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Genetic Resources Communication

– a new paper category in *Tropical Grasslands-Forrajes Tropicales*

As of 30 November 2019, the Journal has established a new paper category, **Genetic Resources Communication** (GRC). Papers in this category document the characterization and evaluation of large/comprehensive collections of germplasm of tropical and subtropical forage species and are accompanied by relevant data sets that are considered, in general, too extensive for publication in other paper categories such as Research Article. GRC papers contribute to safeguarding information which otherwise would not be easily accessible by the research community or even might become lost.

GRC papers are treated in the same way as research papers, so should be structured accordingly, and are peer-reviewed. They are not meant to consist merely of data sets with limited description but should contain all relevant methodological details as in a Research Article and culminate in clear conclusions and suggestions.

Submissions for this paper category are welcome, even when they present data collected years ago.

With the establishment of this paper category, *Tropical Grasslands-Forrajes Tropicales* follows the publication series of the Australian CSIRO *Tropical Agriculture Genetic Resources Communication* (ISSN 0159-6071) published during 1980–2000. By kind permission of CSIRO, 32 issues that deal with tropical and subtropical forages can be accessed at the 'Former Journals' button.

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Genetic Resources Communications

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Research Paper

Animal performance and sward characteristics of Mombaça guineagrass pastures subjected to two grazing frequencies

Desempeño animal y características de pasturas del pasto guinea cv. *Mombaça sometidas a dos frecuencias de pastoreo*

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Abstract

The aim of this work was to compare grazing management practices of Mombaça guineagrass (*Megathyrsus maximus* syn. *Panicum maximum* cv. Mombaça) based on the sward incident light interception (LI) concept. We tested, when the regrowth period in rotationally stocked Mombaça guineagrass ended, if LI (90 or 95%) affected forage accumulation, sward characteristics and animal performance. Both treatments had a common post-grazing canopy height of 50 cm and were replicated 4 times in a randomized complete block design. Pastures were sampled pre- and post-grazing to determine forage mass, morphological composition and forage accumulation rate (FAR). Nutritive value (NV) was estimated in pre-grazing samples. Stocking rate was adjusted twice a week, and animals were weighed every 28 days. Pre-grazing conditions of 90 and 95% LI were reached at pasture heights of approximately 80 and 90 cm, respectively. FAR, sward structure and NV were similar for pastures grazed at 90 and 95% LI. Consequently, stocking rate, average daily gain and liveweight gain/ha were similar for both LI treatments. Data suggest that Mombaça guineagrass can be grazed at pre-grazing heights of 80–90 cm (90–95% LI) without compromising pasture structure and animal performance provided moderate defoliation severity is employed. Further testing of this grazing strategy over longer periods should be carried out with this species as well as other tropical grasses.

Keywords: Canopy structure, forage accumulation, light interception, *Megathyrsus maximus*, nutritive value, stocking rate, tropical forages.

Resumen

El objetivo de este trabajo fue comparar prácticas de manejo de pastoreo de Mombaça (*Megathyrsus maximus* sin. *Panicum maximum* cv. Mombaça) con base en el concepto de intercepción de luz incidente (IL). Al finalizar el período de rebrote de Mombaça manejado de forma rotacional, se evaluó si la IL (90 o 95%) afectaba la acumulación de forraje, la estructura de la pastura y el rendimiento de los animales. En ambos tratamientos la altura del pasto después del pastoreo fue igual (50 cm). Se usó un diseño de bloques completos al azar con 4 repeticiones. Para las mediciones del pasto se tomaron muestras antes y después del pastoreo para determinar la masa forrajera, la composición morfológica y la tasa de acumulación de forraje. El valor nutritivo se determinó antes del comienzo del pastoreo. La carga animal se ajustó 2 veces por semana, y los animales fueron pesados cada 28 días. Las condiciones previas al pastoreo de 90 y 95% de IL se alcanzaron cuando el pasto llegó a una altura aproximada de 80 y 90 cm, respectivamente. La tasa de acumulación de forraje, la estructura de la pastura y el valor nutritivo fueron similares para pasturas con 90 y 95% IL. Por tanto, la carga animal, la ganancia diaria promedio y la ganancia de peso vivo/ha fueron similares para ambos tratamientos. Los datos sugieren que el pasto guinea cv. Mombaça se puede pastorear cuando alcanza una altura de 80–90 cm (90–95% IL), sin comprometer su estructura y el

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rendimiento animal, siempre y cuando la defoliación sea moderada. Se deben realizar pruebas con esta estrategia de pastoreo durante períodos prolongados con esta especie, así como con otros pastos tropicales.

Palabras clave: Acumulación de forraje, carga animal, estructura de la pastura, intercepción de luz, *Megathyrsus maximus* cv. Mombaça, pasturas tropicales, valor nutritivo.

Introduction

Some studies with tropical grasses under intermittent stocking have shown that the point at which the canopy intercepts 95% of photosynthetically active radiation (PAR) approximates an ideal time period to interrupt regrowth. After this point, forage accumulation and nutritive value decrease (Carnevalli et al. 2006; Barbosa et al. 2007; Zanini et al. 2012) as proportions of stem and dead material in pre-grazing forage mass increase (Silva et al. 2009).

However, insistence that an interruption of the rest period must occur precisely when the canopy intercepts 95% of the PAR can be restrictive and impractical for producers. Flexibility of management greatly facilitates the planning of livestock systems because it is common for more than one paddock to reach the ideal grazing condition at the same time during periods of vigorous forage growth (Zanine et al. 2011). On the other hand, when weather conditions are unfavorable for plant growth, the time required to achieve the target of 95% LI can be very long (Carnevalli et al. 2006; Barbosa et al. 2007; Giacomini et al. 2009), hindering the rotation of animals in paddocks available.

Using mathematical models, Parsons et al. (1988) demonstrated that, regardless of variation in management, there was a range in level of interception of PAR by the canopy in which forage production remained relatively stable. In this context, Barbosa et al. (2007) and Zanine et al. (2011) found no difference in the accumulation of leaf blades of guineagrass (*Megathyrsus maximus*) cv. Tanzania when the canopy LI was 90 or 95%. This suggests there could be some flexibility in the definition of pre-grazing targets, i.e. instead of a specific point there could be a range of possible values.

The end point of grazing events is also important. Maximization of short-term forage intake rate was achieved when the reduction in pasture height during grazing did not exceed 40% of the initial height (Fonseca et al. 2012; Mezzalira et al. 2014). This indicates that, regardless of the pre-grazing goals, an important condition for the maintenance of high livestock production is the use of relatively lenient defoliation levels.

Against this background, we aimed to evaluate forage accumulation and nutritive value, canopy characteristics and animal production in Mombaça guineagrass (*Megathyrsus maximus* syn. *Panicum maximum* cv. Mombaça) pastures subjected to 2 grazing frequencies, defined by 90 and 95% LI by the canopy, in conjunction with a common post-grazing canopy height of 50 cm.

Materials and Methods

The experiment was conducted during a single growing season from September 2012 to May 2013 at the National Beef Cattle Research Center in Campo Grande, MS, Brazil (20º25' S, 54º51' W; 530 masl). The climate, according to the Köppen classification, is rainy tropical savanna, corresponding to the Aw subtype, characterized by a seasonal distribution of rainfall with a well-defined dry period during the colder months. Average annual rainfall is about 1,500 mm, of which 80% falls during the 7-month wet period (October–April). The historical average minimum and maximum temperatures (1993–2013) in the coldest month were 15.3 and 27.3 °C, respectively, and during the summer 18.2 and 31.2 °C. Weather data during the experimental period were collected from a meteorological station located 2 km from the research site (Figure 1).

Average temperature and monthly precipitation were used to calculate the water balance (Figure 2). The soil water storage capacity was determined to be 75 mm.

Average chemical characteristics of the soil at the experimental site, a clay soil classified as red dystrophic latosol (Oxisol), were for the 0–10 cm layer: pH $CaCl_2$ = 5.8; OM = 43.4 g/dm^3; P (Mehlich 1) = 7.0 mg/dm^3; Ca = 4.8 cmol/dm^3; K = 0.5 cmol/dm^3; Mg = 1.5 cmol/dm^3; Al = 0.0 cmol/dm^3; sum of bases = 6.7 cmol/dm^3; cation exchange capacity = 9.9 cmol/dm^3; and base saturation = 68.7%.

Based on these analyses, commencing in October 2012 well-established pastures (planted in 2009) were fertilized with 39 kg P, 75 kg K and 200 kg N/ha, divided equally among 4 application times, namely: October, December, January and February. Nitrogen was applied as ammonium sulfate in October and the remaining applications were as urea.

The experimental area was 12.0 ha, divided into 8 pastures measuring 1.5 ha, and these pastures were subdivided into 6 paddocks of 0.25 ha each. A 6.0 ha reserve pasture was used for holding extra animals when they were not grazing experimental pastures.

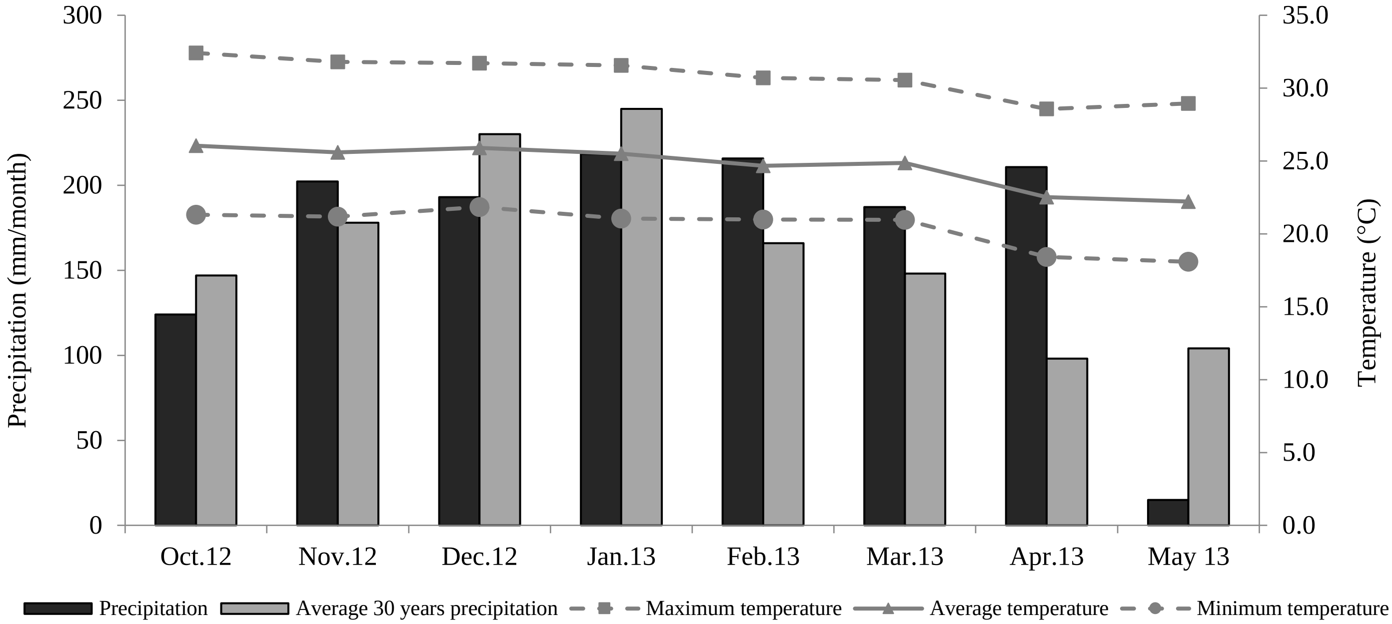

Figure 1. Monthly and historical 30-year rainfall plus maximum, average and minimum temperatures during the experimental period.

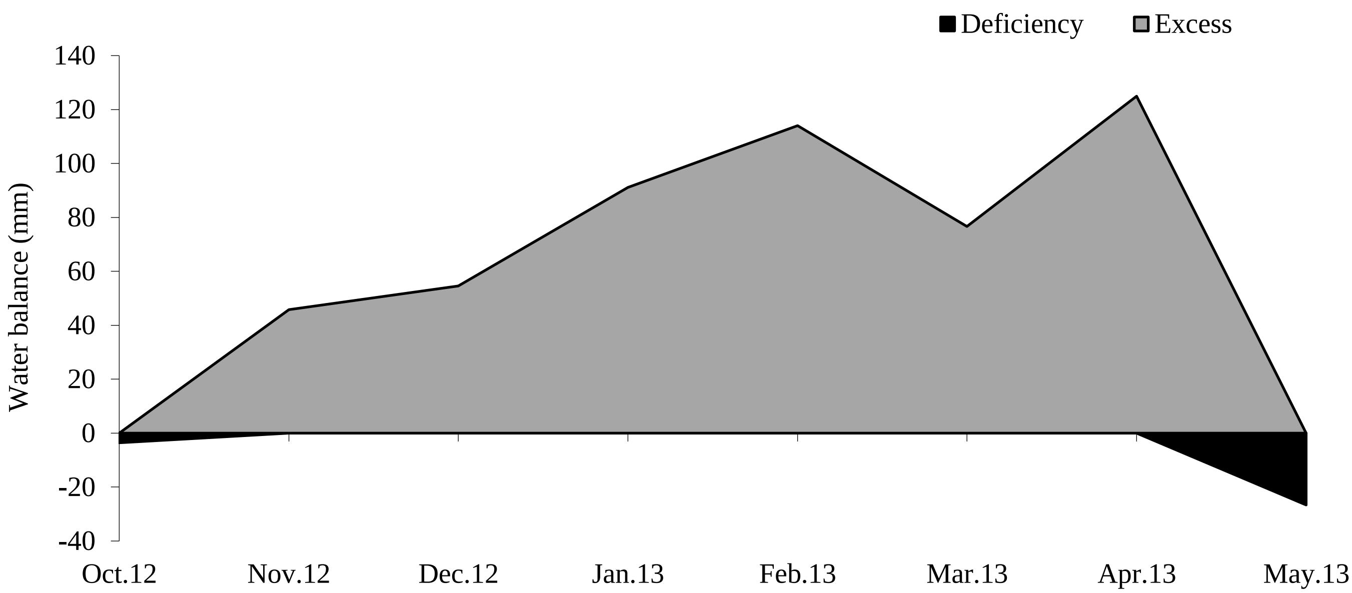

Figure 2. Water balance, i.e. deficit and surplus, in the soil during the experimental period.

The experimental design was a randomized complete block with 2 treatments and 4 replications. The grazing method used was rotational stocking with a variable stocking rate. The treatments comprised 2 grazing frequencies, characterized by pre-grazing conditions in which the canopy intercepted 90 and 95% of PAR at interruption of pasture growth, i.e. introduction of grazing animals. Stock were removed from each paddock of both treatments when grazing height had been reduced to 50 cm. When each pasture reached the predetermined level of light interception it was grazed by 6 Senepol × Caracu (50:50) tester steers (approximately 11 months of age and with an average weight of 224 ± 16 kg initially). The testers were assigned randomly to experimental units at the beginning of the experimental period; the differences in allocation weights across treatments were not significant at the beginning of the growing season. The tester animals grazed the same pasture (1.5 ha divided into 6 paddocks) for the entire experimental period. Fifty-two regulator steers, similar to the tester steers in weight, age, background and breeding, were kept in the reserve pasture and used whenever the stocking rate needed to be increased.

The animals were treated with a broad-spectrum anthelmintic at the beginning of the experiment and with pour-on ectocide during the experiment as needed for the control of ticks and horn flies. Animal health management was performed as recommended by the National Beef Cattle Research Center. All animals received water and a mineral mixture ad libitum.

Sward LI was monitored in 2 paddocks of each pasture, using a canopy analyzer apparatus (AccuPAR Linear PAR/LAI ceptometer, Model PAR-80; DECAGON Devices) at 20 random points per paddock, with one reading being taken above the canopy and one at ground level at each point. The measurements were performed weekly. When LI reached 85%, LI was monitored daily until the target was reached. Concurrently with the LI measurements, canopy height was monitored, using a 1 m ruler graduated in centimeters, at 40 random points per paddock. The readings of sward non-extended leaf height were taken from ground level to the 'leaf horizon' on the top of the sward as a reference, even during periods when plants were reproductive and produced taller flowering stems. Average heights corresponding to 90 and 95% LI were used as target heights for the other 4 paddocks from each pasture. Post-grazing heights were measured as soon as the animals left each paddock, as described above.

Forage mass, morphological composition and total forage and leaf accumulation rates were measured in a single paddock per pasture for each grazing cycle. Pre- and post-grazing forage mass were estimated by cutting 9 randomly selected samples (1 m^2 each) at ground level in each paddock using a manual mower. The samples were divided into 2 subsamples: 1 subsample was weighed and oven-dried at 65 °C until constant weight, and the other subsample was separated into green blades (leaf blades), green stems (stem and sheath) and dead material, and these fractions were dried at 55 °C until constant weight.

Forage accumulation rate was calculated as the difference between the current pre-grazing and the previous post-grazing forage mass, considering only the green portion (leaves and stems), divided by the number of days between samplings. For leaf accumulation rate, we used the same procedure, considering only the leaf portion in the samples. The total herbage accumulated from the entire experimental period, i.e. grazing season, was the sum of forage accumulation values across all grazing cycles.

In a second paddock of each pasture, 3 stratified samples were collected. A 1 m^2 frame was placed in areas that were representative of the average sward condition (based on visual assessment of height and herbage mass). At each location, the canopy was sampled using scissors in 4 vertical strata: >80, 60–80, 40–60 and 0–40 cm, commencing from top to basal layers. Samples from each stratum were weighed and handled as described above to estimate forage mass and its morphological components. Leaf samples were dried, ground and analyzed for crude protein (CP), neutral detergent fiber (NDF) and acid detergent lignin (ADL) concentrations, as well as in vitro organic matter digestibility (IVOMD), using near-infrared spectroscopy (NIRS).

Steers were weighed at 28-d intervals following a 16-hour fasting period to minimize gut-fill effects on liveweight measurements, i.e. fasted from both water and feed. The average daily gain was calculated as the increase in live weight of the testers divided by the number of days between weighings.

The stocking rate per cycle was calculated as the sum of the animal days (tester and regulator steers) spent in each of 6 paddocks (0.25 ha) divided by the total number of grazing days of a complete cycle, and divided by the pasture area (1.5 ha). It was expressed in animal units (AU = 450 kg live weight) per hectare. Liveweight gain/ha was calculated as the product of average daily gain and the number of steers/ha.

The data were grouped by season as follows: spring (15 October–20 December), summer (21 December–20 March) and autumn (21 March–16 May). The experimental unit for both vegetation and animal data was the pasture. The data were subjected to an analysis of variance using the Mixed Procedure in SAS (Statistical Analysis Systems, version 9.4). The choice of the covariance matrix was made using the Akaike Information Criterion (AIC) (Wolfinger 1993), and analysis was performed considering sward light interception levels and season of the year and their interactions as fixed effects and blocks as a random effect (Littell et al. 2000). The season effect (spring, summer and autumn) means were compared using a Tukey test at a 5% significance level. For the stratified herbage samples, the same model was applied, but the effect of the stratum was added and considered fixed. Average daily gain data were analyzed via multivariate analysis with repeated measures according to Littell et al. (2000). Furthermore, we performed analyses of the relationships between the means of pre-grazing sward height and the means of interception of incident light by the canopy for each experimental unit for the entire experimental period

Results

There were no significant (P>0.05) interactions between LI and season for all variables associated with pasture characteristics. However, pastures grazed at 95% LI had longer rest and grazing periods, greater pre-grazing sward heights, forage mass, green stem (GSP) and dead material (DMP) percentages, plus fewer grazing cycles with lower green leaf percentages (GLP) and leaf:stem ratios (LSR) than those managed at 90% LI (Table 1).

Table 1. Means, s.e.m. and significance level (P) for leaf accumulation rate, rest and grazing periods, number of grazing cycles, pre-grazing sward height, forage mass, percentages of green leaf, stem and dead material and leaf:stem ratio in *Megathyrsus maximus* cv. Mombaça pastures subjected to rotational stocking targeting either a light interception (LI) of 90 or 95% pre-grazing.

Parameter	LI[1] 90	LI 95	s.e.m.	P
Rest period (days)	27.1	30.2	0.2	0.0001
Grazing period (days)	4.2	5.5	0.1	0.0001
Grazing cycles (n)	7.0	5.6	0.2	0.0082
Sward height (cm)	82	88	1.1	0.0003
Forage mass (kg DM/ha)	6,610	7,160	111	0.0007
Green leaf (%)	69.8	64.5	0.4	0.0001
Green stem (%)	17.1	21.9	0.4	0.0001
Dead material (%)	12.3	14.0	0.3	0.0002
Leaf:stem ratio	4.2	3.0	0.09	0.0001

[1]Light interception (%).

On the other hand, LI had no significant effect on forage accumulation rates (FAR; P = 0.248) and leaf accumulation rates (P = 0.085). The means and standard errors were: 86.7 ± 4.3 kg DM/ha/d and 59.6 ± 2.2 kg DM/ha/d, respectively.

There was a positive correlation (P = 0.0001; r^2 = 0.86; n = 61) between sward height and LI.

With regard to seasonal effects (Table 2), lengths of rest periods followed the order summer<spring<autumn, while the reverse order (autumn<spring<summer) was observed for forage and leaf accumulation rates. Grazing periods were longer in autumn than during summer, with those in spring being intermediate. During autumn, pastures had greater stem percentages and lesser forage and leaf accumulations per cycle, leaf percentages and leaf:stem ratios than in spring and summer. However, pre-grazing forage mass (P = 0.725) and dead material percentages did not differ (P = 0.6738) between seasons.

There was no effect of LI (P>0.05) on forage dry mass in each layer and the distribution of the various morphological components in the vertical canopy profile. However, a stratum effect was observed for those variables. Forage dry mass and percentages of green stem and dead material decreased, but green leaf percentage increased from the basal to upper strata of the canopy (Table 3). Furthermore, no interactions were observed for LI by stratum (P>0.05), season by stratum (P>0.05) or LI by season by stratum (P>0.05).

Table 2. Means and significance level (P) for rest and grazing periods, forage (FAR) and leaf accumulation rates (LAR), forage (FA) and leaf accumulations (LA) per grazing cycle, pre-grazing green leaf and stem percentages and leaf:stem ratios in *Megathyrsus maximus* cv. Mombaça pastures under rotational stocking from October 2012 to May 2013.

Parameter	Season			P
	Spring	Summer	Autumn	
Rest period (days)	28.9b	24.5c	33.6a	0.0001
	(0.3)	(0.2)	(0.3)	
Grazing period (days)	4.9ab	4.5b	5.9a	0.0007
	(0.3)	(0.2)	(0.4)	
FAR (kg DM /ha/d)	93b	107a	61c	0.0001
	(4.3)	(4.4)	(5.3)	
LAR (kg DM/ ha/d)	64b	74a	38c	0.0001
	(2.9)	(3.0)	(3.6)	
FA (kg DM/ha/ grazing cycle)	2,750a	2,820a	2,300b	0.0012
	(83)	(84)	(101)	
LA (kg DM/ha/ grazing cycle)	1,900a	1,950a	1,450b	0.0001
	(57)	(58)	(70)	
Green leaf (%)	69.3a	68.8a	63.2b	0.0001
	(0.5)	(0.4)	(0.5)	
Green stem (%)	18.2b	18.4b	21.9a	0.0001
	(0.5)	(0.4)	(0.6)	
Leaf:stem ratio	4.0a	3.9a	3.1b	0.0001
	(0.12)	(0.09)	(0.13)	

Means within rows followed by different letters differ significantly at P<0.05. Values in parentheses correspond to the standard error of the mean.

Table 3. Means, standard error of the mean (s.e.m.) and significance level (P) for forage dry mass (FDM) and percentages of green leaf, stem and dead material in the vertical strata of *Megathyrsus maximus* cv. Mombaça pastures under rotational stocking.

Stratum (cm)	FDM (kg/ha)	Leaf (%)	Stem (%)	Dead material (%)
0–40	4,640a	24c	35a	41a
40–60	1,530b	88b	5.5b	6.3b
60–80	660c	98a	1.5c	0.1c
>80	320d	99a	0.1c	0.4c
s.e.m.	127	1.9	1.0	1.4
P	0.0001	0.0001	0.0001	0.0001

Means within columns followed by different letters differ significantly at P<0.05.

Post-grazing residues were maintained close to the target height of 50 cm throughout. Means ± SD were: 47.1 ± 1.3 and 49.7 ± 1.5 cm for pastures grazed at 90 and 95% LI, respectively.

No differences were observed between pastures managed at 90 and 95% LI for post-grazing forage mass (mean ± s.e.m. 4,260 ± 51 kg DM/ha, P = 0.127) and percentages of green leaf (mean 27.5 ± 0.7%, P = 0.564), green stem (mean 30.4 ± 1.0%, P = 0.554) and dead material (mean 42.1 ± 1.2%, P = 0.522). However, post-grazing forage mass was greater in autumn than in spring and summer (Table 4). During spring, the pastures had lesser stem percentage and greater dead material percentage than in the other seasons (Table 4).

Table 4. Means and significance level (P) for forage dry mass and percentages of green stem and dead material in post-grazing residual of *Megathyrsus maximus* cv. Mombaça pastures under rotational stocking.

Parameter	Seasons			P
	Spring	Summer	Autumn	
Forage mass (kg DM/ha)	4,220b (52)	4,102b (53)	4,465a (77)	0.0028
Green stem (%)	27.4b (1.06)	31.0a (1.09)	32.7a (1.57)	0.0179
Dead material (%)	47.1a (1.27)	39.8b (1.30)	39.3b (1.88)	0.0006

Means within rows followed by different letters differ significantly at P<0.05. Values in parentheses correspond to the standard error of the mean.

In the pre-grazing condition, percentages of CP (P = 0.367), IVOMD (P = 0.458), NDF (P = 0.196) and ADL (P = 0.352) of the leaves were similar for pastures grazed at 90 and 95% LI. The means ± s.e.m. were 11.8 ± 0.3%, 61.2 ± 0.5%, 77.2 ± 0.4% and 3.6 ± 0.1%, respectively.

In addition, there were no differences in the percentages of CP (P = 0.194), IVOMD (P = 0.132), NDF (P = 0.626) or ADL (P = 0.321) of green stems from the 2 grazing strategies, with means ± s.e.m. of 5.3 ± 0.3%, 48.3 ± 0.6%, 81.0 ± 0.5% and 4.8 ± 0.1%, respectively. Moreover, there was no effect of season on the percentages of CP, IVDOM, NDF or ADL of either green leaves or stems.

On the other hand, when variables associated with the nutritive value of green leaf were evaluated in the vertical canopy profile, percentages of CP and IVDOM increased and concentrations of NDF and ADL decreased from the basal to the top strata (Table 5). No interactions were observed between LI and stratum, LI and season and season and stratum (P>0.05) for the variables associated with nutritional value of leaves.

There was no interaction between LI and season for stocking rate (SR; P = 0.578) or for average daily gain (ADG; P = 0.671). Moreover, there was no effect of light interception on SR, ADG or liveweight gain/ha (Table 6).

With regard to seasons, ADG was least in autumn and SR and liveweight gain/ha were greater in summer than in spring and autumn (Table 7).

Table 5. Means, standard error of the mean (s.e.m.) and significance level (P) for percentage of crude protein (CP), in vitro organic matter digestibility (IVOMD), neutral detergent fiber (NDF) and acid detergent lignin (ADL) of leaves in the pre-grazing vertical strata of *Megathyrsus maximus* cv. Mombaça pastures under rotational stocking.

Stratum (cm)	CP (%)	IVOMD (%)	NDF (%)	ADL (%)
0–40	8.8d	51.1d	78.5a	3.9a
40–60	10.2c	54.8c	77.3b	3.7b
60–80	11.9b	59.5b	75.9c	3.4c
>80	13.5a	65.1a	74.6d	3.1d
s.e.m.	0.2	0.5	0.3	0.06
P	0.0001	0.0001	0.0001	0.0001

Means within columns followed by different letter differ significantly at P<0.05.

Table 6. Means, standard error of the mean (s.e.m.) and significance level (P) for stocking rate, average daily gain and liveweight gain/ha in *Megathyrsus maximus* cv. Mombaça pastures subjected to rotational stocking targeting either a 90 or 95% LI pre-grazing.

Parameter	LI 90[2]	LI 95	s.e.m.	P
Stocking rate (AU/ha)[1]	3.60	3.87	0.15	0.1042
Average daily gain (kg/hd/d)	0.77	0.72	0.03	0.1363
Liveweight gain/ha (kg/ha)	995	986	52	0.9135

[1]AU = 450 kg live weight; [2]Light interception (%).

Table 7. Means and significance level (P) for seasonal effects on stocking rate, average daily gain and liveweight gain/ha of *Megathyrsus maximus* cv. Mombaça pastures under rotational stocking.

Parameter	Season			P
	Spring	Summer	Autumn	
Stocking rate (AU/ha)[1]	2.9b (0.13)	5.0a (0.11)	3.3b (0.14)	0.0001
Average daily gain (g/hd/d)	780a (25)	800a (23)	655b (29)	0.0006
Liveweight gain/ha (kg/ha)	223b (34)	554a (34)	213b (34)	0.0001

[1]AU = 450 kg live weight.

Means within rows followed by different letters differ significantly at P<0.05. Values in parentheses correspond to the standard error of the mean.

Discussion

Pre-grazing canopy heights for the pastures managed at light interceptions (LI) of 90 and 95% remained relatively stable during the experimental period (Table 1). There was a positive correlation (P = 0.0001; r^2 = 0.86) between LI and sward height, which highlights the potential use of canopy height as a field guide for monitoring grazing management of this cultivar. This result supports Silva and Nascimento Júnior (2007), who suggested that canopy height could be used as a reliable criterion on which to base the optimal time to interrupt pasture regrowth.

Regardless of the LI target used to define the time to re-graze pasture, forage accumulation resumed quickly after defoliation because a lenient grazing strategy was adopted (post-grazing target of 50 cm), which led to 42 and 44% decreases in the pre-grazing heights for pastures managed at 90 and 95% LI, respectively. According to Parsons et al. (1988), the rate of photosynthesis is reduced less by defoliation and the maximum rate of photosynthesis is restored sooner in more leniently defoliated swards. Total forage accumulations were similar for pastures managed at 90 or 95% LI. This was in agreement with the results of Barbosa et al. (2007) and Zanine et al. (2011), who found that leaf accumulation was similar in Tanzania guineagrass pastures managed at 90 and 95% LI, and those of Sbrissia et al. (2013), who observed similar forage accumulation values in kikuyu grass (*Cenchrus clandestinus* syn. *Pennisetum clandestinum*) pastures managed at 15 and 25 cm (25 cm corresponding with 95% LI).

However, pre-grazing green stem and dead material percentages were greater in pastures managed at 95% LI (Table 1), indicating that stem elongation may have started even before the pasture reached 95% LI. Santos et al. (2016) observed up to a 7-fold increase in stem elongation rate in annual ryegrass when the pastures exceeded a height of 17 cm, a condition in which there was still no restriction by high light interception. This supports the hypothesis that stem elongation can be initiated with a LI of the PAR lower than 95%.

In this context, Barbosa et al. (2012) observed that the forage mass of Tanzania guineagrass pasture grazed at 90% LI was composed of younger tillers than that in pastures grazed at 95 and 100% LI. These authors also observed that younger tillers had higher leaf appearance and leaf elongation rates, and consequently a greater leaf length and number of live leaves than mature and/or older tillers.

By contrast, fluctuations in weather conditions (Figures 1 and 2) and the dates of nitrogen application (1/3 in spring and 2/3 in summer) affected forage and leaf accumulation rates throughout the experiment (Table 2). This, in turn, influenced the variation in rest periods (Table 2; Figure 3) and stocking rates (Table 7; Figure 3) of the pastures, throughout the experiment. It is highlighted that weather conditions were similar to the historical 30-year average rainfall.

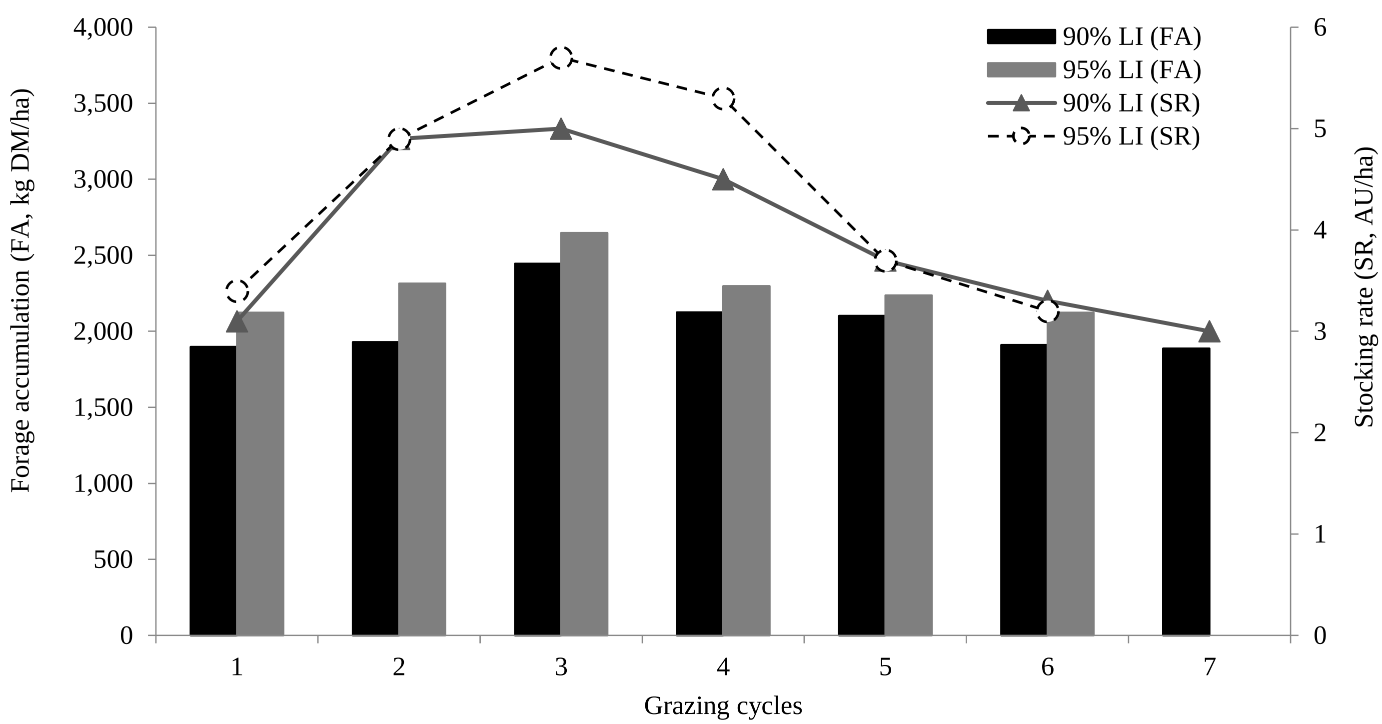

Figure 3. Forage accumulation (FA) per grazing cycle, stocking rate (SR) and rest period in *Megathyrsus maximus* cv. Mombaça pastures subjected to rotational stocking targeting either a 90 or 95% LI pre-grazing from October 2012 to May 2013.

Considering that post-grazing target height was the same for both treatments and forage accumulation rates were similar for these treatments, light interception levels determined the lengths of the resting periods (Table 1; Figure 3). Pastures grazed at 90% LI required less time to reach the pre-grazing target, resulting in an additional 1.4 grazing cycles for these pastures than for pastures managed at 95% LI (Table 1).

The changes in lengths of the grazing periods throughout the study (Tables 1 and 3) could be explained by the variation in forage accumulation rates (Table 3), stocking rate adjustments (Figure 3) to maintain the pre-grazing treatment targets and the need for animals to remain in their current paddocks until the next paddocks to be grazed reached the pre-grazing LI target.

The greater pre-grazing forage mass values for pastures managed at 95% LI (Table 1) did not result in a higher stocking rate in these pastures (Table 6). This can be explained by the need to use fewer animals because the grazing period was longer (Table 1) as a longer resting period was required for these pastures to reach 95% LI (Figure 3).

Despite the greater green stem and dead material percentages in pastures managed at 95% LI, when considering vertical distribution in the canopy profile, we found that about 95% of the green stems and dead material were located in the 0–40 cm stratum (Table 3). This stratum is below the post-grazing target (approximately 50 cm), so theoretically the animals did not have to explore this stratum. This finding supports the results of Zanini et al. (2012), wherein approximately 90% of all stem mass is located in the lower half of the canopy, regardless of the grass species or the targeted pre-grazing height.

Considering only the theoretical grazing horizon (that part of the canopy above 40 cm), green leaf and green stem percentages were 92.3 and 3.9%, respectively (Table 4), resulting in a leaf:stem ratio of 24:1. This indicates that, regardless of the pre-grazing LI target, the canopy structure above 40 cm did not limit the selection and prehension of leaves, and consequently, forage intake by the animals.

Even with the strict control of pre- and post-grazing targets, the morphological composition of the forage varied between seasons. The decrease in leaf percentage and increase in stem percentage during the autumn (Table 2) can be partly explained by the onset of flowering of the Mombaça guineagrass in mid-April. In this period, 6.5% of the forage mass was inflorescences, regardless of the pre-grazing height targets. It is known that, after the inflorescence emerges, the appearance of leaves ceases and stem elongation increases; this was confirmed by the lowest leaf percentage and the highest stem percentage in the pre-grazing forage being recorded in this period of the year (Table 2). This greater growth of stems may explain the high stem percentage in the stubble in autumn (Table 4). On the other hand, regardless of the management strategy used, dead material percentage was higher in spring than in summer and autumn (Table 4). The increased presence of dead material is common in early spring when pastures begin to recover from the dry season (Barbosa et al. 2007; Difante et al. 2009).

The similarity in nutritional value of the leaves and stems in the pastures managed using these 2 grazing strategies could be explained by their very close stage of growth, since the major changes in nutritive value occurring in pasture plants are those that accompany maturation (Van Soest 1994).

The similarity in animal performance in pastures grazed at 90 and 95% LI (Table 6) can be explained by the similarities in the canopy structures (Table 3), percentages of the stratum removed and nutritional value of the forage, indicating that the animals accessed similar pasture conditions. In this context, when analyzing the nutritional value of the leaves in the strata over 40 cm (Table 5) and considering the stem percentages above 40 cm (Table 3) and their nutritional values, the average crude protein concentration and in vitro digestibility of organic matter were 11.5 and 58.6%, respectively, for the forage theoretically available to the animals. The estimated average daily gains of the animals as a function of the amount of protein and energy (NRC 1996) revealed that the daily gain possible from the nutritive value of this grass was 810 g, a value close to those observed in the spring and summer (Table 7).

However, average daily gain in autumn was much lower (Table 7). Since there was no change in pasture nutritive value between seasons, the variation in pasture structure (Table 2) was the probable cause of the decrease in forage intake, and consequently, weight gain of the animals in autumn. According to Benvenutti et al. (2008), in pastures in the reproductive stage stems act as a physical barrier by interfering with the process of bite formation, thus affecting bite dimensions and selectivity, and consequently daily nutrient intake. Recent studies have shown that maximum short-term forage intake rates could be maintained until forage in the upper 40% of the optimal pre-grazing canopy height had been consumed (Fonseca et al. 2012; Mezzalira et al. 2014). In this study, similar (P = 0.258) defoliation severity (in percentage of the height removed) was found for both treatments. The averages and standard errors for extent of reduction in canopy height during grazing were 43.8 ± 0.3 and 42.4 ± 0.3% for the pastures managed at 95 and 90% LI, respectively. Therefore, these results suggest that relatively moderate defoliation levels are more important than pre-grazing goals per se (provided the maximum height limit does not exceed the critical PAR) when the objective is to maximize animal performance.

Similarly, because there was no change in forage accumulation or stocking rate, the similar levels of liveweight gain/ha with the two LIs indicate that Mombaça guineagrass pastures can be managed using either of these management strategies. Thus, instead of basing decisions on a specific LI, some flexibility exists in the pre-grazing target used, without impairment of the productive performance of the animals (Table 6).

Our data indicate that Mombaça guineagrass pastures can be grazed under a rotational system using pre-grazing heights of 80–90 cm (90–95% LI) without compromising the performance of either the pasture or the animals provided a moderate defoliation severity is employed, i.e. approximately 45% of the optimal pre-grazing height of pasture is consumed before animals are removed. This hypothesis should be tested further with this pasture and other erect grass species plus prostrate species.

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

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Research Paper

A survey to assess the value of the legume chimero (*Bouffordia dichotoma* syn. *Desmodium dichotomum*) in mixed farming systems in North and South Wollo Zones, Amhara Region, Ethiopia

Un estudio para explorar el valor de la leguminosa nativa 'chimero' (Bouffordia dichotoma *sin.* Desmodium dichotomum) *en sistemas de producción mixta en Amhara, Etiopía*

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Abstract

This study was conducted to determine the yields and chemical composition of the legume, chimero (*Bouffordia dichotoma* syn. *Desmodium dichotomum*), at its niche in North and South Wollo Zones, Amhara Region, Ethiopia and how it is used by farmers in the region. Dry matter yields of chimero growing as spontaneous intercrop with sorghum in 3 Peasant Associations in each of the 5 sampled districts were determined as was the chemical composition of the forage, based on pooled samples. The average yield of chimero growing as a self-sown legume with sorghum was 4,400 kg DM/ha. Mean chemical composition was 15.4% ash, 22% CP, 31% NDF, 26% ADF and 5.8% ADL, while IVDMD was 61%. Mineral concentrations were: 0.6% Ca, 0.23% P, 1.5% K, 0.78% Mg, 0.01% Na, 0.27% S, 0.16% Fe, 4.4 mg/kg Cu, 45 mg/kg Mn and 12.3 mg/kg Zn. Chimero appears useful as a supplement for feeding to ruminant animals, provided no anti-nutritional factors are present. A self-sown legume that can produce at least 4 t DM/ha with 22% CP when growing with a sorghum crop seems worthy of further investigation. Further studies are needed to assess the impacts on grain and stover yields when chimero is sown with grain crops of sorghum and maize, as well as effects on soil N. The role of this legume in association with grasses warrants investigation. Multi-site evaluation of a range of ecotypes could identify more productive lines.

Keywords: Community use, dry matter yield, nutritive value, tropical legumes.

Resumen

El estudio tuvo como objetivos determinar el rendimiento de forraje y la composición química del chimero (*Bouffordia dichotoma* sin. *Desmodium dichotomum*), familia Fabaceae, en su nicho en las North y South Wollo Zones, Amhara Region, Etiopía, y conocer las formas de uso por los agricultores de la región. Se determinaron los rendimientos del chimero que crecía espontáneamente en los cultivos de sorgo de 3 asociaciones campesinas en cada uno de 5 distritos del estudio y se determinó su composición química con base en muestras agrupadas. El rendimiento promedio fue de 4,400 kg de MS/ha. La composición química promedio fue: ceniza, 15.4%; proteína cruda, 22%; fibra detergente neutro, 31%; fibra detergente ácido, 26%; y lignina detergente ácido, 5.8%. La digestibilidad in vitro de la materia seca fue de 61%. Las concentraciones minerales fueron: 0.6% Ca, 0.23% P, 1.5% K, 0.78% Mg, 0.01% Na, 0.27% S, 0.16% Fe, 4.4 mg/kg Cu, 45 mg/kg Mn y 12.3 mg/kg Zn. El forraje del chimero parece útil como suplemento para rumiantes, siempre y cuando no se presenten factores antinutricionales. Una leguminosa que ocurre en forma espontánea en un cultivo de sorgo y produce 4 t de MS/ha con un 22% de proteína cruda merece más investigación. Se necesitan estudios sobre el efecto de la leguminosa en los rendimientos de grano y biomasa de cultivos de sorgo y maíz, así como el efecto en el nitrógeno del suelo. También se requiere estudiar la factibilidad de asociaciones con gramíneas forrajeras y explorar si existe variabilidad genética en las poblaciones nativas.

Palabras clave: Leguminosas tropicales, producción de materia seca, uso comunitario, valor nutritivo.

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Introduction

Ethiopia is considered to have the largest livestock population in Africa. It is home for about 59.5 million cattle, 30.7 million sheep, 30.2 million goats, 2.16 million horses, 8.44 million donkeys, 0.41 million mules and about 1.21 million camels (CSA 2016/17).

Livestock play vital roles in generating income for farmers, creating job opportunities, ensuring food security, providing services, contributing to asset, social, cultural and environmental values and sustaining livelihoods (Metaferia et al. 2011).

Despite the high livestock population and favorable environmental conditions for animal production, current livestock production and productivity are far below expectations. This is associated with a number of complex and inter-related constraints such as inadequate feed and nutrition, widespread diseases, limited genetic potential of local breeds, marketing issues and inefficiency of livestock development services with respect to credit, extension, marketing and infrastructure (Negassa et al. 2011). Among these constraints, poor nutrition is a major factor limiting livestock performance (Belete et al. 2012).

While supplementing animals with concentrate feeds can increase digestibility, nutrient supply and intake (Preston and Leng 1987), concentrates are expensive and may exceed the financial limits for rural farmers. A logical alternative is to improve the nutrition of livestock by improving the quality of available feed resources like native pastures and crop residues. Another approach is to develop new forage crop varieties by selecting from within local species or through exotic introductions.

One native herbaceous legume, known locally as 'chimero' [*Bouffordia dichotoma* (Willd.) H. Ohashi & K. Ohashi] [syn. *Desmodium dichotomum* (Willd.) DC], family Fabaceae, is recognized by farmers in several districts of North and South Wollo Zones, Amhara Region, Ethiopia as a valuable livestock feed. Chimero is an herbaceous annual self-regenerating legume growing in a wild state. The stem and branches have a trailing growth habit and reach 64–90 cm in length. Leaves are trifoliolate, with the leaflets being ovate (5.8–8 cm long and 4–5 cm wide). Both the dorsal and under-sides of the leaves are hairy and green in color, while flowers are pink to violet and seeds are yellow to light brown (Figure 1). Chimero is known by other common names in the various countries where it is found, including "er qi shan ma huang" in China and "chikta", "asud" or "gander-lapto" in India. It is also found in other parts of Africa (Cameroon, Chad, Eritrea, Sudan, Uganda) and Asia (Indonesia, Myanmar).

On the basis of this scenario, the current study aimed to explore how the community uses this legume, plus identify and evaluate the chemical composition of chimero at its niche.

Figure 1. Morphology of chimero: whole plant; stem, leaves and inflorescence; and pods. (Photos: H. Abebe.)

Materials and Methods

Description of North and South Wollo Zones

South Wollo, situated approximately between 10°15'–11°30' N and 38°25'–39°30' E (Figure 2), has a total landmass of 17,067 km^2. Elevation varies from 1,000 (Chefameda) to 4,247 (Amba Ferit) masl. The annual range of temperature is 10–25 °C and drops with the increase in elevation. Frost is very common at higher elevations, specifically above 2,500 masl. Annual rainfall varies from 900 to 1,000 mm, most falling in Belg (February–May) and Meher (June–September) (Figure 3). Soil types vary with the major type in the western part of the Zone being vertisol followed by luvisol and nitosol. The southern and eastern parts of the Zone have cambisols, vertisols and dark brown silty clay soils. Water-logging occurs as a result of poor surface drainage plus shallow soil depth and soil infertility is common.

North Wollo central area is one of the 11 Zones of Amhara Regional State. It is in the northern part of the country (11°21'–12°20' N, 38°27'–39°57' E) (Figure 2) and shares a border with South Wollo Zone, South Gondar Zone, Wag Hamra Zone, Tigray Region and Afar Region. In addition to these neighboring areas, part of North Wollo's southern border is defined by the Mille River. The districts of North Wollo Zone fall under 4 livelihood zones. These are: the lowland areas, North Wollo East Plain livelihood Zone, Northeast Midland mixed cereal livelihood Zone and North Wollo Highland Belg livelihood Zone. Climatic conditions in the Zone are presented in Figure 4.

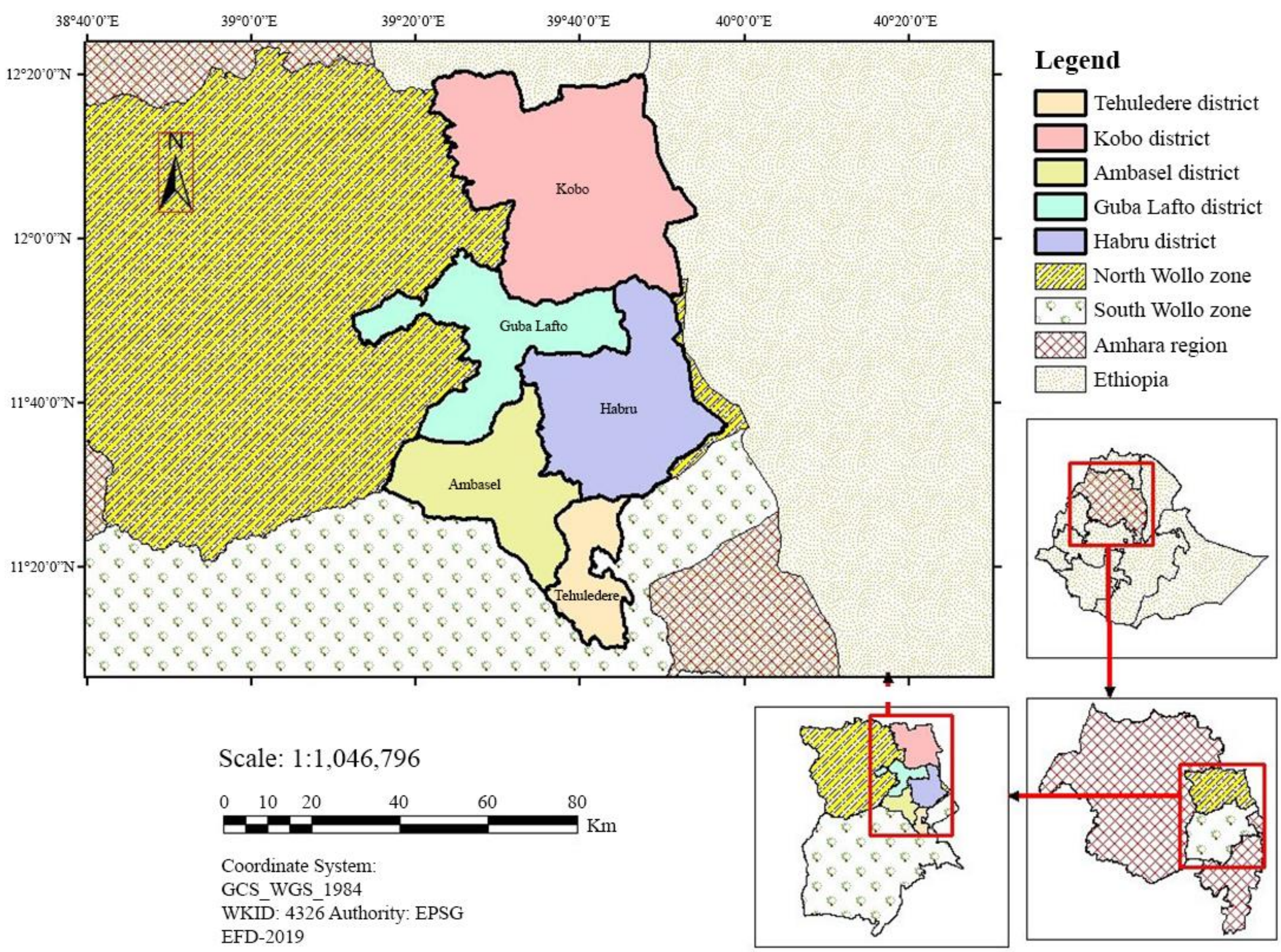

Figure 2. Map of the study area in Ethiopia.

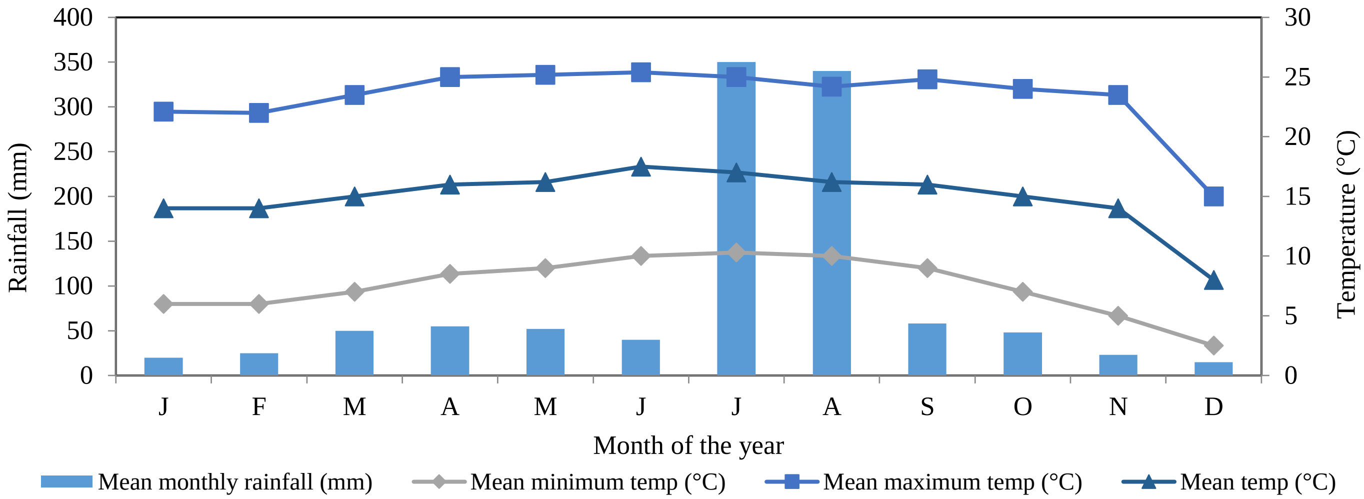

Figure 3. Rainfall distribution and temperature for South Wollo Zone [means of 16 years (2000–2015)]. Source: National Meteorological Service Agency, Kombolcha Station (NMSAKS 2019).

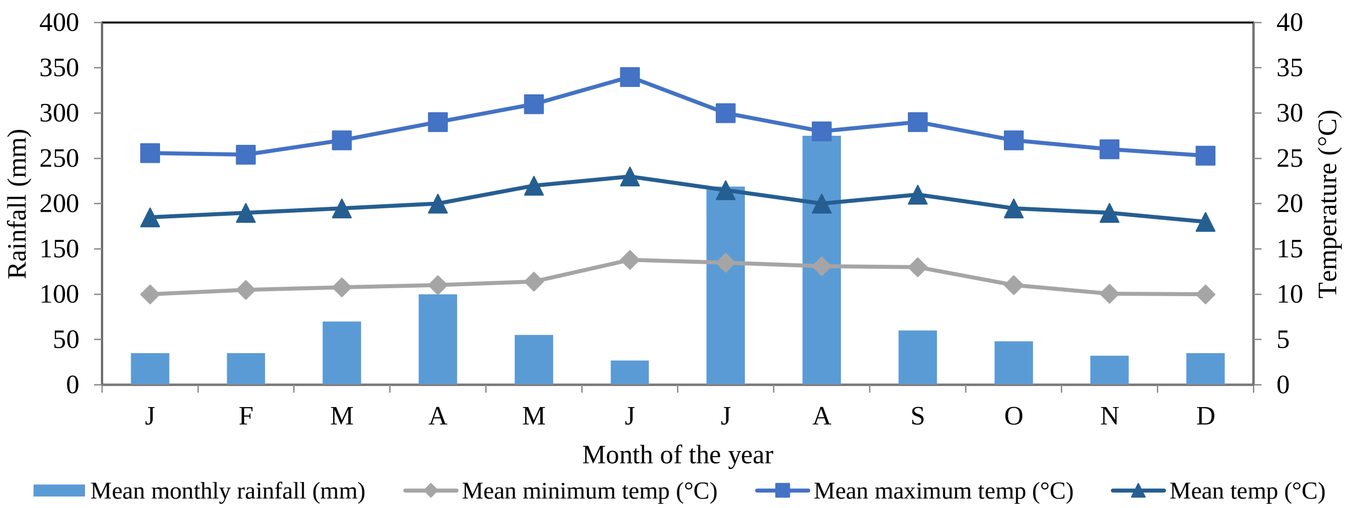

Figure 4. Rainfall distribution and temperature for North Wollo Zone [means of 16 years (2000–2015)]. Source: National Meteorological Service Agency, Kombolcha Station (NMSAKS 2019).

Questionnaire-based survey on community use of chimero

A survey was conducted from 10 October 2018 to 20 November 2018 in North and South Wollo Zones. From North Wollo Zone, Habru, Gubalafito and Kobo districts and from South Wollo Zone, Ambasel and Tehuledere districts were assessed. Three PAs (Peasant Associations) from each district were selected as representative of the study area. Random sampling of households within these PAs was employed. The sample size was determined by using the formula given by Yamane (1967):

$$n = \frac{N}{1 + N * (e)^2}$$

where: n is sample size, N is number of households and e is the desired level of precision (0.05).

A total of 387 households were interviewed from 12,262 households in the population representing the selected PAs. Structured and semi-structured questionnaires were used to collect information on: the season in which chimero is harvested and consumed by livestock; which parts of chimero are preferred and by which animal species; abundance; harvesting and conservation methods; ease of browsing; and additional uses. The questionnaires were pretested prior to commencing the survey to ensure respondents understood all questions clearly.

Sample collection, dry matter yield, chemical composition and in vitro dry matter digestibility of chimero

Samples of chimero were collected from sorghum-growing farmers from each PA, pressed, labeled, dried and transported to the National Herbarium of Addis Ababa University for identification, based on the Flora of Ethiopia (Hedberg 1996). Three samples of vegetative parts of chimero were collected from each Kebele (the lowest administrative unit of a certain area or PA) at random and pooled for chemical composition and in vitro dry matter digestibility (IVDMD) determination.

At the 50% flowering stage chimero was harvested from each PA using 1 × 1 m quadrats (9 quadrats per PA) for dry matter (DM) yield determination. Plants were harvested at ground level and fresh biomass weighed immediately. A subsample of 15–20% of the total weight was taken, weighed and placed in a paper bag for DM determination. The samples were oven-dried at 105 °C for 24 h.

Nutritive value analysis. The oven-dried samples were ground in a Wiley mill to pass through a 1 mm sieve for the determination of chemical composition. To determine ash concentration, samples were ignited in a muffle furnace at 550 °C (AOAC International). Crude protein (CP) concentration was determined using the Kjeldahl method (AOAC International), while neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations were determined according to Van Soest et al. (1991). In vitro dry matter digestibility (IVDMD) was determined according to the 2-stage method outlined by Tilley and Terry (1963). All chemical composition and IVDMD analyses were carried out at the Nutrition Laboratory, Holeta Agricultural Research Center.

Mineral composition analysis. Three samples of chimero were collected from each PA (total of 45 samples) and delivered to the JIJE analytical testing service laboratory, Addis Ababa for the analysis of macro-minerals: calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg) and sulfur (S); and micro-minerals: cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn). Na and K were determined by flame spectrophotometry (AOAC International, Official method 966.16); Ca and Mg by EDTA titration (AOAC International, Official method 962.01); P by spectrophotometry (AOAC International, Official method 965.17); S by magnesium nitrate ashing – turbidimetry; Co, Cu, Fe, Mn and Zn by flame AAS (AOAC International, Official method 985.35); and Se by Graphite Furnace AAS (AOAC International, Official method 985.35).

Statistical analysis

The primary data collected for this survey were analyzed using descriptive statistics such as means, frequency distributions, percentages and standard deviations using SPSS (2007).

Results

Household characteristics

The household characteristics of the respondents are presented in Table 1. Overall, in the present study 84.2% of the respondents were male- and 15.5% female-headed households. The overall average age of the respondents in the study districts was 46.8 years.

Landholding and land use pattern of the households

In the study districts, the average total crop land and private natural grazing land owned by the households was 2.76 ha (range 2.49–3.56 ha) and 0.62 ha (range 0.54–0.8 ha), respectively (Figure 5). No respondents had fallow land or improved pasture land. The average landholding of the respondents in the study was greater than the average national landholding size (0.96 ha/household) (CSA 2011).

The samples of chimero from each Woreda (third-level administrative divisions of Ethiopia) were sent to the National Herbarium of Addis Ababa University for identification and confirmed as being *Desmodium dichotomum* (Willd.) DC. This plant was initially named *Hedysarum dichotomum* by Willdenow in 1802 and

Table 1. Household characteristics of the respondents in the study districts in North and South Wollo Zones, Ethiopia.

Characteristic		District											
		Habru (n=85)		Gubalafito (n=73)		Kobo (n=70)		Ambasel (n=80)		Tehuledere (n=79)		Overall (n=387)	
		Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Gender	Male	73	18.9	62	16.0	58	15.0	68	17.6	65	16.8	326	84.2
	Female	12	3.1	11	2.9	12	3.1	12	3.1	14	3.6	61	16.5
Age (Mean ±SD)		46.9±7.28		46.5±7.46		47.2±7.33		47.0±7.36		46.3±7.33		46.8±7.32	

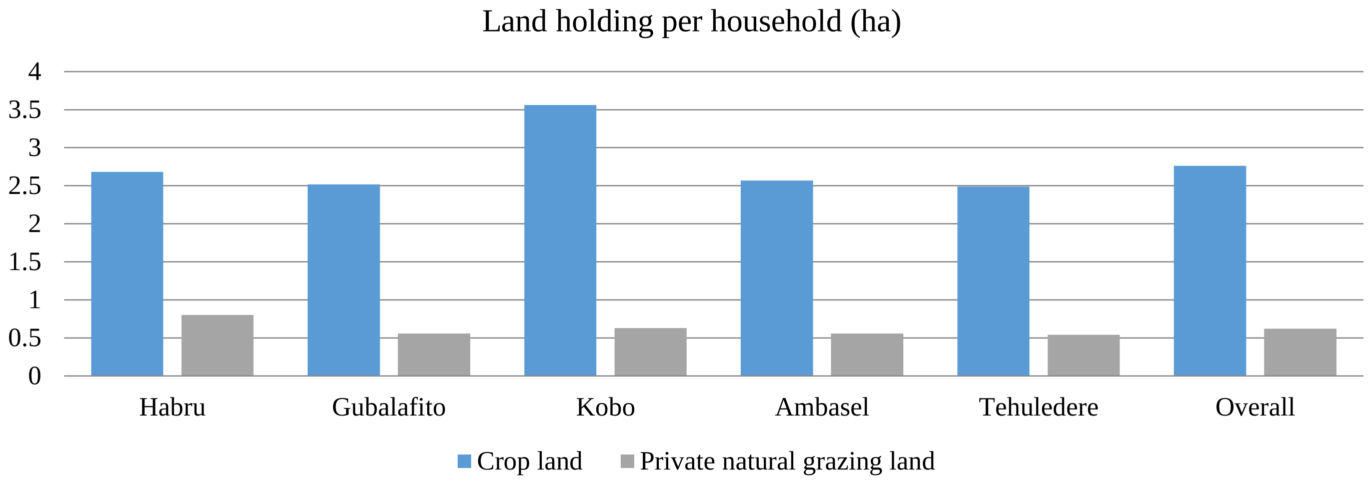

Figure 5. Landholding patterns of the surveyed households in 5 districts of North and South Wollo Zones, Ethiopia.

changed to *Desmodium dichotomum* by de Candolle in 1825. Following recent studies by Ohashi et al. (2018), the new scientific name *Bouffordia dichotoma* (Willd.) H. Ohashi & K. Ohashi has now been accepted as appropriate for this species.

During the wet season, most respondents (80.4%) indicated that their first choice for forage was weeds and green crop chop, with crop residue and natural pasture being the main second choices (40.8 and 39%, respectively) and natural pasture (40.1%) and crop residue (39.5%) the main third choices (Table 2). Green crop chop is defined as a harvested forage crop without allowing it to dry in the field. In the overall ranking, weeds and green crop chop were the most important, crop residues the second most important and natural pasture the third choice.

During the dry season, 100% of respondents used crop residues as the primary feed resource, while crop aftermath was second choice (80.4%) and hay (60.2%) the third choice. Stover was the first choice of fodder in the dry season since it includes crop residue. Crop residues are defined as material left after the crop has been harvested, e.g. teff straw, barley straw, wheat straw, chick pea hulls, sorghum and maize stover, while crop aftermath is a second-growth crop.

Chimero emerges spontaneously under sorghum crops (Figure 6). It is categorized as green chop for immediate feeding to livestock since the farmers have no experience in preserving forage, e.g. in the form of hay, as a feed resource for the dry season. Most farmers do not sow any forage for livestock feeding and prefer to use naturally occurring grass, grass hay, crop residues, green chop,

Table 2. Types of feed resources used in the wet and dry seasons in 5 districts of North and South Wollo Zones, Ethiopia.

Feed resource	Priority level (%)					Overall Ranking
	1st	2nd	3rd	4th	5th	
Wet season						
Natural pasture	-	39	40.1	20.7	-	3
Hay	-	0.4	33.9	65.7	-	4
Crop residues	19.6	40.8	39.5	-	-	2
Agro-industrial by-products	-	-	-	19.6	60.2	5
Green chop, weeds	80.4	19.6	-	-	-	1
Dry season						
Natural pasture	-	19.6	39.4	40.6	-	4
Fodder trees	-	-	-	20.4	-	
Hay	-	-	60.2	19.6	20.2	3
Crop residues	100	-	-	-	-	1
Crop aftermath	-	80.4	-	19.6	-	2
Agro-industrial by-products	-	-	-	-	20.8	5

fodder trees and crop aftermath and do not keep records of how much was sown and produced. It is normally harvested when the sorghum is heading and not progressively throughout the growing season of the crop. The amount of ground covered by the plants at harvesting differed from farm to farm and even within the same farm because farmers broadcast sorghum seed at sowing so there was little consistency. To determine DM yield of chimero in our study, we sampled the legume from within the sorghum crop when chimero was displaying 50% flowering (at approximately 3 months after emergence).

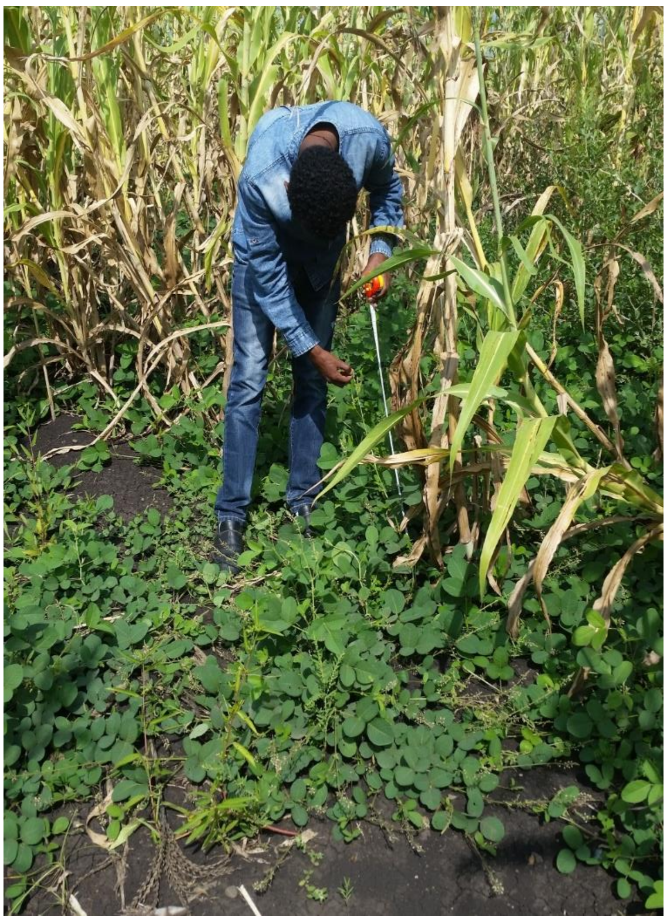

Figure 6. Chimero growing spontaneously under sorghum. (Photo: H. Abebe.)

All categories of animals were fed chimero including oxen, sheep and goats for growth and fattening, cows for higher milk production and mules, horses and camels for energy. While all farmers did not have all classes of animal at the time of the survey, they indicated that at some time they had owned all types and fed them chimero. Farmers rated the animal preference through long periods of observation and experience and considered chimero was most preferred by cattle (273 from 387 respondents) (Table 3). According to the information gained during survey work, there was limited indication that different animal species had a special preference for different parts of chimero, although some farmers said equines preferred to eat stem and small ruminants preferred to eat pods. All respondents (100%) preferred chimero as a feed source over other locally available herbaceous legume feed resources, e.g. *Neonotonia wightii*. No respondent conserved/stored chimero, treated it in any way, sold it or used it for any purpose other than feeding his/her livestock. No farmers have received formal training on how to conserve and preserve important indigenous forage legumes for feeding later.

Table 3. Frequency of feeding chimero, class of animals fed and plant parts preferred in 5 districts of North and South Wollo Zones, Ethiopia (total respondents: 387).

Variable	Frequency	Percentage
Categories of animals fed chimero		
Large ruminants	387	100
Small ruminants	387	100
Equines	387	100
Camels	387	100
By which animal more preferred?		
Large ruminants	273	70.5
Small ruminants	65	16.8
Equines	6	1.6
Camels	43	11.1
Parts of chimero preferred		
Stem	86	22.2
Leaf	258	66.7
Seed pod	43	11.1

All respondents used the self-regenerating chimero with sown crops (78.8% with sorghum and 21.2% with maize) and indicated that it was abundant for harvesting in the months September–November. All farmers used a cut-and-carry system for utilizing the chimero.

The average yield of 3–4-months-old chimero as assessed under the grain crops was 4,400 kg DM/ha with a range of 4,100–4,800 kg DM/ha between districts. Possible factors contributing to variation across districts might be variation in rainfall, soil characteristics and competition from the grain crops.

Chemical analyses revealed that mean concentrations of various components in chimero were: 22% CP (DM basis), 31% NDF, 26% ADF and 5.8% ADL, while IVDMD was 61%. Mineral concentrations were: 0.6% Ca, 0.23% P, 1.47% K, 0.78% Mg, 0.01% Na, 0.27% S, 0.16% Fe, 4.4 mg/kg Cu, 44.9 mg/kg Mn and 12.3 mg/kg Zn. There was no variation between sites in chemical composition.

Discussion

This survey has shown the important role that chimero plays as a self-sown legume with grain crops in this part of Ethiopia, especially for use as a source of feed during the wet season. Not surprisingly, the forage was fed using a cut-and-carry system as it would not be appropriate to allow livestock access to the plants while growing with the sorghum or maize crops. Further studies would seem to be warranted to determine the impacts of growing the legume with the grain crops on grain and stover yields of the crops as well as on soil improvement. Another aspect would be the possible contribution it could make to the diets of livestock during other times of year, especially if sown into native pastures.

The finding from this study that crop residues from sorghum and maize stover plus teff straw were the most important feed sources during the dry season agrees with the report of Abate et al. (2010) that straw from maize, sorghum and teff was used mainly during the dry season in southeastern parts of the country. Contrary to the current study, Desalw (2008) reported that the major dry season feed resources for cattle were natural pasture (55.7%), crop residues (20.7%), stubble (14.3%) and hay (9.3%). Most farmers fed chimero to large ruminant animals and assumed that it would fatten animals rapidly, especially oxen. In this study it has not been possible to locate any data on how well animals perform when fed this legume and how it might compare with other legumes grown under these conditions.

It was of considerable interest that all respondents preferred chimero over other locally available herbaceous legume feed resources, such as *Neonotonia wightii*. In the preference table (Table 3) the percentages of respondents listed indicated that particular animal categories had highest preference for chimero. For example, 6 (1.6%) farmers indicated that equines had the greatest preference and 43 (11.1%) farmers indicated that camels had the greatest preference. Similarly, 86 (22.2%) farmers stated that animals preferred to eat stem over leaf and pods, 258 (66.7%) indicated that animals preferred to eat leaf over stems and pods, while 43 (11.1%) indicated that animals preferred pods over leaf and stems. A preference for stem over leaf and pods is surprising but according to farmers' explanations during survey work, most indicated that equines preferred to eat stem and small ruminants preferred to eat pods. Despite there being surplus production of chimero in September–November, the crop growing season when good rainfall was received and other non-crop residue feed resources should have been most readily available, no respondents conserved and stored chimero as either hay or silage for use during periods of feed scarcity. However, data suggest that considerable amounts of other hays were fed in both wet and dry seasons, more so in the dry season, the major types being sorghum and maize stover, teff residue and natural grasses. The opportunity obviously exists to conserve this relatively high protein source for feeding during the winter-spring period when both quantity and quality of available feed, especially native pastures, stovers etc., are low. There are numerous references in the literature that a supplement of high protein forage increases intake of low quality roughage and improves animal performance (Adu et al. 1990; Melese et al. 2014). However, as no farmers have received formal training on how to conserve and preserve important indigenous forage legumes for feeding later, a technology transfer program would need to be mounted to achieve this end.

Conclusions

This study has shown that many farmers in the study area grow grain crops and chimero is self-sown in these crops from residual seed (soil seed bank). Farmers feed it using a cut-and-carry system to all classes of livestock. Mean yields obtained of 4.4 t DM/ha were quite significant and would provide a valuable source of forage for stock. As mean CP concentration of the forage was 22%, this forage could be used as either a supplement to other feeds or as a complete feed. However, the presence of anti-nutritive factors in the forage should be investigated. As little research has been conducted on this very promising species, much more effort should be devoted to determining if more productive ecotypes are available and how yields of the forage can be maximized. The impacts of sowing this species with grain crops on grain and stover yields of the crops should be examined as well as its role if sown into native pastures. Conservation for feeding at times of low feed quality and availability seems a logical method to utilize the forage and this process should be investigated as well as mounting a technology transfer program to promote conservation.

Post script

Since completing this survey the author has collected seed from 26 populations of *Bouffordia dichotoma* from a number of districts in the South Wollo, North Wollo and Oromia Zones of Ethiopia (10–12º N, 39–40º E; 1,470–1,890 masl) following the Ethiopian biodiversity institute collection format for forage genetic resources conservation.

Mean annual rainfall at collection sites varies from 500 to 1,557 mm. The seeds are currently stored at Wollo University and the author will undertake preliminary evaluations of these populations as part of his Ph.D. studies to assess what degree of variation exists in the natural populations in the region.

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(Note of the editors: All hyperlinks were verified 17 December 2019.)

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Research Paper

Dry matter concentration and corn silage density: Effects on forage quality

Concentración de materia seca y densidad de ensilaje de maíz: Efectos en la calidad del forraje

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Abstract

Considering the hypothesis that density and dry matter (DM) concentration may be used as indicators of silage nutritional quality, the aim of the present study was to determine density and maturation stage (i.e. DM concentration) of corn silages under farm conditions in Brazil, establishing relationships between density and physical and chemical characteristics. In a completely randomized design, 20 bunkers of corn silage, each from a different farm, were used for data collection. Using a coring machine, 5 samples of silage were extracted from an exposed face of each silo and samples were analyzed for density of compaction, plus concentrations of DM, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN), total carbohydrate (TC), non-fiber carbohydrate (NFC) and starch (STA), as well as electrical conductivity. There was significant variation in many of the parameters measured with the greatest variation in density on a natural matter basis. Negative correlations were observed between percentages of DM, NDF and ADF in the silage and silage density on a natural matter basis ($P<0.05$). On the other hand, DM% was positively correlated with concentrations of STA, TDN and TC ($P<0.05$). Density on a DM basis showed positive correlation with STA but was negatively correlated with NDF and ADF ($P<0.05$) indicating that the more fibrous material is harder to compact. A technology transfer program seems warranted to inform Brazilian farmers of these findings and the importance of harvesting forage at a stage of growth when quality would be better to increase the probability of achieving adequate compaction of the ensiled material and hence better quality of material at feeding out.

Keywords: Bunker silos, compaction, forage conservation, silage quality, specific mass.

Resumen

Con base en la hipótesis que la densidad y la concentración de materia seca (MS) son indicadores de la calidad nutritiva del ensilaje, el objetivo del presente estudio fue determinar ambas características en ensilaje de maíz a nivel de fincas en el estado de Paraná, Brasil. La recolección de datos se hizo en 20 silos búnker en diferentes fincas y para su análisis se usó un diseño completamente aleatorio. En cada silo se tomaron 5 muestras y se determinaron la densidad de compactación y las concentraciones de MS, proteína cruda (PC), fibra detergente neutra (FDN), fibra detergente ácida (FDA), nutrientes digestibles totales (TDN), carbohidratos totales (CT), carbohidratos no fibrosos (CNF) y almidón (STA), así como la conductividad eléctrica. La densidad basada en materia natural fue el parámetro que presentó la mayor variación entre silos. Se observaron correlaciones negativas entre los porcentajes de MS, FDN y FDA y la densidad del ensilaje basada en materia natural ($P<0.05$). Por otro lado, el porcentaje de MS se correlacionó positivamente con las concentraciones de STA, TDN y CT ($P<0.05$). La densidad basada en materia seca mostró una

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correlación positiva con STA pero negativa con FDN y FDA (P<0.05), lo que indica que el material más fibroso es más difícil de compactar. Un programa de transferencia de tecnología parece justificado para informar a los ganaderos brasileños de estos resultados y resaltar la importancia de cosechar forraje en una etapa de crecimiento que facilite una compactación adecuada del material y por tanto, una mejor calidad del forraje ensilado.

Palabras clave: Calidad de ensilaje, compactación, conservación de forraje, masa específica, silos búnker.

Introduction

Major challenges for high quality silage production occur at the stages of ensiling, storing and discharging from bunker silos. During these stages, microbial activities may affect fermentation processes in the ensiled forage and consequently its nutritional quality. Thus, to reduce quality losses, parameters related to the forage itself at the harvest stage, such as moisture content, crude protein concentration and particle size, must be evaluated as well as those related to the type of bunker silo, which will determine the exposure of the ensiled material to oxygen and compaction (Cardoso et al. 2016).

One of the most important factors influencing silage quality is its density (Craig and Roth 2005), which is primarily determined, among other things, by the average particle size of the forage plant, its stage of maturity at harvest and how efficiently the compaction of the material is carried out, which usually in bunker silos is done by using packing tractors (Muck and Holmes 2000). Silages with low density often contain high residual air mass, resulting in longer periods of oxygen exposure and consequently increased consumption of soluble carbohydrates, plus reduced production of organic acids and higher pH (McDonald et al. 1991).

In addition, low density values lead to higher porosity and passage of air into the bunker silo, affecting the aerobic stability and increasing losses during utilization of the silage (Jobim et al. 2007). Thus, reduction of porosity/increasing compaction or density during ensiling is a crucial management practice for reducing aerobic deterioration (Bernardes et al. 2009; Hentz et al. 2017). Aerobic deterioration, besides reducing the nutritional value of the ensiled material, can increase the proliferation of pathogenic or undesirable micro-organisms, impairing the performance of animals fed on these forages (Barbosa et al. 2011). Greater compaction results in higher density, allowing a better retention of soluble carbohydrates and reduced proteolysis, resulting in improved acceptability to animals and nutritional quality of the ensiled material (Velho et al. 2007; Sucu et al. 2016).

Based on the above, we hypothesized that measuring density and dry matter (DM) concentration of silages on farm could provide reliable indicators of the nutritional quality of the feed. We designed this study to sample corn silages under farm conditions in Paraná, Brazil, determine their density and DM percentage, and calculate the relationships between these variables and physical and chemical characteristics of silages.

Materials and Methods

Following a completely randomized design, samples of corn silages were collected from 20 bunker silos on 20 typical dairy farms in the ABCW dairy basin, Paraná State, Brazil, specifically in the municipalities of Arapoti (24º09' S, 49º49' W), Piraí do Sul (24º31' S, 49º56' W), Castro (24º47' S, 50º00' W), Carambeí (24º55' S, 50º05' W) and Ponta Grossa (25º05' S, 50º09' W). Before starting the sampling procedure, a slice of silage was removed from the vertical face of each silo panel in order to remove any loose silage, so that the samples were collected from undisturbed material.

Density measurements (i.e. specific mass) were made by employing the methodologies described by Muck and Holmes (2000) and D'Amours and Savoie (2004); a metal cylinder (20 cm long and 10 cm diameter) with a serrated cutting edge and attached to a chainsaw was used as described by Craig and Roth (2005) and Krüger et al. (2017). The force generated by the chainsaw screwed the cylinder horizontally into the vertical face of the silage panel. When the silage sample was withdrawn from the silage, the depth was measured with a rule to calculate the volume of the sample. Silage samples were withdrawn at 5 points (replications) on the vertical face of the silo panel: 3 locations at the top and 2 at the bottom, forming a "W" like pattern. The samples were weighed when withdrawn and from the cylinder volume and the mass of the sample, density of silage in the silo on a natural matter basis (DNM) was calculated, assuming uniform density throughout the silo. Samples were then dried (55 °C for 72 h in a forced air circulation oven) and density on a dry matter basis (DDM) was calculated in order to account for differences in moisture content between silages.

The Penn State particle size separator method (Heinrichs 1996) was used to determine the average particle size (APS) in each composite sample (5 replicates from the silo panel). Calculations of particle size were carried out according to manufacturer's instructions. This particle separator contains sieves of 19 and 8 mm, in which the 19 mm sieve was designed to retain forage or feed particles that would be buoyant in the rumen (form the forage mat) and require substantial cud chewing by the animal; in theory this would supply additional buffering to the rumen and help modify rumen pH. After that, the 8 mm sieve collects primarily forage particles that are also part of the forage mat in the rumen, but will be broken down faster with less cud chewing and will hydrate in the rumen faster to allow more rapid rumen microbial breakdown. Additionally, there is a bottom pan where all the other smaller particles should be collected. Subsequently, APS is calculated according to the percentage (on natural matter mass basis) of material retained in each sieve.

A digital potentiometer was used to measure pH according to Cherney and Cherney (2003) and electrical conductivity was determined as described by Jobim et al. (2007) using a digital conductivity meter.

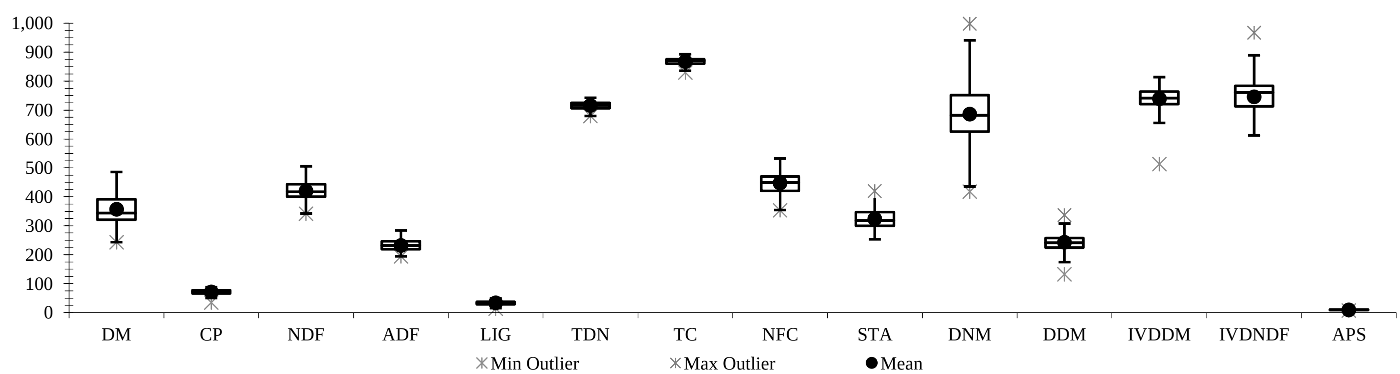
For chemical composition analysis, dried silage samples (55 °C for 72 h in a forced air circulation oven) were ground in a Wiley mill through a 1 mm screen. The concentrations of DM (ID number: 934.01), crude protein (CP; ID number: 2001.11), ether extract (EE; ID number: 2003.5) and ash (ID number: 942.05) were determined according to AOAC International (2011). Starch (STA) determination followed the methodology described by Pereira and Rossi (1995), while neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG) were determined according to Van Soest et al. (1991). Total carbohydrates (TC) were calculated using the equation: TC = 1,000 – [CP (g/kg DM) + EE (g/kg DM) + ash (g/kg DM)]. Non-fiber carbohydrates (NFC) were calculated as the difference between TC and NDF (Hall 2000). Total digestible nutrients (TDN) were calculated using the equation: TDN (g/kg) = 87.84 – (0.70 × ADF) (Undersander et al. 1993). All chemical composition parameters, except for DM, were determined on a DM basis.

The methodology proposed by Tilley and Terry (1963), adapted to a Daisy II incubator system (ANKOM® - Technology Corporation) as described by Santos et al. (2000), was used to determine the in vitro digestibility of DM (IVDDM) and NDF (IVDNDF).

Statistical analysis was performed using the software SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA). Correlation (Proc CORR) and factor analysis (Proc Factor) were performed to verify the relationships between the obtained variables. For all procedures, 5% significance level was adopted.

Results

The means and variation between tested silage samples in physical and chemical composition parameters are presented as a boxplot in Figure 1. The greatest variation was shown for DNM, being much greater than the variation in DDM, while the variation in total carbohydrates, TDN and pH was minimal as shown by standard deviation (SD) values below. Average electrical conductivity and pH for silages were 591 ± 100.9 (μS/cm) and 4.0 ± 0.09 (mean ± SD), respectively.



DM – dry matter (g/kg); CP – crude protein (g/kg DM); NDF – neutral detergent fiber (g/kg DM); ADF – acid detergent fiber (g/kg DM); LIG – lignin (g/kg DM); TDN – total digestible nutrients (g/kg DM); TC – total carbohydrates (g/kg DM); NFC – non-fiber carbohydrates (g/kg DM); STA – starch (g/kg DM); DNM – density on natural matter basis (kg NM/m^3); DDM – density on dry matter basis (kg DM/m^3); IVDDM – in vitro digestibility of dry matter (g/kg); IVDNDF – in vitro digestibility of neutral detergent fiber (g/kg); APS – average particle size (mm).

Figure 1. Boxplot of chemical, physical and nutritional variables of corn silages for dairy cattle on 20 farms in Paraná State, Brazil. Boxplot elements – upper and lower hinges: 75th and 25th quartiles; inner horizontal line: median; vertical lines: extensions of the hinges to the largest and smallest values at most 1.5 times of interquartile range; upper and lower markers: outlier values; internal circle markers: mean values.

Dry matter percentage in silage was negatively correlated with NDF (r = -0.44*), ADF (r = -0.42*) and DNM (r = -0.48*) but was positively related to DDM (r = 0.54*) (Table 1). However, DDM and DNM were positively correlated (r = 0.47*), while DDM was negatively related to NDF (r = -0.46*) and ADF (r = -0.40*). These correlations may be clearly observed in the 2 main factor analysis (Figure 2 – variables next to one another along the axes are positively correlated while those in opposite positions are negatively correlated), which explained 49.1% (i.e. sum of autovectors) of the variance observed between the parameters evaluated.

Discussion

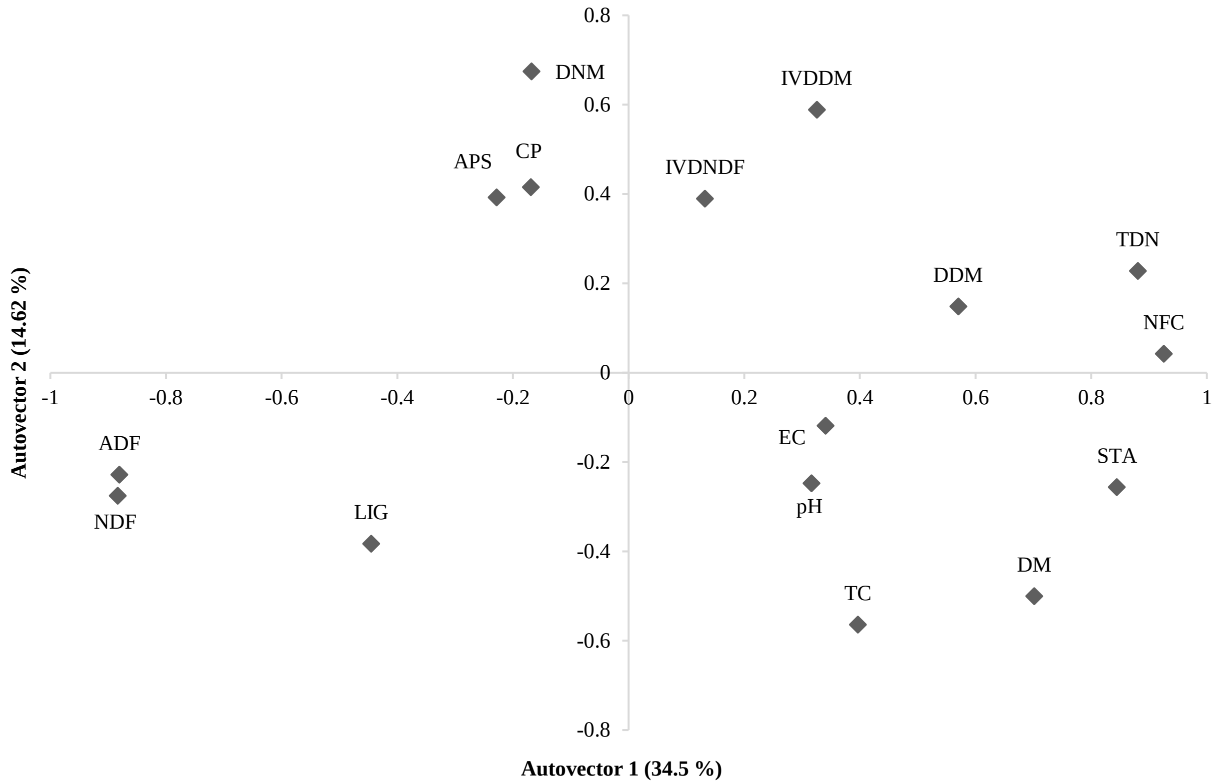
An important outcome of this study was the demonstration that maize silage being made on dairy farms in this region varied greatly in quality, which can probably be related to the stage of maturity of the maize crop at harvesting (Figure 1). The ability to compress the ensiled forage depends greatly on factors like fiber levels, moisture content, particle size etc. High density of compaction can prevent loss of nutrients because more air is expelled from the compacted material and the opportunity for aerobic respiration to occur is reduced. On the other hand, low density of compression can result in an environment favorable for aerobic respiration, increasing the proliferation of molds and mycotoxins (Sucu et al. 2016; Hentz et al. 2017). Amaral et al. (2007) showed that low density of compaction resulted in greater gas production, while high density of compaction resulted in a greater preservation of DM content of *Brachiaria brizantha* cv. Marandu silage. In addition, reduced pH was associated with high density of compaction, indicating that the environment was more suitable for the proliferation of lactic acid-producing bacteria (Amaral et al. 2007). Santos et al. (2010a) demonstrated that high density of compaction in tropical forage silages was possible when NDF and ADF concentrations in the ensiled material were low and the in vitro digestibility was high.

Our results showed that DNM was negatively related to STA, possibly due to the adequate environment for the fermentation process, since the microorganisms prefer low molecular weight carbohydrates as soluble sugars (Hentz et al. 2017). The DM% and density of the ensiled material has significant impacts on the final result of the fermentation process, because when DM concentration exceeds the ideal, it is more difficult to achieve the desired level of compaction in the ensiling process. Average DM observed here was 358 ± 50.3 g/kg (mean ± SD), which was at the upper limit of the ideal range for corn silage (300–350 g DM/kg) suggested by Marafon et al. (2015). The positive correlations between DM% and parameters such as STA, TDN and TC lend weight to this suggestion (Table 1; Figure 2).

Table 1. Pearson correlation coefficients among evaluated variables in corn silages for dairy cattle on 20 farms in Paraná State, Brazil.

	DM	CP	ADF	LIG	NDF	TDN	TC	NFC	STA	DNM	DDM	IVDDM	IVDNDF	pH	EC
CP	-0.14														
ADF	-0.42*	0.00													
LIG	-0.08	0.09	0.40*												
NDF	-0.44*	0.01	0.83*	0.42*											
TDN	0.42*	0.00	-100.00*	-0.40*	-0.83*										
TC	0.39*	-0.77*	-0.16	-0.10	-0.13	0.16									
NFC	0.53*	-0.29*	-0.79*	-0.40*	-0.93*	0.79*	0.48*								
STA	0.68*	-0.25*	-0.64*	-0.32*	-0.65*	0.64*	0.55*	0.78*							
DNM	-0.48*	0.28*	0.06	0.00	0.01	-0.06	-0.30*	-0.11	-0.23*						
DDM	0.54*	0.11	-0.40*	-0.10	-0.46*	0.40*	0.12	0.45*	0.47*	0.47*					
IVDDM	-0.01	-0.08	-0.34*	-0.40*	-0.41*	0.34*	-0.05	0.35*	0.07	0.15	0.14				
IVDNDF	-0.08	-0.03	-0.14	-0.19	-0.13	0.14	-0.07	0.09	-0.01	0.14	0.07	0.31*			
pH	0.49*	0.10	-0.18	-0.09	-0.20*	0.18	-0.06	0.16	0.21*	-0.30*	0.24*	-0.09	-0.01		
EC	0.45*	0.34*	-0.28*	0.01	-0.26*	0.28*	-0.22*	0.16	0.18	-0.20*	0.27*	-0.14	0.12	0.31*	
APS	-0.34*	-0.13	0.19	-0.28*	0.03	-0.19	-0.17	-0.09	-0.24*	0.11	-0.23*	0.23*	0.00	-0.07	-0.37*

DM – dry matter (g/kg); CP – crude protein (g/kg DM); ADF – acid detergent fiber (g/kg DM); LIG – lignin (g/kg DM); NDF – neutral detergent fiber (g/kg DM); TDN – total digestible nutrients (g/kg DM); TC – total carbohydrates (g/kg DM); NFC – non-fiber carbohydrates (g/kg DM); STA – starch (g/kg DM); DNM – density on natural matter basis (kg NM/m^3); DDM – density on dry matter basis (kg DM/m^3); IVDDM – in vitro digestibility of dry matter (g/kg); IVDNDF – in vitro digestibility of NDF (g/kg DM); EC – electrical conductivity (μS/cm); APS – average particle size (mm).



DM – dry matter (g/kg); CP – crude protein (g/kg DM); ADF – acid detergent fiber (g/kg DM); LIG – lignin (g/kg DM); NDF – neutral detergent fiber (g/kg DM); TDN – total digestible nutrients (g/kg DM); TC – total carbohydrates (g/kg DM); NFC – non-fiber carbohydrates (g/kg DM); STA – starch (g/kg DM); DNM – density on natural matter basis (kg NM/m^3); DDM – density on dry matter basis (kg DM/m^3); IVDDM – in vitro digestibility of dry matter (g/kg); IVDNDF – in vitro digestibility of neutral detergent fiber (g/kg); EC – electrical conductivity (µS/cm); APS – average particle size (mm).

Figure 2. Factor analysis evaluating chemical, physical and nutritive parameters of corn silages for dairy cattle on 20 farms in Paraná State, Brazil.

The IVDDM was not related to DM% of silage, but we found evidence that excessive maturity in material at ensiling can result in reduced nutritional quality of silage, since STA, TDN and TC were negatively related to the fiber content (ADF and NDF), a factor which is emphasized by the extremely opposite positions of these variables along the axes in Figure 2. Still concerning the fiber levels in the silages, IVDDM was negatively correlated with NDF and ADF concentrations, indicating the importance of ensiling crops at the most appropriate maturity stage to obtain high quality silage (Souza Filho et al. 2011). In addition, the positive relationship between DDM and STA as well as IVDNDF indicated the importance of ensuring adequate compaction while ensiling forage material. Our results showed that DM% in silage and density are parameters that must be considered by producers to achieve better productivity results, since both are related to good quality indexes of the ensiled material (Figure 2), and are also supported by other studies in the literature (Santos et al. 2010b; Hentz et al. 2017).

It was interesting that CP concentration in ensiled material was negatively related to total carbohydrates, non-fiber carbohydrates and starch, which might all be expected to increase as plants matured, i.e. when cobs were formed and seeds were produced. Crude protein levels would also be expected to fall at this stage of growth, while at the same time, they are positively related to density on a natural matter basis, again following a logical pattern. As the plants mature the forage becomes more fibrous so the material is harder to compact, while CP% declines.

Electrical conductivity measurements have been used in studies carried out in Brazil (Jobim et al. 2007). The EC is defined as the ability that water has to conduct electrical current, which is related to the presence of dissolved ions. The EC values found in our study are lower than those found by Castro et al. (2006) evaluating *Cynodon* sp. silage (965 µS/cm). This measurement does not express specifically what ions are present in a given sample, but is related to the loss of intracellular material, i.e. soluble substances such as pectin, during the ensiling process as evidenced by its significant correlation with TC (r = -0.22*), CP (r = 0.34*) and TDN (r = 0.28*). The effects of fermentation products on EC are still not properly understood; however, the results can be used to draw inferences about adequate APS (r = -0.37*) and desirable DM% in forage at ensiling (Figure 2) (Jobim et al. 2007; Bumbieris Jr et al. 2010).

Adequate APS values for corn silages should be in the range of 8–12 mm, when DM content is in the range of 300–370 g/kg (Weirich Neto et al. 2013), which was the case for most samples we evaluated (Figure 1), limiting the extent of unwanted fermentations and improving preservation of the nutritional quality (Neumann et al. 2007) by allowing higher density of compaction (DDM; r = -0.23*).

In addition, we found that the APS of our samples was associated with higher digestibility of the ensiled material (IVDDM; r = 0.23*); it is important to emphasize that this correlation applies specifically to the data set we worked here (APS of samples in the range of 8–12 mm) since excessively long fiber particles are often correlated with reduced digestibility when compared with shorter particles. Hildebrand et al. (2011) emphasized that finely ground silage led to higher gas production on in vitro batch culture assay, while in a rumen simulation technique (Rusitec) assay, coarsely ground corn silage led to increased organic matter degradability, which the authors attributed to increased degradability of NFC and CP. Despite of observing a positive correlation between IVDDM and APS, the correlation had a relatively low r value (0.23) indicating that particle size explained very little of the variation in IVDDM values.

The APS is also important in diet formulation, since it is directly related to animal selectivity, rumination time, stability of ruminal pH, passage rate and microbial degradation and consequently affects animal production (Santos et al. 2010a; Marafon et al. 2015). Corroborating the idea that silage materials with high DM% usually present higher pH values (Senger et al. 2005), our results showed that pH was affected by compaction levels (DNM = -0.30), with greater pH values in low density samples, which may be associated with poor nutritional quality of the silage and reduced acceptability by the animals.

Conclusions

Our study has demonstrated that the corn silages produced on dairy farms used in our study varied greatly in parameters such as DM% and CP concentration but the most variable characteristic was the degree of compaction, which has such an important influence on the quality of the silage produced. Silages which were well compacted, i.e. with a high DDM, were also high in TDN, NFC and starch, but were low in fiber, i.e. were of better quality. On the other hand, more mature material at ensiling, i.e. with higher fiber levels, was difficult to compact and had lower DDM levels. These findings reveal a need for a technology transfer initiative to inform farmers of the variation which exists in terms of quality of silage produced and deficiencies in the silage making process. In utilization and conservation of forage there is always a trade-off between quantity and quality of material produced. In the case of silage, delaying harvesting until forage is quite mature can result in a poor outcome because of inappropriate levels of compaction combined with reduced quality of the forage ensiled. Farmers should be informed of the need to ensile material at a stage of growth when forage is still of good quality and good compaction of the ensiled material can be achieved. While high quality of the material at ensiling is positive, an added benefit is the reduced losses of nutrients during the fermentation process. When removed from the silo for feeding, the silage would retain much of its better quality at ensiling and resulting production from livestock would be greater.

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

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Research Paper

Biomass accumulation, phenology and seed yield of *Trifolium alexandrinum* ecotypes evaluated in Central India

Acumulación de biomasa, fenología y rendimiento de semilla de ecotipos de Trifolium alexandrinum *en India central*

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Abstract

Berseem or Egyptian clover (*Trifolium alexandrinum*) comprises 3 ecotypes, Miskawi, Fahli and Saidi, with Miskawi being the most widely cultivated. The narrow genetic base coupled with low availability and utilization of genetic resources is hindering genetic improvement of Berseem in India. Exploitation of new and diverse sources of variation is essential for the genetic enhancement of the cultivated genepool of Berseem. In the present study 7 populations of the 3 *T. alexandrinum* ecotypes were evaluated over 2 years to analyze the patterns of biomass accumulation, phenology, nutritional value and seed yield. Results indicate that Fahli and Saidi populations accumulated higher biomass per unit area than the tested populations of Miskawi and were earlier maturing. While crude protein (CP) concentration in forage was higher for Miskawi, Fahli and Saidi ecotypes contained more than 17% CP at 50% flowering. Further, seed yields of Fahli and Saidi populations were significantly higher than those of Miskawi. It is possible that genetic improvement of cultivated populations of Miskawi could be achieved by incorporating genes for dry matter yield and seed yield from the populations of Fahli and Saidi ecotypes.

Keywords: Berseem, dry matter yields, ecotypes, genetic improvement, legumes.

Resumen

El bersín o trébol de Alejandría (*Trifolium alexandrinum*) comprende los ecotipos Miskawi, Fahli y Saidi, siendo Miskawi el más ampliamente cultivado. La estrecha base genética y la baja disponibilidad y utilización de recursos genéticos son un obstáculo para el mejoramiento genético de esta leguminosa en la India. Por tanto la explotación de nuevas y diversas fuentes de variación genética es esencial. En el presente estudio, 7 poblaciones de estos ecotipos de *T. alexandrinum* fueron evaluadas durante 2 años para analizar los patrones de acumulación de biomasa, fenología, valor nutritivo y rendimiento de semilla. Los resultados indican que los ecotipos Fahli y Saidi produjeron más biomasa por unidad de área y fueron más precoces que las poblaciones del ecotipo Miskawi. No obstante en muestras de plantas con 50% de floración, la concentración de proteína cruda en el forraje fue significativamente mayor en las poblaciones de Miskawi (20%) que en las de Fahli y Saidi (17.4 y 18.0%, respectivamente). Por otra parte, los rendimientos de semilla de los ecotipos Fahli y Saidi fueron significativamente mayores que los de las poblaciones de Miskawi. Los resultados sugieren que es posible mejorar los rendimientos de biomasa y la producción de semilla de las poblaciones cultivadas de Miskawi mediante la incorporación de genes procedentes de los ecotipos Fahli y Saidi.

Palabras clave: Bersín, ecotipos, leguminosas, mejoramiento genético, rendimiento de materia seca, trébol de Alejandría.

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Introduction

The genus *Trifolium* comprises more than 250 species and is widely distributed, being best adapted to mesic or humid environments, in soils of moderate to high fertility and slightly acid to alkaline pH. *Trifolium* species play a key role in soil improvement by providing biological nitrogen fixation and a source of green manure. Ten *Trifolium* species are used as the major forage legumes in tropical upland, Mediterranean and temperate regions of the world with the most important being white clover (*T. repens*), red clover (*T. pratense*) and Berseem or Egyptian clover (*T. alexandrinum*) (Zohary and Heller 1984). The eastern Mediterranean region possesses the greatest species diversity and is believed to be the center of origin of the genus *Trifolium* (Vavilov 1926). Berseem or Egyptian clover ($2n = 2x = 16$) is an important annual forage legume and has been introduced from the Mediterranean region to many countries like India, Pakistan, South Africa, USA and Australia.

Trifolium alexandrinum is divided into 3 different bio/ecotypes, Miskawi/Miscavi, Saidi and Fahl/Fahli. The Miskawi, Saidi and Fahli ecotypes differ in morphology, yield and regrowth ability after cutting. Miskawi can be cut 4–6 times in a season, while Saidi can be cut twice and Fahli only once. Fahli berseem is a low branching cultivar and is more adapted to dry areas than the other ecotypes (Suttie 1999; Hannaway and Larson 2018). This species is commonly grown in Egypt and displays high level of morphological and molecular variability compared with Miskawi (Hussain et al. 1977; Muhammad et al. 2014). Miskawi was introduced into India in 1904 and has been widely utilized since 1916 as a major winter fodder due to its multi-cut (4–8 cuts) nature, ability to provide fodder for a long duration (November–May), very high green fodder yields (up to 85 t/ha), good forage quality (20% crude protein), high digestibility (up to 65%) and good palatability (Narayanan and Dabadghao 1972).

While Berseem produces high quality forage in India, aspects requiring improvement are dry matter (DM) yields from early cuts, initial vigor, extended vegetative growth and resistance to stem rot and root rot disease complexes (Malaviya et al. 2004b). However, owing to extensive genetic drift and natural selection over time and space, the genetic base of this crop in India is narrow (Verma and Mishra 1995) and needs to be broadened for targeted traits using different breeding approaches like hybridization, mutation, etc.

There are many species in the secondary and tertiary gene pool of *T. alexandrinum* L. such as *T. apertum* Bobrov, *T. meironense* Zohary & Lerner, *T. resupinatum* L., *T. constantinopolitanum* Ser. and *T. vesiculosum* Savo possessing genes for wide adaptability and resistance to biotic and abiotic stresses (Putiyevsky and Katznelson 1973; Malaviya et al. 2004a). However, cross-incompatibility barriers and linkage drags limit their exploitation for cultivar improvement (Putiyevsky and Katznelson 1973; Malaviya et al. 2018). For the development of interspecific hybrids with these species, the embryo rescue technique is needed (Malaviya et al. 2004b; Kaur et al. 2017). However, the primary gene pool of Berseem needs to be exploited before addressing the interspecific hybrids, which are coupled with problems like linkage drag.

Detecting and exploiting genetic variation in biomass accumulation and phenology is of great importance for increasing Berseem yield as well as development of plant ideotypes for different cropping systems. Therefore, we conducted an investigation to characterize a selection of *T. alexandrinum* lines for targeted traits that could be utilized for genetic improvement of this crop by breeders in the future.

Materials and Methods

Genetic material and location of experiment

Seven lines (populations) of *T. alexandrinum* comprising cvv. Wardan, Bundel Berseem-2 and Bundel Berseem-3 from Miskawi, JHBF-1 and JHBF-2 from Fahli, and JHBS-1 and JHBS-2 from Saidi ecotypes were used. The experiment was conducted for 2 consecutive years during winter (November–April) at the Central Research Farm of the ICAR-Indian Grassland and Fodder Research Institute (25°31' N, 78°32' E; 237 masl), Jhansi, India. The experimental site is characterized by a semi-arid climate with extreme temperatures during summer (43–46 °C) and winter (as low as 2 °C). The soil was deep, moderately well drained, and brown to dark grayish brown with fine loamy texture. Nitrogen (20 kg N/ha), phosphorus (60 kg P/ha) and farmyard manure (30 t/ha) were applied at sowing. The design was a completely randomized block (CRBD) with 3 replications. Each line was planted (second week of November) in 4 × 3 m plots with 10 rows of plants/plot. Line to line distance was maintained at 30 cm. In each plot equal plant populations were maintained by planting of equal numbers of viable seeds. Immediately after sowing a very light irrigation was applied followed by 2 light irrigations at 7 day intervals and 9 subsequent irrigations at intervals of 12–15 days. Meteorological data for the experimental period showed that in the first winter season (November 2016–April 2017) total rainfall was as low as 5.6 mm and 5.0 mm in the second winter season (November 2017–April 2018), while the mean monthly minimum and maximum temperatures during those 6 months were 12.2 and 29.4 °C, respectively, for 2016/17, and 16.6 and 29.8 °C, respectively, for 2017/18.

Data recording and statistical analyses

During the growing period, biomass yields (kg DM/ha) were measured on 4 occasions, viz. 45 days after sowing (DAS), 60 DAS, 75 DAS and at 50% flowering by clipping 2 rows (2.4 m^2) of each plot at 6 cm above ground level. Immediately after harvest, fresh forage yield was determined using a portable balance. A 500 g sample of fresh forage was taken from each plot and dried at room temperature without direct exposure to sunshine. When samples showed equal weight on 3 successive days, the weight recorded was considered as approximate dry weight and DM yields were determined. Dried samples of similar growth stage (50% flowering) material were ground for estimating the nutrient parameters in the 2016/17 cropping season. Crude protein (CP) percentage was estimated as per procedures of AOAC International (2005). Fiber fractions, namely neutral detergent fiber (NDF) and acid detergent fiber (ADF), were determined following the detergent method of Van Soest et al. (1991). Days to initiation of flowering, 50% flowering and maturity were recorded from planting date. Plant height (cm) and leaf:stem ratio were recorded on 5 plants in each plot at the 50% flowering stage. Plant height was measured from soil surface to tip of flower. For estimation of leaf:stem ratio (fresh weight) leaves and stems of clipped plants were hand-separated and weighed immediately. Seed yields (kg/ha) were recorded on open-pollinated plots and thousand-seed weights (g) were assessed.

Data were analyzed using PROC GLM (SAS Institute 2011). Mean data for populations were compared using the t-test. Shapiro-Wilk's test was used for normality of residual effect and homogeneity of variance was tested using Levene's test. Crop growth rate (CGR) was measured as dry biomass (kg/ha/d) accumulated in different growth stages using the following formula:

$$CGR = (W2 – W1)/(T2 – T1)$$

where:
W1 = Dry biomass at T1 of the period (kg/ha);
W2 = Dry biomass at T2 of the period (kg/ha);
T1 = Date at the start of the period; and
T2 = Date at the end of the period.

Results

Analysis of variance for biomass yield (green and dry) at 4 different growth stages, plus phenology and seed yields of the 7 different Berseem populations (lines) belonging to 3 different ecotypes was conducted to determine the variability between populations and years and any interactions. Population × year interactions were rarely significant so main effects only are presented. The majority of the diversity was attributable to differences between lines rather than between years. Year effects were significant only for days to initiation of flowering, 50% flowering and maturity, as well as seed yields.

Biomass yields (green and dry) of the populations belonging to Fahli and Saidi ecotypes were greater than those of Miskawi at all growth stages (Tables 1 and 2) and accumulated progressively with time irrespective of ecotype (Figure 1). At 75 DAS Fahli and Saidi ecotypes yielded 5.80 t DM/ha, while the Miskawi ecotype yielded 4.16 t DM/ha. Number of days to initiation of flowering, 50% flowering and maturity differed significantly between ecotypes with Fahli<Saidi<Miskawi (P<0.05; Table 3) with the main difference being between Fahli

Table 1. Fresh forage yield (kg green herbage mass/ha) of Berseem ecotypes and populations in two cropping seasons at different growth stages in Jhansi, India.

Ecotype	Population	Growth stage											
		45 days after sowing			60 days after sowing			75 days after sowing			50% flowering		
		Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean
Fahli	JHBF-1	11,278	10,978	11,128	26,320	26,220	26,270	38,617	38,285	38,451	39,083	38,301	38,692
	JHBF-2	11,952	11,651	11,801	25,775	25,675	25,725	35,677	35,361	35,519	36,177	35,453	35,815
	Mean	11,615	11,315	11,465	26,048	25,948	25,998	37,147	36,823	36,985	37,630	36,877	37,254
Saidi	JHBS-1	11,194	10,894	11,044	24,549	24,449	24,499	35,805	35,502	35,653	36,538	35,807	36,172
	JHBS-2	11,699	11,399	11,549	25,947	25,847	25,897	35,225	34,930	35,077	36,925	36,186	36,555
	Mean	11,447	11,147	11,297	25,248	25,148	25,198	35,515	35,216	35,365	36,732	35,997	36,364
Miskawi	Wardan	8,439	8,039	8,239	18,141	17,941	18,041	23,700	23,477	23,588	24,133	26,547	25,340
	Bundel Berseem-2	8,953	8,553	8,753	18,536	18,336	18,436	24,546	24,314	24,430	25,113	27,624	26,368
	Bundel Berseem-3	8,993	8,592	8,792	18,799	18,599	18,699	24,847	24,615	24,731	25,330	27,863	26,596
	Mean	8,795	8,395	8,595	18,492	18,292	18,392	24,364	24,135	24,250	24,859	27,345	26,101
Overall mean		10,358	10,015	10,187	22,581	22,438	22,510	31,202	30,926	31,064	31,900	32,540	32,220
LSD (P<0.05)		1,902	1,902	1,219	1,959	1,959	1,255	2,223	2,131	1,420	2,370	2,370	1,519

Table 2. Dry herbage mass (kg DM/ha) of Berseem ecotypes and populations in two cropping seasons at different growth stages in Jhansi, India.

Ecotype	Population	Growth stage											
		45 days after sowing			60 days after sowing			75 days after sowing			50% flowering		
		Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean
Fahli	JHBF-1	1,447	1,394	1,420	3,624	3,666	3,645	6,016	6,120	6,068	7,173	7,006	7,089
	JHBF-2	1,557	1,502	1,529	3,522	3,601	3,561	5,648	5,748	5,698	7,292	7,121	7,206
	Mean	1,502	1,448	1,475	3,573	3,634	3,603	5,832	5,934	5,883	7,233	7,064	7,148
Saidi	JHBS-1	1,423	1,371	1,397	3,494	3,312	3,403	5,749	5,641	5,695	7,139	6,972	7,055
	JHBS-2	1,473	1,420	1,446	3,704	3,651	3,677	5,798	5,690	5,744	6,591	6,437	6,514
	Mean	1,448	1,396	1,422	3,599	3,482	3,540	5,774	5,666	5,720	6,865	6,705	6,785
Miskawi	Wardan	821	766	793	2,664	2,607	2,635	4,197	4,143	4,170	4,367	5,019	4,693
	Bundel Berseem-2	887	830	858	2,631	2,576	2,603	4,225	4,171	4,198	4,332	5,241	4,786
	Bundel Berseem-3	906	848	877	2,678	2,622	2,650	4,151	4,098	4,124	4,249	5141	4,695
	Mean	871	815	843	2,658	2,602	2,629	4,191	4,137	4,164	4,316	5,134	4,725
Overall mean		1,216	1,162	1,189	3,188	3,148	3,168	5,112	5,087	5,100	5,878	6,134	6,005
LSD (P<0.05)		270	266	171	249	482	311	333	456	259	586	567	371

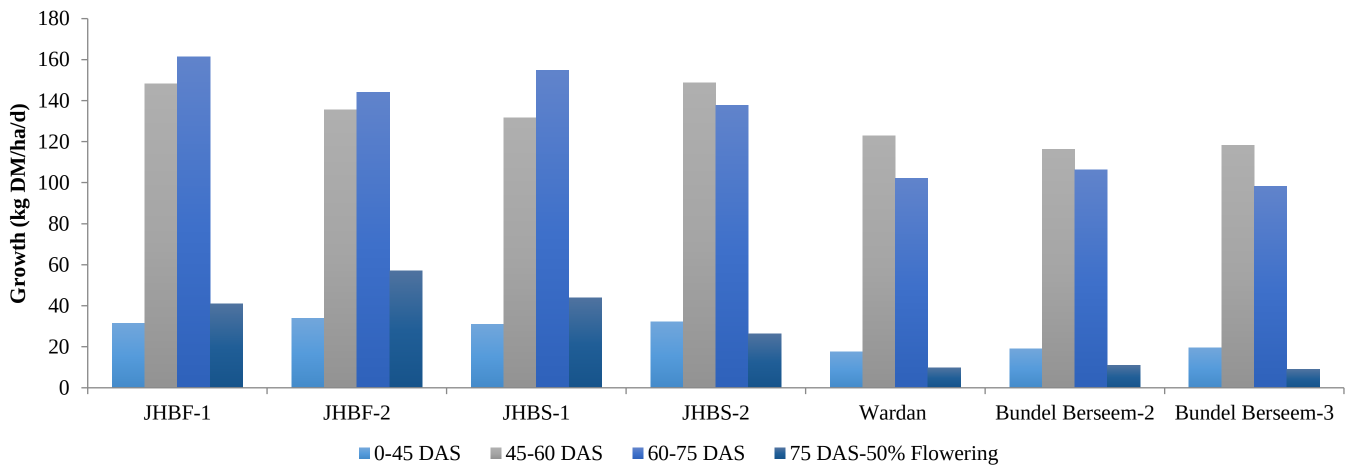

Figure 1. Dry biomass accumulation of Berseem populations at different growth stages in Jhansi, India. DAS = days after sowing.

Table 3. Flowering phenology of Berseem ecotypes and populations in two cropping seasons at different growth stages in Jhansi, India.

Ecotype	Population	Flowering stage								
		Days to initiation of flowering			Days to 50% flowering			Days to maturity		
		Yr I	Yr II	Mean	Yr I	Yr II	Mean	Yr I	Yr II	Mean
Fahli	JHBF-1	91	93	92	105	106	105	121	123	122
	JHBF-2	92	94	93	104	105	104	123	126	125
	Mean	92	94	93	104	105	105	122	124	123
Saidi	JHBS-1	97	99	98	105	109	107	121	134	128
	JHBS-2	96	97	97	104	109	106	123	136	130
	Mean	96	98	97	104	109	107	122	135	129
Miskawi	Wardan	116	118	117	128	129	128	161	162	162
	Bundel Berseem-2	114	117	115	126	129	128	162	163	163
	Bundel Berseem-3	123	123	123	138	140	139	168	171	169
	Mean	117	119	118	130	133	132	164	165	165
Overall mean		104	106	105	116	118	117	140	145	142
LSD (P<0.05)		1.9	2.0	0.7	2.4	1.6	0.8	1.8	1.6	0.6

plus Saidi and Miskawi. This difference increased as the stage of flowering advanced, the difference being 21–25 days at initiation of flowering and 36–42 days at maturity. Seed yields of Fahli and Saidi ecotypes exceeded those of Miskawi (750 vs. 414 kg/ha) as did 1,000-seed weights (P<0.001; Table 4).

Both Fahli and Saidi ecotypes were significantly taller (P<0.001) than Miskawi ecotype (Table 5) but were less leafy as reflected in the lower leaf:stem ratios. Crude protein concentration differed (P<0.05) between ecotypes, being highest in the Miskawi populations, but variation within ecotypes was non-significant (Table 5).

Variation in NDF and ADF concentrations between and within ecotypes was non-significant.

Table 4. Seed yields and 1,000-seed weights of Berseem ecotypes and populations in two cropping seasons in Jhansi, India.

Ecotype	Population	Seed yield (kg/ha)			1,000-seed weight (g)		
		Yr I	Yr II	Mean	Yr I	Yr II	Mean
Fahli	JHBF-1	803	767	785	3.65	3.64	3.65
	JHBF-2	730	730	730	3.65	3.61	3.63
	Mean	767	748	758	3.65	3.63	3.64
Saidi	JHBS-1	770	690	730	3.67	3.66	3.67
	JHBS-2	783	767	775	3.62	3.67	3.65
	Mean	777	728	753	3.65	3.67	3.66
Miskawi	Wardan	473	420	447	2.15	2.21	2.18
	Bundel Berseem-2	477	467	472	2.16	2.18	2.17
	Bundel Berseem-3	330	320	325	3.14	3.15	3.15
	Mean	427	402	414	2.48	2.51	2.50
Overall mean		624	594	609	3.15	3.16	3.16
LSD (P<0.05)		69	63	43	0.07	0.04	0.03

Table 5. Plant height, leaf:stem ratio and nutritive quality parameters of Berseem ecotypes and populations at 50% flowering in Jhansi, India.

Ecotype	Population	Plant height (cm)	Leaf:stem ratio	CP (%)	ADF (%)	NDF (%)
Fahli	JHBF-1	114.3	0.7	17.4	35.5	47.4
	JHBF-2	111.0	0.8	17.3	38.0	46.5
	Mean	112.7	0.8	17.4	36.7	47.0
Saidi	JHBS-1	109.0	0.7	17.8	34.7	44.9
	JHBS-2	109.0	0.7	18.2	34.9	45.9
	Mean	109.0	0.7	18.0	34.8	45.4
Miskawi	Wardan	90.3	0.9	19.9	34.7	44.3
	Bundel Berseem-2	88.3	0.9	20.5	33.7	44.7
	Bundel Berseem-3	81.0	0.8	19.6	34.7	44.4
	Mean	86.6	0.9	20.0	34.4	44.5
Overall mean		100.4	0.8	18.7	35.2	45.4
LSD (P<0.05)		18.7	0.1	1.7	2.5	7.6

CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber.

Discussion

This study has shown that Saidi and Fahli ecotypes of Berseem clover have distinct advantages over Miskawi ecotype in terms of early growth following planting and overall yield in the first growth cycle to flowering. DM yields from Saidi and Fahli ecotypes in the 100 days following planting were 6,800–7,100 kg DM/ha compared with 4,700 kg DM/ha for the Miskawi ecotype, i.e. a 47% increase in yield at 50% flowering. Not only did the Saidi and Fahli ecotypes demonstrate this DM yield advantage over Miskawi but also they did so in a much shorter time as days to 50% flowering were much shorter in the former ecotypes (106 vs. 132 days, respectively). In situations where the window of opportunity for growing these forages is limited to about 3 months, the Saidi and Fahli ecotypes would seem to be the varieties to choose. While CP% in Miskawi populations was higher than that of the Saidi and Fahli ecotypes (20 vs. 17.4 and 18.0%, respectively), the DM yield advantage for the latter ecotypes would offset the small quality benefit.

In fact the Fahli ecotype is grown as a catch crop in Egypt and represents about 20% of the total area sown to Berseem preceding major summer crops. It has high nutritional value and is very palatable in addition to being highly productive (Muhammad et al. 2014). In India, Fahli Berseem could be planted to utilize the gap of 60–70 days between two main crops or in areas where fields are fallowed after harvesting of the rice crop. Rice fallow land represents a huge resource that could be utilized for fodder/nutritional security through planting of high-growth-rate ecotypes of Berseem (Fahli/Saidi), while ensuring sustainability of land resources with the cereal-legume rotational cropping system.

Seed yield potential of cultivated varieties of the Miskawi ecotype proved very low. Owing to its low productivity and greater demand for fodder during the lean period of summer, commercial seed production of clover is not highly successful in India (Vijay et al. 2017). The present study showed that seed yield potential of Fahli and Saidi ecotypes exceeded that of Miskawi. There is possible scope to increase seed yield potential of the cultivated Miskawi ecotype by transfer of genes from Fahli and Saidi. The poorer seed yields of Bundel Berseem-3 relative to Wardan and Bundel Berseem-2 may be due to the tetraploid nature of Bundel Berseem-3.

Seed size is a widely accepted measure of seed quality and an important seed yield component in crop species (Egli et al. 1987). Increased seed size has been positively associated with germination, seedling height, root length, primary leaf size and seedling weight, crop performance and yield potential in different species (Chandra Babu et al. 1990; Bretagnolle et al. 1995; Assis et al. 2018). Seed sizes of Fahli and Saidi ecotypes were much larger than Miskawi, although within Miskawi populations variation existed for seed size. This may be due to ploidy differences, because cultivars Wardan and Bundel Berseem-2 are diploid and the bold-seeded Bundel Berseem-3 is a polyploid ($2n = 4x = 32$) variety (Pandey and Roy 2011). Effects of ploidy on seed size have been reported in other species (Scott et al. 1998; Miller et al. 2012).

Conclusions

Both Saidi and Fahli ecotypes of Berseem seem to have distinct advantages over the Miskawi ecotype with more rapid establishment, more rapid growth and earlier maturity. In situations where short-term crops are needed to fill winter feed gaps, they would seem to be the varieties of choice. The slower establishment of Miskawi ecotype could possibly be improved through genetic methods.

Genetic improvement of Berseem crops in India has not been pursued in the past due to lack of variability for targeted traits in the Miskawi gene pool of *T. alexandrinum*. Researchers have attempted to exploit the secondary and tertiary gene pools of Berseem, which is associated with problems like cross-incompatibility barriers and linkage drags. Our investigation has shown that genetic variability for many agronomic traits exists at the ecotype level in the primary gene pool of Berseem. It is possible that genes present in one ecotype could be transferred successfully into other ecotypes of Berseem without biotechnological tools as needed in interspecific hybridization. Both Fahli and Miskawi ecotypes could be used as parents to develop mapping populations. Such a mapping population could be utilized in future for the development of linkage maps and to map a range of quantitative traits including days to flowering, regrowth potential, dry matter yields etc. Due to their rapid maturation and high biomass accumulation rate, Fahli and Saidi ecotypes could be used as catch crops during the gap between *kharif* (July–October) and *rabi* (November–April) crops in different cropping systems. In some areas where land is fallowed after harvesting of a rice crop, single-cut Berseem could be grown on residual moisture from the rice crop.

Every year India imports a huge quantity of Berseem seed from abroad. For self-sufficiency of Berseem seed, the low productivity of Miskawi varieties could be improved through a seed yield improvement program utilizing Fahli and Saidi ecotypes as parent populations for introduction of traits to enhance seed yield. More sources of germplasm must be targeted to increase diversity beyond the variability available in the Berseem genepool currently available in India to improve the cultivar development program.

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Research Paper

Development, rooting and nodulation of mororó (*Bauhinia cheilantha*) cuttings harvested in different seasons

Desarrollo, enraizamiento y nodulación de esquejes de mororó (Bauhinia cheilantha) *cortados en diferentes estaciones.*

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Abstract

To increase the establishment options of the tropical forage legume tree, mororó (*Bauhinia cheilantha*), a native of the Caatinga vegetation in Northeast Brazil, a vegetative propagation study was carried out. In 2 experiments the performance of cuttings taken from 2 different locations on the mother plant (apical and basal branches) was evaluated on 4 different substrates: washed sand (SA); soil (S); soil in a moist chamber (S+MC); and vermiculite (V), in which cuttings were 'planted'. The variables analyzed were: bud emergence; presence of expanded leaves; length and width of expanded leaves; and development of roots. For the first experiment, cuttings were taken in the dry season (December), for the second experiment in the rainy season (June). Cutting season had the major effect on all variables, particularly emerging buds (37–90% in the dry vs. 1–34% in the rainy season) and expanded leaves (23–60% in the dry vs. 1–13% in the rainy season). The best results were obtained in substrates S+MC and SA, the latter applying particularly for root development. It seems cuttings can be taken from any part of the mother plant but should be taken in the dry season, when an adequate supply of nutrients exists in the branches. Further studies are warranted to determine how to increase the success of root development on cuttings.

Keywords: Caatinga, cutting season, forage trees, tropical legumes, vegetative propagation.

Resumen

Para aumentar las opciones de establecimiento del mororó (*Bauhinia cheilantha*), un árbol forrajero nativo de la vegetación de Caatinga en el noreste de Brasil, se realizó un estudio de propagación vegetativa con esquejes de ramas apicales y basales que fueron plantados en 4 sustratos diferentes: arena lavada (SA); suelo (S); suelo colocado en una cámara húmeda (S+MC); y vermiculita (V). En 2 experimentos, uno en época seca (diciembre) y el otro en época lluviosa (junio), se analizaron las variables: brotes emergentes; presencia de hojas expandidas; largo y ancho de las hojas expandidas; y desarrollo de raíces. La época tuvo el mayor efecto en todas las variables, particularmente el desarrollo de brotes (37–90% en la época seca vs. 1–34% en la época lluviosa) y hojas expandidas (23–60% en la primera vs. 1–13% en la segunda). Los mejores resultados se obtuvieron con el uso de S+MC y SA; especialmente en el desarrollo de raíces en el medio SA. No se observaron diferencias entre esquejes de ramas apicales y basales, pero sí entre épocas de colecta,

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siendo la época seca más adecuada para la toma de esquejes, probablemente debido a mayor disponibilidad de nutrientes en las ramas. Se requieren estudios para determinar cómo aumentar el desarrollo de raíces en los esquejes.

Palabras clave: Árboles forrajeros, Caatinga, época de colecta, leguminosas tropicales, propagación vegetativa.

Introduction

The Brazilian Northeast Caatinga Biome covers an extensive area, characterized by a semi-arid climate, with stochastic rainfall events, 300–1,000 mm/year concentrated in 3–5 months during the year (January–May varying by subregion). The region experiences high evaporation rates (Silva et al. 2012). Its main vegetation is trees and shrubs, with specific adaptations to their harsh habitats, such as loss of leaves in the dry period, small leaves, thorns and other xerophytic adaptations (Silva et al. 2017). As in other tropical and subtropical arid and semi-arid ecosystems, also in the Caatinga Leguminosae make up an important part of the native vegetation (Muir et al. 2019).

Among the leguminous forage tree species in the Caatinga, *Bauhinia cheilantha* (Bong.) Steud. (mororó) is highly palatable to cattle (Ydoyaga-Santana et al. 2011) and is considered potentially important for introduction into pastures (Lira Júnior et al. 2013). However, legume introduction into pastures and rangelands can be hampered by several factors such as lack of commercial seed sources, impermeable seeds, seedlings displaying low vigor resulting in slow establishment, low seed yields, pod dehiscence and low persistence under continuous stocking (Muir 2019). Mororó seed, for example, suffers from integument impermeability (Gutiérrez et al. 2011).

Nevertheless, propagation by cuttings presents an alternative establishment method which can be employed throughout the year, thus circumventing the problems associated with using seeds, and gives uniform stands. Vegetative propagation has distinct advantages for expediting breeding programs, e.g. for distribution of sterile materials and for planting in non-arable locations (Shelton 2019).

Vegetative propagation by cuttings can be influenced by several factors, including the plant's self-inherent characteristics, environmental conditions and the period of the year when cuttings are collected (Ahkami et al. 2013). For each cutting type (apical or basal), optimal size for rooting should be considered, as reserves of nutrients in the cutting and the number of buds on the cutting are important determinants of success (Pizzatto et al. 2011).

The objective of this study was to evaluate the effects of different harvest periods, cutting types and substrates on the initial establishment of *B. cheilantha* cuttings.

Materials and Methods

Two experiments were conducted at the Department of Animal Science of Federal Rural University of Pernambuco, Dois Irmãos, Pernambuco, Brazil: Experiment 1 with cuttings harvested in the dry season (December 2003) and Experiment 2 with cuttings harvested in the rainy season (June 2004).

The experimental treatments were combinations of 2 types of cuttings (apical and basal) and 4 substrates [washed sand (SA), soil (S), soil in a moist chamber (S+MC) and vermiculite (V)], under a completely randomized design with 4 replicates. Whereas SA is a substrate free of salts, silt and clay, V is an industrial mineral substrate with its particular advantage being lightness, cleanness and high water-holding capacity. The soil used came from the Experimental Station of the Agronomic Institute of Pernambuco (IPA) in São Bento do Una municipality, Pernambuco, Brazil, is classified as a Regolithic Neosol with a loamy-sandy texture (Santos et al. 2013) and is considered of medium fertility.

The cuttings were harvested in a mororó forest at the same IPA Experimental Station where the soil originated from, in the dry season (December 2003; accumulated rainfall from August to December 2003: 89 mm) for Experiment 1, and in the rainy season (June 2004; accumulated rainfall from January to June 2004: 763 mm) for Experiment 2.

Apical cuttings were harvested from branches located in the upper part of the plant, and basal cuttings from the lower part. All cuttings, 15–20 cm long, with a horizontal section at the base, no leaves plus similar diameters (mean 7.5 mm) and 8–10 buds, were taken from a single adult plant. After collection, the cuttings were transported in coolers to the experimental site at Dois Irmãos.

Eight PET bottles with a capacity of approximately 1.5 kg were filled with the respective substrate to form each experimental unit. Each bottle had newspaper at the bottom with a hose to drain excess water and was irrigated daily to keep the substrate moist.

The cuttings of the treatments SA, S and V were kept in a standard greenhouse whereas the moist chamber for treatment S+MC consisted of a white 12 L plastic container, closed with a transparent acetate box. Plastic cups with 50 mL of water were kept inside to ensure the environment remained saturated, as well as the PET bottles with the plants from that treatment.

The following variables were measured: emergence of buds; presence of expanded leaves; and length and width of expanded leaves. After 120 days, rooting percentages of the cuttings and presence of nodules were determined.

Statistical analyses were performed separately for each experiment (season of year) using the SAS statistical software with means compared by Tukey test at P<0.05 (SAS 2012).

Results

Experiment 1

For cuttings collected in the dry period, there was no significant effect (P>0.05) of cutting type, i.e. apical or basal, nor any interaction between cutting type and substrate, so data were pooled for both cutting types (Table 1).

Substrate type had a significant effect (P<0.05) on percentage of cuttings which produced buds, expanded leaves and roots plus length and width of expanded leaves. The substrate S+MC promoted the highest percentage of buds (89.7% of cuttings), while S had the lowest number of cuttings which produced buds (37.3%) and fully expanded leaves (22.9%). Rooting of cuttings was significantly higher in V (19.1%) than in both substrates involving soil, where very low percentages of cuttings produced roots (2.4 and 0.8%). Both V and SA produced longer and wider leaves on cuttings than did straight soil.

Some nodules were found on roots in the S+MC after 120 days of culture.

While no statistical comparison between sampling periods was done, higher values were found for bud, expanded leaves and rooting percentages for cuttings obtained during the dry season, without any apparent difference for leaf width or length (Table 1).

Experiment 2

For cuttings taken in the rainy season, no significant effect was observed (P>0.05) for cutting type, nor was there any interaction between cutting type and substrate, so data were pooled for the 2 cutting types. Substrate type had a significant effect (P<0.05) on the percentage of cuttings which produced buds and expanded leaves plus length and width of expanded leaves (Table 1).

Percentage of cuttings producing buds was higher for those grown in S+MC and SA than for those grown in S.

Substrate had no significant effect (P>0.05) on percentage of rainy season cuttings producing roots, the only substrate producing any rooting being S+MC (0.8%).

Discussion

This study has shown that season when cuttings were taken had the greatest overall effect on production of buds, expanded leaves and roots on cuttings of mororó. It is possible that the difference between seasons is linked to amounts of substances stored in the cuttings, as during the rainy season the tendency is for more intense growth, with translocation/consumption of these substances in the branches.

While cuttings taken during the dry season generally produced buds and expanded leaves at an acceptable level, those taken in the rainy season produced at a much lower level, barely better than a third of that in the dry. While root development was also affected by time when cuttings were taken, only SA and V substrates produced acceptable levels of root development and then only for cuttings taken in the dry season.

Table 1. Percentages of mororó (*Bauhinia cheilantha*) cuttings harvested during the dry and rainy seasons which produced buds, expanded leaves and roots in different substrates plus dimensions of leaves produced.

Substrate	Dry Season (Experiment 1)					Rainy Season (Experiment 2)				
	Buds (%)	Expanded leaves (%)	Leaf length (cm)	Leaf width (cm)	Rooting (%)	Buds (%)	Expanded leaves (%)	Leaf length (cm)	Leaf width (cm)	Rooting (%)
Washed sand (SA)	62.4b[1]	47.6a	2.7ab	3.0ab	16.4ab	27.2ab	12.6a	1.7a	2.3a	0.0a
Soil + Moist Chamber (S+MC)	89.7a	62.3a	1.9bc	2.1bc	2.4bc	34.0a	13.4a	1.2ab	1.3ab	0.8a
Soil (S)	37.3c	22.9b	1.7c	1.9c	0.8c	0.5c	0.5b	0.1c	0.1c	0.0a
Vermiculite (V)	56.1b	46.8a	2.9a	3.3a	19.1a	13.0b	5.0ab	0.7bc	0.8bc	0.0a
Mean	61.4	44.9	2.3	2.6	9.7	18.7	7.9	0.9	1.1	0.2
CV (%)	10.0	14.5	10.4	10.4	57.1	30.4	36.4	17.4	43.6	20.5

[1]Means within columns followed by the same letter are not significantly different at P<0.05 by Tukey test.

The role of leaves in the rooting of semi-woody cuttings is related to photosynthesis, and the supply of carbohydrates, auxins and rooting cofactors, which are transported to the base of the cuttings (Lima et al. 2011). In this way, Bowerman et al. (2013) suggested that, providing good soil characteristics, e.g. adequate aeration and drainage, and a relatively consistent but moderate amount of moisture should ensure faster and better quality root development. Despite producing the highest levels of both buds and expanded leaves, cuttings grown in S+MC had poor root development, which suggests that factors other than adequate leaf development determined the level of root development in this study.

The fact that cuttings grown in sand had the second highest budding (>50%) and expanded leaf percentages (47%) (Table 1) plus satisfactory root production (16%) for cuttings taken in the dry season, was opportune as sand has many advantages as a substrate, since it is low-cost, easily available and has positive drainage characteristics (Almeida et al. 2008).

The effects of season when cuttings were taken on success rate agreed with the findings of Santos and Diodato (2017) who worked with algaroba [*Prosopis juliflora* (Sw.) DC.]. They reported that the dry season was the ideal time to collect cuttings in Petrolina, Pernambuco's semi-arid area, independent of cutting type (basal or apical).

The emergence of expanded leaves was lower than the development of buds for all substrates, which may help explain the poor development of roots, since leaves are necessary for the survival of cuttings, as they provide the carbohydrates produced by photosynthesis, plus auxins and other substances for root development and growth (Ahkami et al. 2013).

The substrate which promoted the highest percentage of buds was S+MC; this is probably associated with a greater water availability for the cuttings, a fundamental factor, especially in the initial growth phase.

Although 90% of dry season cuttings cultivated in S+MC produced buds and 60% produced expanded leaves, continued development would likely be compromised by the low degree of root formation (2.4%). While the cuttings presented lots of buds, probably due to the presence of nutrient reserves in the cuttings, these reserves seemed insufficient to promote root development. The failure of cuttings to develop roots may be associated with nutritional deficiency in plants at the collection site, but this is speculation as we have no supporting evidence. It was reassuring that V and SA substrates allowed development of roots (16–19%) on dry season cuttings, as no hormones were applied to stimulate root development. It appears that substrate had an over-riding influence on root development as cuttings in both substrates including soils produced virtually no roots. Rooting of cuttings is dependent on many factors, both internal and external, e.g. the mother plant's nutritional and phytosanitary condition, genetic potential, hormonal balance, collecting period, temperature and humidity (environmental conditions), etc. (Gratieri-Sossella et al. 2008; Pizzatto et al. 2011). Natural climate factors, such as temperature and photoperiod, may also explain the year effects that we observed. In our study all cuttings were obtained from the same mother plant so most of the above factors can be ruled out in explaining why differences in rooting success between substrates were obtained in the dry season. More studies seem warranted to determine if the substrate plays an important role in rooting success. There seems little merit in repeating the seasonal comparisons as other studies, e.g. Santos and Diodato (2017), have previously found that dry season cuttings provide the optimal outcomes.

Although Song et al. (2010) reported that plants of the genus *Bauhinia* are probably non-nodulating, some nodules were found on roots in the S+MC substrate. Sprent et al. (2017) indicate that there are many non-nodulated caesalpinioid legumes in the New World tropics, for example *Bauhinia* and *Caesalpinia*.

The ability to grow uniform mororó cuttings could be of importance in cultivation of this species, which is well adapted to semi-arid conditions and is highly palatable to animals. Further studies are needed to determine appropriate substrates plus additives, e.g. hormones, which might stimulate root development, to expedite the successful adoption of this species by farmers.

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Genetic Resources Communication

Clearing confusion in *Stylosanthes* taxonomy: 1. *S. seabrana* B.L. Maass & 't Mannetje

Aclarando confusiones en la taxonomía de Stylosanthes: *1.* S. seabrana *B.L. Maass & 't Mannetje*

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Abstract

Stylosanthes seabrana was first formally described as a new species in 2002 following extensive morphological and agronomic characterization, accompanied by genetic and molecular studies. Since then it has been proposed as a synonym of *Stylosanthes scabra* Vogel. This paper refutes this synonymization and indicates the indisputable evidence that *S. seabrana*, a diploid, is a likely putative progenitor of the allotetraploid *S. scabra*.

Keywords: Agronomy, cytology, morphology, phylogeny, rhizobiology, *Stylosanthes scabra*.

Resumen

Stylosanthes seabrana fue formalmente descrita como una especie nueva en 2002, como resultado de extensivas caracterizaciones morfológicas y agronómicas, junto con estudios genéticos y moleculares. En 2011 se propuso que se trata de un sinónimo de *Stylosanthes scabra* Vogel. En este trabajo se refuta esta sinonimización y se muestra que existen múltiples estudios para indicar que *S. seabrana*, una especie diploide, es probablemente un progenitor putativo de *S. scabra*, una especie alotetraploide.

Palabras clave: Agronomía, citología, filogenética, morfología, rizobiología, *Stylosanthes scabra*.

Introduction

Since recognition in Australia of the forage value of the adventive species, *Stylosanthes humilis* Kunth, in the early 20th century, there has been continuing focus on the genus, *Stylosanthes*, to determine the commercial pasture potential of other species within the genus. Of the 40 species of *Stylosanthes* currently accepted by the US National Plant Germplasm System (GRIN), 7 have been demonstrated to have commercial agricultural merit. Large collections of a number of species were assembled by CIAT in Colombia and CSIRO in Australia, including shrubby stylo (*Stylosanthes scabra*) that was found to have potential in the acid, infertile soils of subhumid and semi-arid northern Australia. The most recent addition to the list of commercial species in the genus, *S. seabrana* B.L. Maass & 't Mannetje, has proven well-adapted to the slightly acid to alkaline, more fertile clay and clay-loam soils in the same region, but extending into the subtropics.

Taxonomy of *Stylosanthes seabrana*

While characterizing the *S. scabra* collection held by CIAT in Colombia, Maass (1989) identified a group of plants from Bahia state in Brazil that shared a number of morphological characteristics with *S. scabra* but were morphologically and agronomically different from *S. scabra* and other known species of *Stylosanthes*. Following the provisional name given to this form by plant collectors, she referred to the group in her classification as "cf. *scabra*-Type". This

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promising phenotypic group was subsequently referred to as *S.* sp. aff. *scabra* by Jansen and Edye (1996), and eventually as "*Stylosanthes seabrana*" by Edye et al. (1998), accepting that *S.* sp. aff. *scabra* was indeed a different species from *S. scabra*. The name was selected in reference to the town of Seabra in the region of Bahia state, where the earliest accessions of the species were collected. The scientific name, *Stylosanthes seabrana*, was formalized by Maass and Mannetje (2002). The common name, Caatinga stylo, was adopted in Australia, referring to the xerophytic Caatinga vegetation type in northeastern Brazil on the medium- to heavy-textured soils on which the species is largely found. Vanni and Fernandez (2011) disputed the conclusion of Maass and Mannetje (2002), claiming instead that *S. seabrana* is a synonym of *S. scabra*, a claim that is hereby rejected based on a comprehensive assessment of all relevant information/evidence.

Morphology

To help direct future plant evaluation in the sub-humid/semi-arid tropics of Australia, and to obtain a clearer picture of the taxonomic and agronomic boundaries of a number of promising *Stylosanthes* species, morphological and agronomic classificatory experiments involving large numbers of entries of *S. scabra*, *S. hamata* (L.) Taub. and *S.* sp. aff. *scabra* (= *S. seabrana*) were conducted at CSIRO Lansdown Research Station, north Queensland (Jansen and Edye 1996; Date et al. 2010). Each used a numerical classification program, PATN (Belbin 1995) that, at the 5 group level, separated entries largely into homogeneous groups, with *S.* sp. aff. *scabra* separated from *S. scabra*. Shapes of the terminal leaflet and the terminal leaflet apex, the presence or absence of leaflet and stem hairs, the presence or absence of inflorescence bristles, the presence or absence of stipule horn lateral bristles and stipule horn terminal bristles were the most useful attributes defining groups. Maass and Mannetje (2002) used the most consistent of these and other observations to develop a key to distinguish the 3 species morphologically.

Key to *Stylosanthes seabrana*, *S. hamata* and *S. scabra*:

1a. Beak equal to or exceeding the upper article, leaflets without bristles……..…………..……………………… *S. hamata*

1b. Beak shorter than the upper article, leaflets with bristles

- 2a. Leaflets narrowly elliptical, glabrous except for long bristles on the margins and midrib and prominently raised veins on the lower surface…………... *S. seabrana*
- 2b. Leaflets elliptical to obovate, pubescent with bristles at least underneath or on the margins without prominently raised veins on the lower surface... *S. scabra*

Many collections and studies of *Stylosanthes scabra* have been conducted since Vogel (1838) described the specimen from Serra da Moeda, Minas Gerais, Brazil and Mohlenbrock (1957) reviewed the genus, *Stylosanthes*. On this basis, it can be presumed that the Edye and Topark-Ngarm (1992) description based on research experience and the description of Costa and Ferreira (1984) might be more comprehensive than earlier keys. Vanni and Fernandez (2011) provide what they call a "standard description" of *S. scabra*, which differs from those of Vogel (1838), Mohlenbrock (1957) and Costa and Ferreira (1984)/Edye and Topark-Ngarm (1992), all varying somewhat in their choice of descriptors. However, some characteristics provided in the various keys help to further distinguish *S. seabrana* from *S. scabra* morphologically. A characteristic not used in the Maass and Mannetje (2002) key is the length of the axis rudiment, 7–8 mm in their description of *S. seabrana* and 4–5 mm in *S. scabra* (Mohlenbrock 1957; Edye and Topark-Ngarm 1992).

Agronomy

There are clear agronomic differences between *S. seabrana* and *S. scabra*. Early research in the 1960s and 1970s to identify other *Stylosanthes* species to extend the range of *S. humilis* identified the potential of *S. scabra* and the tetraploid form of *S. hamata* (= *S. hemihamata* nom. nud.), resulting in the release of cultivars of each. However, while these were very effective in the light, acid infertile soils of northern Australia, they were not adapted to the heavier, more fertile clay soils in the region. Attention was then turned to the group of *Stylosanthes* sp. aff. *S. scabra* that were collected on broadly similar soils in Brazil (Edye and Maass 1997). These proved well-adapted to heavy- and medium-textured alkaline soils in Australia, and unlike *S. scabra*, were also adapted to the more frost-prone environment of southern Queensland (Edye and Hall 1993; Jansen and Edye 1996). CSIRO applied for Plant Breeders Rights for the 2 most promising lines in 1996 (granted in 1997) as "Caatinga Stylo (*Stylosanthes* sp. nov. aff. *S. scabra*) cvv. Primar and Unica" to provide a legume base for forage systems on neutral to alkaline soils of central and southern Queensland.

Early evaluation highlighted another important difference between the 2 species. While *S. scabra* is promiscuous in its root nodule bacterial requirements, nodulating effectively on native strains of *Bradyrhizobium* in Australia or the broad spectrum CB 756 commercial strain (Date 1997), this was not the case for Caatinga stylo. During field evaluation at a range of sites in Queensland in the 1990s, Caatinga stylo accessions nodulated poorly and ineffectively and frequently failed to nodulate at all (Edye 1994; Edye et al. 1998). Most accessions grew well for 1 or 2 years, before beginning to show classical signs of nitrogen deficiency. Success of the new cultivars was contingent on discovery of an effective and persistent strain of inoculum. Accordingly, nodules were collected during germplasm collections in Brazil, and strains of *Bradyrhizobium* were isolated, tested and released prior to release of cvv. Primar and Unica (Date 2010; 2016).

Ploidy

A major part of the argument advanced by Vanni and Fernandez (2011) revolves around their finding both diploid and tetraploid specimens in the roots of seedlings grown from a sample of commercial seed of *S. seabrana* cv. Unica from Australia. In their Introduction, they make the following confusing statement: "In addition, they (referring to Maass and Mannetje 2002) reported different levels of ploidy in *S. scabra*, 2*n* = 40 chromosomes and *S. seabrana*, 2*n* = 20 chromosomes." The ploidy cited for the 2 species is correct; however it in no way supports their contention of dual ploidy in *S. scabra*. Rather, Vanni and Fernandez (2011) use this confusing statement to support their claim that: "ploidy levels are not valid criteria for species distinction in the genus *Stylosanthes*, as *S. scabra* has been reported to be one of the few species with diploid (2*n* = 20) and tetraploid (2*n* = 40) genotypes (Cameron 1967)." This is not the case. In fact, Cameron (1967) determined the chromosome number for a single accession of *S. tuberculata* (presumably *Stylosanthes tuberculata* S.F. Blake syn. *S. scabra* Vogel), which he found to be tetraploid (2*n* = 40) only. Since then a number of workers (Battistin and Martins 1987; Liu et al. 1999; Lira 2015) have reported tetraploidy in *S. scabra.* No report of diploidy in the species exists in the published literature.

'Unica' was derived from CPI 110361, which has been shown to be diploid (Liu and Musial 1997), so the question arises: how could there have been the 2 ploidy levels in the sample tested by Vanni and Fernandez (2011)? The answer lies in the fact that the seed lot on which Vanni and Fernandez (2011) based their taxonomic revision was a commercial sample. Since seed crops of both *S. scabra* and *S. seabrana* are grown in the same general area in north Queensland, it is probable that a commercial sample of seed may contain both species, either from contamination in the crop (*S. scabra* is now naturalized in the region), in the harvester from a previously harvested crop of *S. scabra* or during post-harvest handling. There is no seed certification scheme for this cultivar in Australia and post-harvest cleaning procedures for harvesting machinery are not as stringent for standard commercial crops as for certified crops.

Phylogeny

Until relatively recently, morphological characters were the only means of describing species, but they have not always provided the level of resolution required to categorically define interspecific and intraspecific differences. Vanni and Fernandez (2011) consider that the form of leaflets, the absence or presence of bristles and hairs on stipules and leaflets and their venation are not sufficient to separate species. Whether or not this is valid is debatable. However, the evidence provided from genetic and molecular studies is indisputable. As discriminatory methodologies improved with the development of molecular technologies, so did the evidence to more clearly define relationships within and between taxonomic groups.

It has been shown that *S. scabra* is an allotetraploid with *S. viscosa* Sw. as one of the putative diploid progenitors (Stace and Cameron 1984; Vander Stappen et al. 2002). The identity of the other diploid progenitor is not so clear-cut. Stace and Cameron (1984) postulated that, since *S. scabra* bears an axis rudiment on the loment, a characteristic governed by a dominant gene, and *S. viscosa* lacks an axis rudiment (section *Stylosanthes*), the other parent must bear an axis rudiment (section *Styposanthes*). Working with chloroplast DNA, Gillies and Abbott (1996) proposed *S. hamata* sensu stricto as the section *Styposanthes* progenitor, while Liu and Musial (1997) provided evidence that the other putative progenitor was *Stylosanthes* sp. aff. *S. scabra* (= *S. seabrana*). These 2 species fell into the same basal genome group A, determined by restriction fragment length polymorphisms (RFLP) and sequence-tagged-sites (STS) analyses by Liu et al. (1999). In the same study, *S. viscosa* fell into basal genome group B and *S. scabra* into group AB. More recent work (Tewari and Chandra 2008; Chandra and Kaushal 2009; Marques et al. 2018) confirms the proposition of allotetraploid origins of *S. scabra* with *S. hamata* or *S. seabrana* as the maternal donor and *S. viscosa* as the paternal donor. However, Marques et al. (2018) point out the difficulty in precise identification of the maternal donor since both the diploid and the polyploid species have diverged since the allopolyploidy event some 0.63 to 0.52 million years ago.

Conclusion

Stylosanthes seabrana is clearly morphologically, agronomically, rhizobially, cytologically and phylogenetically different from *S. scabra* (Appendix I), and taxonomic logic dictates that it must be treated as a separate species. It is no more conspecific with *S. scabra* than is its other putative progenitor, *S. viscosa*. Similar confusion is faced by practitioners in relation to 2 other *Stylosanthes* diploid-allotetraploid derivative pairs, *S. hamata* - *S. hemihamata* nom. nud. and *S. macrocephala* - *S. capitata*, that will be dealt with in subsequent papers in this series.

Taxonomists at the US Germplasm Resources Information Network (GRIN; https://npgsweb.ars-grin.gov/gringlobal/taxon/abouttaxonomy.aspx) have reviewed their earlier decision to accept the Vanni and Fernandez (2011) thesis of synonymy between *S. seabrana* and *S. scabra* and have now listed *S. seabrana* as a valid species. A list of all *S. seabrana* germplasm accessions registered in the major *Stylosanthes* genebanks is presented as Appendix II. All accessions with known origin have been collected in Bahia State, except for ser. nos. 15 and 16 which are from Minas Gerais, Brazil.

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Appendix I. Differences in brief between *Stylosanthes seabrana* and *S. scabra*.

Characteristic/trait	*S. seabrana*	*S. scabra*
Leaflet shape	Narrowly elliptical	Elliptical to obovate
Leaflet indumentum	Glabrous except for long bristles on the margins and midrib	Pubescent with bristles at least underneath or on the margins
Leaflet venation	Prominently raised veins on the lower surface	Without prominently raised veins on the lower surface
Length of axis rudiment	7–8 mm	4–5 mm
Ploidy	Diploid (2*n* = 20)	Tetraploid (2*n* = 40)
Genome	A	AB
Soil pH	Neutral to alkaline	Acid
Soil texture	Medium-heavy	Light
Soil fertility	Moderate to high	Low
Rhizobial specificity	Very specific	Promiscuous

Appendix II: *Stylosanthes seabrana* germplasm accessions registered in the major tropical forages genebanks (January 2020).

Ser. no.	BRA[1]	CIAT[2]	ILRI[3]	APG[4]	Comments, additional information, collector numbers
1		12014		**APG 58185*** CPI 55802	CSIRO collection, April 1971; RLB B69
2		12015		**APG 58187*** CPI 55804	CSIRO collection, April 1971; RLB B77
3		12019		**APG 58190*** CPI 55809	CSIRO collection, April 1971; RLB B97
4		12016		**APG 58191*** CPI 55810	CSIRO collection, April 1971; RLB C23
5		12020		**APG 57821*** CPI 55811A	CSIRO collection, April 1971; RLB C25 CPI 55811 = *S. scabra*
6		12021		**APG 58194*** CPI 55813	CSIRO collection, April 1971; RLB C27
7				**APG 58232*** CPI 55871	CSIRO collection, April 1971; RLB C29
8				**APG 58197*** **APG 57822** CPI 55816A	CSIRO collection, April 1971; RLB C42 CPI 55816 = *S. seabrana*
9	**00145661-5*** 007951	2050*		**APG 57483** CPI 110341	Joint collection Cenargen-CIAT, September 1978; LC 1172
10	**00219732-5*** 007901	2043*	15767	**APG 57482** CPI 110340	Joint collection Cenargen-CIAT, September 1978; LC 5186
11	**00219733-3*** 008095	2070	15768	**APG 56718** CPI 92454 **APG 57484** CPI 110342	Joint collection Cenargen-CIAT, September 1978; LC 5208
12	**00219734-1*** 008206	2085*	15769	**APG 56723** **AGP 57485** CPI 110343 CPI 92463	Joint collection Cenargen-CIAT, September 1978; LC 5221
13	**00219724-2*** 008915	2107*		**APG 56729** CPI 92476 CPI 110344	Joint collection Cenargen-CIAT, October 1978; LC 1234
14	**00219725-9*** 009318	10517	15795	CPI 110372	Cenargen collection, April 1979; LC 1417

Continued

Ser. no.	BRA[1]	CIAT[2]	ILRI[3]	APG[4]	Comments, additional information, collector numbers
15	**00145502-1** 030058			**APG 57165** CPI 105729	IPF 1038* (NSC 933a); an EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais, Brazil) collection ("*S. scabra*") from Itamarandiba, Minas Gerais (June 1979)
16				**APG 56854*** CPI 93099	CSIRO collection, May 1981; DFC 562; accession collected at Mato Verde, Minas Gerais (May 1981)
17	**00219726-7*** 022462	10026*		**APG 56942** CPI 104710	Joint collection Cenargen-CIAT, August 1981; LC 4335
18	**00219727-5*** 022594	10113*			Joint collection Cenargen-CIAT, August 1981; LC 4351
19	**00219728-3*** 022608	10030*			Joint collection Cenargen-CIAT, August 1981; LC 4353
20	**00219729-1*** 022811	10033*		**APG 57502** **APG 58153** CPI 110361	Joint collection Cenargen-CIAT, August 1981; LC 4402 cv. Unica
21	**00219730-9*** 022977	10119*	15793	CPI 110370	Joint collection Cenargen-CIAT, August 1981; LC 4447
22	**00219735-8*** 029220	10537			Joint collection Cenargen-RBG Kew, June 1983; LC 5782a
23	**00219738-2***				MSB 48767 from the RBG Kew Millenium Seed Bank Project; joint collection Cenargen-RBG Kew, June 1983; LC 6171a; LC 6171 (= BRA 00145997-3, former BRA 029335) is *S. macrocephala*
24	**00219736-6*** 029327	10547	15796	**APG 57514** CPI 110373	Joint collection Cenargen-RBG Kew, June 1983; LC 6257
25	**00219737-4*** 028961	10471		**APG 58015** ATF 2350	Joint collection Cenargen-RBG Kew, June 1983; LC 6261; species holotype at herbarium CEN
26	**00145640-9*** 036609	11578		**APG 57579** CPI 115993	Cenargen collection, June 1987; LC 7653
27	**00146011-2*** 036617	11583		**APG 57580** CPI 115994	Cenargen collection, June 1987; LC 7661
28	**00145653-2*** 036625	11585		**APG 57581** CPI 115995	Cenargen collection, June 1987; LC 7666
29	**00219739-0*** 041238			**APG 58052*** ATF 2523	Joint Cenargen-CSIRO collection, May/June 1996; LAE 746
30	**00145697-9*** 041246			**APG 58069*** ATF 2540	Joint Cenargen-CSIRO collection, May/June 1996; LAE 748
31	**00145698-7*** 041254			**APG 58068*** ATF 2539	Joint Cenargen-CSIRO collection, May/June 1996; LAE 749
32	**00145699-5*** 041262			**APG 58067*** ATF 2538	Joint Cenargen-CSIRO collection, May/June 1996; LAE 750
33	**00145700-1*** 041271			**APG 58066*** ATF 2537	Joint Cenargen-CSIRO collection, May/June 1996; LAE 751
34	**00145725-8*** 041289			**APG 58065*** ATF 2536	Joint Cenargen-CSIRO collection, May/June 1996; LAE 752
35	**00145722-5*** 041297			**APG 58064*** ATF 2535	Joint Cenargen-CSIRO collection, May/June 1996; LAE 753
36	**00145702-7*** 041301			**APG 58063*** ATF 2534	Joint Cenargen-CSIRO collection, May/June 1996; LAE 754
37	**00145703-5*** 041319			**APG 58062*** ATF 2533	Joint Cenargen-CSIRO collection, May/June 1996; LAE 755
38	**00145706-8*** 041327			**APG 58061*** ATF 2532	Joint Cenargen-CSIRO collection, May/June 1996; LAE 756
39	**00145705-0*** 041335			**APG 58060*** ATF 2531	Joint Cenargen-CSIRO collection, May/June 1996; LAE 757
40	**00145704-3*** 041343			**APG 58059*** ATF 2530	Joint Cenargen-CSIRO collection, May/June 1996; LAE 758

Continued

Ser. no.	BRA[1]	CIAT[2]	ILRI[3]	APG[4]	Comments, additional information, collector numbers
41	**00145711-8*** 041351			**APG 58051*** ATF 2522	Joint Cenargen-CSIRO collection, May/June 1996; LAE 759
42	**00145726-6*** 041360			**APG 58050*** ATF 2521	Joint Cenargen-CSIRO collection, May/June 1996; LAE 760
43	**00219740-8*** 041378			**APG 58049*** ATF 2520	Joint Cenargen-CSIRO collection, May/June 1996; LAE 762
44	**00145708-4*** 041394			**APG 58047*** ATF 2518	Joint Cenargen-CSIRO collection, May/June 1996; LAE 764
45	**00145707-6*** 041408			**APG 58046*** ATF 2517	Joint Cenargen-CSIRO collection, May/June 1996; LAE 765
46	**00145710-0*** 041416			**APG 58045*** ATF 2516	Joint Cenargen-CSIRO collection, May/June 1996; LAE 766
47	**00219741-6*** 041513			**APG 58036*** ATF 2507B	Joint Cenargen-CSIRO collection, May/June 1996; LAE 776 ATF 2507 = *S. macrocephala*
48		11957		**APG 57614** CPI 105546B	IPF xxxx* (accession no. unknown); EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais, Brazil) collection CPI 105546 = *S. scabra* HMS 6 41
49		**11945** 12629		**APG 56763** APG 58152 CPI 92838B TQ 100	cv. Primar CPI 92838 = *S. tomentosa* DFC 008
50				**APG 57629** CPI 110370B	Isolated from CIAT 10119
51				**APG 57630** CPI 110370C	Isolated from CIAT 10119
52				**APG 58173** TQ 102	No further accession information available
53		12630			Probably a donation from the former CSIRO collection, but no further information available in the CIAT Genetic Resources database

Notes:
a) Some accessions are still registered under species names other than *S. seabrana*.
b) Accession numbers in **bold** are those to be preferably used.
c) Asterisk (*) indicates the most original accession number, i.e. the one assigned by the institution(s) that conducted the respective original collecting mission. This information is useful for eventual enquiries on passport data information, genetic purity and the like.
d) Sources: Databases of the former CSIRO Australian Tropical Forages Genetic Resources Centre (ATFGRC); Embrapa Recursos Genéticos e Biotecnologia; and CIAT; Maass and Mannetje (2002).

[1]BRA: Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil (www.embrapa.br/recursos-geneticos-e-biotecnologia); the first BRA number (in bold) corresponds to the new Alelo code; former BRA numbers (second line) are still in use.
[2]CIAT: International Center for Tropical Agriculture, Cali, Colombia (ciat.cgiar.org).
[3]ILRI (formerly ILCA): International Livestock Research Institute, Addis Ababa, Ethiopia (www.ilri.org).
[4]APG: Australian Pastures Genebank, Adelaide, Australia (https://pir.sa.gov.au/research/australian_pastures_genebank); former Australian plant introduction numbers with CPI and ATF prefixes, also TQ, are still in use.

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