively, compared with 93.9% for Control. Analysis of variance showed significant differences among genotype and treatment means (Table 3). However, the paired t test revealed significant differences only between EC4-EC8 and EC12-EC16 but no differences between DW-EC4 and EC8-EC12 (Table 2). Thus, high EC created by NaCl had some effect on germination. A few genotypes, which showed low germination percentage under saline conditions, also had poor germination in Control treatments (Table 2). Some genotypes showed numerically higher germination than the Control, and their susceptibility index showed a negative value. The regression equation for germination percentage (y) against electrical conductivity of the germination fluid (x) was: y = 94.62 - 0.58**x.

Mean radicle growth of these genotypes was reduced to 5.5 and 4.8 cm, respectively, at EC12 and EC16 compared with 6.6 cm in Control, although radicle growth at EC4 and EC8 was higher than that in Control. The t test showed significant differences between DW-EC4, EC4-EC8, EC8-EC12 and EC12-EC16. The regression equation for radicle length (y) against EC of irrigation water (x) was: y = 7.67 - 0.16**x. Twenty-four genotypes possessed negative SSI values, indicating their salinity-tolerant nature, although most genotypes showed reduced growth at EC12 and EC16.

Mean plumule growth was a little higher at EC4 but reduced to 3.5, 2.6 and 2.2 cm at EC8, EC12 and EC16, respectively, compared with 3.8 cm in distilled water. Twenty-one genotypes displayed salinity-tolerant nature for plumule growth as indicated by negative SSI values. A t test revealed no difference between growth in DW-EC4 and that in EC12-EC16. The regression equation for plumule growth (y) against EC of irrigation water (x) was: y = 4.24 - 0.12**x. Analysis of variance established significant differences among genotypes as well as treatments (Table 3). Twenty genotypes possessed salinity-tolerant nature for both radicle and plumule growth.

Sand culture experiment

Germination percentages at 10 days after sowing fell in the following ranges: 88–92% for Control; 88–92% for 0.5% NaCl; 72–84% for 0.75% NaCl; 92% in scrap soil solution (SSS1) with EC8.0 dS/m; and 60–92% in scrap soil solution (SSS2) with EC10.4 dS/m for JHO2000-5. For OS6x851-1, germination percentage was: 92–96% for Control; 100% for 0.5% NaCl treatment; 88–100% for 0.75% NaCl; 96–100% for SSS1; and 84–100% for SSS2. At Day 25 after sowing, most plants in 0.5 and 0.75% NaCl treatments had survived, while one-third had died in EC8.0 dS/m and two-thirds in EC10.4 dS/m. By Day 45 after sowing, no seedlings in SSS1 and SSS2 survived. Plant height was drastically reduced at EC8.0 and EC10.4 by Day 25 after sowing, whereas there was little effect on height in 0.5 and 0.75% NaCl treatments relative to Control. A similar trend was observed for leaf length and leaf width (Table 4). Even at Day 70 after sowing, seedlings in 0.5 and 0.75% NaCl treatments were growing well, but JHO2000-5 seedlings were 15–28% shorter than those in Control, and OS6x851-1 seedlings were 26–35% shorter than Control plants. Analysis of variance revealed significant differences among treatments and the morphological attributes of the plants. The interaction effects were also significant (Table 3).

Table 2. Germination, radicle length and plumule length in *Avena sativa* genotypes growing at different levels of salinity.

Genotype	Germination (%)						Radicle length (cm)						Plumule length (cm)					
	DW[1]	EC4	EC8	EC12	EC16	Av SSI	DW	EC4	EC8	EC12	EC16	Av SSI	DW	EC4	EC8	EC12	EC16	Av SSI
NGB2114	95.0	100.0	100.0	90.0	90.0	-0.1	2.7	6.9	4.9	7.2	3.0	-6.9	1.6	3.2	2.8	3.0	1.0	-6.2
NGB2117	100.0	100.0	100.0	100.0	100.0	0.0	6.4	7.0	7.7	5.2	9.0	-0.8	3.8	3.6	1.6	0.7	4.8	0.9
NGB2118-1	85.0	70.0	80.0	100.0	70.0	0.4	3.9	9.1	7.1	3.9	3.4	-3.9	1.4	4.5	2.9	1.1	1.5	-6.4
NGB2120-1	90.0	100.0	100.0	100.0	95.0	-1.6	8.3	7.7	6.4	4.1	6.8	1.7	3.5	3.1	5.7	1.3	3.1	0.2
NGB2718	100.0	100.0	100.0	90.0	95.0	0.6	9.2	10.1	7.6	4.3	4.4	1.7	7.2	6.2	3.4	0.7	0.4	2.6
NGB4417	100.0	100.0	100.0	95.0	95.0	0.4	4.6	6.3	4.5	4.0	4.2	-0.1	3.8	3.4	2.7	2.5	0.2	1.0
NGB4467	90.0	95.0	95.0	85.0	75.0	0.2	5.8	8.7	6.7	3.6	2.9	0.0	5.6	2.8	2.9	2.1	1.8	1.9
NGB4470	85.0	85.0	75.0	80.0	80.0	1.2	11.9	7.3	7.7	6.1	3.5	2.9	6.2	3.3	3.3	3.5	2.0	3.2
NGB4474-1	100.0	100.0	100.0	90.0	85.0	0.8	7.0	8.5	4.5	5.0	4.5	1.6	5.5	5.8	1.4	2.3	1.8	2.4
NGB4732	90.0	85.0	80.0	100.0	100.0	0.1	7.9	8.6	5.6	3.4	5.3	2.0	5.3	2.7	1.4	1.0	2.0	3.4
NGB4757	95.0	95.0	80.0	80.0	70.0	2.4	3.7	8.7	8.0	2.8	3.7	-4.7	1.8	4.9	3.9	3.1	2.8	-6.9
NGB4758	100.0	100.0	95.0	85.0	90.0	1.3	6.3	8.0	6.2	4.6	5.1	0.3	1.5	3.1	2.3	0.1	0.1	-1.6
NGB4870	100.0	95.0	100.0	80.0	80.0	1.5	7.6	8.4	5.2	6.9	2.3	1.6	4.3	3.5	2.2	3.1	2.1	2.2
NGB4871	100.0	100.0	100.0	100.0	100.0	0.0	5.5	7.2	8.5	5.6	5.3	-2.1	3.0	1.3	3.4	0.7	0.8	0.0
NGB4872	85.0	75.0	70.0	45.0	60.0	4.3	3.6	7.3	7.1	5.4	4.1	-5.1	1.9	2.3	3.6	2.1	0.7	-4.2
NGB4887	100.0	100.0	100.0	80.0	70.0	1.7	5.3	10.6	9.2	4.3	1.7	-2.4	2.5	7.9	5.4	4.6	1.9	-6.3
NGB6189	15.0	10.0	20.0	20.0	20.0	-3.9	6.3	4.1	4.3	5.9	3.4	1.9	1.5	1.3	1.1	0.9	0.7	1.9
NGB6368	100.0	100.0	90.0	100.0	95.0	0.8	6.8	9.3	7.6	9.8	5.4	-1.3	6.7	6.7	7.6	9.8	2.9	-0.8
NGB6370	100.0	95.0	100.0	95.0	100.0	0.3	4.7	6.9	7.2	4.2	10.1	-3.0	2.1	4.1	3.6	1.8	8.7	-4.9
NGB6374	85.0	70.0	60.0	85.0	75.0	2.6	9.9	7.1	5.9	5.4	3.8	2.8	4.6	3.5	2.2	3.5	0.9	2.9
NGB6963	100.0	100.0	85.0	70.0	80.0	2.9	4.8	8.8	9.7	6.8	9.7	-5.6	2.3	6.5	6.6	6.3	6.2	-9.7
NGB6968	100.0	70.0	90.0	70.0	80.0	3.1	6.5	4.6	7.1	7.2	3.1	0.4	3.1	1.0	3.8	2.4	2.1	0.8
NGB6995	100.0	100.0	95.0	80.0	75.0	1.9	8.8	9.6	6.0	3.7	5.1	2.2	5.0	4.8	1.0	3.6	2.5	2.8
NGB6997	100.0	100.0	100.0	100.0	95.0	0.1	7.6	7.9	7.8	4.7	3.7	0.9	4.1	2.7	5.5	1.9	1.1	0.7
NGB7002	95.0	100.0	100.0	95.0	90.0	-0.3	6.9	11.3	7.7	6.8	5.7	-0.8	3.9	8.3	6.4	2.4	1.9	-2.7
NGB7003	95.0	100.0	100.0	95.0	100.0	-0.6	9.1	8.9	9.9	5.8	5.1	0.7	5.6	5.2	7.2	2.0	3.1	0.3
NGB7007	100.0	100.0	95.0	85.0	55.0	2.1	7.9	7.2	6.2	3.0	2.6	2.3	3.8	3.2	2.8	1.8	1.7	2.2
NGB7013	95.0	95.0	100.0	100.0	95.0	-0.6	7.1	6.9	4.5	5.2	5.0	1.9	4.4	3.9	2.4	2.9	2.3	2.3
NGB7021	100.0	100.0	100.0	95.0	95.0	0.4	4.1	6.5	7.0	5.2	2.7	-2.9	3.4	2.9	2.8	1.0	0.6	-0.4
NGB7026	100.0	95.0	75.0	95.0	85.0	2.4	5.1	9.0	5.4	4.0	3.5	-0.3	4.1	4.7	1.0	0.9	0.5	1.5
NGB7244	45.0	55.0	40.0	50.0	40.0	0.0	3.8	7.4	7.6	4.1	3.0	-3.9	3.8	2.9	4.1	3.2	2.1	-1.6
NGB7245	100.0	90.0	90.0	95.0	90.0	1.3	6.9	6.5	6.8	5.8	4.4	0.7	2.6	2.6	1.6	0.5	0.3	1.6
NGB7247	80.0	95.0	90.0	85.0	90.0	-1.8	3.8	7.2	5.2	6.6	6.7	-3.9	4.3	4.6	2.8	1.1	2.5	-1.1
NGB7252	100.0	95.0	90.0	85.0	90.0	1.7	4.6	8.6	8.2	7.9	6.7	-4.9	2.3	3.3	3.6	5.5	3.1	-4.5
NGB7253	100.0	100.0	100.0	100.0	100.0	0.0	5.0	6.9	8.8	6.8	6.9	-3.7	2.8	3.8	2.4	2.4	2.0	-1.9
NGB7259	100.0	100.0	100.0	95.0	80.0	0.7	6.7	8.6	7.4	5.7	3.1	0.1	4.4	4.1	4.6	3.2	2.9	0.2
NGB7279	85.0	90.0	65.0	80.0	50.0	2.7	7.9	7.2	6.8	4.5	4.6	1.6	3.3	3.0	4.6	1.4	1.1	0.7
JHO2000-3	100.0	100.0	100.0	100.0	95.0	0.1	6.8	8.1	7.0	6.4	4.6	0.1	2.9	3.8	2.2	2.3	0.7	0.4
JHO2000-5	100.0	100.0	95.0	100.0	100.0	0.3	6.9	9.7	7.3	6.8	7.7	-0.7	3.5	7.4	5.0	4.2	5.1	-2.7
JHO2001-2	100.0	95.0	95.0	95.0	90.0	0.9	5.3	11.4	8.4	9.2	1.7	-3.6	4.2	5.0	3.3	3.7	3.1	-1.6
JHO2001-4	100.0	100.0	100.0	100.0	80.0	0.5	8.1	8.6	10.2	7.7	2.5	-0.2	3.2	7.3	6.7	4.7	1.8	-3.6
OS6x851-1	100.0	100.0	100.0	100.0	90.0	0.2	7.4	8.5	9.2	5.9	5.5	-0.4	4.0	4.5	5.4	2.3	1.0	-0.6
OS-7x320	100.0	100.0	100.0	95.0	95.0	0.4	9.3	10.2	8.1	8.2	7.6	0.7	6.9	5.7	3.2	4.2	3.4	1.8
UPO94xIGO-220	100.0	95.0	85.0	95.0	60.0	2.3	9.2	7.5	5.0	4.1	2.6	3.1	3.8	3.1	2.3	2.1	1.8	2.8
JHO822	100.0	100.0	100.0	100.0	100.0	0.0	7.3	8.9	9.6	4.0	9.4	-0.8	3.4	5.0	4.1	2.0	5.0	-1.3
JHO851	100.0	100.0	100.0	100.0	95.0	0.1	4.0	11.7	8.0	6.6	4.0	-6.2	6.3	6.5	4.0	2.7	1.7	-2.1
JHO99-1	95.0	100.0	100.0	100.0	100.0	-0.8	8.5	7.7	8.1	5.9	6.9	0.9	4.5	5.2	4.4	2.6	4.2	0.5
JHO99-2	100.0	100.0	100.0	100.0	100.0	0.0	8.8	7.9	5.6	4.2	2.9	2.7	3.8	3.1	3.1	2.7	2.2	2.1
Mean	93.9	92.7	90.3	88.8	84.2		6.6	8.1	7.1	5.5	4.8		3.8	4.2	3.5	2.6	2.2	
Min	15.0	10.0	20.0	20.0	20.0		2.7	4.1	4.3	2.8	1.7		1.4	1.0	1.0	0.1	0.1	
Max	100.0	100.0	100.0	100.0	100.0		11.9	11.7	10.2	9.8	10.1		7.2	8.3	7.6	9.8	8.7	
SD	14.9	15.8	16.5	16.0	17.4		2.0	1.5	1.5	1.6	2.1		1.5	1.7	1.7	1.7	1.7	
t test probability		0.13	0.02	0.16	0.00			0.00	0.00	0.00	0.02			0.09	0.01	0.00	0.10	
SII		0.01	0.04	0.05	0.10			-0.24	-0.08	0.16	0.27			-0.10	0.07	0.33	0.42	
Regression			Y=94.62-0.58**x						Y=7.67-0.16**x						Y=4.24-0.12**x			

[1]DW = distilled water (Control); EC4 = electrical conductivity 4 dS/m; EC8 = electrical conductivity 8 dS/m; EC12 = electrical conductivity 12 dS/m; EC16 = electrical conductivity 16 dS/m; SSI = salt susceptibility index; SII = salinity intensity index.

Table 3. ANOVA table for various traits of oats genotypes grown in distilled water and solutions with EC4, EC8, EC12 and EC16.

Source of Variation	SS	df	MS	F	F crit
Seed germination (in vitro)					
Genotype	51,299.58	47	1,091.48	20.89**	1.43
Treatment	2,777.71	4	694.43	13.29**	2.41
Error	9,822.29	188	52.25		
Total	63,899.58	239			
Radicle length (in vitro)					
Genotype	209.92	47	4.46	1.61**	1.43
Treatment	337.90	4	84.48	30.41**	2.42
Error	522.31	188	2.78		
Total	1,070.14	239			
Plumule length (in vitro)					
Genotype	252.72	47	5.38	3.02**	1.45
Treatment	66.60	3	22.208	12.46**	2.67
Error	251.22	141	1.788		
Total	570.55	191			
Na, K and Ca concentration (sand culture)					
Genotype	546.20	5	109.24	18.06**	2.48
Treatment	9,121.55	2	4,560.78	754.19**	3.26
Interaction	3,549.09	10	354.91	58.6**	2.11
Error	217.70	36	6.047		
Total	13,434.54	53			
Morphological attributes (sand culture)					
Genotype	923.84	9	102.60	3.45**	1.97
Treatment	139,027.00	4	34,757.00	1,168.00**	2.46
Interaction	27,747.57	36	770.80	25.90**	1.53
Error	2,974.62	100	29.75		
Total	170,673.04	149			

Table 4. Germination, mortality and morphological attributes of 2 oats genotypes growing in sand culture irrigated with solutions containing varying salt levels.

Genotype	Treatment	MG[2]	Mortality[3]	30 days after sowing			45 days after sowing			70 days after sowing		
		(%)	(%)	Height (cm)	LL[4] (cm)	LW[5] (cm)	Height (cm)	LL (cm)	LW (cm)	Height (cm)	LL (cm)	LW (cm)
JHO2000-5	0.50% NaCl	90.7	1.5	30.0	23.7	0.8	42.0	29.3	1.3	51.9	28.2	1.3
	0.75% NaCl	80.0	0.0	24.3	18.1	0.5	34.1	26.1	1.1	45.4	23.4	1.1
	SSS1[1]	92.0	39.1	16.8	13.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	SSS2	72.0	70.1	13.0	10.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0
	Control	90.7	0.0	37.7	29.1	1.0	51.8	34.0	1.5	61.3	32.7	1.4
OS6x851-1	0.50% NaCl	100.0	2.7	31.3	23.7	0.8	36.6	21.4	1.2	52.5	26.5	1.4
	0.75% NaCl	93.3	0.0	26.9	20.8	0.7	40.8	25.8	1.2	47.4	22.6	1.4
	SSS1	97.3	28.7	11.1	9.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0
	SSS2	88.0	64.5	6.5	5.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	Control	93.3	0.0	35.5	27.5	1.2	50.8	36.0	1.4	72.9	36.2	1.8

[1]SSS1 = Saline soil scrap solution, EC8.0 dS/m; SSS2 = Saline soil scrap solution, EC10.4 dS/m; [2]MG = Mean germination; [3]25 days after sowing; [4]LL = Leaf length; [5]LW = Leaf width.

Plants irrigated with 0.5 and 0.75% NaCl solution showed concentrations of Na ranging from 36.5 to 43 mg/g DM compared with 7.2–14.9 mg/g DM in Control (Table 5), while concentrations of K ranged from 17.4 to 25.5 mg/g DM for saline treatments and 26.2–34.7 mg/g DM for Control. Concentrations of Ca ranged from 0.26 to 0.33 mg/g DM in saline conditions and 0.12–0.14 mg/g DM in Control (Table 5). Analysis of variance revealed significant differences among treatments and for the accumulation of Na, K and Ca in plants growing in saline

and Control treatments. The interaction effects were also significant (Table 3).

Proline concentrations in leaves increased with increasing salt concentration and mean values increased from 0.036 × 10^{-5} µg/g fresh weight for Control to 0.166 × 10^{-5} µg/g fresh weight for 0.75% NaCl treatment (Table 5).

Evaluation for field level salinity tolerance

Of the 44 genotypes sown into saline soil (i.e. at EC3.3 and pH 9.6) as well as normal non-saline soil condition (Control), NGB7259 showed highest germination percentage (>75%), whereas 5 genotypes, viz. NGB2718, NGB2120-1, NGB7002, NGB7253 and JHO2001-4, showed 50–75% germination. Twenty-seven genotypes showed 25–50% germination, while 11 genotypes showed less than 25% germination (Table 6). Growth of the plants was drastically impacted and only NGB7259 recorded >50% survival, with the remaining genotypes showing <20% survival. Morphological observations at Day 38 after sowing revealed that most genotypes had poor growth at this salinity level. Eleven genotypes (NGB6368, NGB6968, NGB6995, NGB7003, NGB7013, NGB7021, NGB7245, NGB7247, NGB7252, JHO2001-2, JHO851) showed 100% mortality. Hence, survival percentage and growth parameters presented in Table 7 are for 33 genotypes only. The mean survival of plants in the saline treatment was only 16.3% compared with 76.6% in Control. Plant growth was adversely affected and the average plant height of the genotypes growing under salt stress was 7.2 cm compared with 52.5 cm in non-stressed Control condition (Table 7). Leaf growth was also badly affected and mean leaf length and width in saline soil were 6.3 and 0.4 cm, respectively, compared with 38.0 and 1.3 cm in Control (Table 7). Proline accumulation was higher among plants growing under saline conditions than in Control (Table 8).

Table 5. Na, K, Ca and proline concentrations in 2 oats genotypes growing in Control and saline conditions in sand culture.

Genotype	Treatment (% NaCl)	Na (mg/g dry wt)	K (mg/g dry wt)	Ca (mg/g dry wt)	Proline (µg/g fresh wt)
JHO2000-5	0.50	43.04	17.40	0.31	0.064 ×10^{-5}
	0.75	41.83	19.75	0.26	0.115 ×10^{-5}
	Control	14.87	26.18	0.14	0.034 ×10^{-5}
OS6x851-1	0.50	36.50	21.92	0.33	0.058 ×10^{-5}
	0.75	38.75	25.54	0.29	0.217 ×10^{-5}
	Control	7.17	34.67	0.12	0.039 ×10^{-5}

Table 6. Grouping of oats genotypes based on germination at EC3.3 and pH 9.6 (field tolerance).

Germination percentage			
<25%	25–50%	50–75%	>75%
NGB4470, NGB4871, NGB4872, NGB6189, NGB6370, NGB6975, NGB7013, NGB7249, NGB7252, NGB7279, JHO2000-3	NGB2114, NGB2117, NGB4417, NGB4467, NGB4474-1, NGB4732, NGB4757, NGB4758, NGB4870, NGB6368, NGB6374, NGB6963, NGB6968, NGB6997, NGB7003, NGB7021, NGB7026, NGB7247, JHO2001-2, JHO2000-5, OS6x851-1, OS-7x320, UPO94xIGO-220, JHO822, JHO851, JHO99-1, JHO99-2	NGB2120-1, NGB2718, NGB7002, NGB7253, JHO2001-4	NGB7259

Table 7. Survival percentage and morphological attributes of oats genotypes grown in soil at EC3.3 and pH 9.6 (stressed, field tolerance) as well as in Control soil, at 38 days after sowing.

Genotype	Surviving plants (%)		Plant height (cm)		Leaf length (cm)		Leaf width (cm)	
	Stressed	Control	Stressed	Control	Stressed	Control	Stressed	Control
NGB2114	24.5	72.0	7.3	60.8	6.2	44.9	0.39	1.27
NGB2117	3.5	68.0	4.8	52.7	4.4	40.4	0.30	1.13
NGB2120-1	7.0	92.0	5.8	36.8	5.3	24.8	0.37	1.20
NGB2718	31.0	68.0	7.6	59.1	6.7	34.3	0.43	1.20
NGB4417	4.0	92.0	4.1	45.6	4.0	32.9	0.33	1.47
NGB4470	9.7	32.0	6.3	36.5	5.4	34.6	0.31	1.32
NGB4474-1	2.0	88.0	5.0	47.7	4.7	33.1	0.35	1.43
NGB4732	24.0	80.0	7.4	58.6	6.5	38.4	0.34	1.12
NGB4757	12.0	60.0	4.3	31.3	3.6	32.6	0.27	1.10
NGB4758	10.0	80.0	7.6	64.2	7.0	44.9	0.37	1.23
NGB4870	12.0	72.0	6.9	42.1	5.9	30.2	0.35	0.90
NGB4871	14.0	96.0	6.2	55.5	5.9	36.2	1.53	1.24
NGB6189	2.0	8.0	6.8	55.5	5.8	40.7	0.30	1.30
NGB6370	7.0	52.0	7.3	54.6	6.2	39.9	0.30	1.63
NGB6374	10.0	40.0	4.6	35.7	4.2	27.1	0.29	0.80
NGB6963	35.5	84.0	9.6	47.9	8.8	36.7	0.48	0.97
NGB6975	7.0	80.0	9.6	65.0	7.6	44.2	0.40	1.70
NGB6997	17.0	88.0	7.3	64.6	6.2	48.1	0.28	1.18
NGB7002	22.0	76.0	6.8	44.8	6.3	32.4	0.40	0.93
NGB7026	22.7	84.0	8.1	61.0	7.3	43.9	0.45	1.50
NGB7249	10.0	92.0	7.7	46.0	7.4	42.0	0.33	1.07
NGB7253	9.0	96.0	4.2	57.4	3.6	39.7	0.30	1.37
NGB7259	57.0	100.0	13.5	58.8	10.7	46.3	0.57	1.07
NGB7279	6.5	36.0	4.7	49.6	4.0	36.3	0.27	1.38
JHO2000-3	4.0	72.0	6.4	42.6	5.4	33.6	0.28	1.07
JHO2000-5	10.0	84.0	6.2	54.9	5.7	39.2	0.30	1.30
JHO2001-3	11.0	76.0	12.8	55.4	10.7	35.6	0.48	0.98
JHO2001-4	21.0	100.0	8.0	56.4	6.8	39.9	0.51	1.60
OS6x851-1	9.0	100.0	5.8	54.1	5.4	36.8	0.57	1.67
UPO94xIGO-220	39.0	68.0	8.8	57.5	7.9	40.9	0.47	1.30
JHO822	42.0	96.0	10.7	61.9	8.7	43.3	0.47	1.20
JHO99-1	15.0	96.0	6.7	60.7	5.4	42.4	0.50	1.40
JHO99-2	27.0	100.0	8.1	55.9	7.0	38.0	0.47	1.47
Mean	16.3	76.6	7.2	52.5	6.3	38.0	0.42	1.26
Min	2.0	8.0	4.1	31.3	3.6	24.8	0.27	0.80
Max	57.0	100.0	13.5	65.0	10.7	48.1	1.53	1.70
SD	12.95	22.20	2.23	9.03	1.76	5.48	0.22	0.23

Table 8. Estimation of proline concentration among oats genotypes growing in Control and saline soil.

Genotype	Proline (µmol/g fresh weight)	
	Control soil	Saline soil
NGB2718	0.074×10^{-5}	0.079×10^{-5}
NGB4470	0.038×10^{-5}	0.084×10^{-5}
NGB4732	0.045×10^{-5}	0.057×10^{-5}
NGB6370	0.071×10^{-5}	0.313×10^{-5}
NGB6963	0.077×10^{-5}	0.091×10^{-5}
NGB6975	0.073×10^{-5}	0.081×10^{-5}
NGB7026	0.058×10^{-5}	0.103×10^{-5}
NGB7259	0.047×10^{-5}	0.094×10^{-5}
JHO2001-4	0.079×10^{-5}	0.085×10^{-5}
JHO822	0.068×10^{-5}	0.085×10^{-5}

Discussion

For assessment of salinity tolerance of crops, it is essential that studies continue from the critical germination stage (Wang et al. 2011) through all different growth stages (Zhu et al. 2016). Hence, the present study used both germination under controlled conditions as well as germination and growth under field conditions for screening of the genotypes.

Genotypic differences for salt tolerance were observed in our study and some genotypes displayed tolerance at the germination stage, whereas other genotypes possessed tolerance at the seedling growth stage. Bai et al. (2018) also found 21 out of 248 oats genotypes to be tolerant of both

salinity and alkalinity during germination, with no correlation between tolerances at germination and adult stages or between tolerances of salt and alkali. Verma and Yadava (1986) evaluated 12 varieties of oats for relative tolerance of increasing levels of salinity using combinations of salts similar to those in natural salt-affected soils. Seeds were sown in petri dishes and were exposed to 5 salinity levels (40, 80, 120, 160 and 200 meq salts/L). Germination percentage, root and shoot lengths and dry weight of seedlings decreased with increase in salinity. In general, varieties JHO815, JHO802, JHO816 and UPO201 were found to be more tolerant at germination and seedling stages than other varieties.

Based on the in vitro germination test in our study, the best 5 salinity-tolerant genotypes, which possessed tolerance for both plumule and radicle growth, were: NGB2114, NGB4757, NGB4872, NGB6963 and NGB7252. A few genotypes, e.g. NGB2118-1, NGB4887, NGB6370 and JHO851, showed tolerance in terms of either plumule or radicle growth and moderate tolerance for the other trait. The field-level tolerance study revealed genotypes NGB2120-1, NGB2718, NGB7002, NGB7253, NGB7259 and JHO2001-4 showing salt tolerance during the germination phase. However, of these genotypes NGB7259 was the only one showing survival >50%. These results demonstrate that tolerance of salinity created specifically by NaCl is quite different from natural salinity, where many other salts may be present in the soil and result in more toxic effects.

Cultivation of oats is considered a valuable strategy to utilize saline lands due to its high capacity to accumulate salt ions in its straw, which is widely used as forage for livestock (Han et al. 2013). Therefore, tolerance of abiotic stresses such as salt and drought is a highly important trait in oat breeding. Abiotic stress tolerance is a quantitative trait controlled by multiple genes (Munns and Tester 2008; Deinlein et al. 2014) and identification of lines possessing tolerance of salinity at various growth stages is important for developing salt-tolerant lines in breeding of any crop.

Oats is considered to be a moderately salt-tolerant crop (Grattan 2016). However, it has been rated as having low salt tolerance by Ogle and St. John (2010) and reported to tolerate up to EC4 with an upper limit of EC8, at which it will not even germinate (USDA 1996). The degree of salt tolerance of the crop varies not only with plant species but also with different varieties of the same species (Hernández 2019; Malaviya et al. 2019). Germination and seedling stages have an important bearing on plant development at later stages of growth and ultimately crop yield. Soluble salts in high concentration interfere with a balanced absorption of essential nutritional ions by the plants, resulting in wilting, desiccation and stunted growth.

In the present study, salinity up to EC16 had little effect on germination of any accession. However, in this situation the raised EC was due to NaCl only, and under natural saline sodic conditions responses may be different, as in the field level tolerance experiment only a few genotypes showed germination >50%. The toxic effect of natural saline sodic soil was also apparent in poor germination in the experiment with SSS1 and SSS2 solutions. Earlier reports showed no reduction in yield up to EC3.3, 10% reduction at EC3.6 and 25% reduction in yield at EC4.1 (NSW-DPI 2017). Bai et al. (2018) also found that 68.5 mmol salt/L and 22.5 mmol alkali/L were appropriate concentrations for determining oat tolerance of salinity and alkalinity during germination, whereas Na_2SO_4:NaCl (1:1, 150 mmol/L each) was found optimal for screening oat tolerance of salinity during plant growth and development. For alkalinity tolerance, Na_2CO_3:$NaHCO_3$ (1:1, 75 mmol/L each) was found to be optimal. The field level tolerance experiment indicated that pH level of saline soils is far more important for growth of plants than EC level. Even genotypes showing good germination could not survive after a few weeks. A high NaCl concentration in soil causes a reduction in growth parameters (Sixto et al. 2005) such as fresh and dry weight of leaves, shoots and roots along with a decrease in moisture content (Parvaiz and Riffat 2005). High salinity stress also delays the emergence of nodal roots, leaves and tillers (Córdoba et al. 2001).

Correlation analysis revealed that ion accumulation is positively correlated with biomass and accumulation of Na^+ and Cl^- in straw is negatively ($P<0.05$) correlated with accumulation of K^+ (Wu et al. 2017). Sodium (Na^+) and chloride (Cl^-) are the key ions responsible for both osmotic and ion-specific damage, which significantly reduces crop growth and yield (Munns and Tester 2008). Using comprehensive transcriptome and functional analyses, Wu et al. (2017) showed that salinity stress in oats affects a variety of genes involved in different biological processes, osmotic adjustment and regulatory networks. Bai et al. (2018) found there was no correlation between tolerance of salinity and alkalinity during germination and plant growth stages. Alkalinity mainly decreases chlorophyll concentration, while salinity mainly disrupts water absorption and water balance. With increasing soil salt concentration, production of oats biomass decreases, which coincides with increasing Na^+ and Ca^{2+} concentrations (Zhao et al. 2007).

The finding of higher concentrations of proline in salt-stressed plants than in those growing under Control conditions confirms the usefulness of this biochemical indicator (Ashraf and Harris 2004; Ashraf and Foodlad 2007) and suggests that also in oats this concentration can be used as an indicator for salt tolerance.

Salinity is best characterized by EC of the irrigation water or EC of the saturated soil solution in distilled water. The higher the concentration of dissolved salts, the higher was the EC value. In the field study EC was 3.3, whereas in nature in general, soils with EC≤5 have total dissolved salts of 640 mg/L, while at EC>8 total dissolved salts are above 800 mg/L. In addition, soil in our study was highly alkaline. Few genotypes showed tolerance of both salinity and alkalinity, although genotype NGB7259 was an exception. In an earlier study by Bai et al. (2018), although 3 genotypes proved tolerant of both salt and alkali at both germination and adult stages, tolerance of salinity and alkalinity during germination and plant growth were not correlated. The identification of lines tolerant of different forms of salts as well as at different growth stages provides an opportunity for further gene pyramiding.

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Research Paper

How does agro-pastoralism affect forage and soil properties in western Serengeti, Tanzania?

¿Cómo afectan actividades agro-pastoriles el forraje y las características del suelo en Serengeti occidental, Tanzania?

PIUS YORAM KAVANA[1,2], EPHRAIM J. MTENGETI[2], ANTHONY SANGEDA[2], CHRISTOPHER MAHONGE[3], ROBERT FYUMAGWA[4] AND BUKOMBE JOHN[4]

[1]*Tanzania Wildlife Research Institute, Mahale-Gombe Wildlife Research Centre, Kigoma, Tanzania.* tawiri.or.tz
[2]*Sokoine University of Agriculture, College of Agriculture, Department of Animal, Aquaculture & Range Sciences, Morogoro, Tanzania.* sua.ac.tz
[3]*Sokoine University of Agriculture, College of Social Sciences and Humanities, Department of Policy Planning and Management, Morogoro, Tanzania.* sua.ac.tz
[4]*Tanzania Wildlife Research Institute, Arusha, Tanzania.* tawiri.or.tz

Abstract

The impacts of agro-pastoral activities on soil properties, plus nutritive value and residual standing biomass of herbaceous plants in areas of different land uses in western Serengeti, were evaluated. Vegetation and soil were sampled along 4,000 m transects laid across fallow land, areas grazed only by livestock, mixed grazing (livestock and wildlife) and wildlife grazing only. A total number of 123 plant species were encountered during sampling. Analyses of soil and vegetation samples were conducted at Sokoine University of Agriculture laboratories. The estimated average density of grazing animals encountered was 160 TLU/km^2 on transects within livestock-dominated grazing lands, 129 TLU/km^2 for mixed grazing and 83 TLU/km^2 for wildlife grazing only. Results indicated that ADF, IVDMD, IVOMD, ME and TDN in residual herbaceous forage at flowering were significantly ($P<0.05$) affected by land use type but CP, NDF and ADL were not affected. Soil pH, OC, CEC, C:N ratio and Ca differed significantly ($P<0.05$) between land use types. An overall evaluation indicated that regardless of climatic conditions, residual biomass of herbaceous plants in western Serengeti is determined by intensity of grazing, soil C:N ratio and concentrations of Ca and P in the soil. We conclude that agro-pastoral practices conducted in western Serengeti affected residual standing biomass of herbaceous plants and soil properties. We recommend that grazing pressure in communal grazing lands be reduced by either reducing number of grazing animals or duration of grazing in a particular grazing area, and specific studies be conducted to establish stocking rates appropriate for specific communal grazing lands in villages.

Keywords: Grazing pressure, land use type, nutritive value, residual standing biomass.

Resumen

En el oeste de la región de Serengeti, Tanzania, se evaluaron los impactos de diferentes actividades agropastoriles en las características del suelo, la biomasa residual y el valor nutritivo de las plantas herbáceas. Para el efecto se tomaron muestras de la vegetación y del suelo a lo largo de transectos de 4,000 m en áreas con diferentes sistemas de uso: (1) barbecho; (2) pastoreo con ganado (vacunos, caprinos y ovinos); (3) pastoreo mixto con ganado y animales silvestres; y solo (4) pastoreo por animales silvestres. En total fueron identificadas 123 especies diferentes de plantas. Los análisis de las muestras de suelo y plantas fueron realizados en los laboratorios de la University of Agriculture de Sokoine. Se encontró que la densidad promedio de animales estimada fue de 160 unidades tropicales de ganado (TLU)/km^2 en áreas

Correspondence: P.Y. Kavana, Tanzania Wildlife Research Institute, Mahale-Gombe Wildlife Research Centre, P.O. Box 1053, Kigoma, Tanzania. Email: pius.kavana@tawiri.or.tz, pykavana@gmail.com

de pastoreo por solo ganado, 129 TLU/km^2 en áreas de pastoreo mixto, y 83 TLU/km^2 en áreas de pastoreo solo por animales silvestres. Los resultados mostraron que en la época de floración de la vegetación utilizada para pastoreo, la fibra detergente ácida, la digestibilidad in vitro de la materia seca, la digestibilidad in vitro de la materia orgánica, la energía metabolizable y el total de nutrientes digestibles en la biomasa herbácea residual fueron afectados (P<0.05) por el tipo de uso del suelo. Por el contrario, la proteína cruda, la fibra detergente neutra y la lignina detergente ácida no fueron afectados. El pH del suelo, la capacidad de intercambio catiónico, las concentraciones de carbono orgánico y calcio (Ca) y la relación C:N fueron diferentes (P<0.05) en los diferentes tipos de uso del suelo. Una evaluación general indicó que, independiente de las condiciones climáticas, la biomasa residual de las plantas herbáceas en el oeste de Serengeti está determinada por la intensidad del pastoreo, la relación C:N del suelo y las concentraciones de Ca y P en el suelo. Los resultados permiten concluir que las prácticas agropastoriles en el oeste de Serengeti afectan la biomasa residual de las plantas herbáceas utilizadas por los animales en pastoreo, y las características del suelo. Los resultados sugieren (1) la necesidad de reducir la intensidad de pastoreo en las tierras comunales de la región, bien disminuyendo el número de animales en pastoreo o la duración del pastoreo en un área en particular, y (2) realizar estudios específicos para determinar ciclos de uso y cargas animal apropiadas en zonas de pastoreo comunal específicas.

Palabras clave: Biomasa residual, presión de pastoreo, uso de la tierra, valor nutritivo.

Introduction

Agro-pastoralism as a livelihood strategy involves some traditional and contemporary 'best-bet' practices such as deferred grazing, in Tanzania traditionally known as Ngitiri or Alalili, grass band cultivation, zay pit cultivation, traditionally known as Ngoro system, and controlled grazing. The best-bet agro-pastoral practices are considered to contribute to sustainable systems due to reduced disturbance to soil and native plants, resulting in retention of diverse plant species that contribute to high primary production. However, some agro-pastoral practices, such as keeping large herds of livestock within a small grazing area, exert high grazing pressure on plant species and soil (Veblen 2008), affecting species composition and abundance. Other practices, such as unlimited expansion of cultivated land, affect availability of herbaceous species due to land clearing, thereby reducing the feed resource base for grazing animals. Both livestock keeping and cultivation are important for the livelihood of people in western Serengeti, so good land use planning is needed to accommodate both activities.

Both land clearing and cultivation disrupt stable ecosystems (Cassman and Wood 2005) and result in changes in species composition of vegetation that consequently influence the quantity and quality of herbaceous plants available (Butt and Turner 2012). Herbaceous plants are the primary feed resource for grazing animals in western Serengeti, so any significant disturbance to herbaceous vegetation affects performance of grazing animals in the ecosystem. This suggests a need for careful consideration when allocating specific areas for either grazing or cropping as establishing cultivation within grazing lands might reduce availability of natural feed resources but availability of crop residues could offset the reduction.

Both the human population and conversion of pasture lands to cropping are increasing in western Serengeti (Estes et al. 2012). However, little is known (Nortjé 2015; Lankester and Davis 2016) regarding the effects of agro-pastoralism on soil properties, livestock and wildlife performance, forage richness and diversity and biomass production. Increased human and livestock populations around the Serengeti National Park resulted in progressive livestock encroachment in the western part of the Park. Currently, no scientific study has been conducted to evaluate contradicting views between conservationists and agro-pastoralists on the effects of agro-pastoralism on conservation of wildlife in protected areas of the western part of the Serengeti ecosystem.

This work was designed to evaluate the impacts of agro-pastoral activities on soil properties plus standing biomass and quality of the herbaceous plant layer in western Serengeti. It was hypothesized that there are no variations in quantity and quality of residual standing biomass of herbaceous plants and soil properties as a result of agro-pastoral activities in fallow, livestock, mixed and wildlife-dominated land use types.

Materials and Methods

Study area

The study was conducted in western Serengeti, which is part of the Serengeti ecosystem as shown in Figure 1. Average annual rainfall ranges between 500 and 1,200 mm, declining towards the Serengeti National Park boundary and increasing towards Lake Victoria to the west (Sinclair et al. 2000). However, rainfall during the study period ranged from 400 to 900 mm. Western Serengeti is occupied by agro-pastoralists and is one of the most densely settled areas in the

Greater Serengeti ecosystem with human population growth rates exceeding those to the north, east and south of the National Park (Kideghesho 2010). The study was conducted in 4 districts by selecting villages that were adjacent to protected areas as shown in brackets, namely: Serengeti district (Park Nyigoti), Bunda district (Nyamatoke), Meatu district (Makao) and Bariadi district (Mwantimba and Matale). While the western Serengeti is considered to be unsuitable for arable agriculture, the subsistence economy depends mainly on agro-pastoralism (Emerton and Mfunda 1999), which is constrained by inadequate inputs of resources, e.g. fertilizers, and poor delivery of agricultural extension services, and people in villages practice extensive cropping and livestock keeping, which encroaches on protected areas (Mfunda and Røskaft 2011).

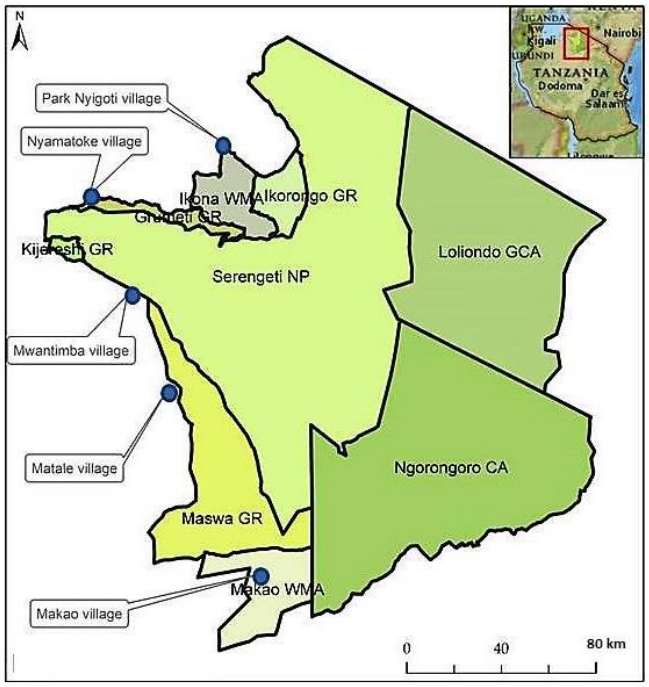

Figure 1. A map of Serengeti ecosystem showing the study sites and protected areas in western Serengeti. CA = Conservation Area; GCA = Game Controlled Area; GR = Game Reserve; NP = National Park; WMA = Wildlife Management Area.

Field data collection

Vegetation was sampled at the peak blooming period of herbaceous plants during April and May 2016 and 2017 to enable field identification by inflorescences. Herbaceous plants were sampled along 4,000 m transects in selected villages that were adjacent to protected areas. Transects were aligned in each village to cross different land use types in such a way that each transect started in village land and progressively traversed 0–1,000 m in lands dominated by cropping, 1,000–2,000 m in lands dominated by livestock grazing, 2,000–3,000 m crossing the boundary between village land and protected areas, where mixed grazing of livestock and wildlife occurred, and the remaining 3,000–4,000 m was in the protected areas dominated by wildlife grazing. A 0.25 m^2 quadrat was used to sample herbaceous plants at 100 m intervals along each transect. The sampling distance was established during a reconnaissance survey as this frequency ensured that 80–100% of the herbaceous plant species in the study areas would be encountered. Before harvesting, overall herbaceous plant ground cover in each quadrat was estimated visually and expressed as percentage cover. All plant species within quadrats were identified, clipped and weighed for determination of standing dry matter available. Species not identified in the field were taken to the National Herbarium in Arusha for identification. Samples were air-dried in the field and then re-dried to a constant weight in a vacuum oven at 50 °C for 48 hours in a laboratory. The dry samples were ground in a Wiley mill to pass through a 1-mm screen for subsequent laboratory analyses. Following harvesting of forage, soil sampling was conducted at the central point of each quadrat to a depth of 30 cm at every 300 m along each transect.

Densities of both livestock and wildlife in the study areas were estimated based on observations made along transects during sampling periods. Livestock species commonly observed in study sites included cattle, goats and sheep, while wildlife included wildebeest, zebra, topi, impala, Grant's gazelle, reedbuck and Thomson's gazelle; elephant were encountered once on the border between Maswa Game Reserve and Matale village. Throughout the sampling process, all wildlife and domestic grazing animals spotted within 200 m either side of each transect were identified and counted. Animal counts were converted to tropical livestock units (TLU) based on the respective species average weights, where 1 TLU = 250 kg live weight according to LEAD/FAO (1999).

Laboratory analyses of plant samples

Laboratory analyses included neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP), in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD); they were performed in the laboratory at the Sokoine University of Agriculture. Standard laboratory methods were used as described by Van Soest et al. (1991) for NDF, ADF and ADL, and AOAC (1990) for CP. IVDMD was determined by the Tilley and Terry (1963) method. Total digestible nutrients and metabolizable energy were estimated according to Undersander and Moore (2004) and Spörndly (1989), respectively.

Laboratory analysis of soil samples

Soil samples were taken for determination of soil texture, pH, organic carbon (OC), total N, available P, Ca and CEC according to standard procedures (Okalebo et al. 2002).

Data analysis

Variation of residual standing biomass and nutritive value of herbaceous plants plus soil properties among different land use/grazing types were analyzed by using R statistical software version 3.5.3. Assessment of collinearity among explanatory variables was performed using stepwise variance inflation factor (VIF), whereby all predictor variables were initially included in the linear regression equation. Variables with VIF greater than 4 were eliminated from the model progressively, while the predictor variables with VIF less than 4 were retained. The resulting linear regression model was then used to assess variables that were significantly associated with the response variable standing biomass. Herbaceous plant species association was analyzed by using null model according to Griffith et al (2016). Prominence of herbaceous plant species in different land use types was categorized into 4 groups based on the range of occurrence of all species (0–10.7%). The groups were classified as less common (0–2.7%), common (2.8–5.5%), more common (5.6–8.3%) and most common (8.4–11.1%). Analyses were performed using pooled data for respective land use type with type III sum of squares in ANOVA. Distribution of herbaceous plant species on identified soil texture classes was analyzed according to Heberle et al. (2015).

Results

Herbaceous plant community properties

Average density of grazing animals observed on the various land use types was estimated as 160 TLU/km^2 on livestock-dominated grazing lands, 129 TLU/km^2 on transects dominated by mixed grazing and 83 TLU/km^2 on wildlife grazing areas. A total of 123 plant species (Appendix 1) were encountered during sampling; occurrence of common species is shown in Table 1.

Table 1. Occurrence (%) of common herbaceous plant species in different land use types in western Serengeti.

Species[1]	Land use type			
	Fallow	Livestock grazing	Mixed grazing	Wildlife grazing
Aristida kenyensis Henrard (Poaceae)	0.3	2.3	6.3*[2]	0.0
Bidens schimperi Sch.Bip. ex Walp. (Compositae)	0.0	2.0	4.7*	1.0
Blepharis linariifolia Pers. (Acanthaceae)	0.0	0.3	4.7*	0.7
Bothriochloa insculpta (A. Rich.) A. Camus (Poaceae)	1.0	1.7	0.3	3.7*
Brachiaria semiundulata (Hochst.) Stapf (Poaceae)	1.3	6.0**[3]	7.3**	4.3*
Chloris pycnothrix Trin. (Poaceae)	0.7	5.7**	10.7***[4]	6.0**
Chrysochloa orientalis (C.E. Hubb.) Swallen (Poaceae)	0.0	0.7	0.0	0.7
Cynodon dactylon (L.) Pers. (Poaceae)	4.0*	7.0**	2.0	0.7
Dactyloctenium aegyptium (L.) Willd. (Poaceae)	0.7	4.7*	8.0**	0.3
Digitaria macroblephara (Hack.) Paoli (Poaceae)	0.0	0.3	0.0	3.7*
Eragrostis racemosa (Thunb.) Steud. (Poaceae)	0.3	0.3	2.3	1.3
Eragrostis patula (Kunth) Steud. (Poaceae)	1.0	1.0	0.0	0.7
Euphorbia inaequilatera Sond. (Euphorbiaceae)	0.0	0.7	1.3	1.7
Heteropogon contortus (L.) P. Beauv. ex Roem. & Schult. (Poaceae)	0.0	0.0	0.0	4.7*
Hyperthelia dissoluta (Nees ex Steud.) Clayton (Poaceae)	0.0	0.0	0.0	4.3*
Indigofera hochstetteri Baker (Leguminosae)	0.0	1.3	1.3	3.3
Indigofera volkensii Taub. (Leguminosae)	0.0	1.3	0.7	3.3
Microchloa kunthii Desv. (Poaceae)	0.0	1.0	3.7*	3.7*
Panicum coloratum L. (Poaceae)	0.0	1.0	3.7*	3.7*
Portulaca quadrifida L. (Portulacaceae)	0.7	0.7	1.0	0.7
Sporobolus festivus Hochst. ex A. Rich. (Poaceae)	0.0	0.0	3.7*	5.0*
Sporobolus ioclados (Trin.) Nees (Poaceae)	0.0	2.3	1.3	2.0
Sporobolus pyramidalis P. Beauv. (Poaceae)	0.0	2.3	1.0	3.3
Themeda triandra Forssk. (Poaceae)	0.0	0.7	0.3	8.3**
Tragus berteronianus Schult. (Poaceae)	1.0	0.7	2.3	0.3

[1]Taxonomy according to The Plant List (theplantlist.org). [2]* = Common. [3]** = More common. [4]*** = Most common. Values without asterisks indicate less common.

Chloris pycnothrix was the most prominent in mixed grazing land use type and was more apparent in livestock and wildlife-dominated grazing land use types. *Aristida kenyensis*, *Bidens schimperi*, *Blepharis linariifolia*, *Microchloa kunthii*, *Panicum coloratum* and *Sporobolus festivus* were common in mixed grazing land use type, while *Brachiaria semiundulata* was apparent in wildlife-dominated land use type and appeared more commonly in both livestock- and mixed grazing land use types. *Dactyloctenium aegyptium* was noticeable in livestock-dominated grazing land use type and more common in mixed grazing land use type. *Digitaria macroblephara*, *Heteropogon contortus* and *Hyperthelia dissoluta* were prominent in wildlife-dominated grazing land use type. *Themeda triandra* was more common only in wildlife-dominated land use type. *Cynodon dactylon* was apparent in cultivated land use type and appeared more commonly in livestock-dominated grazing land use type. Association of herbaceous plant species was analyzed using 325 species pairs combinations that provided the results presented in Figure 2.

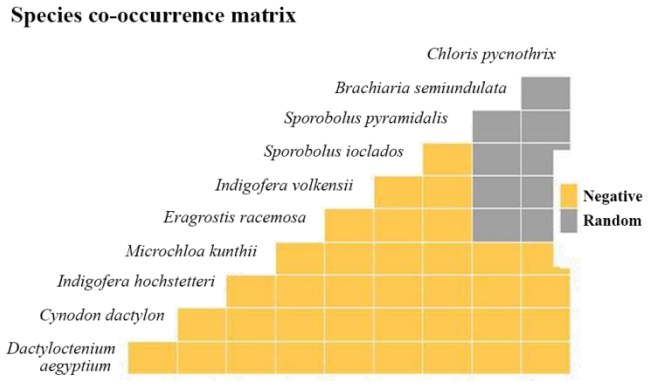

Figure 2. Association of herbaceous plant species in western Serengeti.

Results from Figure 2 show that *Dactyloctenium aegyptium*, *Cynodon dactylon*, *Indigofera hochstetteri* and *Microchloa kunthii* were negatively associated with other herbaceous plant species in the community. The negative association of *Cynodon dactylon* with other herbaceous plant species becomes more prominent under the influence of livestock grazing, while negative association of *Dactyloctenium aegyptium* with other herbaceous plant species became more prominent under the influence of mixed grazing of livestock and wildlife. The negative association of *Microchloa kunthii* with other herbaceous plant species became noticeable under the influence of wildlife grazing. However, the negative association of *Indigofera hochstetteri* with other herbaceous plant species is slightly apparent under the influence of wildlife grazing.

Results shown in Table 2 indicate that standing above-ground biomass of herbaceous plants in grazing lands at flowering was significantly ($P<0.05$) affected by land use type. Wildlife-dominated grazing lands carried 50% more standing above-ground biomass than livestock-dominated land. While ADF, IVDMD, IVOMD, ME and total digestible nutrients were significantly ($P<0.05$) affected by land use type, CP, NDF and ADL were unaffected.

Soil properties

Soil samples collected in different land use types revealed that clay is a major component in all soils of western Serengeti (Figure 3).

Five soil texture classes, namely: clay, sandy clay, sandy clay loam, clay loam and sandy loam, were identified from soil samples collected in this study with the former 3 types being most common (about 95%). Distribution of herbaceous plant species in the different soil texture classes is shown in a Venn diagram (Figure 4).

Table 2. Effects of land use type on residual standing biomass and nutritive value of herbaceous plants at flowering in western Serengeti.

Variable	Land use type				P value	Significance
	Fallow	LG	MG	WG		
Biomass (kg DM/ha)	2,320b	2,126b	2,575ab	3,188a	0.02	*
CP (%)	9.2	9.0	8.4	8.4	0.19	NS
NDF (%)	62.9	62.4	60.7	60.0	0.76	NS
ADF (%)	33.4b	33.9ab	35.4ab	36.2a	0.01	**
ADL (%)	3.7	3.7	3.9	3.8	0.96	NS
IVDMD (%)	47.0a	39.5b	39.5b	40.1b	0.00	***
IVOMD (%)	49.0a	44.1ab	42.4b	42.2b	0.00	***
ME (MJ/kg DM)	5.6a	4.4b	4.4b	4.5b	0.00	***
TDN (%)	57.8a	57.6a	55.6ab	54.6b	0.00	***

Values within rows followed by different letters differ significantly ($P<0.05$). LG = Livestock grazing; MG = Mixed grazing; WG = Wildlife grazing.

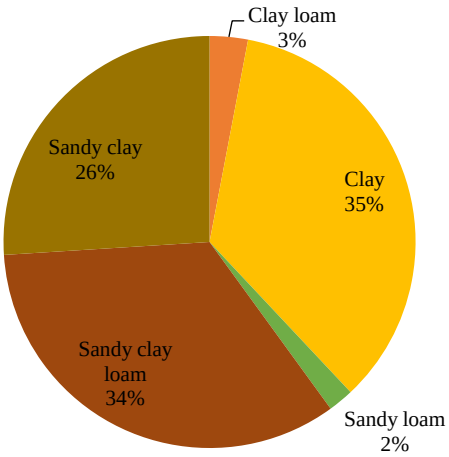

Figure 3. Proportions of soil texture classes in western Serengeti.

Figure 4 indicates that 12 herbaceous plant species were most common in clay soil, including: *Eragrostis tenuifolia*, *Achyranthes aspera*, *Brachiaria semiundulata*, *Commelina benghalensis*, *Digitaria milanjiana*, *Ocimum basilicum*, *Justicia exigua*, *Tragus berteronianus*, *Justicia matammensis*, *Cynodon dactylon*, *Chloris gayana* and *Lactuca capensis*. Three herbaceous plant species, i.e. *Oxygonum sinuatum*, *Sporobolus cordofanus* and *Digitaria eriantha*, were most common in sandy clay soil, while *Cycnium tubulosum*, *Setaria sphacelata*, *Heteropogon contortus*, *Indigofera hochstetteri*, *Chrysochloa orientalis*, *Euphorbia inaequilatera* and *Kyllinga nervosa* occurred mainly in sandy clay loam soil. *Corchorus aestuans* grew in sandy loam soil only. The species observed in both clay and clay loam soils was *Panicum coloratum*, while *Portulaca quadrifida* occurred in sandy loam and sandy clay loam soils. *Sporobolus festivus*, *Sporobolus ioclados* and *Dactyloctenium aegyptium* were found in 4 soil texture classes, namely: clay, clay loam, sandy clay loam and sandy loam, while *Bothriochloa insculpta* and *Themeda triandra* occurred in clay, clay loam and sandy clay loam soils. *Aristida kenyensis*, *Bidens schimperi* and *Blepharis linariifolia* were found in clay and sandy clay soils. A universal herbaceous plant species that was growing in all 5 soil texture classes was *Microchloa kunthii*.

Table 3 shows the average values of the measured soil parameters and reveals that soil pH, soil OC, CEC, soil Ca and soil C:N ratio were significantly ($P<0.05$) different among land use types.

Using stepwise variance inflation factor (VIF) of distance from communal grazing land towards protected areas, herbaceous ground cover and soil variables indicated that distance, cover, soil C:N ratio and Ca and P concentrations in the soil had VIF values below the threshold that sufficed development of a linear model (Table 4) for prediction of residual standing biomass (Figure 2) in western Serengeti.

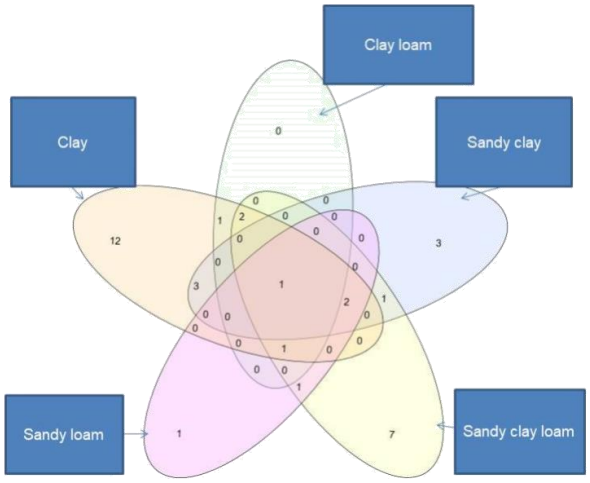

Figure 4. Distribution of herbaceous plant species in different soil texture classes in western Serengeti.

Figure 3 presents examples of the accuracy of this model in predicting residual standing biomass with the following equation:

$y = 1{,}278 + 0.49x$ ($r^2 = 0.46$), where:

y = predicted biomass; and x = actual biomass.

Table 3. Soil properties of different land use types in western Serengeti.

Parameter	Land use type				P value	Significance
	Fallow	LG	MG	WG		
pH	7.2b	7.9ab	8.3a	7.4ab	0.0453	*
OC (%)	0.78b	1.64a	1.64a	1.32a	1.46a	**
P (mg/kg)	1.36	1.26	1.76	1.63	0.1620	NS
CEC (cmol/kg)	16.14b	23.94a	22.68a	23.73a	0.0008	**
Ca (cmol/kg)	10.52b	12.63b	18.10a	13.08b	0.0077	**
Total N (%)	0.10	0.13	0.12	0.12	0.5240	NS
C:N ratio	7.74b	14.10a	11.20	12.72a	0.0442	*

Values within rows followed by different letters differ significantly ($P<0.05$). LG = Livestock grazing; MG = Mixed grazing; WG = Wildlife grazing; NS = Not significant.

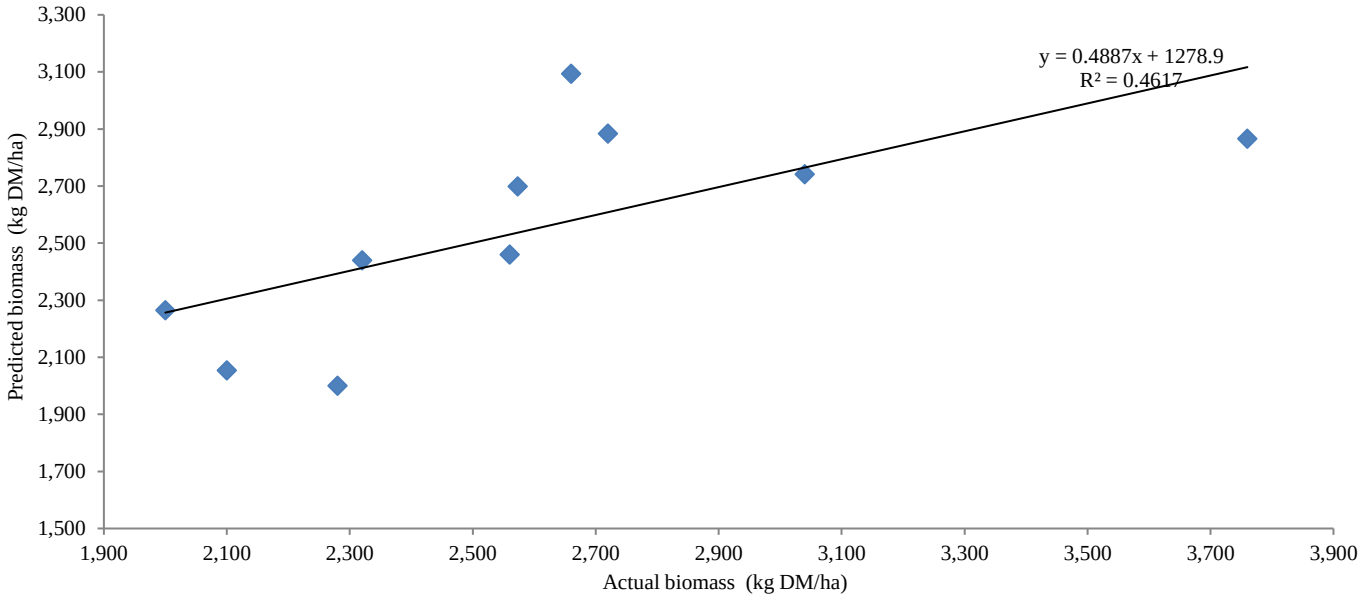

Figure 3. Residual standing biomass prediction model validation output.

Table 4. Variables for herbaceous plant residual standing biomass prediction model.

Variable	Coefficient (estimate)	VIF
Intercept	1,059.5	
Distance (m)	0.45	1.44
Cover (%)	11.67	1.40
Soil C:N ratio	16.70	1.25
Soil Ca (cmol/kg)	1.10	1.08
Soil P (mg/kg)	8.64	1.07

Model: Residual Standing Biomass = 0.45 Distance + 11.67 Cover + 16.70 C:N + 1.10 Ca + 8.64 P + 1059.54.

Discussion

Effects of agro-pastoralism on residual standing biomass

This study showed that grazing areas under high density of animals and continuous livestock grazing had lower residual standing biomass than areas with low animal density and intermittent animal grazing, e.g. in protected areas. This result was not surprising, given the different densities of animals grazing the different areas and hence grazing pressure applied. Low residual standing biomass levels on heavily grazed areas observed in this study are consistent with observations by Ngatia et al. (2015) in Kenya and Mbatha and Ward (2010) in South Africa. However, other studies on effects of grazing on standing biomass showed that the effect is site-specific, influenced by environmental conditions and grazing history (Osem et al. 2002; Jia et al. 2018). Livestock at high density tend to graze herbaceous plants to ground level without strong plant selection (Adler et al. 2001; Hayes and Holl 2003), which reduces the ability of livestock to graze out more desirable species. However, pastures need periodic rest periods to allow species to recover and it is up to herders to control these grazing patterns. In village lands, high density of domestic animals occurs during the rainy season and extends until late dry season, when communal grazing lands become bare. Herders then shift groups of animals to more remote areas in search of pastures, including trespassing in protected areas based on independent decisions of livestock owners. As a result grazing pressure on the village lands is reduced at this time. Wildlife, in contrast, move freely on grazing areas to select nutritious herbaceous plants depending on their mouth width and body weight (Fynn 2012; Bukombe et al. 2017) but at much lower grazing pressures. These differences in grazing pressure and duration of grazing on specific areas for livestock and wildlife obviously contributed to the big differences in residual standing biomass observed between livestock- and wildlife-dominated grazing lands. Cultivation resulted in low standing biomass of herbaceous plants due to removal of herbaceous plants in crop farms as they are viewed as weeds in the crops.

Effects of agro-pastoralism on nutritive value of herbaceous plants

While some significant differences in nutritive parameters for forage from the different land use types were recorded, the magnitude of most differences was scarcely significant from an overall perspective.

IVDMD and IVOMD were highest in herbaceous plants found in cultivated lands as compared with other land use types, which is possibly a function of release of nutrients

from the soil during plowing/hoeing etc. plus plants not having been grazed and the more digestible components still being present. Energy is an important indicator of the nutritive value of feeds and considerably more nutrient is required to maintain normal energy metabolism than for all other purposes combined (Dietz 1970). The most common nutritional deficiency affecting range animals is lack of available energy in feeds, digestible energy or both (Michalk and Savile 1978; Corbett and Ball 2002). Results from this study showed that herbaceous plants found in cultivated lands and lands grazed by livestock had highest metabolizable energy and total digestible nutrients.

Effects of agro-pastoralism on soil properties

Clay formed the major texture component of soils of the study area in western Serengeti with a range from straight clay to sandy loams. As would be expected, different herbaceous plant species were found on the different soil types, which produced a mosaic pattern of herbaceous plants in the Serengeti ecosystem. The aggregation of herbaceous plants according to soil texture classes supports findings reported by Kavana et al. (2019), which showed soil texture as an important input variable in herbaceous plant ground cover models. *Microchloa kunthii* was the only herbaceous species present in all soil texture classes, highlighting the versatility of this species and its ability to compete with other herbaceous plant species by exhibiting negative association as shown in Figure 2.

While in general wildlife distribute their faeces and urine at random, except for camping areas where there is some accumulation of faeces, livestock deposit much of their faeces in specific areas such as kraals and other resting areas, where they are generally held at night. Returning of this manure to cultivated areas would help counteract the rundown of nutrients on fallow where lowest CEC and equally lowest soil OC, Ca, P, C:N ratio and pH were measured. Juo et al. (1995) in Nigeria and Lian et al. (2013) in China reported a decline in fertility on cultivated areas in the absence of fertilizer inputs.

Broader implications of agro-pastoralism on grazing land systems

In addition to weather conditions, residual standing biomass production in western Serengeti relies on a range of variables that affect the complex soil and plant systems. The finding that distance from the protected areas, ground cover, C:N ratio, soil Ca and P were key factors in determining amount of standing biomass was of interest. Distance from protected areas was possibly merely a reflection of the grazing pressure applied to the relevant areas as was ground cover. The C:N ratio indicates whether or not mineralization of N is taking place in the soil and the amount available to plants and is significantly correlated (Appendix 2) with CEC ($r = 0.51$), so is important. Soil Ca is important as building blocks for herbaceous plant cells as Ca has a structural role in the cell wall membranes and as a counter cation for inorganic and organic anions in the cell vacuole (Marschner 1995). The importance of P for fundamental processes of photosynthesis, flowering, fruiting (including seed production) and maturation of herbaceous plants is well understood (Weil and Brady 2017).

Conclusion

This study contributes to the understanding of the ecological effects of agro-pastoralism on the herbaceous vegetation and soil properties in Western Serengeti. The results indicate that decrease in residue standing biomass and soil properties as a result of agro-pastoral activities is significant, highlighting the need for sustainable agro-pastoralism. It was shown that persistence and successful production of herbaceous plants in western Serengeti requires consideration of agro-pastoral activities that are not detrimental to adequate C:N ratio, and Ca and P concentrations in soil. Grazing pressure appeared to affect seriously residual standing biomass in communal grazing lands that requires reduction in order to allow recovery of herbaceous plants. Grazing pressure should be reduced by either reducing number of animals or duration of grazing on these lands. Specific studies should be conducted by respective local government authorities to establish appropriate stocking rates and grazing patterns for specific communal grazing lands in villages. Based on the findings, appropriate grazing strategies can be developed. Manure accumulated in kraals should be returned to at least cultivated areas to reduce soil run-down.

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(Note of the editors: All hyperlinks were verified 15 December 2020.)

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Appendix 1. Herbaceous plant species occurrence (%) in different land use types (taxonomy according to The Plant List; theplantlist.org).

No.	Species (Family)	Fallow	Livestock	Mixed	Wildlife	Overall
1.	*Abutilon mauritianum* (Jacq.) Medik. (Malvaceae)	0	0.283	0.243	0	0.189
2.	*Achyranthes aspera* L. (Amaranthaceae)	0	0.283	0.973	0	0.472
3.	*Aeschynomene indica* L. (Leguminosae)	1.136	0	0	0	0
4.	*Albuca kirkii* (Baker) Brenan (Asparagaceae)	0	0	0.243	0	0.094
5.	*Alternanthera pungens* Kunth (Amaranthaceae)	0	0	0	0.678	0.189
6.	*Amaranthus graecizans* L. (Amaranthaceae)	0	0	0.243	0	0.094
7.	*Andropogon greenwayi* Napper (Poaceae)	0	0.85	0.243	0	0.378
8.	*Aristida adoensis* Hochst. ex A. Rich. (Poaceae)	0	0	0.243	0.339	0.189
9.	*Aristida kenyensis* Henrard (Poaceae)	2.273	1.983	4.623	0	2.455
10.	*Asparagus africanus* Lam. (Asparagaceae)	0	0.567	0.487	0.339	0.472
11.	*Aspilia mossambicensis* (Oliv.) Wild (Compositae)	0	0	0	1.356	0.378
12.	*Bidens schimperi* Sch.Bip. ex Walp. (Compositae)	2.273	1.7	3.406	1.017	2.172
13.	*Blepharis linariifolia* Pers. (Acanthaceae)	0	0.283	3.406	0.678	1.605
14.	*Blepharis maderaspatensis* (L.) B. Heyne ex Roth (Acanthaceae)*	0	0	0.486	1.695	1.699
15.	*Bothriochloa insculpta* (A. Rich.) A. Camus (Poaceae)	4.545	3.966	2.676	3.729	3.399
16.	*Brachiaria brizantha* (A .Rich.) Stapf (Poaceae)	0	2.833	1.46	1.017	1.794
17.	*Brachiaria jubata* (Fig. & De Not.) Stapf (Poaceae)	0	0.567	0	0.678	0.378
18.	*Brachiaria semiundulata* (Hochst.) Stapf (Poaceae)	4.545	5.099	5.353	4.407	5.005
19.	*Brachiaria serrata* (Thunb.) Stapf (Poaceae)[1]	0	0.85	0.73	0	0.567
20.	*Cenchrus ciliaris* L. (Poaceae)	0	0.283	0	0.339	0.189
21.	*Centrapalus pauciflorus* (Willd.) H. Rob. (Compositae)	1.136	0	0	0.339	0.094
22.	*Chamaecrista mimosoides* (L.) Greene (Leguminosae)	1.136	0	0	0	0
23.	*Chloris gayana* Kunth (Poaceae)	2.273	0	0.73	1.356	0.661
24.	*Chloris pycnothrix* Thrin. (Poaceae)	4.545	5.949	8.029	6.102	6.799
25.	*Chloris virgata* Sw. (Poaceae)	0	0	0.243	0	0.094
26.	*Chrysochloa orientalis* (C.E. Hubb.) Swallen (Poaceae)	0	1.416	0.487	0.678	0.85
27.	*Cleome monophylla* L. (Cleomaceae)	2.273	0	0	0	0
28.	*Clitoria ternatea* L. (Leguminosae)	0	0.283	0	0	0.094
29.	*Commelina africana* L. (Commelinaceae)	0	0.567	0.243	0.339	0.378
30.	*Commelina aspera* G. Don ex Benth. (Commelinaceae)	0	0	0.243	0	0.094
31.	*Commelina benghalensis* L. (Commelinaceae)	2.273	0.567	0.487	0.678	0.567
32.	*Corchorus aestuans* L. (Malvaceae)	0	0.283	0.243	0	0.189
33.	*Corchorus trilocularis* L. (Malvaceae)	1.136	0	0	0	0
34.	*Craterostigma plantagineum* Hochst. (Linderniaceae)	0	0.85	0.487	0.339	0.567
35.	*Crotalaria spinosa* Benth. (Leguminosae)	0	1.416	0.243	0.339	0.661
36.	*Cycnium tubulosum* (L.f.) Engl. (Orobanchaceae)	0	0	0	1.017	0.283
37.	*Cymbopogon caesius* (Hook. & Arn.) Stapf (Poaceae)	3.409	0	0	0	0
38.	*Cynodon dactylon* (L.) Pers. (Poaceae)	13.636	7.082	1.946	0.678	3.305
39.	*Cynodon plectostachyus* (K. Schum.) Pilg. (Poaceae)	0	0	0	0.339	0.094

Continued

No.	Species (Family)	Fallow	Livestock	Mixed	Wildlife	Overall
40.	*Cyperus pulchellus* R.Br. (Cyperaceae)	1.136	1.216	0.73	1.017	1.138
41.	*Cyphostemma serpens* (Hochst. ex A. Rich.) Desc. (Vitaceae)	0	0	0.243	0	0.094
42.	*Dactyloctenium aegyptium* (L.) Willd. (Poaceae)	5.682	4.816	6.083	0.339	4.06
43.	*Desmodium tortuosum* (Sw.) DC. (Leguminosae)	0	0.283	0	0	0.094
44.	*Digitaria abyssinica* (A.Rich.) Stapf (Poaceae)	0	0	0.243	0	0.094
45.	*Digitaria bicornis* (Lam.) Roem. & Schult. (Poaceae)	0	0	0.243	0	0.094
46.	*Digitaria eriantha* Steud. (Poaceae)	0	0	0.243	0	0.094
47.	*Digitaria longiflora* (Retz.) Pers. (Poaceae)	2.273	0.85	0.73	0	0.567
48.	*Digitaria macroblephara* (Hack.) Paoli (Poaceae)	0	0.283	2.676	3.729	2.172
49.	*Digitaria milanjiana* (Rendle) Stapf (Poaceae)	1.136	0.567	1.703	0	0.85
50.	*Digitaria ternata* (A. Rich.) Stapf (Poaceae)	0	2.266	0.243	0.339	0.944
51.	*Dyschoriste radicans* (Hochst. ex A. Rich.) Nees (Acanthaceae)	0	0.567	0.487	0.678	0.567
52.	*Echinochloa pyramidalis* (Lam.) Hitchc. & Chase (Poaceae)	0	0.283	0	1.356	0.472
53	*Eleusine indica* (L.) Gaertn. (Poaceae)	0	0.283	0.243	0	0.189
54.	*Eragrostis aspera* (Jacq.) Nees (Poaceae)	1.136	0.283	0.243	0	0.189
55.	*Eragrostis cilianensis* (All.) Janch. (Poaceae)	1.136	0	0.243	0	0.094
56.	*Eragrostis patula* (Kunth) Steud. (Poaceae)	3.409	2.266	0	0.678	0.944
57.	*Eragrostis racemosa* (Thunb.) Steud. (Poaceae)	1.136	1.416	2.92	1.356	1.983
58.	*Euphorbia inaequilatera* Sond. (Euphorbiaceae)	0	2.266	0.973	1.695	1.605
59.	*Eustachys paspaloides* (Vahl) Lanza & Mattei (Poaceae)	0	0	0.243	0.339	0.189
60.	*Gomphrena globosa* L. (Amaranthaceae)	3.409	1.133	0.243	0	0.472
61.	*Gutenbergia cordifolia* Benth. ex Oliv. (Compositae)	0	0.283	0.973	0.339	0.567
62.	*Gutenbergia petersii* Steetz (Compositae)	1.136	0	0	1.017	0.283
63.	*Harpachne schimperi* A. Rich. (Poaceae)	0	0.567	0.73	1.017	0.755
64.	*Heliotropium nelsonii* C.H. Wright (Boraginaceae)[1]	0	0.567	0	0	0.189
65.	*Heliotropium steudneri* Vatke (Boraginaceae)	0	0.567	0	0	0.189
66.	*Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult. (Poaceae)	1.136	0	2.19	4.746	2.172
67.	*Hygrophila auriculata* (Schumach.) Heine (Acanthaceae)	0	0.85	0	0.678	0.472
68.	*Hyparrhenia hirta* (L.) Stapf (Poaceae)	0	0	0	0.678	0.189
69.	*Hyperthelia dissoluta* (Nees ex Steud.) Clayton (Poaceae)	1.136	0	0.73	4.407	1.511
70.	*Hypoxis hirsuta* (L.) Coville (Hypoxidaceae)	0	0	0.243	0	0.094
71.	*Indigofera basiflora* J.B. Gillett (Leguminosae)	2.273	0	0.243	0	0.094
72.	*Indigofera hochstetteri* Baker (Leguminosae)	0	2.55	0.973	3.39	2.172
73.	*Indigofera spicata* Forssk. (Leguminosae)	1.136	1.133	0	0	0.378
74.	*Indigofera volkensii* Taub. (Leguminosae)	0	1.983	1.703	3.39	2.266
75.	*Ipomoea mombassana* Vatke (Convolvulaceae)[2]	0	0	0	0.678	0.189
76.	*Justicia betonica* L. (Acanthaceae)	0	0.85	0.73	0.339	0.661
77.	*Justicia exigua* S. Moore (Acanthaceae)	0	0	0.73	0.339	0.378
78.	*Justicia glabra* K.D. Koenig ex Roxb. (Acanthaceae)	0	0	0.243	0	0.094
79.	*Justicia matammensis* (Schweinf.) Oliv. (Acanthaceae)	0	0.283	0.73	0	0.378
80.	*Kyllinga nervosa* Steud. (Cyperaceae)	1.136	1.983	0.73	0.678	1.133
81.	*Lactuca virosa* Habl. (Compositae)	1.272	0.283	0	0	0.094

Continued

No.	Species (Family)	Fallow	Livestock	Mixed	Wildlife	Overall
82.	*Lepidagathis scabra* C.B. Clarke (Acanthaceae)	0	0.567	0	0	0.189
83.	*Leucas aspera* (Willd.) Link (Lamiaceae)	0	0	0.243	0.678	0.283
84.	*Leucas deflexa* Hook.f. (Lamiaceae)	3.409	0	0.243	0	0.094
85.	*Leucas martinicensis* (Jacq.) R.Br. (Lamiaceae)	1.136	0	0	0	0
86.	*Macroptilium atropurpureum* (DC.) Urb. (Leguminosae)	0	0	0	0.339	0.094
87.	*Melhania ovata* Spreng. (Malvaceae)	1.136	0	0	0	0
88.	*Microchloa kunthii* Desv. (Poaceae)	0	4.249	5.839	3.729	4.721
89.	*Mollugo nudicaulis* Lam. (Molluginaceae)	0	0.283	0	0	0.094
90.	*Ocimum basilicum* L. (Lamiaceae)	1.136	0.283	0.487	0.339	0.378
91.	*Ocimum gratissimum* L. (Lamiaceae)	0	0	0	0.339	0.094
92.	*Ormocarpum kirkii* S. Moore (Leguminosae)	0	0	0.243	0	0.094
93.	*Ormocarpum trichocarpum* (Taub.) Engl. (Leguminosae)	0	0	0	0.339	0.094
94.	*Oxygonum sinuatum* (Hochst. & Steud. ex Meisn.) Dammer (Polygonaceae)	1.136	0.283	0.487	0	0.283
95.	*Panicum coloratum* L. (Poaceae)	0	1.133	1.46	3.729	1.983
96.	*Panicum maximum* Jacq. (Poaceae)	0	0	0.243	1.356	0.472
97.	*Pennisetum mezianum* Leeke (Poaceae)	0	0.283	0.973	1.695	0.944
98.	*Portulaca oleracea* L. (Portulacaceae)	0	0.283	0	0.339	0.189
99.	*Portulaca quadrifida* L. (Portulacaceae)	2.273	2.266	0.73	0.678	1.228
100.	*Rhynchosia minima* (L.) DC. (Leguminosae)	0	0	0.243	0	0.094
101.	*Senna occidentalis* (L.) Link (Leguminosae)	0	0.283	0	0.339	0.189
102.	*Sesbania sesban* (L.) Merr. (Leguminosae)	0	0.567	0	0	0.189
103.	*Setaria pumila* (Poir.) Roem. & Schult. (Poaceae)	1.136	1.7	0	0	0.567
104.	*Setaria sphacelata* (Schumach.) Stapf & C.E. Hubb. ex Moss (Poaceae)	1.136	0.283	0.487	1.356	0.661
105.	*Setaria verticillata* (L.) P. Beauv. (Poaceae)	2.273	0.283	0.243	0	0.189
106.	*Sida acuta* Burm.f. (Malvaceae)	0	0.283	0	0	0.094
107.	*Solanum incanum* L. (Solanaceae)	1.136	0.283	0.487	0.678	0.472
108.	*Sphaeranthus suaveolens* (Forssk.) DC. (Compositae)	0	2.266	0.243	0	0.85
109.	*Sporobolus africanus* (Poir.) Robyns & Tournay (Poaceae)	0	0	0.243	0.678	0.283
110.	*Sporobolus cordofanus* (Hochst. ex Steud.) Hérincq ex Coss. (Poaceae)	0	0.283	0	0.339	0.189
111.	*Sporobolus festivus* Hochst. ex A. Rich. (Poaceae)	0	0.567	6.569	5.085	4.155
112.	*Sporobolus ioclados* (Trin.) Nees (Poaceae)	0	5.382	0.973	2.034	2.738
113.	*Sporobolus kentrophyllus* (K. Schum.) Clayton (Poaceae)[2]	0	0.567	0.73	0	0.472
114.	*Sporobolus pyramidalis* P. Beauv. (Poaceae)	0	2.266	1.946	3.39	2.455
115.	*Tagetes minuta* L. (Compositae)	2.273	0	0	0	0
116.	*Talinum portulacifolium* (Forssk.) Asch. ex Schweinf. (Talinaceae)	0	0	0	0.339	0.094
117.	*Tephrosia pumila* (Lam.) Pers. (Leguminosae)	0	0.567	1.46	1.356	1.133
118.	*Themeda triandra* Forssk. (Poaceae)	1.136	3.966	5.596	8.475	5.855
119.	*Tragus berteronianus* Schult. (Poaceae)	3.409	0.85	1.703	0.339	1.039
120.	*Tribulus terrestris* L. (Zygophyllaceae)	0	0.567	0.243	0	0.283
121.	*Triumfetta rhomboidea* Jacq. (Malvaceae)	0	0	0	0.339	0.094
122.	*Urochloa brachyura* (Hack.) Stapf (Poaceae)	0	0.283	0	0	0.094
123.	*Xanthium strumarium* L. (Compositae)	0	0.283	0	0	0.094

[1]Taxonomy according to Global Plants (JSTOR); plants.jstor.org. [2]Taxonomy according to African Plants data base (ville-ge.ch/musinfo/bd/cjb/africa).

Appendix 2. Correlation analysis for soil and plant properties in western Serengeti.

	Distance	Biomass	Soil pH	Clay	Silt	Sand	Soil N	Soil OC	Soil C:N	Soil P	Soil C:P	Soil N:P	CEC	Soil Ca	Forage CP	NDF	ADF	ADL	IVDMD	IVOMD	TDN	ME
Distance	1.00																					
Biomass	0.68***	1.00																				
Soil pH	0.10	0.06	1.00																			
Clay	0.18	0.03	0.24	1.00																		
Silt	0.06	0.13	-0.59***	-0.06	1.00																	
Sand	-0.18	-0.08	0.03	-0.91***	0.37	1.00																
Soil N	0.20	-0.18	-0.07	0.37	0.05	-0.36	1.00															
Soil OC	0.13	-0.09	0.04	0.59***	0.15	-0.61***	0.58***	1.00														
Soil C:N	-0.04	0.03	0.04	0.38	0.15	-0.42*	-0.10	0.73***	1.00													
Soil P	0.04	-0.05	-0.17	-0.35	0.06	0.31	0.18	-0.23	-0.43	1.00												
Soil C:P	0.19	-0.07	0.01	0.59***	0.13	-0.60***	0.67***	0.97***	0.61***	-0.18	1.00											
Soil N:P	0.07	-0.07	0.14	0.61***	0.01	-0.57***	0.53**	0.61***	0.30	-0.67***	0.63***	1.00										
CEC	0.08	0.01	0.46*	0.90***	-0.18	-0.76***	0.27	0.63***	0.51**	-0.44*	0.59***	0.64***	1.00									
Soil Ca	0.12	0.05	0.73***	0.63***	-0.49**	-0.38	0.24	0.36	0.17	-0.32	0.26	0.49**	0.81***	1.00								
Forage CP	-0.28	-0.12	-0.10	0.05	0.18	-0.13	-0.27	0.20	0.52**	-0.19	-0.02	0.00	0.17	-0.05	1.00							
NDF	-0.15	-0.15	-0.15	0.00	0.12	-0.05	0.30	-0.07	-0.38	0.14	-0.04	0.15	-0.02	0.08	-0.11	1.00						
ADF	0.27	0.20	0.02	-0.11	0.01	0.11	0.02	-0.22	-0.33	0.05	-0.10	-0.09	-0.20	0.03	-0.51	0.20	1.00					
ADL	0.07	0.18	0.19	0.32	0.20	-0.38	0.13	0.05	-0.10	-0.03	0.11	0.18	0.24	0.15	-0.24	0.06	0.18	1.00				
IVDMD	0.03	-0.02	0.05	0.06	0.27	-0.17	0.23	0.32	0.20	0.21	0.25	0.10	0.12	0.01	0.34	0.08	-0.66***	0.11	1.00			
IVOMD	0.17	0.12	-0.07	0.28	0.12	-0.31	0.16	0.13	0.00	0.01	0.15	0.18	0.18	0.09	0.05	0.19	-0.40	0.09	0.60***	1.00		
TDN	-0.27	-0.20	-0.02	0.11	-0.01	-0.11	-0.02	0.22	0.33	-0.05	0.10	0.09	0.20	-0.03	0.51**	-0.20	-1.00***	-0.18	0.66***	0.40*	1.00	
ME	0.17	0.12	-0.07	0.28	0.12	-0.31	0.16	0.13	-0.01	0.01	0.15	0.18	0.18	0.08	0.05	0.19	-0.40	0.09	0.60***	1.00***	0.40*	1.00

Values with asterisks indicate significant correlation (* = P<0.05; ** = P<0.01; *** = P<0.001).

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Short Communication

Chlorophyll concentration and production of *Urochloa decumbens* treated with diazotrophic bacteria and thiamine in the Brazilian Cerrado

Concentración de clorofila y producción del pasto Urochloa decumbens *tratado con bacterias diazotróficas y tiamina en el Cerrado brasileño*

EDUARDO PRADI VENDRUSCOLO[1], PAULO RICARDO DE OLIVEIRA[2], ALINY HELOÍSA ALCÂNTARA RODRIGUES[2], SÁVIO ROSA CORREIA[2], LUIZ FERNANDES CARDOSO CAMPOS[2], ALEXSANDER SELEGUINI[3] AND SEBASTIÃO FERREIRA DE LIMA[4]

[1]*Universidade Estadual de Mato Grosso do Sul, Cassilândia, MS, Brazil.* uems.br
[2]*Escola de Agronomia, Universidade Federal de Goiás, Goiânia, GO, Brazil.* agro.ufg.br
[3]*Universidade Federal do Triângulo Mineiro, Iturama, MG, Brazil.* uftm.edu.br
[4]*Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, MS, Brazil.* ppgagronomiacpcs.ufms.br

Abstract

The effects of application of *Azospirillum brasilense* and thiamine on chlorophyll concentration and forage mass of *Urochloa decumbens* were evaluated in a small plot experiment conducted in Goiânia, Goiás, Brazil. The treatments were applications of: *A. brasilense* (concentration of 10 mL/L); thiamine at 2 concentrations: 50 and 100 mg/L; combinations of *A. brasilense* and thiamine at the 2 concentrations; and a Control treatment (untreated grass). At the first harvest there was a trend for applying *A. brasilense*, either alone or in combination with thiamine, to increase the concentrations of chlorophyll, but differences were not always significant at $P<0.05$. Dry mass of forage from applying *A. brasilense* plus thiamine at 100 mg/L was greater than that for Control and thiamine at both concentrations. At the second harvest, chlorophyll concentrations were not affected by treatment ($P>0.05$), while dry matter production of forage from applying *A. brasilense* alone and thiamine at 100 mg/L was greater ($P<0.05$) than that of Control and thiamine at 50 mg/L. Larger-scale and longer-term studies to validate these preliminary findings are needed.

Keywords: Biological fixation, forage, plant protection, tropical grasses, vitamin B1.

Resumen

En un experimento de campo conducido en Goiânia, Goiás, Brasil, se evaluó la producción de forraje y la concentración de clorofila en *Urochloa decumbens* en respuesta a la aplicación de *Azospirillum brasilense* y tiamina (vitamina B1). Los tratamientos consistieron en aplicaciones de: *A. brasilense* en concentración de 10 mL/L; tiamina en concentraciones de 50 y 100 mg/L; las combinaciones de *A. brasilense* con ambas concentraciones de tiamina; y un tratamiento testigo (gramínea no tratada). En el primer corte, la aplicación de *A. brasilense*, tanto sola como combinada con tiamina, incrementó la concentración de clorofila, pero en algunos casos las diferencias entre tratamientos no fueron significativas ($P<0.05$). La producción de forraje seco fue más alta con la aplicación de *A. brasilense* más 100 mg/L de tiamina que la de los tratamientos testigo y tiamina en ambas concentraciones. En el segundo corte, las concentraciones de clorofila no presentaron diferencias ($P>0.05$), mientras que la producción de materia seca fue más alta ($P<0.05$) cuando se aplicó *A. brasilense* sola o tiamina en dosis de 100 mg/L**,** en comparación con el testigo y tiamina en dosis de 50 mg/L. Para validar estos resultados preliminares se necesitan estudios a escala mayor y plazo más largo.

Palabras clave: Fijación biológica, forraje, gramíneas tropicales, protección de plantas, vitamina B1.

Correspondence: E.P. Vendruscolo, Mato Grosso do Sul State University, Cassilândia, Brazil.
Email: agrovendruscolo@gmail.com

Introduction

Brazil has the largest cattle herd in the world, with about 218 million animals which represent approximately 15% of the world's total cattle population (FAO 2017). Most of these animals are raised on pasture, generating a product in great global demand (Jacinto et al. 2005). The production of cattle on pasture is dependent on both forage quantity and quality. As for other grasses, applying N fertilizer has a significant impact on the production of species of the genus *Urochloa* (Duarte et al. 2020). Economically, applying N fertilizer is a significant component of the costs of grass production (Costa et al. 2015).

Although N is commonly applied as chemical fertilizer, biological fixation through diazotrophic bacteria of the *Azospirillum* genus has been studied as a technique for the provision of atmospheric N to grasses (Hungria 2011), e.g. maize (Longhini et al. 2016). Nitrogen plays a major role in plant development, participating in the synthesis of several compounds, such as nucleic acids and proteins, as well as photosynthetic activity (Taiz et al. 2017). Chlorophyll concentration in a grass is generally accepted as a suitable indicator of a plant's N status and consequently crude protein (N × 6.25) concentration (Rocha et al. 2010; Rincón Castillo et al. 2019).

In addition to N fertilizer, other compounds with bio-stimulating and protective effects, such as vitamins, can improve physiological and morphological characteristics of species of commercial interest. Among the vitamins studied, thiamine (vitamin B1) has the capacity to stimulate the production of secondary metabolites, which exert antioxidant activity and avoid the degradation of the photosynthetic system (Goyer 2010; Kaya et al. 2015). This vitamin is also related to the ability to stimulate the accumulation of energy reserves in plant tissues (Barakat 2003); its application has been shown to provide productive gains in beans (*Phaseolus vulgaris*) (Vendruscolo et al. 2018).

The present study aimed to evaluate the effect of inoculation with *Azospirillum brasilense* and treatment with thiamine, either alone or in combination, on forage mass production and chlorophyll concentration of *Urochloa decumbens*, an economically important grass in the Brazilian Cerrado.

Materials and Methods

The study was conducted in Goiânia, Goiás, central Brazil (16°40' S, 49°15' W; 750 masl). The climate in the experimental area is tropical with a rainy season from October to April and a dry season from May to September. The monthly average temperatures range from 20.8 °C in June and July to 25.3 °C in October (Cardoso et al. 2014).

The soil of the experimental area was classified as Latossolo Vermelho (Oxisol) and had the following chemical characteristics: Organic matter = 12.0 g/kg; pH ($CaCl_2$) = 5.3; P (Mehlich) = 2.0 mg/dm^3; K = 80.0 mg/dm^3; Ca = 3.8 cmol$_c$/dm^3; Mg = 0.8 cmol$_c$/dm^3; H+Al = 2.6 cmol$_c$/dm^3; Al = 0.0 cmol$_c$/dm^3; cation exchange capacity = 7.4 cmol$_c$/dm^3; and base saturation = 65.0%.

The experiment was conducted in a pasture of *U. decumbens* cv. Basilisk using a randomized complete block design with 5 replicates. The 6 treatments used in the study were as follows: 1 - Control; 2 - *Azospirillum brasilense* (NITRO 1000 Gramineae, NITRO 1000, Cascavél, PR, Brazil) at a concentration of 10 mL/L of water; 3 - Thiamine (Neon, Suzano, SP, Brazil) at a concentration of 50 mg/L; 4 - Thiamine at a concentration of 100 mg/L; 5 - Thiamine at a concentration of 50 mg/L + *A. brasilense*; 6 - Thiamine at a concentration of 100 mg/L + *A. brasilense*. The plots were established in an area of existing 5-year-old pasture, and each plot was 1 × 1 m. Products were applied via a manual sprayer at a volume equivalent to 200 L/ha.

The pasture had previously been mown quarterly. Prior to the application of the treatments, on 29 November 2016, the area was mown, leaving a stubble height of approximately 10 cm. Treatments were applied 5 days after mowing. For treatments that combined *A. brasilense* and thiamine, the products were mixed before application.

The evaluations were carried out when plants started flowering, on 2 occasions, the first at 50 days after the initial mowing (first harvest, 18 January 2017) and the second after a further 45 days (second harvest, 4 March 2017). At this time, the concentrations of chlorophyll a, chlorophyll b and total chlorophyll were evaluated using a digital chlorophyllometer (CFL1030; Falker, Porto Alegre, RS, Brazil) on 5 flag leaves per plot. All forage on each plot was harvested at about 10 cm above ground level and weighed immediately. Samples (200 g) of green forage were selected, packed in brown paper bags and placed in a forced-ventilation oven at 65 °C until constant weight and weighed with a digital scale (ML 600, Marte, São Paulo, SP, Brazil) to determine dry matter yields. The data obtained for each variable were submitted to analysis of variance and compared by the Tukey test at $P<0.05$. For data analysis the computer program System for Variance Analysis – SISVAR was used (Ferreira 2014).

Results

At the first harvest there was a trend for treatments that included *A. brasilense*, either alone or in combination with thiamine, to increase the relative concentrations of chlorophyll, but differences were not always significant at $P<0.05$ (Table 1). Green mass of forage was greater

Table 1. Mean concentrations of chlorophyll a, chlorophyll b and total chlorophyll (CLA, CLB and CLT, respectively) and green and dry mass (GM and DM) production of *Urochloa decumbens* plants treated with *Azospirillum brasilense* (Azos) and thiamine.

Treatment	CLA	CLB	CLT	GM	DM
	(SPAD value)			(Mg/ha)	
	First harvest (50 days after mowing)				
Control	35.5b	11.4b	46.9b	36.9b	7.89b
Azos 10 mL/L	39.9ab	14.5a	54.4a	49.8ab	10.22ab
Thiamine 50 mg/L	38.5ab	13.0ab	51.5ab	39.9ab	7.84b
Thiamine 100 mg/L	39.4ab	13.6ab	53.0ab	38.3b	8.09b
Azos + Thiamine 50 mg/L	40.7a	14.6a	55.4a	43.4ab	8.44ab
Azos + Thiamine 100 mg/L	40.3a	14.3ab	54.5a	51.5a	10.80a
CV (%)	10.8	20.3	12.4	15.3	15.2
LSD	4.50	2.93	6.93	13.13	2.69
	Second harvest (45 days after first harvest)				
Control	30.1a	7.80a	37.9a	31.9c	6.26c
Azos 10 mL/L	30.8a	8.14a	39.0a	52.6a	10.76a
Thiamine 50 mg/L	29.0a	7.39a	36.4a	35.5bc	6.55bc
Thiamine 100 mg/L	29.4a	7.36a	36.8a	39.4bc	9.48a
Azos + Thiamine 50 mg/L	29.9a	8.09a	38.0a	41.6abc	8.68abc
Azos + Thiamine 100 mg/L	29.5a	7.56a	37.0a	45.4ab	8.93ab
CV (%)	7.88	17.14	9.66	14.65	14.94
LSD	3.11	1.76	4.81	11.96	2.51

Within columns means followed by the same letters do not differ significantly by the Tukey test (P>0.05).

(P<0.05) for *A. brasilense* plus thiamine at 100 mg/L than for Control and thiamine at 100 mg/L. Dry mass of forage for *A. brasilense* plus thiamine at 100 mg/L was greater than that for Control and thiamine at both concentrations.

At the second harvest, chlorophyll concentrations were totally independent of treatment. Application of *A. brasilense* alone produced more green mass than the Control and thiamine at 100 mg/L (Table 1). However, dry matter production of forage was greater (P<0.05) for *A. brasilense* alone, and for thiamine at 100 mg/L was greater than that of Control and thiamine at 50 mg/L.

Discussion

The positive response to the inoculation with *Azospirillum* is due to the bacteria's capacity to biologically fix atmospheric N through the enzyme nitrogenase (Hungria 2011), leading to increased synthesis of nucleic acids, proteins and hormones, which are essential to plant development, as well as to increased photosynthetic activity (Taiz et al. 2017). This is shown by the increase in the chlorophyll concentrations at the first harvest, since these concentrations are directly related to the N concentrations in the leaves (Rocha et al. 2010).

The superior results obtained at the first harvest for the combined application of *A. brasilense* and thiamine are also related to the ability of the vitamin to improve the conditions of the photosystem by protecting it, avoiding its degradation by oxidation (Goyer 2010; Kaya et al. 2015). Consequently, with the improvement of physiological conditions, there is an increase in the accumulation of energy reserves of tissues (Barakat 2003), which can be used for plant development and tissue maintenance during periods of stress (Taiz et al. 2017).

Although treatments had no effects on chlorophyll concentration at the second harvest, applying *A. brasilense* and thiamine at 100 mg/L either alone or in combination significantly increased dry mass yields over that of Control. This response may be related to the priming effect of both the bacteria and the vitamin. Both are recognized for their ability to mitigate stress caused by water restriction (Leite et al. 2019; Vendruscolo et al. 2020) and improve the nutritional condition of plants (Kaya et al. 2015; Galindo et al. 2020). However, under adequate growth conditions, including e.g. good availability of water, their effect is reduced (Goyer 2010; Naoe et al. 2020). In the present study, this became evident, since the second harvest occurred at the end of the rainy season, while the first harvest was on plant regrowth that occurred under conditions of erratic rainfall.

Inoculation with *A. brasilense* and application of thiamine, alone or in combination, seemed to have some potential for the maintenance and improvement of physiological and productive characteristics of *U. decumbens*. Larger-scale studies to test the repeatability of these findings seem warranted as well as longer-term studies to endeavor to clarify the medium- and long-term effectiveness of these treatments.

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(Note of the editors: All hyperlinks were verified 11 January 2020.)

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Short Communication

Hybrids of *Paspalum plicatulum* × *P. guenoarum*: Selection for forage yield and cold tolerance in a subtropical environment

Híbridos de Paspalum plicatulum × *P. guenoarum: Selección para rendimiento de forraje y tolerancia al frío en ambiente subtropical*

KARLA M. SARAIVA[1], MIGUEL DALL'AGNOL[1], EDER A. M. DA MOTTA[1], EMERSON A. PEREIRA[2], CLEBER H. L. DE SOUZA[1], CARINE SIMINONI[1], ROBERTO L. WEILER[1], MAURÍCIO M. KOPP[3], RAQUEL SCHNEIDER-CANNY[4] AND MARLON R. BARBOSA[5]

[1]*Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.* ufrgs.br/agronomia
[2]*Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Ijuí, RS, Brazil.* unijui.edu.br
[3]*Embrapa Pecuária Sul, Bagé, RS, Brazil.* embrapa.br/pecuaria-sul
[4]*Noble Research Institute, Ardmore, USA.* noble.org
[5]*Instituto Nacional de Investigación Agropecuaria (INIA), Tacuarembó, Uruguay.* inia.uy

Abstract

Selection of improved genotypes is important for pasture-based feeding systems in subtropical regions. Our goal was to identify hybrids of *Paspalum* with enhanced forage yield and cold tolerance across 2 sites [Bagé and Eldorado do Sul (ES)], in Rio Grande do Sul, Brazil. We evaluated 19 *P. plicatulum* × *P. guenoarum* hybrids, *P. plicatulum* genotype 4PT, *P. guenoarum* cultivars Azulão and Baio and, as Control, *Megathyrsus maximus* cv. Aruana. At both sites, the experimental design was a completely randomized block with 4 replications. Total dry mass (total-DM), leaf-DM and cold tolerance (ColdT) were recorded. At Bagé, hybrid 102069 produced higher total-DM and leaf-DM than the progenitors and cv. Aruana, while at ES, hybrids 102069 and 10308 produced higher total-DM than 4PT, Azulão and Aruana; hybrid 102069 had higher leaf-DM. At Bagé, 16 hybrids displayed ColdT similar to their progenitors and higher than Aruana, while at ES, 12 hybrids showed ColdT similar to Azulão and Baio and higher than 4PT and Aruana. This study demonstrated that hybrids of *Paspalum* with superior forage yield to their progenitors and Aruana, and hybrids with higher ColdT than 4PT and Aruana are in existence. The hybridization technique shows potential for producing alternative genotypes with higher forage yield and ColdT for sowing in subtropical regions.

Keywords: Biomass production, genetic variability, hybridization, native grasses.

Resumen

La selección de genotipos forrajeros mejorados es fundamental para garantizar la productividad y sostenibilidad de sistemas de producción animal basados en pasturas en regiones subtropicales. El objetivo de este trabajo fue identificar híbridos del género *Paspalum* con alto rendimiento de forraje y tolerancia al frío en Bagé y Eldorado do Sul, Rio Grande do Sul, Brasil. Fueron evaluados 19 híbridos de *P. plicatulum* × *P. guenoarum*, *P. plicatulum* genotipo 4PT y los cultivares (cv.) Azulão y Baio de *P. guenoarum*; como testigo se utilizó *Megathyrsus maximus* cv. Aruana. En ambos sitios se utilizó un diseño de bloques al azar, con cuatro repeticiones, para evaluar las variables producción de materia seca total (PMS-total), producción de materia seca de hojas (PMS-hojas) y tolerancia al frio (Tfrío). En Bagé, el híbrido 102069 produjo mayor PMS-total y PMS-hojas que los progenitores y el testigo. En Eldorado do Sul, los híbridos 102069

Correspondence: E.A.M. da Motta, Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 7712, Porto Alegre, RS, Brazil. Email: edermotta87@yahoo.com.br

y 10308 presentaron mayores PMS-total, mientras que el híbrido 102069 produjo más PMS-hojas que el genotipo 4PT, el cv. Azulão y el testigo. En Bagé, 16 híbridos presentaron Tfrío similar a los progenitores pero mayor que el testigo, En Eldorado do Sul, 12 híbridos mostraron Tfrío similar a los cvs. Azulão y Baio pero superior al genotipo 4PT y al testigo. El estudio permitió identificar híbridos de *Paspalum* con rendimiento de forraje superior a los progenitores y el testigo cv. Aruana, e híbridos con mayor Tfrío que el genotipo 4PT y el testigo. La técnica de hibridación tiene potencial para obtener genotipos con mayor rendimiento de forraje y tolerancia al frío para regiones subtropicales.

Palabras clave: Adaptación climática, gramíneas nativas, hibridación, producción de biomasa, variabilidad genética.

Introduction

The production of cultivars adapted to subtropical edapho-climatic conditions with higher potential for biomass production than existing cultivars has been the objective of forage breeding programs throughout the southern region of Brazil. Motta et al. (2016) suggested that the genetic variability among species belonging to the genus *Paspalum* in natural ecosystems in South America represented a broad and important germplasm source to be exploited, with the possibility of increasing the efficiency of production systems and recovery of degraded pasture areas.

The genus *Paspalum* comprises many diverse native species with forage attributes for animal production and adaptability to different ecosystems (Novo et al. 2016). These characteristics demonstrate the potential for use in breeding programs and the establishment of cultivated pastures (Motta et al. 2017). However, the predominant reproductive mode for *Paspalum* species is apomixis (Acuña et al. 2009) and therefore, sexual reproduction is necessary for obtaining genetic variability. With the attainment of tetraploid sexual *P. plicatulum* plants (Sartor et al. 2009), the exploitation of the segregating populations through breeding between apomictic tetraploids and a sexual progenitor was made possible. The progeny resulting from this breeding segregate into sexual and apomictic individuals. When selected and identified, the apomictic hybrids may be used in field assays for agronomic evaluation and then established as cultivars. Alternatively, the selected sexual hybrids may be used as female progenitors for further breeding (Aguilera et al. 2011). The main reason for using hybridization in apomictic species is to fix superior breeds through apomixis (Zilli et al. 2015).

Temperature is a conditioner of vegetative development because damage by low temperatures may result in growth reduction, leaf lesions and basic functional disorders (Taiz and Zeiger 2013). The southern region of Brazil is characterized by the occurrence of low temperatures and frosts in the winter, which may impair forage supply to grazing animals since natural and some cultivated pastures are susceptible to these conditions, making little if any growth during this time.

Hybridization performed by the Forage Breeding Group (FBG) of the Federal University of Rio Grande do Sul (UFRGS), using the apomictic *P. guenoarum* cultivars Azulão and Baio (as pollen donors) and the sexual tetraploid genotype 4PT of *P. plicatulum*, produced *Paspalum* interspecific hybrids that may have a significant positive impact on animal production in southern Brazil. Field assays are necessary to determine the forage production traits of these novel genetic resources, to provide a basis for selection of superior genetic material and consequently, the establishment of novel cultivars for subtropical conditions. Therefore, the goal of this research was to identify new hybrids of *Paspalum* with enhanced forage yield and cold tolerance across different edaphoclimatic regions located in Rio Grande do Sul state, Brazil.

Materials and Methods

The study was conducted from December 2012 to April 2014 in 2 regions of Rio Grande do Sul (RS) state, Brazil: 'Depressão Central' and 'Campanha'. The Depressão Central experimental area is located within the Agronomic Experimental Station (AES) of the UFRGS, located in Eldorado do Sul, RS, Brazil (30°06' S, 51°41' W; 32 masl). The soil is classified as a typical Dystrophic Red Argisol (Streck et al. 2008) and had the following characteristics during the experiment: clay content - 22.0%; pH in water - 5.5; SMP index - 6.5; P - 8.9 mg/dm^3; K - 105 mg/dm^3; OM - 1.5%; exchangeable Al - 0.0 cmol$_c$/dm^3; and effective CEC - 6.2 cmol$_c$/dm^3. According to Köppen's classification, this region has a humid subtropical Cfa climate with warm summers. The mean maximum temperature is 30.2 °C in January (hottest month), while mean minimum temperature is 8.5 °C in June (coldest month). Mean annual rainfall is approximately 1,450 mm.

The Campanha experimental area is located within Embrapa Pecuária Sul, located in Bagé, RS, Brazil (31°25' S, 54°07' W; 212 masl). The soil is classified as a typical Orbicular Hypochromic Luvisol (Streck et al. 2008) and had the following characteristics: clay content - 24.6%; pH in water - 5.7; SMP index - 6.0; P - 7.9 mg/dm^3; K - 45 mg/dm^3; OM - 1.5%; exchangeable Al - 0.0 cmol$_c$/dm^3; and effective

CEC - 7.9 $cmol_c/dm^3$. The regional climate, following Köppen's classification, is also humid subtropical Cfa with warm summers. Mean maximum temperature is 29.7 °C in January (hottest month), while mean minimum temperature is 7.9 °C in July (coldest month). Mean annual rainfall is approximately 1,260 mm.

Maximum and minimum temperatures and rainfall were monitored at both sites and are presented in Figure 1. At both sites, the soil was fertilized according to technical indications for perennial warm season grasses (CQFS 2004). Urea, triple superphosphate and potassium chloride were applied to supply 200 kg N/ha, 100 kg P/ha and 80 kg K/ha.

Hybrids used in this experiment were obtained in 2010 through breeding by the FBG of the Department of Forage Plants and Agrometeorology (DFPA) of the UFRGS. Hybridization was performed in greenhouses using apomictic *Paspalum guenoarum* cvv. Azulão and Baio as male progenitors (pollen donors) and the sexual ecotype 4PT of *P. plicatulum* as the female progenitor. Azulão and Baio originate from the subtropical and temperate regions of southern Brazil, Argentina and Paraguay (Steiner et al. 2017), while ecotype 4PT was collected in northeastern Argentina and had its chromosome number duplicated, resulting in the generation of a sexual tetraploid plant (Sartor et al. 2009). The progeny obtained from this breeding were evaluated (individual plants) in the field to identify and select genotypes with highest forage production.

This study evaluated the hybrids 10202, 1020104, 102084, 102080, 1020133, 102058 and 102069, resulting from crosses between *P. plicatulum* genotype 4PT and *P. guenoarum* cv. Azulão, and the hybrids 103063, 10308, 103042, 103040, 103061, 103077, 103087, 103093, 103031, 103020, 103084 and 103037 resulting from crosses between *P. plicatulum* ecotype 4PT and *P. guenoarum* cv. Baio. *P. guenoarum* cvv. Azulão and Baio and *P. plicatulum* genotype 4PT, as well as *Megathyrsus maximus* cv. Aruana, were included in the evaluation for comparison. The latter was used as Control because it is high yielding and is widely cultivated in southern Brazil.

At both sites, a completely randomized block design with 4 replicates was adopted. The experimental unit was composed of a 1.0 m row containing 5 plants spaced 20 cm apart. Inter-row spacing was 0.80 m and spacing between blocks was 1.5 m, giving a total area of 149.6 m^2 (17.6 × 8.5 m). The experiment was established using seedlings obtained from tussocks collected in the AES during April 2012. Seedlings were grown in plastic packages (300 ml), containing commercial substrate, inside DFPA's greenhouses where they remained until field transplanting in October 2012 at both sites. In Eldorado do Sul, evaluations started in December 2012, while in Bagé they started in January 2013. Evaluations were performed at harvesting, when 80% of the genotypes had leaves with an average length of 35–40 cm, leaving 10 and 15 cm of stubble height for *Paspalum* and Aruana, respectively. After harvest and for morphological evaluation, each sample was sorted into leaves, stems and inflorescences, which were then placed in a forced-air oven at 65 °C until a constant weight was reached.

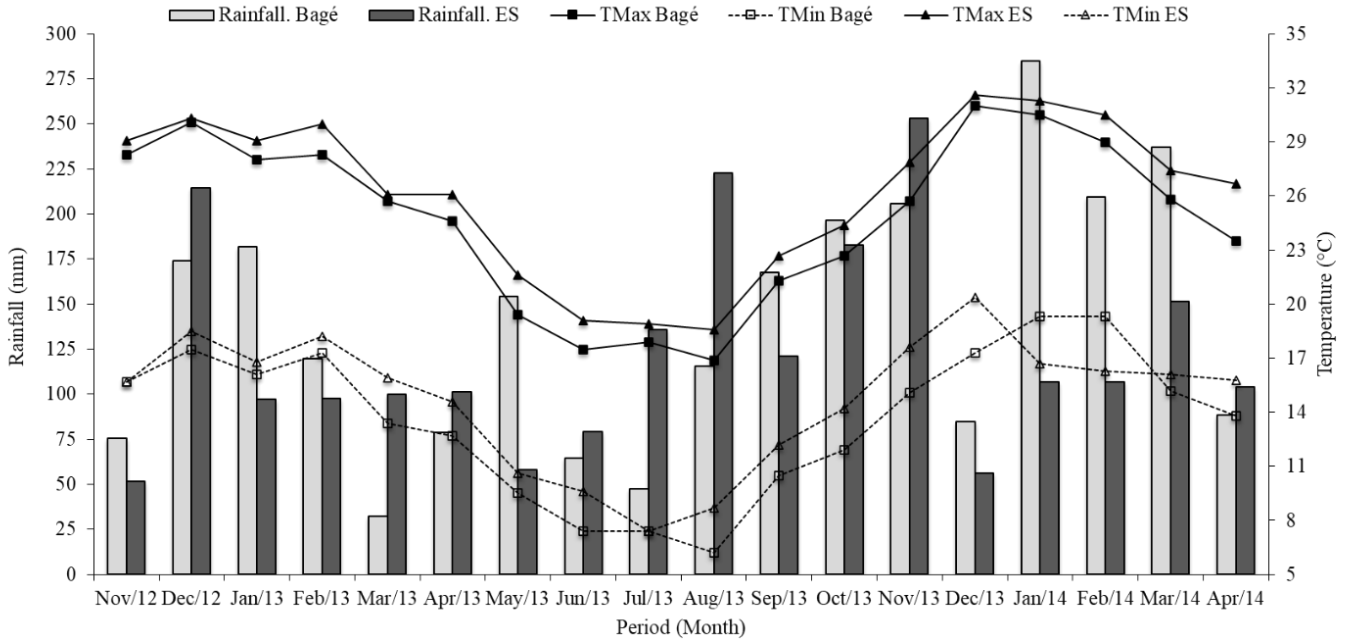

Figure 1. Mean maximum (TMax) and minimum (TMin) temperatures and rainfall recorded between November 2012 and April 2014 in Bagé and Eldorado do Sul (ES), Rio Grande do Sul, Brazil.

The following agronomic characters were evaluated: total dry mass (total-DM) and leaf-DM. After frost events during winter in 2013, visual scores were allocated for cold tolerance (ColdT). Rating system was 1–5, with 1 (many dead leaves) being the lowest and 5 (few dead leaves) being the highest tolerance rating (Acuña et al. 2009; Motta et al. 2017).

At each site, total-DM and leaf-DM data were submitted to analysis of variance involving an F test using the PROC GLM procedure in SAS software (SAS 2001). When differences among genotypes were detected, a means comparison was performed using Tukey's test. The GENES software was used for the ColdT data analysis (Cruz 2007) and when genotype differences were detected, a means comparison was performed using the Scott-Knott test. All differences refer to significance at P<0.05.

Results and Discussion

There was significant variability among genotypes for the characters total-DM, leaf-DM and ColdT. In Bagé, hybrid 102069 had highest (P<0.05) total-DM among the evaluated genotypes, accumulating 652 g/row (Table 1). The forage yield of this hybrid was 76, 93, 76 and 115% greater than that of Baio, Azulão, Aruana and 4PT, respectively. There were few significant differences in total DM for the remaining hybrids and cultivars. At Eldorado do Sul, hybrid 102069 (623 g/row) also had the highest (P<0.05) total-DM but was not significantly different (P>0.05) from hybrid 10308 (567 g/row) (Table 1). There were no significant differences in total-DM for the remaining hybrids and cultivars.

These total-DM values for 102069 and 10308 are important because they exceeded those of Azulão and Baio, which produced more than 15 t DM/ha across different environments (Pereira et al. 2012). They also outyielded Aruana, which was selected as Control because it is a perennial grass used widely for ruminant feeding in RS, Brazil, with productivity between 13 and 24 t DM/ha (Motta et al. 2017); it also has good ColdT among tropical pasture species. Our findings indicate that interspecific hybridization produced hybrid vigor for total-DM, which exceeded those of Azulão, Baio and 4PT, which were used as progenitors.

Table 1. Accumulated total dry mass (Total-DM, g/row) and leaf dry mass (Leaf-DM, g/row) production, and leaf percentage (Leaf %) for *Paspalum* hybrids and their progenitors and *Megathyrsus maximus* cv. Aruana, at two sites in Rio Grande do Sul, Brazil.

Genotype	Bagé	Eldorado do Sul	Bagé		Eldorado do Sul	
	Total-DM		Leaf-DM	Leaf %	Leaf-DM	Leaf %
102069	652a	623a	351a	54	371a	60
10308	430b	567ab	288ab	67	337ab	59
1020133	421bc	481bcd	282ab	67	279abc	58
103084	422bc	479bcd	288ab	68	306abc	64
103061	414bc	480bcd	296ab	71	323abc	67
103087	415bc	470bcd	283ab	68	275abc	59
102080	412bc	466bcd	269ab	65	283abc	61
103031	370bcd	474bcd	247b	67	315abc	66
103063	401bcd	421d	241b	60	234c	56
102084	403bcd	414d	280ab	69	236c	57
103037	345bcde	458cd	241b	70	273abc	60
103093	338bcde	456cd	235b	70	247bc	54
103020	351bcde	441d	269ab	77	279abc	63
103042	364bcd	427d	279ab	77	262bc	61
cv. Baio	371bcd	415d	239b	64	283abc	68
cv. Azulão	337bcde	449cd	248b	74	266bc	59
cv. Aruana	370bcd	410d	247b	67	235c	57
103040	357bcd	417d	263ab	74	295abc	71
1020104	328cde	445cd	229b	70	271bc	61
10202	346bcde	425d	229b	66	275abc	65
103077	351bcde	415d	243b	69	255bc	61
4PT	303de	393d	229b	76	250bc	64
102058	255e	419d	204b	80	270bc	64
Mean	381	454	260	69	279	62

Within columns, means followed by the same letters do not differ by Tukey test at P<0.05.

As for total-DM, leaf-DM for 102069 exceeded those of Azulão, Baio, 4PT and Aruana at Bagé, but differed from only Azulão, 4PT and Aruana at Eldorado do Sul. Many of the hybrids produced similar leaf-DM as 102069 at both locations (Table 1). Results showed that leaf proportion of hybrid 102069 was 54 and 60% of total-DM at Bagé and Eldorado do Sul, respectively. These values are similar to those reported by Huber et al. (2016), who evaluated interspecific *Paspalum* hybrids and observed that this component's percentage ranged between 60 and 68%. Leaf percentage is an important consideration when selecting genotypes for animal feeding, because leaf is the most nutritionally valuable morphological component of a pasture-based production system. Relative amounts of leaf and stem in available forage impacts significantly on nutritive value as well as ingestive behavior of cattle (Fernandes et al. 2014).

The ColdT evaluation of the genotypes revealed the existence of significant genetic variability for this characteristic at both locations (Table 2). At Bagé, all hybrids ($P<0.05$) were more tolerant of cold weather than the Control, Aruana; however, when compared with the progenitors Azulão, Baio and 4PT, only hybrids 103077, 102080 and 103037 had lower tolerance. At Eldorado do Sul, Aruana again had the lowest ($P<0.05$) ColdT among the tested genotypes, whereas 12 hybrids plus Azulão and Baio were most tolerant. Azulão and Baio are known to persist through winter and are considered tolerant of the lower temperatures and frosts that occur in subtropical environments (Fachinetto et al. 2012). The large number of hybrids tolerant of cold weather indicates the transmission of this characteristic from the ecotypes to the progeny. Of all available cultivars of *M. maximus*, Aruana is considered to be among the most tolerant of cold conditions (Corrêa 2002). The higher ColdT displayed by hybrids, when compared with Aruana, agrees with results obtained by Motta et al. (2017), who also observed higher ColdT among *Paspalum* progenies, when compared with the commercial cultivar. This result suggests that use of hybridization between ecotypes adapted to subtropical environments may produce progeny with higher levels of resistance to cold conditions. The ColdT characteristic is essential in subtropical regions, because cold-tolerant ecotypes may have higher persistence and higher herbage allowance for grazing animals during winter along with more rapid regrowth in spring.

Paspalum species with desirable agronomic characteristics obviously exist in natural ecosystems and represent a pool of genetic material for use in pasture breeding programs to enhance ruminant production. The results obtained in this study demonstrate that there are *Paspalum* interspecific hybrids with total-DM and leaf-DM superior to those from Azulão, Baio and 4PT used as progenitors, as well as from Aruana, across both sites. The fact that several hybrids showed ColdT similar to that of Azulão and Baio and considerably higher than that of Aruana, indicates that these hybrids should not suffer from cold temperatures during winter to a greater degree than the existing ecotypes. Hybridization techniques can be used to obtain superior genotypes and facilitate the breeding process in the attainment of novel perennial forage cultivars with higher yields and ColdT in subtropical weather than the existing ecotypes.

Table 2. Means for cold tolerance in *Paspalum* hybrids and their progenitors and *Megathyrsus maximus* cv. Aruana, at two sites in Rio Grande do Sul, Brazil.

Genotype	Cold tolerance score[1]	
	Bagé	Eldorado do Sul
102069	3.5a	3.1a
10308	3.2a	3.5a
1020133	3.3a	3.1a
103084	3.4a	3.5a
103061	2.6a	2.4b
103087	3.2a	3.2a
102080	2.5b	2.3b
103031	3.5a	3.6a
103063	2.8a	2.5b
102084	3.5a	3.1a
103037	2.2b	2.4b
103093	3.5a	3.4a
103020	3.1a	2.2b
103042	2.9a	2.3b
cv. Baio	4.1a	3.7a
cv. Azulão	3.7a	3.6a
cv. Aruana	1.5c	1.1c
103040	3.2a	3.3a
1020104	3.3a	3.3a
10202	3.2a	3.5a
103077	2.4b	2.5b
4PT	2.8a	2.1b
102058	3.1a	3.1a
Mean	3.1	2.9

Within columns, means followed by the same letter do not differ by Scott-Knott test at $P<0.05$.
[1]Scoring scale from 1 (many dead leaves) to 5 (few dead leaves).

Conclusions

We conclude that hybridization can be an alternative tool for obtaining genotypes with higher forage yield and ColdT for subtropical regions. Based on the combination of superior total-DM and leaf-DM, as well as ColdT at 2 sites, hybrids 102069, 10308, 1020133, 103084, 103061, 103087, 103031 and 102080 hybrids are recommended

for further evaluation, such as seed production, response to fertilizer application and animal performance.

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TGFT Editorial Team
A.A. 6713, Km 17 Recta Cali-Palmira, Cali, Valle del Cauca, Colombia.
Phone: +57 2 4450100 Ext. 3084
Email: CIAT-TGFT-Journal@cgiar.org