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Research Paper

Molecular markers as a tool for germplasm acquisition to enhance the genetic diversity of a Napier grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*) collection

Marcadores moleculares como herramienta de obtención de germoplasma para incrementar la diversidad genética en una colección del pasto Napier

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Abstract

At the International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, 171 germplasm accessions of Napier grass were studied using 20 SSR markers with the objective of assessing the allelic richness and genetic diversity of the collections held at ILRI and the Brazilian Agricultural Research Corporation (EMBRAPA), and to determine distinct accessions to be introduced to enhance the diversity in each of the genebank collections. A total of 148 alleles were observed in the whole collection, of which 140 and 93 alleles were observed in the ILRI and EMBRAPA collections, respectively. Fifty-five and 8 alleles were found to be unique to the ILRI and EMBRAPA collections, respectively, while 85 alleles were shared between the collections. The number of alleles per marker ranged from 1 to 23 with an average value of 7.4 across both collections. The heterozygosity per locus ranged from 0.000 to 0.808 with an average value of 0.463. A principal coordinate analysis grouped accessions into 3 main groups, whereas a hierarchical cluster analysis indicated 4 main clusters. From a genebank management and conservation perspective, the marker profile of the accessions was used in the process of selecting and acquiring distinct lines to be added to the ILRI and EMBRAPA collections. Accordingly, 54 accessions and elite lines were selected and introduced from EMBRAPA to the ILRI collection, while 8 distinct accessions from ILRI were added to the EMBRAPA collection. In general, a useful marker profile of an expanded Napier grass collection has been generated which could be used to enhance the conservation, use and management of the available genetic resources of this important forage crop.

Keywords: Conservation, elephant grass, genetic resources, SSR.

Resumen

En el International Livestock Research Institute (ILRI), Addis Ababa, Etiopía se caracterizaron 171 accesiones de germoplasma del pasto Napier (*Cenchrus purpureus* sin. *Pennisetum purpureum*) utilizando 20 marcadores SSR, con el objeto de evaluar la riqueza alélica y la diversidad genética de las colecciones existentes en ILRI y la Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), e identificar accesiones con potencial para incrementar la diversidad genética en cada una de las colecciones. En la colección entera se observaron 148 alelos, de los cuales 140 y 93 alelos se observaron en las colecciones de ILRI y EMBRAPA, respectivamente. De ellos, 55 fueron únicos en la colección de

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ILRI y 8 en la de EMBRAPA, mientras que 85 alelos resultaron compartidos entre ambas colecciones. A través de ambas colecciones el número de alelos por marcador varió entre 1 y 23 con un valor promedio de 7.4. La heterocigosidad por locus varió desde 0.000 hasta 0.808 con un valor promedio de 0.463. El análisis de coordenadas principales permitió agrupar las accesiones en 3 grupos principales, mientras que el análisis de conglomerados jerárquico indicó 4 grupos principales. El perfil de marcador de las accesiones fue utilizado para el manejo y la conservación de germoplasma, dentro del proceso de selección y obtención de líneas genéticamente distintas con el fin de agregarlas a las colecciones en ILRI y EMBRAPA. Como resultado fueron seleccionadas 54 accesiones y líneas élite de EMBRAPA que fueron introducidas a la colección en ILRI, y 8 accesiones de ILRI que fueron agregadas a la colección en EMBRAPA. Con el estudio se generó un perfil de marcador de una colección ampliada del pasto Napier que podría utilizarse para mejorar la conservación, el uso y el manejo del recurso genético disponible de esta importante especie forrajera.

Palabras clave: Conservación, pasto elefante, recursos genéticos, SSR.

Introduction

Livestock play a crucial role in the socio-economic development of many developing countries in tropical and subtropical regions of the world. Year-round availability and supply of forages have always been a huge challenge for sustainable livestock production at the household level, where smallholder farmers are dependent on small plots of land for mixed farming (Ayantunde et al. 2005; Smith et al. 2013). The challenge is becoming even more difficult with the current trends of climate change, increasing population density per unit area with the associated growing demand for meat, milk and eggs, an alarming rate of urbanization and consequently limited availability of arable land (Smith et al. 2013). Achieving the sustainable development goals of eradicating poverty, ending hunger, enhanced food security and promoting climate-smart action at the household level of smallholder farmers would be very difficult without addressing the feed and forage component of livestock production. The lack of sufficient forage negatively affects livestock production and productivity with consequences for smallholder farmers' incomes and their everyday livelihood. Consequently, forage species which can produce high yields per unit area, particularly under stressed conditions (reduced water availability, increased diseases and insect pests, producing on marginal land, etc.), are in greater need than ever to address the increasing demand for forage.

Napier grass [*Cenchrus purpureus* (Schumach.) Morrone (syn. *Pennisetum purpureum* Schumach.)] is an allotetraploid (2n=4x=28, A'A'BB) perennial C4 flowering plant (Harris et al. 2010; Kandel et al. 2016) native to Sub-Saharan Africa and widely grown across the tropical and subtropical regions of the world (Singh et al. 2013). Selection and breeding in the Americas have resulted in new higher-yielding genotypes, such as the elite lines from the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Napier grass breeding program (Pereira et al. 2017). These elite lines are adapted to their local climate and farming system but could also have potential for use by smallholder farmers in Sub-Saharan Africa. Napier grass is a multi-purpose crop used primarily for feeding animals (Farrell et al. 2002; Ishii et al. 2013; Heuzé et al. 2016) but also as a biofuel crop (Singh et al. 2013), for the control of weeds and soil erosion (Kabirizi et al. 2015) and as a trap plant in an integrated pest management strategy (Khan et al. 2006; 2007).

The conservation and use of the available germplasm requires an understanding of the level and structure of genetic diversity of a crop (Laidò et al. 2013). To enhance the conservation and use of Napier grass germplasm, a number of studies have been conducted to characterize its genetic diversity using various types of molecular markers (Negawo et al. 2017 and the references therein) revealing a moderate level of diversity in the studied populations. Molecular information has also been used to support the identification of duplicates and incorrect labeling during the handling of accessions maintained in a genebank (Lowe et al. 2003). A recent review paper by Negawo et al. (2017) provides an up-to-date summary of the molecular, agronomic and feed quality information collected on Napier grass to date and suggests some of the areas where current advances in molecular genetics could be used to enhance the research, conservation and use of the available genetic resources.

Napier grass germplasm is usually distributed as stem cuttings because of poor seed set and the outcrossing nature of seed production (Negawo et al. 2017). Movement of cuttings across borders increases the risk of spreading pests and diseases and requires extensive and expensive testing to ensure the materials are free of diseases for phytosanitary clearance before dispatch and extensive quarantine on arrival. This is especially important when transferring germplasm between continents, where plants may be subjected to different pests and diseases. Gap filling, a targeted acquisition or collection to fill the trait and genetic diversity gaps identified from gap analysis, is also an important activity in genebanks and molecular studies that can identify genotypes that are genetically distinct can be used to reduce the numbers introduced and the associated risks and costs of disease testing and transfer as vegetative cuttings, capturing expanded diversity while minimizing risks.

In the current study, simple sequence repeat (SSR) markers were used to assess the Napier grass collections held in the International Livestock Research Institute (ILRI) forage genebank and the Brazilian Agricultural Research Corporation, Embrapa Gado de Leite. The aim of the study was to assess the genetic diversity and allelic richness in both collections and to use the generated information to assist in the identification of genetically distinct accessions from the collections to supplement those already held by both genebanks.

Materials and Methods

Plant materials

A total of 171 accessions of Napier grass: 83 accessions from the EMBRAPA Napier grass active genebank at Embrapa Gado de Leite; 28 elite lines from the EMBRAPA breeding program; and 60 accessions from the ILRI genebank collection, were studied at ILRI, Addis Ababa, Ethiopia using SSR markers. Eight of the accessions in the ILRI collection are hybrids (*P. purpureum* x *P. glaucum*). Table 1 shows the origin and/or sources of the accessions in both collections.

DNA extraction

Leaf samples of each germplasm accession from the ILRI genebank were collected and dried in silica gel. The dried leaf samples were ground to a fine powder using a tissue grinder (Geno grinder, SPEX SamplePrep, Metuchen, NJ, USA) and DNA was extracted from 30–50 mg of ground leaves using a modified CTAB method (Doyle and Doyle 1987). Briefly, DNA was extracted from the leaf powder with a CTAB buffer followed by chloroform/isoamyl alcohol (24:1) and then precipitated with isopropanol. The re-suspended DNA was treated with RNAse A at 37 °C for 1 h, and then purified using chloroform/isoamyl alcohol followed by sodium acetate precipitation. Following centrifugation (20,000 g) for 30 min at 4 °C the resultant pellet was washed with 100% ethanol (EtOH), followed by 70% EtOH (ice-cooled) and then air-dried for 5 min at room temperature. Finally, the pellet was dissolved in 40 µL TE buffer. The DNA from EMBRAPA accessions was also extracted using the CTAB method and shipped to ILRI in 96 well plates (1µg dry DNA per sample) at room temperature, where it was re-suspended in sterile deionized water. The quantity and purity of the DNA were determined using a Nano Drop spectrophotometer and, to determine quality and integrity, the DNA of a few random samples was also separated by gel electrophoresis. Finally, the DNA was diluted to a working concentration of 50 ng/µL.

Table 1. Origin and/or source of the accessions in both collections.

Origin/collected from	ILRI[1]	EMBRAPA[2]
Brazil		39
Brazilian elite lines		28
Burundi	1	
Colombia		5
Costa Rica		1
Cuba	1	4
Ethiopia	1	
India		3
Malawi	1	
Mozambique	2	
Namibia	1	
Nigeria	1	
Panama		1
Swaziland	6	
Tanzania	6	
USA	17	1
Zimbabwe	11	
Unknown	12	29
Total	**60**	**111**

[1]ILRI: International Livestock Research Institute.
[2]EMBRAPA: Brazilian Agricultural Research Corporation.

SSR markers

Twenty SSR markers, previously developed for pearl millet (Allouis et al. 2001; Budak et al. 2003; Mariac et al. 2006) and demonstrated to be transferable to Napier grass (Azevedo et al. 2012) were used for genotyping accessions from ILRI and EMBRAPA (Table 2).

Amplification and electrophoresis

The PCR was performed in a reaction volume of 15 µL containing 1 x PCR Taq polymerase buffer (Fermentas), 0.2 mM dNTPs (Fermentas), 0.5 µM primers [labeled with different fluorescent dyes: 6-FAM (blue), VIC (green), NED (black), and PET (red)] (Applied Biosystems, Foster City, CA, USA), 0.6 U Taq polymerase (Fermentas) and 150 ng DNA.

Table 2. SSR markers used for the study: marker code; chromosome number, location of the markers on chromosomes of pearl millet; marker type, source from which the marker was derived; sequence of forward and reverse primers; repeat motif and length; annealing temperature (Ta); and source reference.

Marker Code	CN[1]	Marker Type	Forward Primer (5'---3')	Reverse Primer (5'---3')	Repeat motif and length	Ta (°C)	Reference
CTM10	3[2]	Genomic	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA	(CT)22	54	Budak et al. 2003
CTM12	1[2]	Genomic	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT	(CT)12	53	Budak et al. 2003
CTM27	1[2]	Genomic	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT	(CT)71	53	Budak et al. 2003
CTM59	NK	Genomic	TCCTCGACATCCTCCA	GACACCTCGTAGCACTCC	(CT)11	53	Budak et al. 2003
CTM8	7[2]	Genomic	GCTGCATCGGAGATAGGGAA	CTCAGCAAGCACGCTGCTCT	(CT)8	54	Budak et al. 2003
PGIRD21	1[3]	EST	GCTATTGCCACTGCTTCACA	CCACCATGCAACAGCAATAA	(ACC)8	54	Mariac et al. 2006
PGIRD25	6[3]	EST	CGGAGCTCCTATCATTCCAA	GCAAGCCACAAGCCTATCTC	(GA)9	58	Mariac et al. 2006
PGIRD46	3[3]	EST	GAACAATTGCTTCTGTAATATTGCTT	GCCGACCAAGAACTTCATACA	(CTC)6	48	Mariac et al. 2006
PGIRD5	1[3]	EST	CAACCCAACCCATTATACTTATCTG	GCAACTCTTGCCTTTCTTGG	(GA)7	58	Mariac et al. 2006
PGIRD56	3[3]	EST	ATCACTCCTCGATCGGTCAC	ACCAGACACACGTGCCAGT	(TG)6	58	Mariac et al. 2006
PGIRD57	7[3]	EST	GGCCCCAAGTAACTTCCCTA	TCAAGCTAGGGCCAATGTCT	(AG)7	56	Mariac et al. 2006
PRIRD13	1[3]	EST	CAGCAGCGAGAAGTTTAGCA	GCGTAGACGGCGTAGATGAT	(AGC)8	60	Mariac et al. 2006
PSMP2235	5[3]	Genomic	GCTTTTCTGCTTCTCCGTAGAC	CCCAACAATAGCCACCAATAAAGA	(TG)9	54	Allouis et al. 2001
PSMP2248	6[2]	Genomic	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC	(TG)10	58	Allouis et al. 2001
PSMP2255	6[2]	Genomic	CATCTAAACACAACCAATCTTGAAC	TGGCACTCTTAAATTGACGCAT	(TG)34	54	Allouis et al. 2001
PSMP2266	7[2]	Genomic	CAAGGATGGCTGAAGGGCTATG	TTTCCAGCCCACACCAGTAATC	(GA)17	58	Allouis et al. 2001
PSMP2267	3[3]	Genomic	GGAAGGCGTAGGGATCAATCTCAC	ATCCACCCGACGAAGGAAACGA	(GA)16	60	Allouis et al. 2001
Xipes0219	4[2]	EST	GGGGAAGAGATAGGGTTGGT	AGCTGGGCAATAGCGAGAT	TTT(CT)8TT	57	Rajaram et al. 2013
Xipes0093	5[2]	EST	GGATCTGCAGGTTTGGACAT	CCAAGCACTGAAACATGCAC	(TGA)10	57	Rajaram et al. 2013
Xipes0191	5[2]	EST	GAAGAACCTCCAGCTTTCCC	TTCTTTCCTTCAGCCTCTGC	(AG)13	53	Rajaram et al. 2013

[1]CN=Chromosome number.
[2]Genetic location of the markers based on pearl millet linkage groups (Rajaram et al. 2013); NK=Not known.
[3]Predicted location of the markers on pearl millet chromosomes (https://www.ncbi.nlm.nih.gov/assembly/GCA_002174835.1/) based on blast alignment of the primers.

The PCR program consisted of an initial denaturation step at 95 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing using primer-specific annealing temperatures for 1 min and elongation at 72 °C for 2 min. The final elongation step was performed at 72 °C for 30 min followed by a holding step at 4 °C.

The PCR products were assessed by capillary gel electrophoresis. PCR products (1.2 µL each) were mixed with 8.87 µL Hi-Di-formamide and 0.107 µL fluorescent-labeled GeneScan™ LIZ size standard (Applied Biosystems, Foster City, CA, USA) in a 96-well microtiter plate. Then, the aliquot solution was denatured at 95 °C for 3 min and quickly chilled on ice for 5 min to avoid the formation of double-stranded DNA. The products were loaded and run on a 3730 x l DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Data scoring and analysis

The marker data were captured using Genescan® collection software (Applied Biosystems, Foster City, CA, USA) and the resulting fragments (allele sizes in nucleotides) were scored using Genemapper® software version 4.1 (Applied Biosystems, Foster City, CA, USA). The allelic data were scored (rearranged) taking into account the ploidy level of the Napier grass genome (= tetraploid) where a maximum of 4 copies of alleles per locus would be expected. Where there was a single allele, 4 copies were scored and where 2 alleles per locus were observed, an equal dosage (2 copies for each allele) was recorded. Where there were 3 alleles per locus, 2 copies of an allele were scored for the allele with the largest peak area, while the other alleles were scored as a single copy, and whenever there were 4 different alleles, each allele was scored as a single copy. Then, the data were used to calculate the different measures of genetic variability as suggested by Laurentin (2009) for co-dominant markers. The calculated measures include allele size range, the number of alleles per locus and effective number of alleles. In addition, genetic diversity (heterozygosity), a measure of genetic variation of a population, was calculated from the allele frequency using the formula: $H=1-\sum P^2_{ij}$ (where P^2_{ij} is frequency of *i* allele at *j* locus) as described by Nei (1973).

A Bayesian clustering approach implemented in STRUCTURE software (Pritchard et al. 2000) was used to infer presence of population stratification in the population. Burn-in period as well as Monte Carlo Markov Chain (MCMC) iteration number were set to 100,000 testing the probability of 20 K, each with 10 repetitions. An admixture model with correlated allele frequencies was selected. The results of the run were uploaded to the online software, Structure Harvester (Earl and von Holdt 2012), and the most likely number of subpopulations was determined by the Evanno Δk method (Evanno et al. 2005). The SSR data were also subjected to hierarchical cluster analysis using the Ward hierarchical clustering based on the Euclidean distance method in the R package pvclust (Suzuki and Shimodaira 2006). Principal coordinate analyses (PCoA) were conducted using the genetic distance, TriDistance matrix and covariance-standardized options of the software GenAIEx v.6.5 (Peakall and Smouse 2012).

Results

SSR marker polymorphism

SSR data for 166 of the 171 accessions were obtained with missing data points constituting around 13% of the generated data. Table 3 shows the number of alleles (Na), effective number of alleles (Ae) and heterozygosity (H) of each marker for the EMBRAPA active genebank collection, the ILRI genebank collection and both collections combined. The number of alleles per marker ranged from 1 to 15 and 1 to 22 with averages of 4.65 and 7.0 in the EMBRAPA and ILRI collections, respectively. For the whole collection, the number of alleles per marker ranged from 1 to 23 with an average of 7.45. The effective numbers of alleles were 5.011, 5.058 and 5.138 with averages of 2.518, 2.544 and 2.512 in the collections from EMBRAPA, ILRI and combined, respectively. Similarly, the level of heterozygosity ranged from 0.000 to 0.800 and from 0.000 to 0.821 in the EMBRAPA and ILRI collections with averages of 0.436 and 0.456, respectively. Across the whole collection, the heterozygosity value ranged from 0.000 to 0.808 with an average of 0.463. Of the 20 markers, marker PSMP2248 displayed no heterozygosity across the whole collection and markers PGIRD5 and Xipes0191 displayed no heterozygosity for the EMBRAPA collection.

Allelic richness and uniqueness of the collections

A total of 148 alleles representing 20 SSR markers were scored in the whole collection, with 93 and 140 alleles in the EMBRAPA and ILRI collections, respectively. Eighty-five of the alleles were shared between both collections, while 8 (5.4%) and 55 (37.2%) alleles were unique to the EMBRAPA and ILRI collections, respectively (Figure 1). Of the 140 alleles observed in the ILRI collection, 21 and 56 alleles were recorded for the hybrid and pure Napier grass accessions, respectively. Of the 21 alleles recorded for hybrid accessions, 18 alleles contributed to the unique allelic richness of the ILRI collection.

Table 3. Number of alleles (Na), effective number of alleles (Ae) and heterozygosity (H) for each marker from the EMBRAPA active genebank collection, the ILRI genebank collection and both collections combined.

Marker code	Fragment length (bp)	EMBRAPA collection			ILRI collection			Both collections		
		Na	Ae	H	Na	Ae	H	Na	Ae	H
CTM10	120-201	3	1.015	0.015	6	1.178	0.151	7	1.073	0.068
CTM12	292-299	2	2.000	0.500	3	2.066	0.516	3	2.027	0.507
CTM27	291-324	2	1.999	0.500	7	2.204	0.546	7	2.084	0.520
CTM59	170-175	2	1.124	0.110	3	1.084	0.078	3	1.108	0.098
CTM8	233-280	9	4.189	0.761	13	3.470	0.712	13	4.080	0.755
PRIRD13	217-271	8	3.614	0.723	11	4.723	0.788	11	4.547	0.780
PGIRD21	184-220	4	2.247	0.555	6	2.439	0.590	6	2.326	0.570
PGIRD25	149-169	2	1.116	0.104	5	1.179	0.152	5	1.071	0.067
PGIRD46	85-193	4	1.932	0.483	4	2.366	0.577	5	2.126	0.530
PGIRD5	160-164	1	3.580	0.000	2	1.048	0.046	2	1.509	0.337
PGIRD56	136-155	5	3.580	0.721	8	2.928	0.658	8	3.437	0.709
PGIRD57	106-136	2	1.904	0.475	6	2.928	0.530	6	2.009	0.502
PSMP2235	134-243	4	1.533	0.348	6	1.186	0.157	8	1.390	0.280
PSMP2248	159	1	1.000	0.000	1	1.000	0.000	1	1.000	0.000
PSMP2255	210-313	15	5.011	0.800	22	5.058	0.802	23	5.134	0.805
PSMP2266	158-176	7	3.890	0.743	6	4.443	0.775	7	4.175	0.761
PSMP2267	172-229	11	4.769	0.790	14	5.571	0.821	15	5.208	0.808
Xipes0219	124-137	3	1.696	0.410	4	1.473	0.321	4	1.602	0.376
Xipes0093	112-135	7	3.158	0.683	6	3.274	0.695	7	3.236	0.691
Xipes0191	99-127	1	1.000	0.000	7	1.269	0.212	7	1.097	0.088
Average		4.65	2.518	0.436	7	2.544	0.456	7.45	2.512	0.463

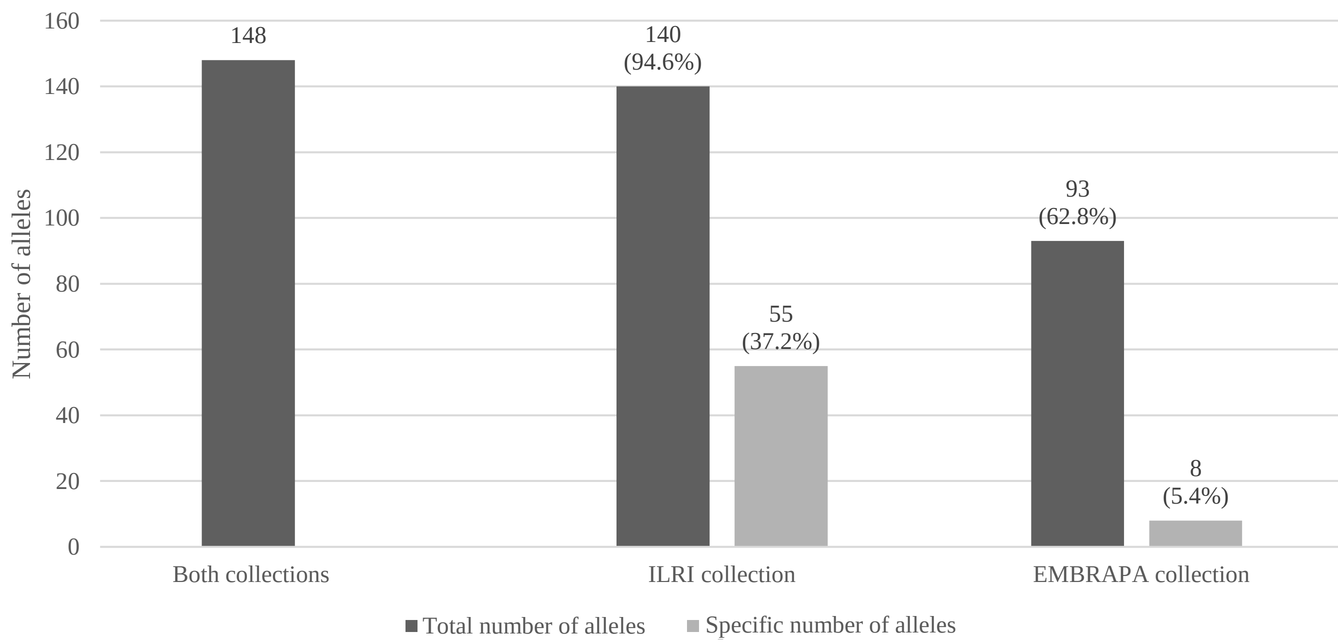

Figure 1. Allelic richness of Napier grass collections from EMBRAPA and ILRI.

Population structure and cluster analysis

In the population stratification assessment and cluster analysis, accessions with more than 30% missing data points were removed and the resultant analysis was performed on 148 accessions (89 from EMBRAPA and 59 from ILRI). As shown in Figure 2, the distribution of the ΔK (Evanno et al. 2005) gave the highest peak at K=2 suggesting the presence of 2 major subpopulations. However, bar plots showed that up to 4 subpopulations, with both the EMBRAPA and ILRI collections represented in each subpopulation, are possible.

The hierarchical cluster analysis assembled the collections into 4 main clusters with further subclusters (Figure 3). Most of the EMBRAPA accessions were captured in Clusters II and IV, while the majority of the individuals in the other 2 clusters came from the ILRI genebank collection. A principal coordinate analysis (PCoA) was plotted based on the first 2 coordinates, which explained approximately 23% of the genetic variation. Here, 3 main groups were identified (Figure 4). Two of the groups (Groups I and II) were similar to Clusters I and II identified by the hierarchical cluster analysis with a few outliers, while the third group contained accessions in Clusters III and IV. Some genotypes (e.g. 16822, 16818, 16809 and 16810 in Cluster I; 16800 and 16801 in Cluster III; and 16785 and 16787 in Cluster IV) appeared to have very similar profiles and could not be discriminated by either analysis.

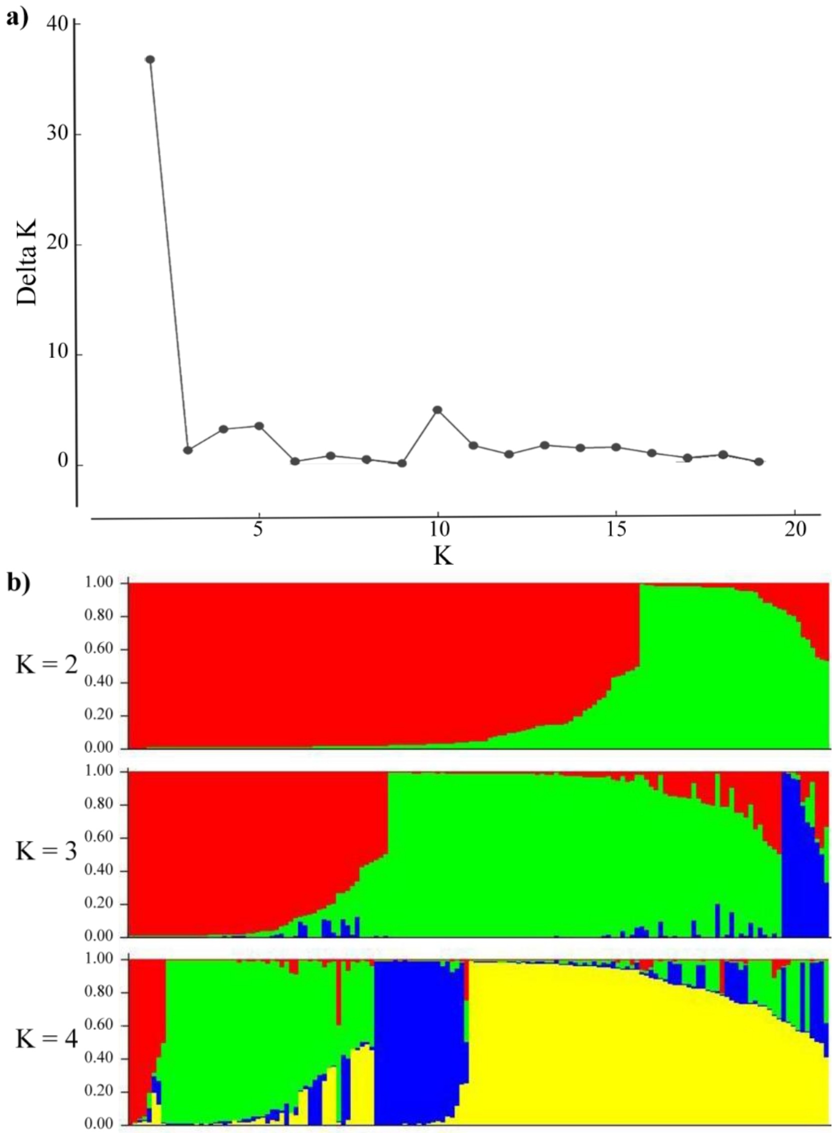

Figure 2. Population structure analysis: **(a)** Inferred using the Evano ΔK method; and **(b)** Bar plots showing the inferred subpopulations (K=2 to K=4) and their probability of group membership.

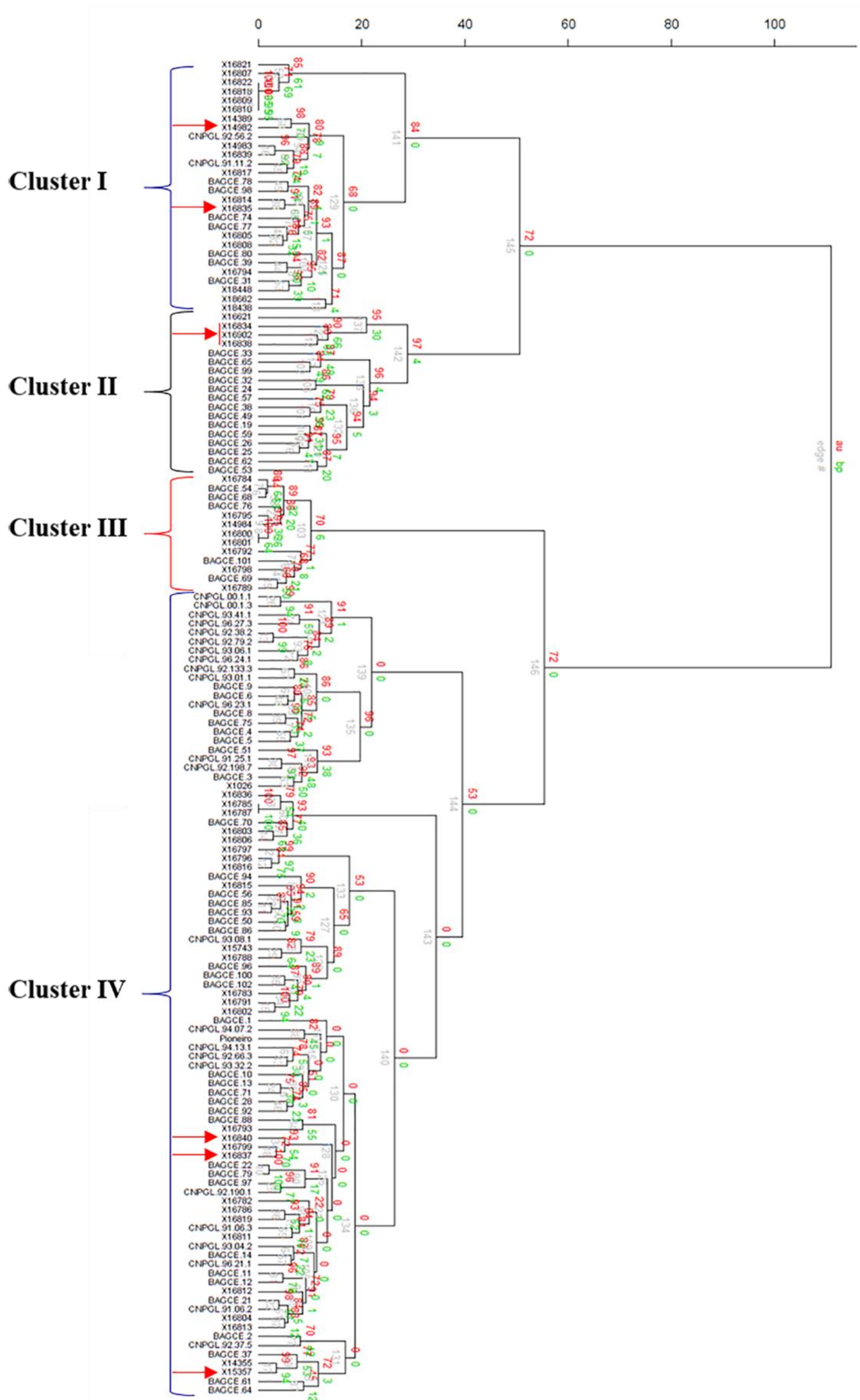

Figure 3. Cluster analysis of 148 accessions of Napier grass from the ILRI and EMBRAPA collections using 20 SSR markers based on Ward hierarchical clustering (Euclidean distance) method in the R package pvclust (the red arrows indicate the hybrid accessions). Au = Approximately Unbiased p-value, bp = Bootstrap probability value, and edge# = numeric vector of edge numbers. Accession codes starting with X are for accessions from the ILRI genebank, CNPGL are for Brazilian elite lines and BAGCE are for accessions from the EMBRAPA Napier grass active genebank.

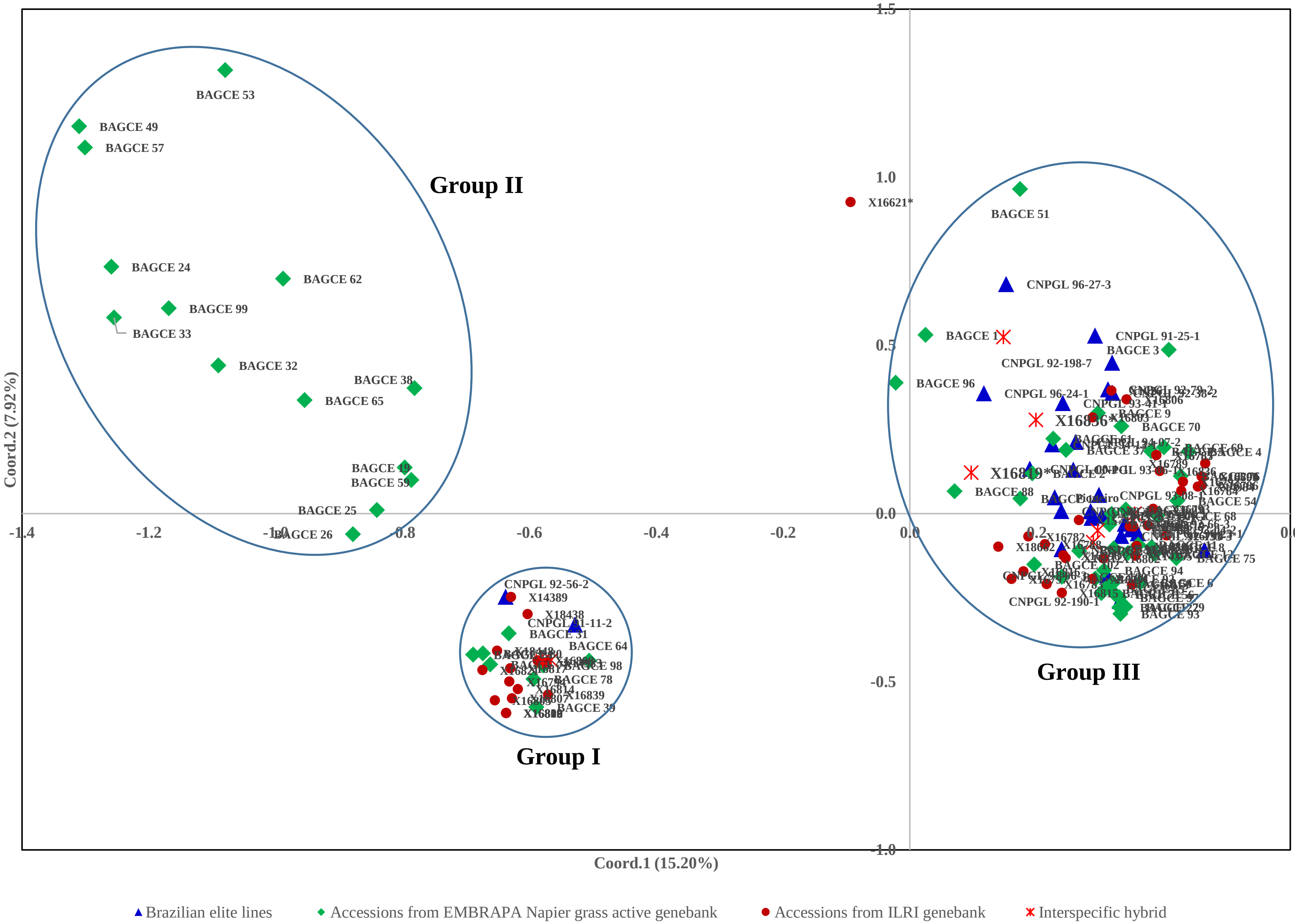

Figure 4. PCoA plot generated using GelALEx 6.5 showing grouping of 148 Napier grass accessions from the ILRI and EMBRAPA collections. *Indicates outlier based on the hierarchical clustering method.

Discussion

Efforts to explore Napier grass genetic diversity in support of better livestock performance and productivity are crucial in order to reduce poverty and enhance food security. By integrating advances in molecular biology, we can support and enhance the process of exploring the available genetic resources for efficient decision making during conservation and utilization. In this study, SSR markers were used to fingerprint a Napier grass collection maintained at the ILRI forage genebank as well as accessions from EMBRAPA, Brazil. The results revealed the existence of diverse genetic resources in the collections maintained by both centers and the presence of some distinct material in each of the collections. These results were used to support the transfer of some materials, representing greater genetic diversity, between Brazil and Ethiopia in order to enhance both genebank collections.

Comparing the two collections, greater allelic richness was discovered in the ILRI genebank collection, despite the fact that it contains only 59 accessions compared with 107 accessions from the EMBRAPA collection. This could be explained by the diverse origins of the accessions maintained in the ILRI genebank, as they were collected from 13 different countries, 8 of which are in Africa from where Napier grass is believed to have originated (Cook et al. 2005; CABI 2013), and the presence of hybrid accessions which contributed a number of unique alleles (12% of the observed alleles) to the collection. The EMBRAPA collection was obtained from only 7 different countries, mainly in Central and South America, where Napier grass has been introduced and become naturalized (Cook et al. 2005; Singh et al. 2013; CABI 2013) and presence of elite lines developed through the plant breeding process. Since most of these accessions were represented in 2 clusters, we assume that a limited range of genetic material was introduced to produce the naturalized material, resulting in a genetic bottleneck of material in this region. However, interestingly there was no clear correlation of clustering of the accessions based on their geographical origin across the 2 collections. Previous studies have provided conflicting results on this point with clustering of genotypes according to their geographical origin using Random Amplification of Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) markers (Lowe et al. 2003; Harris et al. 2010) and no such observation using AFLP markers (Struwig et al. 2009; Wanjala et al. 2013). There were also 8 hybrid Napier grass accessions in the ILRI collection and, unlike in the study of Lowe et al. (2003) using RAPDs, where 6 of the hybrids clustered separately from the pure Napier grass accessions, the hybrids were dispersed into different clusters on the phylogenetic tree.

A literature search on Napier grass pedigrees and variety descriptions yielded little information that could be used to enhance our understanding of the relationship between genotypes and guide the selection of distinct genotypes for gap filling. There is also limited information available on the history of germplasm transfer from institutes that supplied the original samples and only a single accession (the dwarf elephant grass, cv. Mott) could be definitively identified as being in both collections. Molecular markers have been used to assist in the conservation and management of plant genetic resource collections over the last few decades (Spooner et al. 2005) with a positive cost-benefit implication indicated. In this study, a number of accessions with novel DNA fingerprints were identified in each collection and this information was used to support the decision of which accessions to exchange between the centers. From a genebank management perspective, the use of DNA fingerprint information to support this decision helps to avoid the acquisition of duplicates and/or very similar accessions as well as the identification of distant accessions and novel clusters.

In the future, the generated information could also be used to help establish a 'core' collection with the minimum number of accessions representing the maximum possible diversity in the species and to select parent material for future crosses in plant breeding programs. By undertaking this research, the cost of confirming a large collection of materials is free of diseases, and/or of importing duplicates and closely related accessions could be avoided. For example, DNA of 83 accessions from EMBRAPA were acquired and fingerprinted alongside the ILRI genebank collection. Based on the DNA profile, a decision was made to import planting materials of 25 accessions which were found to be distinct from the accessions held in the ILRI genebank collection in order to capture the maximum genetic diversity possible, together with the elite lines which are likely to have potential for production in East African countries such as Ethiopia and Kenya. Furthermore, 8 distinct accessions from the ILRI collection were also added to the EMBRAPA collection. Thus, there was no need to import all accessions and establish them in the field, which would be expensive in terms of time, space and labor required. In addition, confusion among lines is common as they are moved around and given new names and identification numbers so that the same genotype may be found under different names and even different genotypes can be found under the same name. This underscores the multitude of benefits of using molecular information in the management of genetic resources.

In the current trend of genetic resource utilization, where plant variety rights and intellectual property have become of growing importance, it is also necessary to document the genomic profile of accessions maintained in the genebanks where the materials are held as an international public good. The DNA profile is very useful for tracing the distribution and use of true-to-type accessions held in these genebanks. Consequently, the result of the SSR profile analysis can contribute substantially to management, conservation, research and use of the Napier grass accessions. The marker panel used in this work could be applied to further exchanges of Napier grass accessions between genebanks.

In conclusion, the study has been able to identify the presence of diverse genetic variability in the two collections of Napier grass while also demonstrating the importance of integrating molecular tools in the cost-effective determination of genetically distinct germplasm for gap filling, germplasm exchange and enhancing collections of Napier grass.

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References

Allouis S; Qi X; Lindup S; Gale M; Devos KM. 2001. Construction of a BAC library of pearl millet, *Pennisetum glaucum*. Theoretical and Applied Genetics 102:1200–1205. DOI: 10.1007/s001220100559

Ayantunde AA; Fernández-Rivera S; McCrabb G. 2005. Coping with feed scarcity in smallholder livestock systems in developing countries. ILRI (International Livestock Research Institute), Nairobi, Kenya. hdl.handle.net/10568/855

Azevedo ALS; Costa PP; Machado JC; Machado MA; Pereira AV; Lédo FJDS. 2012. Cross species amplification of *Pennisetum glaucum* microsatellite markers in *Pennisetum purpureum* and genetic diversity of Napier grass accessions. Crop Science 52:1776–1785. DOI: 10.2135/cropsci2011.09.0480

Budak H; Pedraza F; Cregan PB; Baenzinger PS; Dweikat I. 2003. Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. Crop Science 43:2284–2290. DOI: 10.2135/cropsci2003.2284

CABI (Centre for Agriculture and Biosciences International). 2013. *Pennisetum purpureum* (elephant grass). CABI, Wallingford, UK. www.cabi.org/isc/datasheet/39771

Cook BG; Pengelly BC; Brown SD; Donnelly JL; Eagles DA; Franco MA; Hanson J; Mullen BF; Partridge IJ; Peters M; Schultze-Kraft R. 2005. Tropical Forages: An interactive selection tool. CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia. www.tropicalforages.info

Doyle JJ; Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

Earl DA; von Holdt BM. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359–361. DOI: 10.1007/s12686-011-9548-7

Evanno G; Regnaut S; Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14:2611–2620. DOI: 10.1111/j.1365-294X.2005.02553.x

Farrell G; Simons SA; Hillocks RJ. 2002. Pests, diseases and weeds of Napier grass, *Pennisetum purpureum*: A review. International Journal of Pest Management 48:39–48. DOI: 10.1080/09670870110065578

Harris K; Anderson W; Malik R. 2010. Genetic relationships among Napier grass (*Pennisetum purpureum* Schum.) nursery accessions using AFLP markers. Plant Genetic Resources 8:63–70. DOI: 10.1017/S1479262109990165

Heuzé V; Tran G; Giger-Reverdin S; Lebas F. 2016. Elephant grass (*Pennisetum purpureum*). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. feedipedia.org/node/395

Ishii Y; Hamano K; Kang DJ; Kannika R; Idota S; Fukuyama K; Nishiwaki A. 2013. C_4-napier grass cultivation for cadmium phytoremediation activity and organic livestock farming in Kyushu, Japan. Journal of Agricultural Science and Technology 3:321–330.

Kabirizi J; Muyekho F; Mulaa M; Msangi R; Pallangyo B; Kawube G; Zziwa E; Mugerwa S; Ajanga S; Lukwago G; Wamalwa ENI; Kariuki I; Mwesigwa R; Nannyeenya-Ntege W; Atuhairwe A; Awalla J; Namazzi C; Nampijja Z. 2015. Napier grass feed resource: Production, constraints and implications for smallholder farmers in Eastern and Central Africa. EAAPP (The Eastern African Agricultural Productivity Project), Naivasha, Kenya. ir-library.mmust.ac.ke/handle/190/314

Kandel R; Singh HP; Singh BP; Harris-Shultz KR; Anderson WF. 2016. Assessment of genetic diversity in Napier grass (*Pennisetum purpureum* Schum.) using microsatellite, single-nucleotide polymorphism and insertion-deletion

markers from pearl millet (*Pennisetum glaucum* [L.] R. Br.). Plant Molecular Biology Reporter 34:265–272. DOI: 10.1007/s11105-015-0918-2
Khan ZR; Midega CAO; Hutter NJ; Wilkins RM; Wadhams LJ. 2006. Assessment of the potential of Napier grass (*Pennisetum purpureum*) varieties as trap plants for management of *Chilo partellus*. Entomologia Experimentalis et Applicata 119:15–22. DOI: 10.1111/j.1570-7458.2006.00393.x
Khan ZR; Midega CAO; Wadhams LJ; Pickett JA; Mumuni A. 2007. Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stemborer (*Busseola fusca*) in a push–pull strategy. Entomologia Experimentalis et Applicata 124:201–211. DOI: 10.1111/j.1570-7458.2007.00569.x
Laidò G; Mangini G; Taranto F; Gadaleta A; Blanco A; Cattivelli L; Marone D; Mastrangelo AM; Papa R; De Vita P. 2013. Genetic diversity and population structure of tetraploid wheats (*Triticum turgidum* L.) estimated by SSR, DArT and pedigree data. PLoS ONE 8:e67280. DOI: 10.1371/journal.pone.0067280
Laurentin H. 2009. Data analysis for molecular characterization of plant genetic resources. Genetic Resources and Crop Evolution 56:277–292. DOI: 10.1007/s10722-008-9397-8
Lowe AJ; Thorpe W; Teale A; Hanson J. 2003. Characterisation of germplasm accessions of Napier grass (*Pennisetum purpureum* and *P. purpureum* × *P. glaucum* hybrids) and comparison with farm clones using RAPD. Genetic Resources and Crop Evolution 50:121–132. DOI: 10.1023/A:1022915009380
Mariac C; Luong V; Kapran I; Mamadou A; Sagnard F; Deu M; Chantereau J; Gerard B; Ndjeunga J; Bezançon G; Pham JL; Vigouroux Y. 2006. Diversity of wild and cultivated pearl millet accessions (*Pennisetum glaucum* [L.] R. Br.) in Niger assessed by microsatellite markers. Theoretical and Applied Genetics 114:49–58. DOI: 10.1007/s00122-006-0409-9
Negawo AT; Teshome A; Kumar A; Hanson J; Jones CS. 2017. Opportunities for Napier grass (*Pennisetum purpureum*) improvement using molecular genetics. Agronomy 7:28. DOI: 10.3390/agronomy7020028
Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America 70:3321–3323. goo.gl/ZQVdAn
Peakall R; Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. Bioinformatics 28:2537–2539. DOI: 10.1093/bioinformatics/bts460
Pereira AV; Lédo FJS; Machado JC. 2017. BRS Kurumi and BRS Capiaçu - New elephant grass cultivars for grazing and cut-and-carry system. Crop Breeding and Applied Biotechnology 17:59–62. DOI: 10.1590/1984-70332017v17n1c9
Pritchard JK; Stephens M; Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959. goo.gl/HyxWm1
Rajaram V; Nepolean T; Senthilvel S; Varshney RK; Vadez V; Srivastava RK; Shah TM; Supriya A; Kumar S; Ramana Kumari B; Bhanuprakash A; Lakshmi Narasu M; Riera-Lizarazu O; Hash CT. 2013. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] consensus linkage map constructed using four RIL mapping populations and newly developed EST-SSRs. BMC Genomics 14:159. DOI: 10.1186/1471-2164-14-159
Singh BP; Singh HP; Obeng E. 2013. Elephantgrass. In: Singh BP, ed. Biofuel Crops: Production, physiology and genetics. CAB International, Fort Valley, GA, USA. p. 271–291. DOI: 10.1079/9781845938857.0271
Smith J; Sones K; Grace D; MacMillan S; Tarawali S; Herrero M. 2013. Beyond milk, meat and eggs: Role of livestock in food and nutrition security. Animal Frontiers 3:6–13. DOI: 10.2527/af.2013-0002
Spooner DM; van Treuren R; de Vicente MC. 2005. Molecular markers for genebank management. IPGRI technical bulletin No. 10. IGPRI (International Plant Genetic Resources Institute), Rome, Italy. goo.gl/NLE2yV
Struwig M; Mienie CMS; Van Den Berg J; Mucina L; Buys MH. 2009. AFLPs are incompatible with RAPD and morphological data in *Pennisetum purpureum* (Napier grass). Biochemical Systematics and Ecology 37:645–652. DOI: 10.1016/j.bse.2009.09.010
Suzuki R; Shimodaira H. 2006. Pvclust: An R package for assessing the uncertainty in hierarchical clustering. Bioinformatics 22:1540–1542. DOI: 10.1093/bioinformatics/btl117
Wanjala BW; Obonyo M; Wachira FN; Muchugi A; Mulaa M; Harvey J; Skilton RA; Proud J; Hanson J. 2013. Genetic diversity in Napier grass (*Pennisetum purpureum*) cultivars: Implications for breeding and conservation. AoB Plants 5:plt022. DOI: 10.1093/aobpla/plt022

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Research Paper

Agronomic characterization of *Paspalum atratum* Swallen and *P. lenticulare* Kunth

Caracterización agronómica de Paspalum atratum *Swallen y* Paspalum lenticulare *Kunth*

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Abstract

Paspalum atratum and *P. lenticulare* are perennial grasses, native to South America. The objective was to evaluate seasonal growth, frost tolerance, cattle preference, nutritional characteristics, seed yield and germination of 11 ecotypes of *P. atratum* and *P. lenticulare* with regard to their potential as alternatives to introduced species. Forage yield was evaluated during 3 growing seasons between 2011 and 2014. Digestibility and CP concentration were assessed in summer 2014, cattle preference in winter 2013 and summer 2015, frost tolerance after the first frosts in June 2012, flowering time during 2013 and 2014 and seed yield and germination in 2014. A marked warm-season growing period was observed for both species with annual forage yields varying from 5 to 12 t DM/ha. Flowering did not occur until autumn, with mean seed yield of 280 kg/ha and germination of 16% for *P. atratum* and 19% for *P. lenticulare*. Most ecotypes were able to tolerate winter temperatures and behaved as perennials. Although no differences were observed for DM digestibility and CP concentration among accessions, cattle preferentially grazed accession *P. atratum* U44. Further studies are needed to evaluate persistence under grazing and animal performance.

Keywords: Cattle preference, forage yield, frost tolerance, nutritive value, seed yield.

Resumen

En Corrientes, Argentina, se evaluaron algunas características agronómicas y de calidad de 11 ecotipos de *Paspalum atratum* y *P. lenticulare*, especies nativas de Sur América. La producción de forraje fue determinada entre 2011 y 2014, la digestibilidad y concentración de proteína en verano 2014 y la preferencia animal en invierno 2013 y verano 2015. La tolerancia al frío fue estimada en junio de 2012, el momento de floración durante 2013 y 2014, y la producción de semilla y la germinación en 2014. Los ecotipos de ambas especies mostraron un crecimiento estival marcado y la producción anual de forraje varió entre 5 y 12 t MS/ha. La floración ocurrió a comienzos del otoño, la producción de semillas fue de 280 kg/ha en promedio, y la germinación de 16% (*P. atratum*) y 19% (*P. lenticulare*). Con excepción de un ecotipo de *P. lenticulare* el germoplasma evaluado persistió en la época de invierno y se comportó como perenne. No se observaron diferencias entre los ecotipos en la concentración de proteína cruda y digestibilidad; sin embargo el ecotipo *P. atratum* U44 fue pastoreado preferencialmente. Futuros estudios serán necesarios para determinar la persistencia en pastoreo y la producción animal de los diferentes ecotipos.

Palabras clave: Preferencia animal, producción de semilla, rendimiento de forraje, tolerancia al frío, valor nutritivo.

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Introduction

Beef-cattle production systems in South America are based mostly on natural rangelands with only a small proportion of cultivated forage species (Berretta 2001; Maraschin 2001; Royo Pallarés et al. 2005). For example, in Argentina, only 10–20% of the total area used for livestock production is under sown species. Furthermore, in Northeastern Argentina, which is a subtropical region, only 4.5% of the total area is sown to predominantly warm-season grasses (Arelovich et al. 2011; Feldkamp 2011). The exception is Brazil, where 61% of the total area devoted to livestock production is sown with cultivated forage species, predominantly *Brachiaria* spp. (Jank et al. 2014). In South America, most forages sown in pasturelands have been introduced from Africa (Moser et al. 2004). However, there are several forage species, indigenous to South America, that have been widely sown in improved beef cattle production systems in the subtropics. For example, the grass *Paspalum notatum* (Gates et al. 2004) and the legume *Stylosanthes guianensis* (Skerman et al. 1991) were selected directly from wild populations, based on their agronomic qualities.

Since wild species have evolved in this environment, it seems logical to evaluate wild grasses and legumes to assess their potential as cultivated forages. The native genus *Paspalum* appears to possess great forage potential in South America. This genus contains about 330 species that grow in the New World (Zuloaga and Morrone 2005). The Plicatula group is an informal taxonomic group of *Paspalum* (Chase 1929) that contains about 30 species, most of which are recognized as good quality forages (Zuloaga and Morrone 2005). This group contains those species related to *P. plicatulum* Michx., characterized by spikelets with transversely wrinkled lemma and shining dark brown anthecium. Species within the Plicatula group vary in many characters, notably morphological characteristics, with the geographic center of variation of the group occupying Central and Western Brazil, Eastern Bolivia and Paraguay (Quarin et al. 1997). Most species of the Plicatula group are important native forages and some have become cultivated pasture plants (Quarin et al. 1997).

Species of the Plicatula group reproduce primarily by apomixis, which is an asexual mode of reproduction through seed, present in more than 500 species distributed in 40 families (Savidan 2007). The genus *Paspalum* contains the highest number of apomictic species in the Poaceae family (Ortiz et al. 2013).

Genetic improvement of apomictic species has been achieved mostly through ecotype selection (Blount and Acuña 2009). In apomictic species ecotypes are individual genotypes that have been able to colonize a particular area (Brugnoli et al. 2014) and new cultivars resulting from the selection of superior ecotypes have the distinction of being highly stable in their agronomic characteristics (Acuña et al. 2007).

Within the Plicatula group, *P. atratum* Swallen and *P. lenticulare* Kunth are characterized by rapid growth. *Paspalum atratum,* a warm-season grass grown on seasonally waterlogged, infertile, acid soils in the tropics and subtropics (Evers and Burson 2004), is a perennial bunchgrass that is larger and more robust than *P. plicatulum*. It is an apomictic tetraploid (2n=4x=40) native species from Brazil with high potential forage yield, good palatability and good seed yield (Quarin et al. 1997).

On the other hand, *P. lenticulare* is a multiploid species with diploid (2n=20), tetraploid (2n=40) and hexaploid (2n=60) cytotypes. Diploids reproduce sexually while polyploids are apomictic (Galdeano et al. 2016). The most common cytotype is tetraploid (Norrmann et al. 1994; Espinoza et al. 2001). The species is distributed throughout seasonally inundated savannas in Eastern Bolivia, Paraguay and the Center-West region of Brazil.

There is growing interest in developing native species as cultivated forages. Regional evaluation of native ecotypes of promising apomictic species may offer a valuable approach for identifying potential new cultivars. Protection and preservation of native species is also of importance and this is simultaneously addressed through the submission of native ecotype seed or vegetative collections to various national and international germplasm banks. At present, germplasm of many *Paspalum* species from different locations in South America and the rest of the world is conserved at the Instituto de Botánica del Nordeste (IBONE) in Northeastern Argentina.

The objectives of this research were to evaluate seasonal growth, frost tolerance, cattle preference, nutritional characteristics, seed yield and field germination of wild accessions of *P. atratum* and *P. lenticulare* collected from different locations in South America and grown in Northeastern Argentina. Additionally, the chromosome numbers in 2 accessions were determined to ensure all conserved germplasm is characterized by its ploidy level.

Materials and Methods

Plant material

Five accessions of *P. atratum* and 6 of *P. lenticulare* collected from different sites in South America were used in this study. The origins and chromosome numbers of these accessions are described in Table 1. Seeds have been maintained in seed storage at moisture content below 10% and approximately 5 ºC.

Table 1. Origin and chromosome number of *Paspalum atratum* and *P. lenticulare* accessions used in the study.

Species and accession	Origin	Chromosome number and reference	
P. atratum			
U39	Brazil, Miranda, Mato Grosso do Sul	40	Freitas et al. 1997
U42	Brazil, Aquidauana, Mato Grosso do Sul	40	Pagliarini et al. 2002
U44	Brazil, Cáceres, Mato Grosso do Sul	40	Freitas et al. 1997
N153	Paraguay, Guayaibí, Dpto. Caa Guazú	40	Espinoza et al. 2001
N164	Paraguay, Bella Vista, Arroyo Alegre and route 3, Dpto. Amambay	40	Espinoza et al. 2001
P. lenticulare			
L347	Paraguay, near the Aguaray-mí River, Dpto. San Pedro	40	This work
U41	Brazil, Miranda, Mato Grosso do Sul	40	Freitas et al. 1997
U43	Paraguay, Bella Vista, Dpto. Amambay	40	Pagliarini et al. 1999
U54	Cultivated in FCA-UNNE. Selection from TK2417	60	This work
TK2396	Bolivia, Estancia El Recreo, Concepción, Prov. Ñuflo de Chávez, Dept. Santa Cruz	40	Espinoza et al. 2001
TK2417	Bolivia, Estancia Viera, Concepción, Prov. Ñuflo de Chávez, Dept. Santa Cruz	40	Espinoza et al. 2001

Seed of each accession was sown in plots in the greenhouse in September 2011, and germinated seedlings were transplanted to planting trays 2 weeks later. These plants were transferred to a field site, previously native rangeland, near the city of Corrientes, Argentina (27º38' S, 58º44' W) on 7 November 2011 in plots of 2.7 m^2 (1.7 x 1.6 m) using a randomized complete block design with 3 replications. Each plot contained 30 plants uniformly distributed over the plot. The soil was classified as Aquic Argiudoll, a fine loam with slope between 1 and 1.5% (Escobar et al. 1996). At the beginning of the experiment the soil pH was 5.5, the P concentration 4.8 ppm (Bray II method), the K concentration 0.14 meq/100 g, and OM 1.18%. Fertilizer was not applied in this study following a low-input pasture system which is typical management in livestock production of tropical and subtropical regions.

Chromosome counting

Chromosome numbers of 9 of the 11 accessions used in this study were reported in past publications (Table 1), and chromosome numbers of the remaining 2 accessions were determined using root tips of young plants grown in pots in the greenhouse. Root tips were collected in the morning, washed and pretreated in a saturated solution of α-bromonaphthalene for 2 h, and hydrolyzed in 1 N HCl for 10 min at 60 ºC. They were then macerated in a drop of acetocarmine stain, heated, pressed under a cover-slip, and observed using a light microscope (Leica DM 2500®) at 200 and 1000 X magnification.

Seasonal growth

Seasonal growth was determined by harvesting plots using a sickle bar mower leaving a 10-cm stubble height. An 80 x 100 cm strip was cut from the middle of each plot, the forage was collected, weighed and a subsample of approximately 300 g was taken immediately. The subsample from each entry was weighed and oven-dried at 60 °C for 48 h to determine percent DM and forage yield. Plots were harvested on 15 March and 3 May 2012 during the 2011/2012 growing season; 28 November 2012, 23 January and 28 March 2013 during the 2012/2013 growing season; and 4 December 2013, 8 January and 8 April 2014 during the 2013/2014 growing season.

Crude protein concentration, in vitro dry matter digestibility and cattle preference

At the harvest in January 2014, clipped subsamples were taken for determination of in vitro dry matter digestibility (IVDMD) and crude protein (CP) concentration in the harvested forage. Samples were analyzed for IVDMD using the technique described by Tilley and Terry (1963). Crude protein concentration was determined following the method of Association of Official Analytical Chemists (AOAC 1990) by determining nitrogen concentration (Kjeldahl method) and multiplying the value obtained by 6.25.

The plots were grazed by cattle (cows) in September 2013 and February 2015 using mob grazing for 4 h duration at a stocking rate of 60 cows/ha. Cattle preference was evaluated using a visual scale from 1 to 5 where 1 represented lightly grazed and 5 heavily grazed. The observations were performed on plots by 2 observers immediately after the cows were removed from the experimental area.

Cool-season regrowth and frost tolerance

Cool-season regrowth and frost tolerance were evaluated after the first frosts occurring on 7 and 8 June 2012 with a minimal temperature of -1.8 °C, and temperatures below 0 ºC for 4 h on each day. The regrowth was estimated visually 30 days after the frost event using a 1–5 scale, where 1 = plants showing limited regrowth and 5 = plants showing high amounts of regrowth as described by Acuña et al. (2009; 2011). Frost tolerance was estimated visually 3 days after the frost using a 1–5 scale, where 1 = the least frost-tolerant and 5 = the most frost-tolerant plant as described by Zilli et al. (2015) and Novo et al. (2017).

Flowering time

Flowering date was determined by making weekly observations until the complete emergence of inflorescences in all plots. This evaluation was performed during the second and third growing seasons (2012/2013 and 2013/2014).

Seed yield and germination

Mature inflorescences were harvested by hand on 20 May 2014 (Figure 1). Inflorescences were air-dried, threshed and the fully formed, mature caryopses were separated from empty ones using a seed blower (Seedburo Equipment Company 1022W) and weighed to assess yields.

Figure 1. Hand-harvesting of seed of *Paspalum atratum* and *P. lenticulare* ecotypes in May 2014.

A field germination test was conducted in October 2014 in a plot previously cultivated with *Avena strigosa*. The sowing was done with an experimental no-till seeder a week after applying glyphosate at 3 L/ha. The seed was sown superficially using 2.3 m rows separated by 1 m. Rows were arranged following a randomized complete block design with 3 replications. Emerged plants were counted 15 days after sowing, and percentage germination was calculated.

Statistical analysis

The generated data were analyzed using Info-Gen software (Balzarini and Di Rienzo 2004) as a randomized complete block design. Means, coefficients of variation, analysis of variance (ANOVA) and mean separations by the Tukey test were calculated. Unless otherwise stated in the text, all differences refer to significance at P<0.05.

Results

Seasonal growth

There were no significant differences among accessions for annual forage accumulation (Table 2) but differences occurred between years with yields for the first, second and third years of 5.70, 11.72 and 12.29 t DM/ha, respectively. Growth rate varied with season of the year (Figure 2) with lowest growth during establishment, which was related to an unusually dry period (Figure 3), and between the end of the fall and the beginning of spring, while highest growth rates occurred during the warm season (December–March) through to mid-fall.

Crude protein concentration, in vitro dry matter digestibility and cattle preference

No significant differences among the accessions were observed for CP concentration and IVDMD of 4-week regrowth (Table 3); mean CP concentration was 87 g/kg DM and mean IVDMD, 525 g/kg DM. Accession L347 of *P. lenticulare* was not evaluated for nutritive value because it did not survive during winter 2013.

Differences between accessions in terms of cattle preference were observed in both years of evaluation (September 2013 and February 2015) (Figure 4). Cattle preferentially grazed accession *P. atratum* U44 in both years (Figure 5). However, correlations between IVDMD or CP concentration and cattle preference were not significant.

Cool-season regrowth and frost tolerance

After 2 consecutive frost events (7 and 8 June 2012) with temperatures below 0 ºC for 4 h on both days, the leaves of all accessions were uniformly frosted and plants did not regrow for a period of 1 month after the initial frost. Therefore, we were unable to evaluate and rate frost tolerance and subsequent winter-time regrowth. While most accessions survived the winter, and regrowth occurred in October when mean air temperature was 24 ºC, one accession, *P. lenticulare* L347, did not survive the second winter period.

Table 2. Forage yields (t DM/ha) of accessions of *Paspalum atratum* and *P. lenticulare* during 3 consecutive growing seasons.

Species and accession	Growing season								Accumulation		
	2011/2012		2012/2013			2013/2014			1st period	2nd period	3rd period
	15-mar	03-may	28-nov	23-jan	28-mar	04-dec	08-jan	08-apr	2011/12	2012/13	2013/14
P. atratum											
U 39	2.92	4.44	3.02	6.47	3.93	2.0	1.70	8.70	7.40	14.40	10.95
U 42	2.89	2.98	2.82	3.51	3.30	2.37	2.84	8.51	5.86	9.22	13.10
U 44	2.01	4.07	1.78	5.66	5.16	2.46	2.43	8.31	6.08	12.91	12.76
N 153	2.05	3.20	2.42	4.91	3.71	3.50	2.70	7.04	5.25	10.10	12.78
N 164	1.85	2.63	1.88	4.63	4.20	2.20	3.03	10.01	4.48	10.15	16.46
P. lenticulare											
L 347	3.36	2.97	4.76	4.09	3.44	2.00	-	5.40	6.33	12.94	6.76
U 41	1.62	3.90	2.44	4.47	5.28	2.00	2.10	8.02	5.52	12.57	11.50
U 43	1.33	3.58	2.76	5.38	4.39	3.49	3.38	7.86	4.91	13.40	14.32
U 54	2.11	3.12	3.15	5.37	4.61	3.31	2.57	8.30	5.23	12.61	13.24
TK 2396	1.54	3.66	2.23	4.17	2.72	1.60	1.65	7.38	5.20	8.80	9.65
TK 2417	2.18	4.27	2.52	5.74	3.81	3.25	2.22	9.10	6.46	11.89	13.65
Mean	2.17	3.53	2.71	4.95	4.05	2.56	2.46	8.06	5.70	11.72	12.29
CV[1]	0.37	0.40	0.43	0.25	0.25	0.49	0.28	0.21	0.29	0.26	0.21
MSD[2]	2.36	4.12	3.42	3.60	3.99	3.70	2.00	6.80	4.76	12.04	10.65
P. atratum	2.34	3.46	2.38	5.03	4.06	2.50	2.53	8.51	5.81	11.35	13.21
P. lenticulare	2.00	3.58	2.98	4.87	4.04	2.60	2.38	7.68	5.57	12.03	11.52
CV	0.44	0.38	0.45	0.27	0.27	0.50	0.33	0.21	0.28	0.25	0.25
MSD	0.68	0.94	0.87	0.96	0.96	0.90	0.60	1.50	1.12	2.58	2.74

[1]Coefficient of variation; [2]Minimum significant difference.

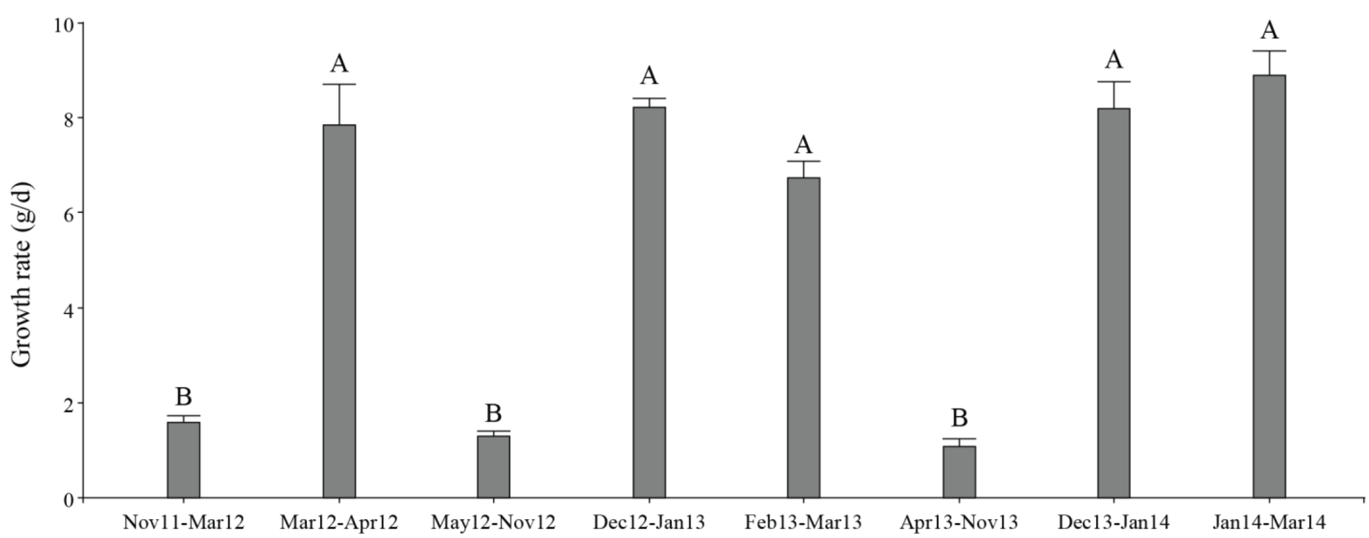

Figure 2. Growth rates of accessions of *Paspalum atratum* and *P. lenticulare* between November 2011 and March 2014. Different letters indicate significant statistical differences (P<0.05).

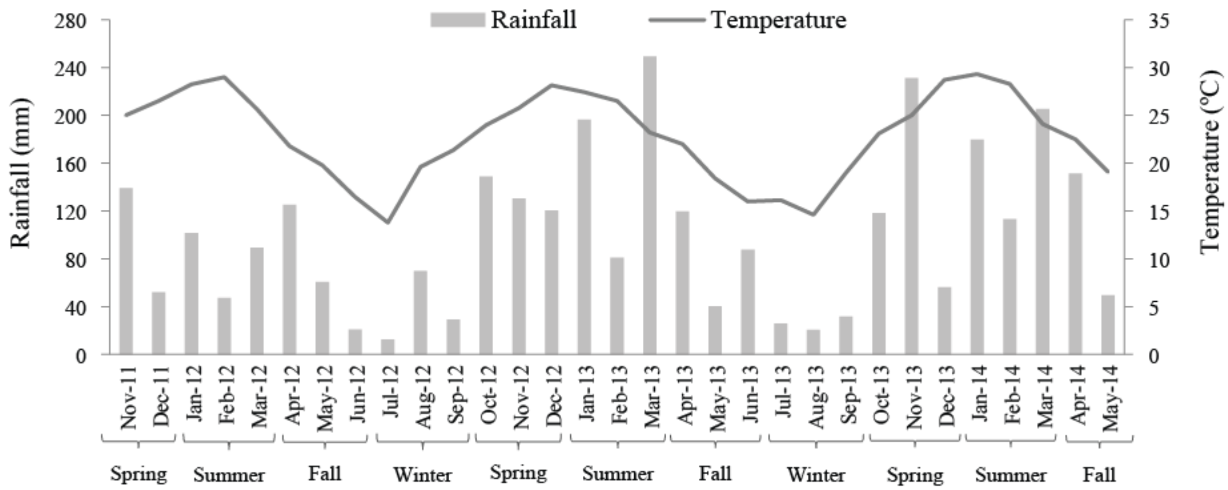

Figure 3. Monthly rainfall and mean temperature for each of the 3 growing seasons at Riachuelo, Corrientes, Argentina (27º38' S, 58º44' W).

Table 3. Crude protein (CP) concentration and in vitro dry matter digestibility (IVDMD) of *Paspalum atratum* and *P. lenticulare* accessions.

Species and accession	Nº observations	CP		IVDMD	
		(g/kg)	CV[1] (%)	(g/kg)	CV (%)
P. atratum					
U 39	3	81	0.5	583	1.2
U 42	3	77	4.8	516	5.6
U 44	3	77	5.4	542	20.4
N 153	3	78	7.1	438	13.0
N 164	3	101	49.4	464	10.9
P. lenticulare					
U 41	3	76	5.8	508	6.6
U 43	3	70	10.1	514	13.8
U 54	3	85	6.0	595	8.8
TK 2396	3	108	5.1	565	2.1
TK 2417	3	94	3.7	542	14.0
Mean		87	20.1	525	12.9
MSD[2]		50		170	

[1]Coefficient of variation; [2]Minimum significant difference.

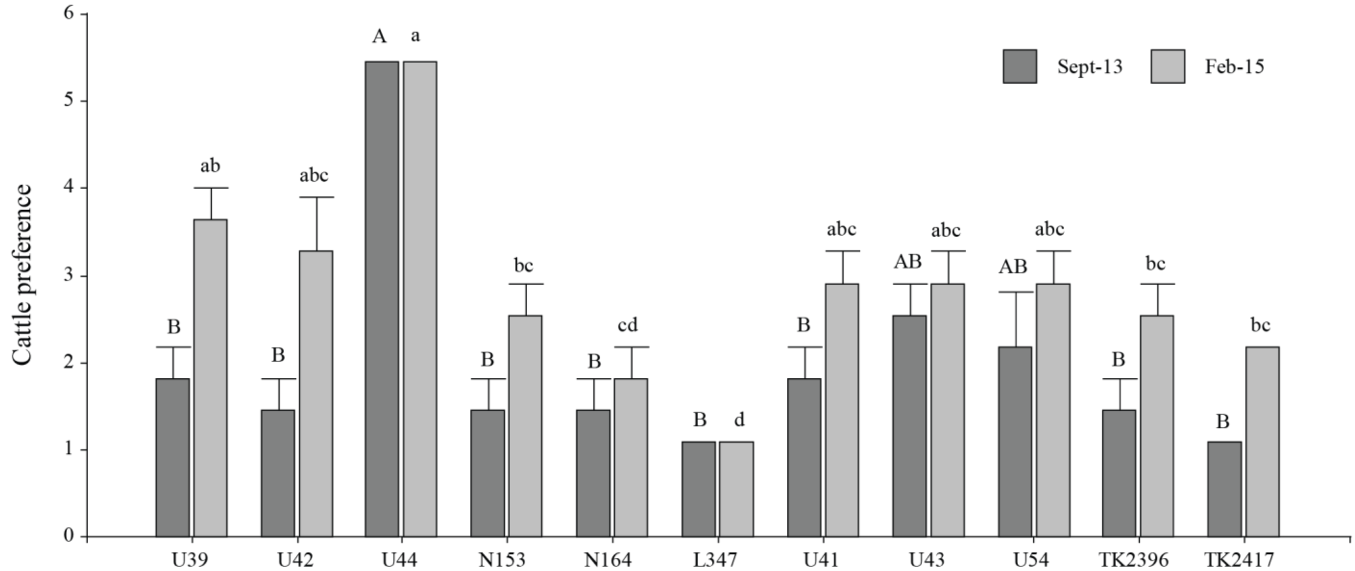

Figure 4. Cattle preference for *Paspalum atratum* and *P. lenticulare* accessions based on a visual scale (1–5) in September 2013 and February 2015. Different letters on columns indicate significant statistical differences (P<0.05). Uppercase letters belong to Sep-13 and lowercase letters to Feb-15.

Figure 5. Preferential grazing of accession *Paspalum atratum* U44 (left plot) over the other ecotypes during summer 2015.

Flowering time

Accession L347 (*P. lenticulare*) began flowering in early spring through September 2012, while all other accessions remained vegetative until late March 2013 (data not shown).

Seed yield and germination

Significant differences were observed among all evaluated accessions for seed yield (Figure 6). Accession *P. lenticulare* L347 had the lowest yield with 90 kg/ha and accession *P. lenticulare* U43 exhibited the highest production with 540 kg/ha. Mean seed yield of the group was 280 kg/ha. There were no differences in seed yields between *P. atratum* and *P. lenticulare* (310 vs. 260 kg/ha) (P>0.05).

Germination under field conditions was significantly different among accessions (Figure 7). Accession *P. atratum* U39 displayed the highest germination (28%) while *P. lenticulare* L347 and *P. atratum* U44 exhibited the lowest (11%). Overall mean germination percentage was 16% for *P. atratum* and 19% for *P. lenticulare*.

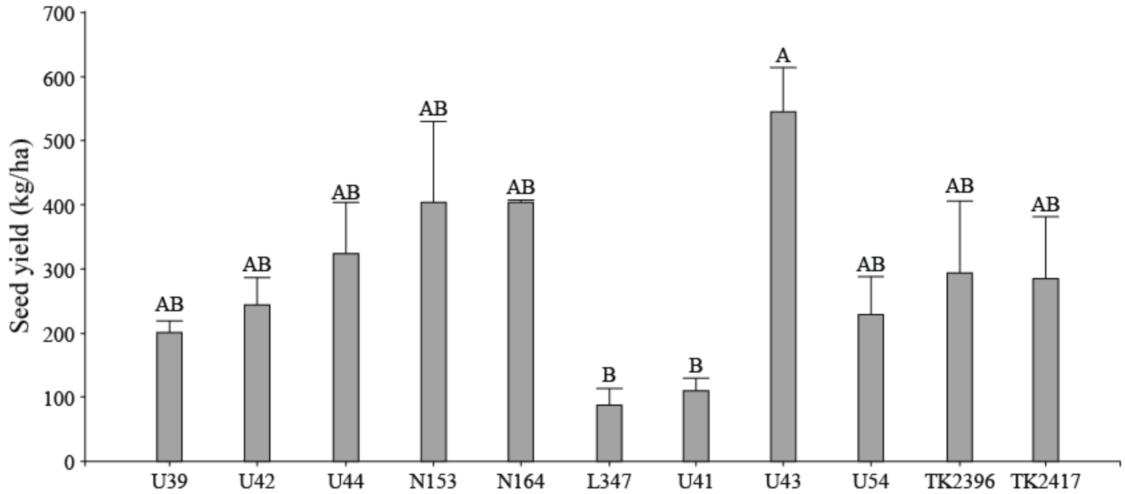

Figure 6. Seed yield of *Paspalum atratum* and *P. lenticulare* accessions evaluated during fall 2014. Different letters on columns indicate significant statistical differences (P<0.05).

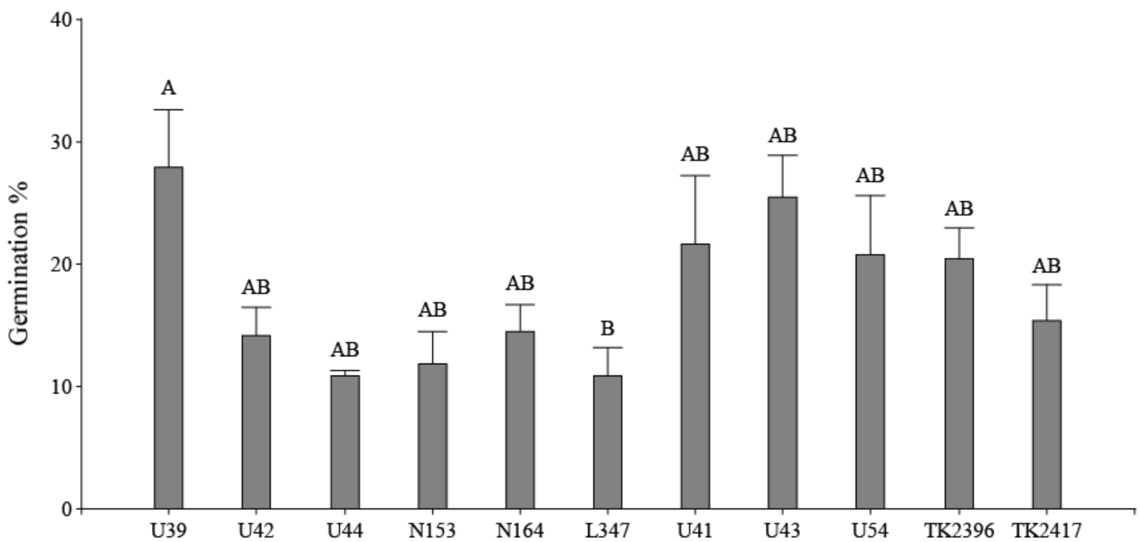

Figure 7. Field germination percentages of *Paspalum atratum* and *P. lenticulare* accessions in October 2014. Different letters on columns indicate significant statistical differences (P<0.05).

Discussion

We hypothesized that the introduction of native forage species into rangeland-based beef cattle production systems in the subtropics could increase the productive potential of these rangelands. To test this hypothesis the first step was to evaluate the growth and quality of some promising native grass species. In this research we evaluated a group of wild apomictic ecotypes of *Paspalum atratum* and *P. lenticulare* for a series of agronomic and forage quality characteristics.

Forage yield is the most important trait to consider before introducing a new cultivar into a pasture system. The mean yields we obtained during the second and third years from *P. atratum* and *P. lenticulare* (up to 11.7 and 12.8 t DM/ha) were quite substantial and quality was acceptable at 8.7% CP and 52.5% IVDMD. This was despite the fact that soils in this area of subtropical Argentina are generally inherently low in fertility and are acidic as they are in many pasturelands of South Brazil, Paraguay, Uruguay, Bolivia and Peru, and no fertilizer was applied. Typically pastures utilized by grazing cattle in the tropics and subtropics are not fertilized, so accessions evaluated in this study were not fertilized to obtain a more realistic evaluation of their potential under low-input pasture systems. As was expected, accessions in this study behaved as perennials in this region of Argentina, except for *P. lenticulare* accession L347, which behaved as a biennial.

The yield and quality data are similar or superior to those recorded for cultivated grass species frequently used in South America. For example, *Setaria sphacelata* cv. Narok is one of the most cultivated forages in North-eastern Argentina because of its ability to regrow rapidly after the winter and produce acceptable yields of good quality forage. Llamas et al. (2016) reported that this cultivar produced 5.1 t DM/ha/yr in Chaco, Argentina on an unfertilized Oxic Argiudoll soil with a harvest frequency of 40 days. Another example is *Brachiaria brizantha* cv. Marandu, the most cultivated warm-season grass in subtropical South America because of its high production in the acid soils of this region. This cultivar produced 3.75 t DM/ha/yr on infertile soils at Campo Grande, MS, Brazil with the addition of 50 kg N + 40 kg P + 40 kg K/ha (Euclides et al. 2008). Forage production of *P. atratum* and *P. lenticulare* is similar to or better than that of other species of the same genus such as *P. notatum* cv. Argentine, which is one of the principal cultivated forage species in the Coastal Plain region of Southeastern United States of America. Vendramini et al. (2013) reported annual forage yields of Argentine bahiagrass between 4.3 and 6.4 t DM/ha at the University of Florida Range Cattle Research and Education Center, Ona, FL, following the addition of 56 kg N + 14 kg P + 24 kg K/ha 2 weeks after planting and 60 kg N/ha annually. Similarly, *P. atratum* cv. Suerte produced 8.9–9.8 t DM/ha and 9.9–11.0 t DM/ha/yr following nitrogen fertilization of 180 kg N/ha and 220 kg N/ha, respectively, in north and central Florida (Kalmbacher et al. 1997).

The concentration of growth of *P. atratum* and *P. lenticulare* during late spring, summer and early fall was not surprising, as these are tropical-subtropical grasses. With the exception of *P. lenticulare* L347 all accessions of both species had exclusive vegetative growth during the spring-summer period with a concentrated flowering and seed production during the fall. Both species were considered to be frost-sensitive, but able to survive the mild winters of the subtropics. This is a common response of warm-season perennial grasses when grown in the subtropics (Moser et al. 2004). These characteristics allow for overseeding with annual winter forages, like *Avena strigosa* or *Lolium multiflorum*, to maintain adequate levels of forage production throughout the year. However, attempts are being made to breed strains of *Paspalum* species with increased forage production during the cool-season (Blount and Acuña 2009).

The mean CP concentration and IVDMD values for the accessions were similar to those of other warm-season grasses cultivated in the subtropics like *B. brizantha* cv. Marandu or *S. sphacelata*. Euclides at al. (2009) reported leaf CP of 9.2% and IVDMD of 53.2% in Marandu when fertilized with 50 kg N + 120 kg P + 40 kg K/ha prior to grazing in Campo Grande, MS. The mean CP percentage and IVDMD values of *S. sphacelata* in South Sulawesi, Indonesia without fertilization were 7.2% and 68.2%, respectively (Nasrullah et al. 2004).

While cattle preferentially grazed *P. atratum* accession U44, differences in acceptability were not related to the CP and IVDMD levels or the growing seasons, and may be due to differences in tactile and visual leaf texture, as the more palatable accession had softer leaves. However, further evaluations are needed to determine why cattle preferred U44, and if intakes were correspondingly higher on this grass.

Time to flowering was considered an important factor in this study. Ten of the 11 accessions we studied had a long vegetative phase and a concentrated reproductive phase at the end of the growing season. Only one accession, *P. lenticulare* L347, flowered early. This characteristic has also been observed in other species and cultivars of *Paspalum,* like *P. atratum* cv. Cambá FCA and *P. guenoarum* cv. Chané FCA (Evers and Burson 2004). However, this behavior is not displayed by other grasses like *P. notatum* (Blount and Acuña 2009) and *S. sphacelata* (Jank and Hacker 2004) that commence flowering early in November-December. An extended vegetative phase is considered advantageous as a longer duration of vegetative growth could result in a higher leaf percentage and better nutritive value at any given time. A concentrated reproductive phase may result in more uniform ripening of seed and better seed harvest.

The mean seed yield obtained in this study (280 kg/ha) can be considered most acceptable for a native warm-season perennial grass which is undomesticated. The most common cultivated species in the tropics and subtropics actually produce similar seed yields. Seed yields reported in the literature are: 57 kg/ha/yr for *S. sphacelata* cv. Narok receiving 50 kg N/ha at monthly intervals at Lawes, Australia over 2 years (Hacker 1991); 75 kg/ha for *P. dilatatum* (Evers and Burson 2004); and 110–220 kg/ha for *P. atratum* cv. Suerte (Kalmbacher et al. 1997). The mean field germinations for the *Paspalum* accessions, evaluated 5 months after seed harvest, of 16 and 19%, would allow good grass stands to be achieved with low amounts of seed sown (Newman and Moser 1988;

Masters et al. 2004). However, further research is needed to determine if seed dormancy is limiting germination soon after harvest, a common characteristic in species of Paniceae (Loch et al. 2004).

Conclusions

This study has shown that *P. atratum* and *P. lenticulare* have an extended warm-season growth and, despite being sensitive to low temperature, survived frosting. Their high forage and seed yields, as well as their capacity to survive the winter season in the subtropics, suggest that these undomesticated species can produce well in the northeast of Argentina and with their good seed yields may be potential candidates for sowing in the humid subtropics of South America as well as similar locations around the world. Most accessions are late-flowering, with a concentrated reproductive phase during the fall. This results in an extended period when forage quality should be acceptable, with the added benefit that seed harvesting should be more efficient with concentrated seed-set and ripening in the fall.

The extended growing period and persistence during the cold season should be compatible with over-sowing of the stands with winter-growing species to extend the period of productive forage availability. Considering that the accessions did not differ in forage yield, CP percentage and IVDMD, other factors like the high palatability of *P. atratum* U44 may determine the accession to sow in a given situation. Additional research is warranted to evaluate these accessions further in terms of persistence under grazing and animal performance in a beef cattle production system.

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References

Acuña CA; Blount AR; Quesenberry KH; Hanna WW; Kenworthy KE. 2007. Reproductive characterization of bahiagrass germplasm. Crop Science 47:1711–1717. DOI: 10.2135/cropsci2006.08.0544

Acuña CA; Blount AR; Quesenberry KH; Kenworthy KE; Hanna WW. 2009. Bahiagrass tetraploid germplasm: Reproductive and agronomic characterization of segregating progeny. Crop Science 49:581–588. DOI: 10.2135/cropsci2008.07.0402

Acuña CA; Blount AR; Quesenberry KH; Kenworthy KE; Hanna WW. 2011. Tetraploid bahiagrass hybrids: Breeding technique, genetic variability and proportion of heterotic hybrids. Euphytica 179:227–235. DOI: 10.1007/s10681-010-0276-y

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis. 15th Edn. AOAC Inc., Arlington, VA, USA.

Arelovich HM; Bravo RD; Martínez MF. 2011. Development, characteristics, and trends for beef cattle production in Argentina. Animal Frontiers 1:37–45. DOI: 10.2527/af.2011-0021

Balzarini M; Di Rienzo J. 2004. Info-Gen: Software para análisis estadístico de datos genéticos. Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina.

Berretta EJ. 2001. Ecophysiology and management response of the subtropical grasslands of southern South America. Proceedings of the XIX International Grassland Congress, São Pedro, SP, Brazil, 11–21 February 2001. p. 939–946. goo.gl/MLQwwT

Blount AR; Acuña CA. 2009. Bahiagrass. In: Singh RJ, ed. Genetic resources, chromosome engineering, and crop improvement series: Forage crops - Volume 5. CRC Press, Boca Raton, FL, USA. p. 81–101. DOI: 10.1201/9781420047400.ch4

Brugnoli EA; Urbani MH; Quarin CL; Zilli AL; Martínez EJ; Acuña CA. 2014. Diversity in apomictic populations of *Paspalum simplex*. Crop Science 54:1656–1664. DOI: 10.2135/cropsci2013.11.0780

Chase A. 1929. The North American species of *Paspalum.* Contributions of the United States National Herbarium 28:1–310. hdl.handle.net/10088/27051

Escobar E; Ligier HD; Melgar R; Matteio H; Vallejos O. 1996. Suelos de la provincia de Corrientes 1:500,000. Instituto Nacional de Tecnología Agropecuaria (INTA), Corrientes, Argentina. goo.gl/VgyS99

Espinoza F; Urbani MH; Martínez EJ; Quarín CL. 2001. The breeding system of three *Paspalum* species with forage potential. Tropical Grasslands 35:211–217. goo.gl/JCTbFG

Euclides VPB; Macedo MCM; Valle CB do; Barbosa RA; Gonçalves WV. 2008. Produção de forragem e características da estrutura do dossel de cultivares de *Brachiaria brizantha* sob pastejo. Pesquisa Agropecuária Brasileira 43:1805–1812. DOI: 10.1590/S0100-204X2008001200023

Euclides VPB; Macedo MCM; Valle CB do; Difante GS; Barbosa RA; Cacere ER. 2009. Valor nutritivo da forragem e produção animal em pastagens de *Brachiaria brizantha*. Pesquisa Agropecuária Brasileira 44:98–106. DOI: 10.1590/S0100-204X2009000100014

Evers GW; Burson BL. 2004. Dallisgrass and other *Paspalum* species. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C_4) grasses. ASA, CSSA, SSSA, Madison, WI, USA. p. 681–713. DOI: 10.2134/agronmonogr45.c20

Feldkamp CR. 2011. Beef production in Argentina: Situation and challenges. In: O'Rourke J, ed. Proceedings of the 9th

International Rangeland Congress, Rosario, Argentina, 2-8 April 2011. CD-ROM. p. 26–30.

Freitas PM de; Takayama SY; Pagliarini MS; Batista LAR. 1997. Evaluation of meiotic behavior in polyploid accessions of *Paspalum* (Plicatula group). The Nucleus 40:47–52. goo.gl/aKMz8R

Galdeano F; Urbani MH; Sartor ME; Honfi AI; Espinoza F; Quarin CL. 2016. Relative DNA content in diploid, polyploid, and multiploid species of *Paspalum* (Poaceae) with relation to reproductive mode and taxonomy. Journal of Plant Research 129:697–710. DOI: 10.1007/s10265-016-0813-4

Gates RN; Quarin CL; Pedreira CGS. 2004. Bahiagrass. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C_4) grasses. ASA, CSSA, SSSA, Madison, WI, USA. p. 651–680. DOI: 10.2134/agronmonogr45.c19

Hacker JB. 1991. Seed production potential in bred populations and cultivars of *Setaria sphacelata*. Tropical Grasslands 25:253–261. goo.gl/sfp1qf

Jank L; Hacker JB. 2004. Setaria. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C_4) grasses. ASA, CSSA, SSSA, Madison, WI, USA. p. 785–807. DOI: 10.2134/agronmonogr45.c23

Jank L; Barrios SC; Valle CB do; Simeão RM; Alves GF. 2014. The value of improved pastures to Brazilian beef production. Crop and Pasture Science 65:1132–1137. DOI: 10.1071/cp13319

Kalmbacher RS; Brown WF; Colvin DL; Dunavin LS; Kretschmer Jr. AE; Martin FG; Mullahey JJ; Rechcigl JE. 1997. Suerte atra paspalum, its management and utilization. Circular S-397. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, USA. goo.gl/D8MVKj

Llamas JO; Leonhard EAA; Yfrán EMM; Pereira MM; Bernardis AC; Fernández JA. 2016. Evaluación de la fertilización nitrogenada sobre la producción y el valor nutritivo de *Setaria sphacelata* cv. Narok. Agrotecnia 24:17–21. goo.gl/R7JKAY

Loch DS; Adkins SW; Heslehurst MR; Paterson MF; Bellairs SM. 2004. Seed formation, development, and germination. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C_4) grasses. ASA, CSSA, SSSA, Madison, WI, USA. p. 95–144. DOI: 10.2134/agronmonogr45.c4

Maraschin GE. 2001. Production potential of South America grasslands. Proceedings of the XIX International Grassland Congress, São Pedro, SP, Brazil, 11–21 February 2001. p. 5–15. goo.gl/KXsRXv

Masters RA; Mislevy P; Moser LE; Rivas-Pantoja F. 2004. Stand establishment. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C_4) grasses. ASA, CSSA, SSSA, Madison, WI, USA. p. 145–178. DOI: 10.2134/agronmonogr45.c5

Moser LE; Burson BL; Sollenberger LE, eds. 2004. Warm-season (C_4) grasses. ASA, CSSA, SSSA, Madison, WI, USA. DOI: 10.2134/agronmonogr45.c1

Nasrullah; Niimi M; Akashi R; Kawamura O. 2004. Nutritive evaluation of forage plants grown in South Sulawesi, Indonesia II. Mineral composition. Asian-Australian Journal of Animal Sciences 17:63–67. DOI: 10.5713/ajas.2004.63

Newman PR; Moser LE. 1988. Grass seedling emergence, morphology, and establishment as affected by planting depth. Agronomy Journal 80:383–387. DOI: 10.2134/agronj1988.00021962008000030001x

Norrmann GA; Quarin CL; Killeen TL. 1994. Chromosome numbers in Bolivian grasses (Gramineae). Annals of the Missouri Botanical Garden 81:768–774. DOI: 10.2307/2399921

Novo PE; Acuña CA; Quarin CL; Urbani MH; Marcón F; Espinoza F. 2017. Hybridization and heterosis in the Plicatula group of *Paspalum*. Euphytica 213:198. DOI 10.1007/s10681-017-1983-4

Ortiz JPA; Quarin CL; Pessino SC; Acuña C; Martínez EJ; Espinoza F; Hojsgaard DH; Sartor ME; Cáceres ME; Pupilli F. 2013. Harnessing apomictic reproduction in grasses: What we have learned from *Paspalum*. Annals of Botany 112:767–787. DOI: 10.1093/aob/mct152

Pagliarini MS; Takayama SY; Freitas PM de; Carraro LR; Adamowski EV; Silva N; Batista LAR. 1999. Failure of cytokinesis and 2n gamete formation in Brazilian accessions of *Paspalum*. Euphytica 108:129–135. DOI: 10.1023/A:1003660327223

Pagliarini MS; Carraro LR; Freitas PM de; Adamowski EDV; Batista LAR; Valls JFM. 2002. Cytogenetic characterization of Brazilian *Paspalum* accessions. Hereditas 135:27–34. DOI: 10.1111/j.1601-5223.2001.00027.x

Quarin CL; Valls JFM; Urbani MH. 1997. Cytological and reproductive behavior of *Paspalum atratum*, a promising forage grass for the tropics. Tropical Grasslands 31:114–116. goo.gl/DBiQSj

Royo Pallarés O; Berreta EJ; Maraschin GE. 2005. The South American Campos ecosystem. In: Suttie JM; Reynolds SG; Batello C, eds. Grasslands of the world. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. p. 171–219. goo.gl/qJZCAq

Savidan Y. 2007. Apomixis in higher plants. In: Hörandl E; Grossniklaus U; van Dijk PJ; Sharvel TF, eds. Apomixis. Evolution, mechanisms and perspectives. A.R.G. Gantner Verlag, Ruggell, Liechtenstein. p. 15–22.

Skerman PJ; Cameron DG; Riveros F. 1991. Leguminosas forrajeras tropicales. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Tilley JMA; Terry RA. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science 18:104–111. DOI: 10.1111/j.1365-2494.1963.tb00335.x

Vendramini JMB; Sollenberger LE; Blount AR; Aguiar AD; Galzerano L; Valente ALS; Alves E; Custodio L. 2013. Bahiagrass cultivar response to grazing frequency with limited nitrogen fertilization. Agronomy Journal 105:938–944. DOI: 10.2134/agronj2012.0404

Zilli AL; Brugnoli EA; Marcón F; Billa MB; Rios EF; Martínez EJ; Acuña CA. 2015. Heterosis and expressivity of apospory in tetraploid bahiagrass hybrids. Crop Science 55:1189–1201. DOI: 10.2135/cropsci2014.10.0685

Zuloaga FO; Morrone O. 2005. Revisión de las especies de *Paspalum* para América del Sur austral (Argentina, Bolivia, sur del Brasil, Chile, Paraguay y Uruguay). Monographs in Systematic Botany from the Missouri Botanical Garden 102:1–297.

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Research Paper

Pre-breeding studies in *Panicum coloratum* var. *coloratum*: Characterization using agro-morphological traits and molecular markers

Estudios de pre-mejoramiento genético en Panicum coloratum *var.* coloratum: *Caracterización usando marcadores moleculares y caracteres agro-morfológicos*

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Abstract

Panicum coloratum var. *coloratum* is a native African perennial C4 grass, introduced to Argentina. It is tolerant of salinity and cold and has good forage production. The scarce genotypic and phenotypic information about this grass limits its breeding in order to satisfy market demands. The aim of this study was to evaluate the variability in a collection of *P. coloratum* var. *coloratum* formed by 8 accessions and grown at EEA INTA Rafaela during the summer of 2011, based on 15 ISSR molecular markers and 17 morphological characters. For all morphological characters, the distribution of variability observed in the collection was high and not homogenous. The characters that showed greater variation were related to forage and seed production. Eight ISSRs, selected according to their reproducibility, showed 127 bands with 100% polymorphism and allowed grouping of populations according to their site of collection. AMOVA study indicated that more than 58% of the molecular variation existed within accessions; this would be consistent with the predominant allogamous form of reproduction. The results showed that the combined use of molecular and morphological markers offer complementary information. The high variability detected in this collection will allow for the initiation of a breeding program to improve important characters like those related to DM yield and seed production.

Keywords: ISSRs, variability, warm season grass.

Resumen

Panicum coloratum var. *coloratum* es una gramínea C4 perenne, originaria de África e introducida en Argentina alrededor de 1990. Se destaca por su buena producción de forraje y tolerancia a la salinidad y frío. La escasa información genotípica y fenotípica de este pasto ha limitado su uso en programas de mejoramiento y su demanda en el mercado. El objetivo del estudio fue evaluar la variabilidad en una colección de *P. coloratum* var. *coloratum* formada por 8 accesiones establecidas en la Estación Experimental Agropecuaria Rafaela del INTA en Argentina. El trabajo se realizó en la época de verano de 2011, utilizando 15 marcadores moleculares ISSR y 17 marcadores morfológicos. Todos los caracteres morfológicos evaluados presentaron una amplia distribución de la variabilidad, siendo esta no homogénea en la colección. Los caracteres con mayor variación fueron los relacionados con la producción de semilla y de forraje. Ocho

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ISSRs fueron seleccionados de acuerdo con su grado de reproducibilidad. Estos reprodujeron 127 bandas con 100% de polimorfismo y permitieron agrupar las poblaciones de acuerdo con su sitio de recolección. El análisis AMOVA indicó que más del 58% de la variación se presenta dentro de las accesiones, lo cual sería consecuente con la forma de reproducción alógama predominante en la especie. Estos resultados demuestran que el uso combinado de marcadores moleculares y morfológicos ofrece información complementaria de gran utilidad para evaluar la variabilidad de esta especie. Los resultados del estudio podrían ser utilizados para el comienzo de un programa de mejoramiento de caracteres importantes, entre ellos los relacionados con la producción de materia seca y semilla.

Palabras clave: Gramínea de clima cálido, ISSRs, variabilidad.

Introduction

Panicum coloratum L. (subfamily Panicoideae, tribe Paniceae) is a warm season perennial grass originating in Africa and introduced to Argentina around 1990 to be used as forage (Tischler and Ocumpaugh 2004). It is referred to in the literature as an allogamous cross-pollinated species (Pritchard and De Lacy 1974), and chromosome numbers are variable, although the most frequently reported is the tetraploid 2n=4X=36. The species is represented in Argentina by 2 varieties: var. *makarikariense* Gooss. (Goossens 1934) and var. *coloratum* (Bogdan 1977). It has been introduced to the country in multiple events that have been partially documented; collections are scarcely preserved in active genebanks (Armando et al. 2013). There has been renewed interest in the species recently following the relocation of livestock production to less productive areas and the subsequent need to produce forage of reasonably good quality and quantity in these situations (Rearte 2007; Manuel-Navarrete et al. 2009). As a result breeding efforts have been initiated in Argentina, since *P. coloratum* is one possible option for overcoming forage shortage in areas with climatic and edaphic constraints.

Breeding of perennial grasses is a lengthy process that involves several phases, such as gathering basic information about aspects of reproductive biology and cytology. It is essential to acquire a range of genetic material by compiling germplasm sources from different collections and evaluating it prior to performing selection and breeding (Vogel and Burson 2004). Breeding efforts with *P. coloratum* var. *coloratum* have led to cultivars with increased seedling weight (Hussey and Holt 1986) and the development of several lines with improved agronomic characteristics such as good seed retention (Young 1986), seed dormancy (Tischler and Young 1983) and mesocotyle length (Tischler and Voigt 1995). Although the species shows promise for use in subtropical areas, several traits still need to be improved to increase its adoption by ranchers. In fact, only a single cultivar, cv. Klein, is currently available to farmers in Argentina, and seed quality is frequently diminished by shattering problems.

Prior to commencing breeding activities, this study aimed to: a) evaluate the genetic diversity in the germplasm collection at the Instituto Nacional de Tecnología Agropecuaria (INTA) in Argentina, using inter-simple sequence repeat markers (ISSRs) and agronomically relevant morphological characters; b) compare the variability within and between accessions in the collection and with the commercial cv. Verde Kleingrass; and c) harmonize configurations rendered by the 2 types of traits and use them for the simultaneous characterization of accessions to be further used in conservation and breeding.

Materials and Methods

Plant material

The germplasm of *P. coloratum* var. *coloratum* used for this research was collected in 2006 from 7 different locations in 3 provinces in Argentina: Córdoba (accession DF), La Pampa (accessions CH, AN, EM, SO, UL) and Entre Rios (accession CU). The collection is preserved at the INTA Rafaela Experimental Station (31°11'41" S, 61°29'55" W; 957 mm mean annual precipitation). Collection sites differed in terms of soil characteristics, annual rainfall, original seed source, grazing management, etc. (Table 1). Individual accessions in the collection consisted of 32 plants in a common garden, separated at 0.60 m from one another. Different accessions were separated by a distance of 15 m to reduce the possibility of cross-pollination. The collection also included a plot of individuals from the commercial cv. Verde Kleingrass (CM; from here on referred to as 'cv. Klein'). Plant morphological characterization was performed between January and April 2011, during which 821 mm accumulated rainfall was recorded, a figure well in excess of the historical precipitation for that period (485 mm); mean temperature was 23.4 °C. The soil at this location is classified as Argiudoll (loamy, approximately 3% organic matter content, neutral pH, medium nutrient content and very low electrical conductivity).

Table 1. Accessions of *Panicum coloratum* var. *coloratum* and description of collection sites.

Accession code	Description	Site	Coordinates	Rainfall (mm/yr)
DF	12-year-old pasture, heavily grazed by cattle	Dean Funes (150 km northwest from Córdoba city)	30°25' S, 64°21' W	620
CH	Intersown in native rangeland, grazed by cattle, seeds produced in Argentina	Farm near Chacharramendi, west of La Pampa Province	37°20' S, 65°39' W	442
AN	9-year-old pasture, sown with seeds from USA, grazed by cattle	Anguil, close to Santa Rosa, La Pampa	36°30' S, 63°59' W	652
EM	3-year-old pasture, grazed by cattle	El Mirador, farm close to Gral. Acha, La Pampa	36°33' S, 64°50' W	556
SO	9-year-old pasture, sown with seeds from USA, grazed by cattle	Sol de Mayo, farm close to Gral. Acha, La Pampa	36°24' S, 64°21' W	556
UL	12-year-old pasture, grazed by cattle	Ultracán, south of Gral. Acha, La Pampa	37°22' S, 64°54' W	625
CU	9-year-old pasture, grazed by cattle	Concepción del Uruguay INTA Experiment Station	32°29' S, 58°20' W	1,188
CM	Seeds currently distributed by a private company	Cultivar 'Verde Kleingrass'	-	-

Morphological and agronomic evaluation

Measurements were performed on 10 individual plants for each accession, giving a total of 80 plants. A group of 17 morphological variables were recorded, related to forage production, forage quality and characters associated with seed production. Descriptions of variables and abbreviations used are displayed in Table 2. All observations were performed on adult plants. Three tillers per plant were tagged before starting measurements to calculate leaf elongation rate (LER). After an initial harvest at 15 cm height, a single growing leaf per tiller was measured every 5–7 days from 25 January to 17 February 2011. Daily growing degree units (GDUs) were calculated as

$$GDU = [(\text{Daily maximum temperature} - \text{Daily minimum temperature})/2] - Tb$$

where Tb was set to 10 °C base temperature (Ferri et al. 2006). Total aerial biomass was obtained by weighing the total biomass produced after 1,016 GDUs from the initiation of the measurements. The dried (60 °C for 72 hours) material was further milled using a 2 mm mesh before determination of quality parameters as described in Table 2.

Molecular evaluation

Genomic DNA was determined on 3–5 young fresh leaves from each of the plants used for morphological evaluation following the protocol CTAB described by Sambrook et al. (1989). The genomic DNA quality was evaluated by electrophoresis in agarose 0.8% gels with ethidium bromide (0.5 µg/mL) and 0.5X TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0) in 1X TBE buffer at 70 V during 1 h, and visualized under UV light and photographed with the G-box (Syngene®). Specific primers from 5.8s ribosomal gene were designed and used to confirm that the extracted DNA actually belonged to *P. coloratum.*

PCR reactions were performed in a Gene Amp PCR System 9700 thermocycler. Fifteen ISSR primers were tested for amplification (Garayalde et al. 2011). The reactions were performed in a final volume of 25 mL of a mixture containing buffer [75 mM Tris-HCl of pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of primer, 0.05 U/µL of Taq polymerase (Invitrogen) and ~15 ng/µL of genomic DNA (dilution 1/100 of DNA stock genomic)]. Simultaneously with each PCR reaction, a mock sample was added plus 2 controls, consisting of product amplifications of 2 individuals (CH9 and CH10) for each primer (with the aim of revealing the presence of non-specific bands). The program used in the PCR reactions was the same for all 15 primers tested and consisted of an initial period of DNA denaturation at 95 °C for 5 min, followed by 32 cycles of 94 °C for 40 sec, 52–42 °C for 45 sec (the corresponding annealing temperature depending on each pair of primers, see Supplementary Table 1), and 72 °C

Table 2. Morphological traits used to characterize *Panicum coloratum* var. *coloratum* and sampling methods.

Variable	Code	Character	Description
Vegetative characters	PH	Plant height (cm)	From soil surface to average leaf height at the end of the growing season
	DW	Dry weight per plant (g/plant)	At the end of the growing season, both vegetative and reproductive tillers cut and oven-dried to constant weight
	LER	Leaf elongation rate (cm/tiller/day)	Difference in blade length between measurements divided by the time elapsed between measurements
	TN	Number of tillers per plant	Count at the end of growing season, 1,016 GDUs after the last cut
Characters associated with seed production	1,000 SW	1,000 seed weight (g)	Calculated from the average of 3 samples of 100 mature seeds from 10 plants of each accession group
	SN	Number of seeds per panicle	Number of mature caryopsis bracts collected in a trap to avoid loss by shattering
	PaL	Panicle length (cm)	Measured from crown to base of flag leaf; average of 3 panicles per plant
	Shat	Seed retention	Seeds retained in the panicle 379 GDUs after anthesis as a percentage of the total number of seeds produced per panicle
	PN	Number of panicles per plant	Counted at the end of the growing season after seed had shattered
	%ES	Percentage of empty seeds	White caryopses as a percentage of the total caryopses produced per panicle
	RL	Rachis length (cm)	From flag leaf ligule up to the end; average of 3 panicles per plant
	FLL	Flag leaf blade length (cm)	Measured when fully expanded; average of 3 panicles per plant
	FLW	Flag leaf blade width (cm)	Measured when fully expanded; average of 3 panicles per plant
Quality parameters	CP	Crude protein concentration (g/100 g DM)	Determined by Kjeldahl procedure (AOAC 1990, No. 976.05)
	ADF	Acid detergent fiber concentration (g/100 g DM)	Determined according to Van Soest et al. (1991) in an ANKOM Daisy in vitro system (Ankom Technology, Method 5; 4/13/11)
	NDF	Neutral detergent fiber concentration (g/100 g DM)	Determined according to Van Soest et al. (1991) in an ANKOM Daisy in vitro system (Ankom Technology, Method 5; 4/13/11)
	LDA	Lignin concentration (g/100 g DM)	Determined according to Van Soest et al. (1991) in an ANKOM Daisy in vitro system (Ankom Technology, Method 5; 4/13/11)

for 40 sec, finally maintained at 72 °C for 5 min. Eight primers were selected for further analysis based on polymorphism and good band resolution. PCR products were supplemented with 5 µL of loading buffer (40% w/v sucrose, 0.25% bromophenol blue, 0.25% w/v xylene cyanol). Electrophoretic separation of the PCR products was performed on 1% agarose gels at 70 V for 1 h in 1X TBE buffer with ethidium bromide (0.5 µg/mL). The molecular profiles were visualized under UV light, photographed and stored for further analysis with the G-box (Syngene®).

Statistical analysis of the data

The analysis of 17 morphological variables was performed through analysis of variance (ANOVA). Mean separation tests were done using the Tukey test with the Bonferroni correction. Variables were transformed to meet normality and/or homoscedasticity as needed. Mean Euclidean Distance (MED) among individuals in each accession was calculated to estimate phenotypic variation within accessions. Principal component analysis (PCA) based on the standardized Euclidean distance was performed.

ISSR profiles were scored for presence (1) or absence (0) of homologous DNA bands. Genetic diversity was estimated in each accession by means of percentage of polymorphic loci (%P) and number of specific bands per accession. Dissimilarity between pairs of individuals was estimated through genetic distances (GD). Mean genetic distance (MGD) was calculated as the average of the genetic distances among individuals within accessions. Principal coordinates analysis (PCoA) was performed and individuals were plotted in a 2-dimensional graph representing the first 2 axes. All calculations were done using

Infogen (Balzarini et al. 2010). Total genetic variation was partitioned via analysis of molecular variance (AMOVA). The statistical significance of the proportion of the total variance attributed to correlation between individuals within accessions relative to that of the total (PhiPT) was tested through 1,000 random permutations using the software GenAlEx (Excoffier et al. 1992; Peakall et al. 1995; Peakall and Smouse 2006).

Joint analysis

Both agro-morphological and molecular traits were used for the simultaneous characterization of accessions by means of a Generalized Procrustes Analysis (GPA) based on the components obtained from the PCA and PCoA analysis previously described (Bramardi et al. 2005).

Mantel's test t (Mantel 1967) was used to establish the relationship between both types of data. Statistical significance was determined using 1,000 random permutations. Analyses were conducted in R version 3.3.2, using package 'vegan'.

Results

Agro-morphological variation

Ten of the 17 agro-morphological variables studied here showed significant ($P<0.05$) differences among accessions (Table 3), while 3 of the remaining 7 showed differentiation at $P<0.10$ (data not shown). Within accessions, MED indicated that accession CU had the highest variability, EM presented the lowest distance among individuals, and the remaining 6 presented values close to the lowest one (Table 3).

Regarding accession characteristics, on average, CH plants showed the highest forage production and number of tillers, while EM presented the lowest dry weight per plant and DF the least number of tillers. However, differences in leaf elongation rate were not so pronounced, and populations presenting the highest values were DF, CU and SO. Forage quality showed a different pattern, with CU plants having highest crude protein concentration and EM plants the lowest, while differences between accessions in NDF, ADF and lignin concentrations were generally less than 10% ($P>0.05$). All materials produced similar numbers of seeds per panicle ($P = 0.32$) and showed only marginal differences in the percentage of empty seeds ($P = 0.08$).

A PCA biplot based on phenotypic variability among individuals explained 37.7% of the variation in the first 2 axes. Nine variables attained percentages of reconstruction approaching or exceeding 50%, while 3 variables showed very low reconstruction in the first 2 axes (Figure 1A). In general, individuals did not congregate according to their origin.

Comparing the relative performance of accessions with that of cv. Klein (CM), currently commercialized in Argentina, some accessions out-performed Klein in LER, PaL and CP, demonstrating that there is some room for selection.

Molecular variation

Analysis of 80 plants of *P. coloratum* var. *coloratum* rendered 127 ISSR reproducible bands, and 65 of them showed polymorphism (51.2%). The number of amplified bands per primer ranged from 10 to 24, representing a polymorphism range from 40.1 to 67.5% (data not shown).

The amount of variability among individuals within accessions did not differ between accessions: the percentage of polymorphic loci ranged from 42.5 in DF and SO to 59.1 in AN (Table 4). Cultivar Klein (CM) showed an intermediate level of variability. The same pattern was observed regarding mean genetic distance (MGD), with accession AN being the most variable, while SO displayed the least MGD between individuals. Accession-specific ISSR bands were apparent only in AN, SO and CM, indicating that most bands were shared by at least 2 accessions.

The first 2 axes of the PCoA analysis based on the ISSR distance matrix explained 20.0% of the variation (Figure 1B). Four groups could be identified in the plot, although the groups were spread and showed some level of overlapping between them. A rather distinct group was formed by individuals of SO, well separated from the rest. Individuals from AN, EM, CU and CH clustered in the center and formed a group. DF appeared related to CM, and UL showed dispersed individuals forming a separated group.

The dendrogram based on the individual GD ISSR distance matrix clearly conglomerated individuals from the same origin but at a high distance (Figure 2, $r = 0.83$). AMOVA performed on ISSR data showed that only 42% of the molecular variability is attributable to differences between accessions; therefore, most of the variation in this variety would be among individuals within accessions. In agreement with that, only 2 of the assayed primers showed more that 50% of the differentiation between accessions, while another 2 initiators indicated that more than 75% of the total genetic variation was explained by differences between individuals within accessions.

Table 3. Mean (M), Bonferroni test (Bt), relative performance (Rp) and mean Euclidean distance (MED) in *Panicum coloratum* var. *coloratum* accessions for 17 agro-morphological traits.

Trait[1]	Accession[2]																						
	AN			CH			CU			DF			EM			SO			UL			CM	
	M	Bt[3]	Rp[4]	M	Bt	Rp	M	Bt	Rp	M	Bt	Rp	M	Bt	Rp	M	Bt	Rp	M	Bt	Rp	M	Bt
PH	86	AB		100	C		99	BC		106	C	+15	83	A	-10	92	ABC		96	ABC		92	ABC
DW	252	AB	-13	422	B	+45	332	AB	+14	244	AB	-16	172	A	-41	220	AB	-25	282	AB		291	AB
LER	4.8	ABC	+10	4.8	ABC	+10	5.5	BC	+26	6.1	C	+39	3.6	A	-18	5.3	BC	+22	4.8	ABC	+11	4.4	AB
TN	148	B	+29	176	B	+54	140	AB	+22	55	A	-52	85	AB	-26	97	AB	-16	120	AB		115	AB
1,000-SW	940	AB		920	AB		850	A	-14	1140	BC	+15	1040	AB		1020	AB		1010	AB		990	AB
SN	113	A		90	A	-21	113	A		62	A	-46	139	A	+21	104	A		110	A		115	A
PaL	69.6	AB		82.3	BC	+19	78.8	ABC	+14	89.7	C	+30	66.2	A		81.6	BC	+18	70.6	AB		69.2	AB
Shat	0.7	A	-17	0.74	A	-11	0.79	A		0.82	A		0.68	A	-17	0.69	A	-16	0.71	A	-13	0.82	A
PN	251	AB		331	B	+35	324	B	+32	135	A	-45	196	AB	-20	171	AB	-30	246	AB		245	AB
%ES	38.8	A		33.7	A	-13	42.8	A	+11	55.1	A	+43	27.9	A	-28	30.3	A	-22	48.7	A	+26	38.6	A
RL	20.7	AB		22.9	AB		23.3	AB		27.1	A	+26	20.9	AB		18.7	B	-13	21.7	AB		21.5	A
FLL	18.9	A		16.9	A		16.4	A		20.0	A	+14	18.6	A		15.2	A	-13	18.2	A		17.5	A
FLW	0.49	AB		0.47	AB		0.55	AB	+10	0.56	AB	+12	0.45	A	-10	0.46	A		0.60	B	+20	0.5	AB
CP	5.9	AB	+16	5.7	A	+12	7.5	B	+48	6.2	AB	+22	4.6	A		5.5	A		6.1	AB	+21	5.1	A
NDF	70.1	A		70.6	A		69.8	A		69.1	A		72.8	A		70.8	A		68.9	A		71.6	A
ADF	35.5	A		37.3	A		36.9	A		35.6	A		37.5	A		36.6	A		35.2	A	-15	37.0	A
LDA	4.1	A		4.3	A		5.1	A	+17	3.9	A	-10	4.1	A		4.7	A		3.7	A		4.4	A
MED	4.88			4.82			7.12			5.13			4.18			4.42			5.15			5.16	

[1]**Evaluated traits** (codes, units): PH: Plant height (cm); DW: Dry weight per plant (g); LER: Leaf elongation rate (cm/tiller/d); TN: Number of tillers per plant; 1,000-SW: 1,000 seed weight (g); SN: Number of seeds per panicle (SN); PaL: Panicle length (cm); Shat: Seed retention; PN: Number of panicles per plant; %ES: Percentage of empty seeds; RL: Rachis length (cm); FLL: Flag leaf blade length (cm); FLW: Flag leaf blade width (cm); CP: Crude protein (g/100 g DM); ADF: Acid detergent fiber (g/100 g DM); NDF: Neutral detergent fiber (g/100 g DM), LDA: Lignin (g/100g DM). [2]**Accession codes**: AN: Anguil; CH: Chacharramendi; CU: Concepcion del Uruguay; DF: Dean Funes; EM: El Mirador; SO: Sol de Mayo; UL: Ultracán; CM: cv. Klein. [3]**Bonferroni test**: Different letters at each population indicate significant differences among populations at P<0.05. [4]**Rp**: Positive and negative numbers indicate higher or lower values than CM (cv. Klein), respectively.

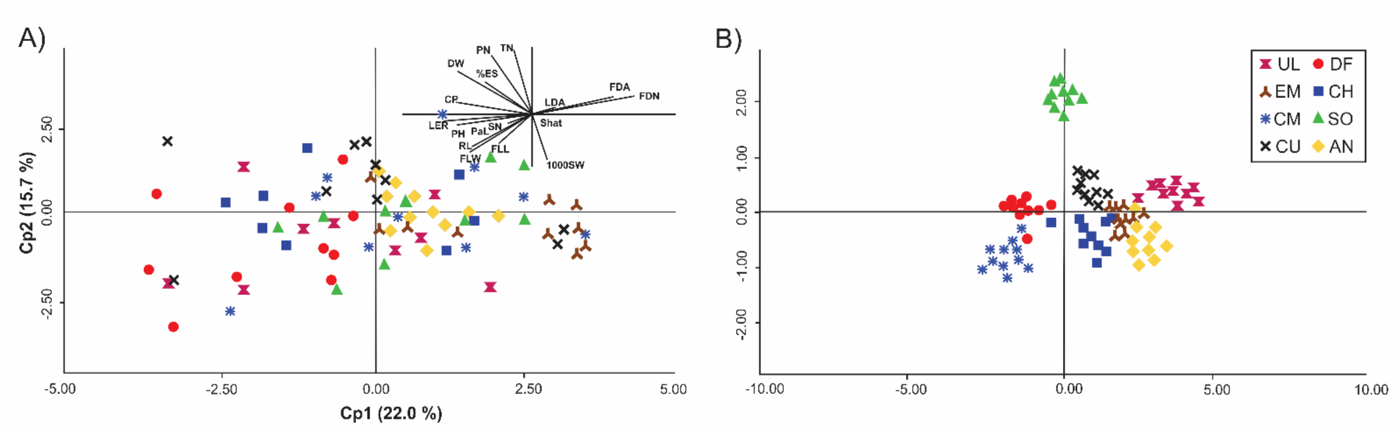

Figure 1. **A**) Individual and variable plots of principal component analysis (PCA) based on the individual distance matrix of *Panicum coloratum* var. *coloratum* calculated from 17 agro-morphological traits. **B**) Principal coordinates analysis (PCoA) plot based on the individual ISSR distance matrix of *P. coloratum* var. *coloratum*. See Tables 1 and 2 for a fuller description of the accessions and variables evaluated.

Table 4. Genetic diversity parameters for ISSR markers in accessions of *Panicum coloratum* var. *coloratum*.

Parameter[1]	Accession[2]							
	AN	CH	CU	DF	EM	SO	UL	CM
NI	78	75	71	56	69	58	79	70
%P	59.1	54.3	53.5	42.5	50.4	42.5	56.7	51.2
MGD	31.3	27.6	26.7	23.7	28.0	23.1	28.5	26.9
NISp	1	0	0	0	0	1	0	1

[1]NI: total number of loci; %P: percentage of polymorphic loci; MGD: mean genetic distance; and NISp: number of accession-specific loci. [2]Accession codes see Table 1.

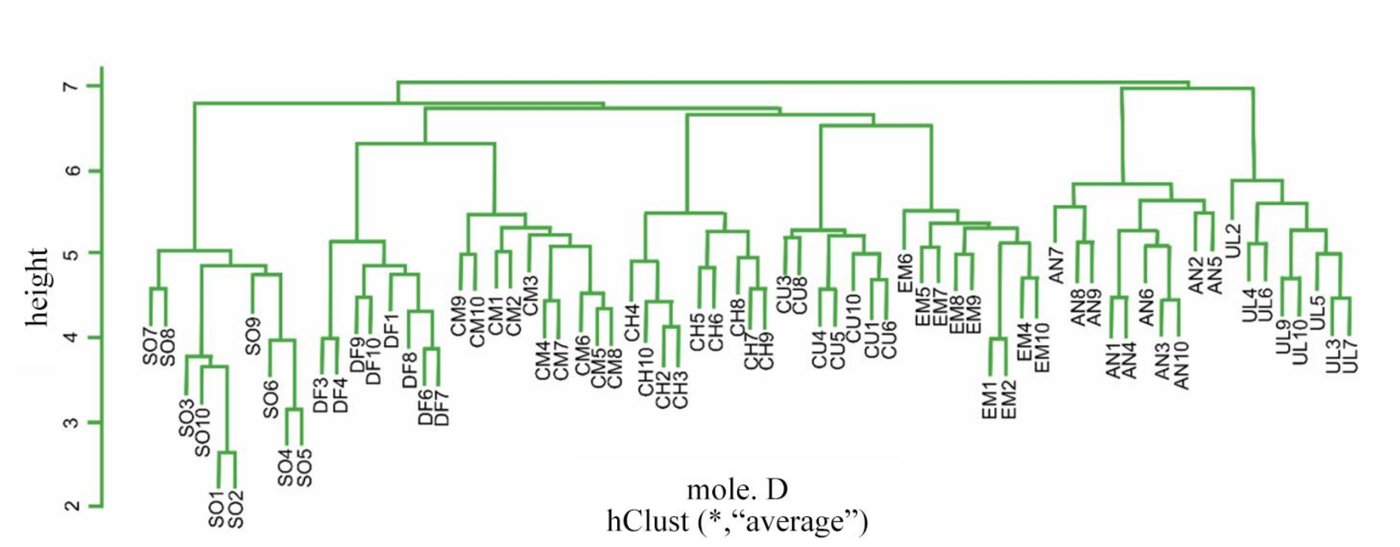

Figure 2. Unweighted pair-group method with arithmetic average (UPGMA) dendrogram based on the GD ISSR distance matrix of *Panicum coloratum* var. *coloratum*. See Table 1 for a fuller description of the accessions evaluated.

Joint characterization

From the Generalized Procrustes Analysis, the first 2 components were responsible for 20.5 and 16.3% of the total variation, respectively, adding up to 36.8%. Breaking down the total sum of squares showed that 77.3% corresponded with the consensus configuration, implying that only 22.7% was linked to the divergence between agronomic and molecular points and the points of the consensus configuration. In addition, the results of the Mantel test showed that the correlation between the 2 sets of data was very low (r = 0.057; P = 0.082).

Discussion

This study reported significant variation for the 17 morphological and molecular characters evaluated in the collection of *P. coloratum* var. *coloratum* at INTA in Argentina, a collection allotted to germplasm preservation and breeding. Even though the species has been an introduced forage to Argentina and introductions were not well documented, it can be argued that no reduction in variability due to factors such as, for example, founder effect occurred. The collection was assembled from ramets of adult plants, sometimes from quite old pastures that might have been under high selection pressure exerted by grazing, but no reduction of variability relative to the original populations was observed (Silvertown and Lovett Doust 1993). The most variable characters were related to forage production (number of tillers and dry weight per plant) and seed production (number of panicles per plant and number of seeds per panicle), as has been reported for other forages (Casler 2005; Morales-Nieto et al. 2008; Wójtowicz et al. 2009; Abbott and Pistorale 2010). As plants were grown in a common garden setting, environmental conditions were similar for all.

Variability in agro-morphological traits was found both within and between accessions that were in general quite variable, with a certain degree of overlapping. Although some accessions were significantly different for some characteristics, it may be difficult to allocate individuals to accessions based only on morphology. The same degree of variability observed within accessions was registered in the commercial material, CM (cv. Klein). Clearly seed of cv. Klein marketed in Argentina is from a rather genetically wide stabilized population of an allogamous species and identification of the origin of the seeds by a governmental agency may not be simple. This cultivar was developed in the USA (Carr 2014) through a process of recurrent selection to increase seed weight (Hussey and Holt 1986). In fact, the collection at INTA comprises accessions with heavier seeds, opening the possibility of a new cycle of selection. An important short-coming of the species is the difficulty in seed production due to shattering (Young 1994), which causes major losses of seed at harvesting. Improving this characteristic would be a worthwhile aim for a breeding program. While some materials with greater seed retention have been registered in the USA (Tischler and Ocumpaugh 2004), we are unaware of any attempts to improve this character through breeding programs. Since variability in this collection is located within accessions it is possible that, even though the commercial material and all accessions showed comparable levels of seed shattering, some individuals with higher seed retention may be identified for use as parents in future breeding programs.

In this study, there was some variability among populations in terms of CP concentration in the forage and accession CU actually produced forage of better quality than that from the commercial cultivar being marketed in the country. Although forage production does not necessarily predict secondary production (meat, milk or wool), forage with higher CP might be more digestible and give higher animal performance (Meissner 1997; Smith et al. 1997). Increments of 1% in in vitro digestibility of forage can lead to an increase in animal liveweight gain of 3.2% (Casler and Vogel 1999).

The high levels of variability within and among accessions of the collection as shown by the use of 8 ISSRs (Table 4) are similar to the genetic diversity observed in other species of open-pollinated grasses, e.g. zoysiagrass (Xie et al. 2012b) and bermudagrass (*Cynodon dactylon*) (Huang et al. 2010). Accession AN showed the highest level of variability, with approximately 59% of polymorphism, the highest MGD and 1 of the 3 accession-specific loci. Combining these features, AN emerged as a significant reservoir of variability in the collection.

As previously discussed, analysis using agro-morphological data failed to detect any type of association between accessions. In contrast, PCoA analysis showed some degree of grouping among accessions according to their site of collection as previously reported in the literature (García et al. 2007; Najaphy et al. 2012). In addition, the dendrogram obtained from the molecular data grouped individuals from the same origin, although the grouping was attained at a high distance, supporting the fact that 58% of the variation was found between individuals within groups, with only 42% between groups. A rather cohesive group was formed by accessions CM and DF, the latter being a recently sown pasture, so it could be hypothesized that individuals of DF retained characteristics of the commercial material, given that they were not exposed to grazing or environmental conditions for enough time to change their genetic composition. On the opposite side of the plot, UL could be identified as an accession from a rather old pasture that had probably been under heavy grazing pressure for several years. Accessions in the center of the plot aggregated into 2 groups: the one below showed populations CH, AN and EM in clusters; CH and EM had been recently sown from seeds produced at the AN site, so shared the same genetic material. A quite separate group was formed by individuals of accession SO obtained from imported seeds with a history of heavy grazing that might have increased the proportion of individuals with grazing tolerance. Finally, the history of CU is undocumented.

The AMOVA study indicated that more than 58% of molecular variation in *P. coloratum* resides within accessions. This finding is consistent with the predominant allogamous form of reproduction in the species (Hamrick and Godt 1990). Similar patterns of genetic variation distribution were reported in other cross-pollinated species. Xie et al. (2012a) reported 65% variation within accessions of orchardgrass (*Dactylis glomerata*) using SSR. *Panicum coloratum* var. *coloratum* is reported as having a high rate of sexual reproduction (Tischler and Ocumpaugh 2004), although the ability to reproduce asexually by apomixis should not be disregarded. In addition, vegetative reproduction through rhizomes and rooting at the nodes when they come into contact with the ground has been observed (Petruzzi et al. 2003; Komatsu et al. 2007).

Results of characterization using molecular markers and agro-morphological descriptors produced different configurations (Figures 1A and 1B). In addition, results of the Mantel test (r = 0.057; P = 0.082) confirmed low correlations between the two types of data, indicating that each individual characterization offers information that can be considered complementary. Semagn (2002) attributed the discrepancy between morphological and molecular variation to 2 possible factors: 1) molecular markers covering a large portion of the genome that includes coding and non-coding regions; and 2) molecular markers being less subject to artificial selection than morphological markers. According to Holderegger et al. (2006), most DNA markers are a random sample whose genome polymorphism sites have no phenotypic effects corresponding to neutral genetic variation, while quantitative morphological characters are usually affected by selection and variability within them is ultimately responsible for adaptation. Interestingly, joint characterizations rendered a consensus configuration explaining 77.3% of the variability, emphasizing the importance of studying the different types of descriptors together in order to get the best characterization and interpretation of the genetic diversity of the collection.

It is widely acknowledged that good characterization of a live collection is an important first step towards germplasm management and preservation. The collection of *P. coloratum* var. *coloratum* at INTA Rafaela was found to be a good resource and a reservoir of genetic variation for valuable characters to be further used in breeding for different objectives such as augmentation of forage production, forage quality or seed weight. The use of both molecular and agro-morphological traits was a valuable tool as both types of characterizations were complementary and jointly rendered a good discrimination of accessions.

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References

Abbott L; Pistorale S. 2010. Determination of variance components and heritability in prairie grass (*Bromus catharticus* Vahl.). Agriscientia 27:115–123. goo.gl/rA9baM

Armando LV; Carrera AD; Tomas MA. 2013. Collection and morphological characterization of *Panicum coloratum* L. in Argentina. Genetic Resources and Crop Evolution 60:1737–1747. DOI: 10.1007/s10722-013-9982-3

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis. 15th Edn. AOAC Inc., Arlington, VA, USA.

Balzarini M; Bruno C; Peña A; Teich I; Di Rienzo JA. 2010. Estadística en biotecnología. Aplicaciones en Info-Gen. Editorial Brujas, Córdoba, Argentina. goo.gl/aCyQE7

Bogdan AV. 1977. Tropical pasture and fodder plants. Grasses and legumes. p. 175–181. Longman, London and New York. lib.ugent.be/catalog/rug01:000083517

Bramardi SJ; Bernet GP; Asíns MJ; Carbonell EA. 2005. Simultaneous agronomic and molecular characterization of genotypes via the Generalised Procrustes Analysis. Crop Science 45:1603–1609. DOI: 10.2135/cropsci2004.0633

Carr BC 2014. Plant guide for kleingrass (*Panicum coloratum* L.). USDA Natural Resources Conservation Service, James E. "Bud" Smith Plant Materials Center, Knox City, TX, USA. https://goo.gl/AUhjiS

Casler MD. 2005. Ecotypic variation among switchgrass populations from the Northern USA. Crop Science 45:388–398. DOI: 10.2135/cropsci2005.0388

Casler MD; Vogel KP. 1999. Accomplishments and impact from breeding for increased forage nutritional value. Crop

Science 39:12–20. DOI: 10.2135/cropsci1999.0011183X003900010003x

Excoffier L; Smouse PE; Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479–491. goo.gl/kR7WGB

Ferri CM; Stritzler NP; Pagella JH. 2006. Tasa de aparición de hojas en *Panicum coloratum* L. cv. Verde. 29º Congreso Argentino de Producción Animal, Mar del Plata, Argentina. Revista Argentina de Producción Animal. goo.gl/qanrU3

Garayalde AF; Poverene M; Cantamutto M; Carrera AD. 2011. Wild diversity in Argentina revealed by ISSR and SSR markers: An approach for conservation and breeding programmes. Annals of Applied Biology 158:305–317. DOI: 10.1111/j.1744-7348.2011.00465.x

García MV; Balatti PA; Arturi MJ. 2007. Genetic variability in natural populations of *Paspalum dilatatum* Poir. analyzed by means of morphological traits and molecular markers. Genetic Resources and Crop Evolution 54:935–946. DOI: 10.1007/s10722-006-9147-8

Goossens AP. 1934. Notes on African grasses: XVI. Bulletin of Miscellaneous Information 5:195–202. DOI: 10.2307/4111662

Hamrick JL; Godt MJW. 1990. Allozyme diversity in plant species. In: Brown ADH; Clegg MT; Kahler AL; Weir BS, eds. Plant populations genetics, breeding and germplasm resources. Sinauer Associates Inc., Sunderland, MA, USA. p. 43–63. goo.gl/Neqrbj

Holderegger R; Kamm U; Gugerli F. 2006. Adaptive vs. neutral genetic diversity: Implications for landscape genetics. Landscape Ecology 21:797–807. DOI: 10.1007/s10980-005-5245-9

Huang CQ; Liu GD; Bai CJ; Wang WQ; Zhou SY; Yu DQ. 2010. Estimation of genetic variation in *Cynodon dactylon* accessions using the ISSR technique. Biochemical Systematics and Ecology 38:993–999. DOI: 10.1016/j.bse.2010.09.013

Hussey MA; Holt EC. 1986. Selection for increased seed weight in Kleingrass. Crop Science 6:1162–1163. DOI: 10.2135/cropsci1986.0011183X002600060017x

Komatsu T; Ubi BE; Enoky H. 2007. Crossability and amplified fragment length polymorphism variation among four types of *Panicum coloratum* L. and meiotic chromosome pairing of their F1 hybrids. Grassland Science 53:97–102. DOI: 10.1111/j.1744-697X.2007.00079.x

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220. goo.gl/iFZYHs

Manuel-Navarrete D; Gallopín GC; Blanco M; Díaz-Zorita M; Ferraro D; Herzer H; Laterra P; Murmis MR; Podestá GP; Rabinovich J; Satorre EH; Torres F; Viglizzo EF. 2009. Multi-causal and integrated assessment of sustainability: The case of agriculturization in the Argentine Pampas. Environment, Development and Sustainability 11:621–638. DOI: 10.1007/s10668-007-9133-0

Meissner HH. 1997. Recent research on forage utilization by ruminant livestock in South Africa. Animal Feed Science and Technology 69:103–119. DOI: 10.1016/S0377-8401(97)81626-4

Morales-Nieto C; Quero-Carrillo A; Pérez-Pérez J; Hernández-Garay A; Le-Blanc O. 2008. Morphological characterization of native populations of sideoat grama [*Bouteloua curtipendula* (Michx.) Torr.] in Mexico. Agrociencia 42:767–775. goo.gl/fRAdTk

Najaphy A; Parchin RA; Farshadfar E. 2012. Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. Australian Journal of Crop Science 6:326–332. goo.gl/7sN4eL

Peakall R; Smouse PE; Huff DR. 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalo grass *Buchloë dactyloides*. Molecular Ecology 4:135–148. DOI: 10.1111/j.1365-294X.1995.tb00203.x

Peakall R; Smouse PE. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295. DOI: 10.1111/j.1471-8286.2005.01155.x

Petruzzi HJ; Stritzler NP; Adema EO; Ferri CM; Pagella JH. 2003. Mijo perenne - *Panicum coloratum*. Instituto Nacional de Tecnología Agropecuaria (INTA), Anguil, La Pampa, Argentina. goo.gl/CeTx7y

Pritchard AJ; Lacy IHD. 1974. The cytology, breeding system and flowering behaviour of *Panicum coloratum*. Australian Journal of Botany 22:57–66. DOI: 10.1071/BT9740057

Rearte D. 2007. Situación de la ganadería argentina en el contexto mundial. Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina. goo.gl/UdKrWF

Sambrook J; Fritsch EF; Maniatis T. 1989. Molecular cloning: A laboratory manual. 2nd Edn. Cold Spring Harbor Laboratory Press, New York, USA. DOI: 10.1016/0092-8674(90)90210-6

Semagn K. 2002. Genetic relationships among ten endotypes as revealed by a combination of morphological, RAPD, and AFLP markers. Hereditas 137:149–156. DOI: 10.1034/j.1601-5223.2002.01600.x

Silvertown J; Lovett Doust J. 1993. Introduction to plant population biology. Blackwell Scientific Publications, Oxford, UK.

Smith KF; Reed KFM; Foot JZ. 1997. An assessment of the relative importance of specific traits for the genetic improvement of nutritive value in dairy pasture. Grass and Forage Science 52:167–175. DOI: 10.1111/j.1365-2494.1997.tb02347.x

Tischler CR; Young BA. 1983. Effects of chemical and physical treatments on germination of freshly harvested kleingrass seed. Crop Science 23:789–792. DOI: 10.2135/cropsci1983.0011183X002300040045x

Tischler CR; Voigt PW. 1995. Modifying seedling photomorphogenesis in kleingrass by recurrent selection. Crop Science 35:1613–1617. DOI: 10.2135/cropsci1995.0011183X 003500060016x

Tischler CR; Ocumpaugh WR. 2004. Kleingrass, blue panic and vine mesquite. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C_4) grasses. Agronomy Monograph

45. ASA, CSSA, SSSA, Madison, WI, USA. p. 623–649. DOI: 10.2134/agronmonogr45.c18

Van Soest PJ; Robertson JB; Lewis B. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583–3597. DOI: 10.3168/jds.S0022-0302(91)78551-2

Vogel KP; Burson BL. 2004. Breeding and genetics. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C_4) grasses. Agronomy Monograph 45. ASA, CSSA, SSSA, Madison, WI, USA. p. 51–94. DOI: 10.2134/agronmonogr45.c3

Wójtowicz T; Binek A; Moś M. 2009. Correlations and path analysis of the components of *Festuca pratensis* seed yield. Grassland Science in Poland 12:223–232. goo.gl/wNvvCQ

Xie WG; Lu XF; Zhang XQ; Huang LK; Cheng L. 2012a. Genetic variation and comparison of orchardgrass (*Dactylis glomerata* L.) cultivars and wild accessions as revealed by SSR markers. Genetics and Molecular Research 11:425–433. DOI: 10.4238/2012.February.24.1

Xie Y; Liu L; Fu J; Li H. 2012b. Genetic diversity in Chinese natural zoysiagrass based on inter-simple sequence repeat (ISSR) analysis. African Journal of Biotechnology 30: 7659–7669. goo.gl/qzZzSF

Young BA. 1986. A source of resistance to seed shattering in kleingrass, *Panicum coloratum*. Euphytica 35:687–694. DOI: 10.1007/BF00028576

Young BA. 1994. Genetic variation in a *Panicum coloratum* L. population with a limited germplasm base. Euphytica 75: 71–76. DOI: 10.1007/BF00024533

Supplementary Table 1: Polymorphic inter-simple sequence repeats (ISSR) markers used in this study.

Primer	Sequence (5´ ⟶ 3´)	Annealing temperature
ISSR-4	(GA)9T	51 °C
SSR-20	(ACTG)2ACCGACTG	45 °C
ISSR-9	(CAA)5	35 °C
ISSR-8	(GTG)3GC	33 °C
ISSR-7	(CT)8TG	49 °C
ISSR-14	(CAC)4GC	43 °C
ISSR-10	(AGC)5	45 °C
ISSR-19	(GATA)2(GACA)2	39 °C
ISSR-16	(GACA)4	43 °C
ISSR-5	(AG)7 TC	43 °C
ISSR-11	(ACC)5	45 °C
ISSR-18	(AAAG)4	35 °C
ISSR-3	(CT)8T	45 °C
ISSR-15	(GTAT)4	35 °C
ISSR-2	(GT)8G	47 °C
Pan5.8-F	CCCCTTCACCTTTTGTTGGAG	56 °C
Pan5.8-R	AAATGGGTCCTTGAGGCTATGGAG	58 °C

The Pan5.8-F and Pan5.8-R primers from 5.8s ribosomal gene were designed and used to confirm *Panicum coloratum*.

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Research Paper

Pastoralists' grazing systems and eco-related outcomes in Yewa Division of Ogun State, Nigeria

Sistemas de pastoreo y su impacto en el ecosistema en Yewa Division, Estado de Ogun, Nigeria

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Abstract

In Nigeria heavy dependence of cattle on natural pasture for grazing has resulted in the emergence of a range of grazing systems and ecosystem challenges. Consequently this study appraised the grazing systems in use and their eco-relational outcomes for sustainable cattle management in Yewa Division of Ogun State. A total of 143 pastoralists agreed to take part in the survey and provided data on the commonly practiced grazing systems and ecological effects through the use of an interview format, interactive discussions plus field observations. The study outcome showed continuous, unpatterned rotational and transverse grazing systems were commonly practiced. The study also revealed that the employed grazing systems were independent of size of the cattle herds. While cattle were healthy under the grazing systems employed, soil degradation occurred in some instances. While the grazing systems employed by the pastoralists were satisfactory from the animal perspective, studies are needed to minimize the extent of soil degradation in the area.

Keywords: Ecosystems, natural pasture, sedentary pastoralists, sustainable cattle management.

Resumen

En Nigeria, la alta dependencia del ganado de pasturas naturales ha resultado en el surgimiento de una amplia gama de sistemas de pastoreo e impactos en el ecosistema. El estudio se realizó en Yewa Division, estado de Ogun, Nigeria, con el objeto de evaluar los diferentes sistemas de pastoreo en uso y sus implicaciones ecológicas para el manejo sostenible del ganado. En total se entrevistaron 143 productores pastoriles utilizando formularios, discusiones presenciales y observaciones en el campo. El estudio mostró que los sistemas más frecuentes fueron el pastoreo continuo, el pastoreo rotacional sin patrón definido, y un pastoreo llamado 'transverso' (en búsqueda de forraje, animales son llevados a áreas distantes, regresando el mismo día). El estudio también mostró que los sistemas de pastoreo son independientes del tamaño de los rebaños de ganado. Aunque los animales presentaban buenas condiciones de salud, la degradación de los suelos era evidente en algunos casos. Si bien los sistemas de pastoreo practicados son satisfactorios en términos de producción animal, se necesitan estudios para minimizar el grado de degradación de los suelos en el área.

Palabras clave: Degradación del suelo, manejo sostenible de ganado, pastos naturales, productores de ganado sedentarios.

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Introduction

Cattle production, which traditionally entails breeding and management of cows and bulls for meat, milk and possibly skin production, alongside raising of calves, constitutes the economic resource-base of the Fulani pastoralists and is vital for survival and livelihood sustenance (Otchere 1984; Ingawa 1986; Rota et al. 2012). Sustainable production depends on feed quality and adequate feeding of the stock. Although feed rations vary, pasture is crucial to sustainable management of ruminants (Atanga et al. 2013). According to Buckingham et al. (2013), pasture is the cheapest source of feed for ruminant livestock and a well-managed pasture can supply more than 90% of the energy requirements for sheep and beef cattle and 70% for dairy cows.

In contrast with the situation in developed countries where land is owned/leased by farmers and cattle are confined to paddocks, in developing countries, especially those in sub-Saharan Africa, pastoralists are often nomadic or transhumant and solely dependent on the natural grassland for cattle, moving about in search of pasture for grazing. The quantity and quality of the natural pasture depends on soil type, the duration of the rainy season, amount of rainfall received and grazing pressure exerted by cattle grazing the grassland (Kemp et al. 2013). According to Awa et al. (2003), regions with annual rainfall of 750–1,500 mm can potentially support cattle rearing because of the length of the growing season and the resulting pasture production. As pastures deteriorate during the dry season, pastoralists must go further afield to seek pasture and water for their stock (ILC 2007; Rota et al. 2009). While transhumant pastoralists traverse large tracts of land and/or long distances in search of forage for their stock (Ndathi et al. 2011), the sedentary pastoralists remain within or close to their communities placing significant pressure on the available pastures.

According to Roberson (1996) and Belsky and Blumenthal (1997), livestock grazing can reduce herbaceous plant cover and litter, result in soil disturbance and compaction, reduce rate of water infiltration and increase rate of soil erosion. In the same vein Greenwood and McKenzie (2001) indicated that cattle grazing, especially under permanent pasture or rangeland, can lead to soil compaction. According to Howery et al. (2000), light stocking rates not only allow the grazing animal increased dietary selectivity throughout the year, but also greatly reduce grazing pressure on grassland.

As sedentary pastoralism in Yewa Division of Ogun State is widespread, owing to the ready availability of natural pasture in the host communities, pastures have been grazed continuously for many years. This grazing system(s) might have been an intuitive strategy for ensuring their economic survival and maintaining social relationships with their host communities (Omotayo et al. 1999; Adebayo and Olaniyi 2008; Blench 2010; Oladele and Oladele 2011). In the light of this, we deemed it necessary to document the grazing systems adopted by the settled pastoralists in Yewa Division of Ogun State and the eco-related outcomes, which have permitted continuous cattle management in the area. The strategy adopted had the following objectives:

1. Describe the socioeconomic characteristics of the settled pastoralists in Yewa Division of Ogun State;
2. Document natural pasture distribution in the study area;
3. Ascertain the grazing systems employed by the settled pastoralists; and
4. Ascertain the eco-related outcomes of the grazing systems for cattle management.

Research methodology

Study area

The study was conducted in Yewa Division of Ogun State. The Division is composed of 5 so-called Local Government Areas, namely Yewa South, Yewa North, Imeko-Afon, Ado-Odo/Ota and Ipokia, with a total land mass of about 5,878 km² (Figure 1). Rainfall in Yewa Division has a bi-modal distribution (early rains from April to July and late rains in September and October) with a total of about 1,300 mm/yr (Apantaku et al. 2003). The Division is largely characterized by wide distribution of natural grasses/pasture and other herbaceous plants and shrubs (derived savanna), and is considered as attractive to pastoralists for grazing or for settlement. Economic activities are largely farming with maize, cassava, vegetables and spices as dominant crop production. Cattle production in the area is by sedentary Fulani pastoralists and transhumant pastoralists.

The unit of analysis for the study comprised the settled Fulani pastoralists in Yewa Division of Ogun State. Due to non-availability of a comprehensive list of the pastoralists in the study area, a non-probability sampling technique based on selection of as many respondents as were willing to interact with the researchers (Bailey 1982) was employed. A total of 143 pastoralists were eventually selected across the surveyed 13 communities (Oja-Odan, Ebute, Iselu, Igbo-Iro, Egua, Gbogo, Oke-Odan, Seke-Aje, Agbo, Asa, Ibeku, Moro and Igbokoto). Primary data on the pastoralists' socioeconomic characteristics, the

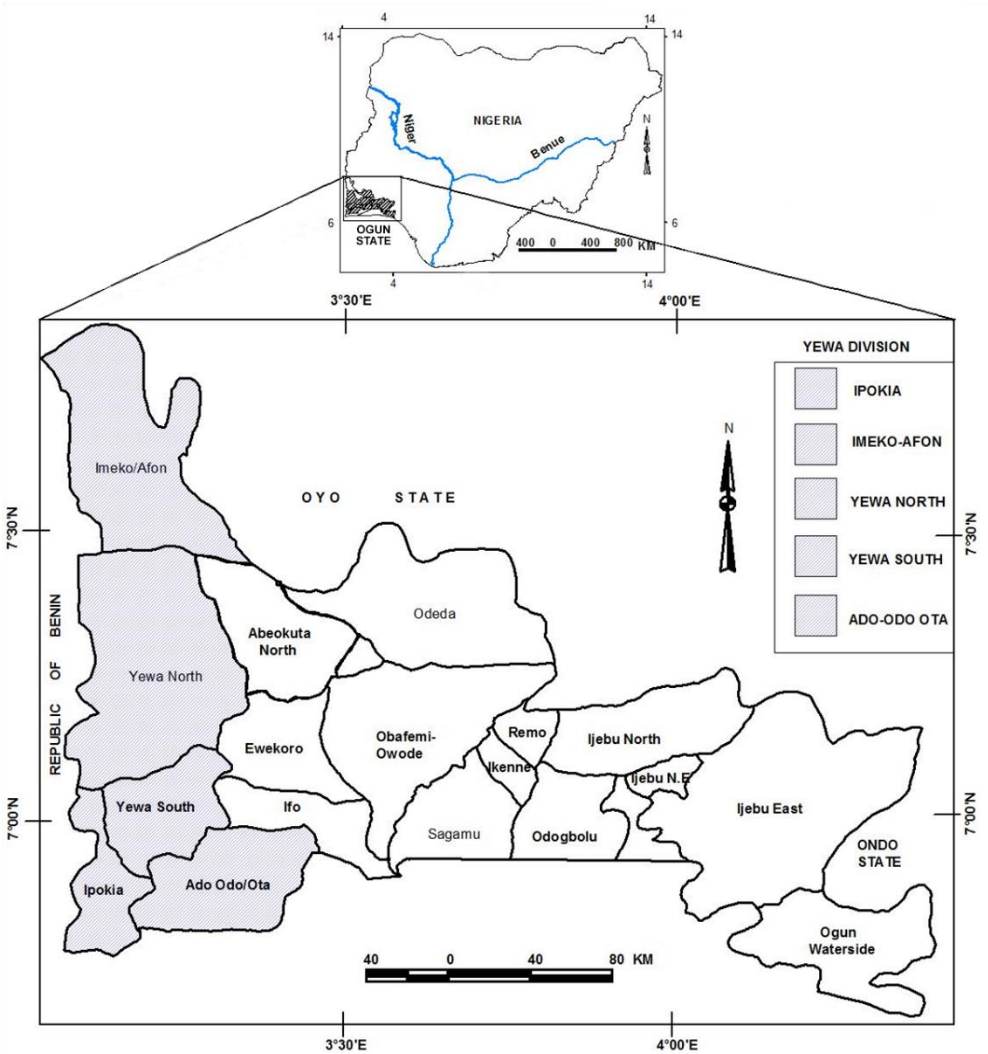

Figure 1. Map of Ogun State showing the study area, Yewa Division.

natural pasture distribution across the designated areas for grazing, grazing systems employed by the pastoralists and grassland management of the designated grazing areas were collected by means of an interview, interactive discussion and field observation.

The interview format was subjected to validity testing before commencement with a view to ensuring it would accurately measure the concepts of grazing system and grassland management. To achieve this, items of the developed interview format were carefully considered by members of the research team using acquired experiences on similar studies and current developments in the literature. The reliability of the instrument was established by pre-testing the interview guide on trial-sampled pastoralists in the study area. The outcomes allowed researchers to modify the instrument by eliminating ambiguous questions to ensure all respondents would interpret questions in a similar way.

The data collected were subjected to both descriptive and inferential statistical analyses. Descriptive tools such as frequency counts and percentages in tables form the basis for summarizing the data collected in relation to the respondents and discussion of the results.

Results

Respondents' personal characteristics

Assessment of the personal characteristics of the surveyed sedentary pastoralists (Table 1) shows that: all were male; 70% were within the age range of 31–50 years; and 90% of households contained 5–10 persons. While 54.5% of the pastoralists had Qur'anic education, a further 30% had no formal education and all practiced the Islamic religion. The data also show that more than 85% of the pastoralists had lived in the study area for at least 11 years.

Table 1. Respondents' personal characteristics (n = 143).

Variable	Frequency	%
Gender		
Male	143	100
Female	0	0
Age		
≤30	22	15.3
31–40	57	39.9
41–50	43	30.1
≥51	21	14.7
Educational level		
No formal education	43	30.1
Qur'anic education	78	54.5
Vocational education	0	0
Primary school	15	10.5
Secondary school	7	4.9
Tertiary school	0	0
Religion		
Islam	143	100
Christianity	0	0
Traditionalist	0	0
Years of residence		
≤10	21	14.7
11–20	71	49.6
21–30	36	25.2
31–40	15	10.5
≥41	0	0

Cattle production characteristics among the Fulani pastoralists

Table 2 shows the commonly reared breeds of cattle among the settled pastoralists in Yewa Division of Ogun State. White Fulani (Bunaji) were most common, being reared by 84.6% of respondents, Sokoto Gudali by 68.7%, Adamawa Gudali by 55.2% and Muturu by 28% of respondents. Size of cattle herds varied widely with the most common herd size being 61–80 head (44.1%), while a further 20.3% had 81–100 head, and only a few (13.3%) had more than 100 head of cattle (Table 2). Assessment of ownership of cattle by respondents showed that 79.7% of them actually owned the cattle in their custody, while a further 12.6% jointly owned the cattle with others and the remaining 7.7% were share keepers of the animals or keeping the animals on behalf of the owners. The majority of respondents (79%) had more than 11 years of experience in cattle management, with more than 15% having managed cattle for more than 20 years (Table 2).

Table 2. Cattle production characteristics among Fulani pastoralists (n = 143).

Variable	Frequency[1]	%
Breed of cattle		
White Fulani (Bunaji)	121	84.6
Sokoto Gudali	98	68.5
Adamawa Gudali	79	55.2
Muturu	41	28.7
Herd size (head)		
≤40	9	6.3
41–60	23	16.1
61–80	63	44.1
81–100	29	20.3
≥101	19	13.3
Ownership of cattle		
Self-owned	114	79.7
Jointly-owned	18	12.6
Share keepers	11	7.7
Experience of cattle management (years)		
≤10	30	21.0
11–15	52	36.4
16–20	39	27.2
≥21	22	15.4

[1]Multiple responses.

Pasture distribution on the designated grazing areas

Table 3 shows the common grasses, *Pennisetum purpureum*, *Panicum maximum*, *Andropogon gayanus* and *Cynodon dactylon*, available for cattle grazing and found in Yewa Division as well as the naturalized legume *Stylosanthes hamata*. Among the grasses, *Pennisetum purpureum* and *Panicum maximum* were the most common as indicated by 93.0 and 90.2% of respondents. Almost 82% of the respondents indicated the presence of *Stylosanthes hamata* in the grazed areas, while *Andropogon gayanus* (72.0%) and *Cynodon dactylon* (67.8%) were also present.

Table 3. Pasture species distribution in the designated grazing areas (n = 143).

Species	Frequency[1]	%
Pennisetum purpureum	133	93.0
Panicum maximum	129	90.2
Andropogon gayanus	103	72.0
Cynodon dactylon	97	67.8
Stylosanthes hamata	117	81.8

[1]Multiple responses.

Grazing systems adopted by the pastoralists

Table 4 lists the grazing systems practiced by the surveyed pastoralists. It shows that all (100%) pastoralists practiced continuous grazing, while 83, 96 and 16% practiced also unpatterned rotational, transverse and zero grazing (cut-and-carry), respectively.

Table 4. Grazing systems adopted by pastoralists (n = 143).

Variable	Frequency[1]	%
Continuous grazing	143	100
Unpatterned rotational grazing	119	83.2
Transverse grazing	131	91.6
Zero grazing (cut-and-carry)	23	16.1

[1]Multiple responses.

The grazing systems are defined as follows:

- Continuous grazing: Animals graze a pasture for a whole grazing season with no rest to the pasture (e.g. for regrowth, reseeding).
- Rotational grazing: Animals move from one pasture to the next, allowing the grazed pasture a planned rest period for forage plants to regrow and accumulate storage carbohydrates. 'Unpatterned' refers to the fact that the duration and frequency of grazing and rest periods is managed flexibly.
- Transverse grazing: Animals are moved farther away from the pastoralist's homestead in search of pasture to graze and return home on the same day.
- Zero grazing (cut-and-carry): Animals do not graze at all; rather they are stall-fed with forage that is cut and carried to them.

Outcome of the grazing systems practiced by pastoralists

Examination of the eco-related outcomes of the pastoralists' grazing systems for cattle management and sustenance (Table 5) showed that the adopted grazing systems, namely continuous, rotational, transverse and zero (cut-and-carry), allowed for periodic regeneration of pasture over time ($\overline{X}$= 3.08; SD = 0.62) and made pasture available for a considerable period of the year ($\overline{X}$= 3.30; SD = 0.31). Cattle remained in good health ($\overline{X}$= 4.63; SD = 0.60) with good marketability ($\overline{X}$= 4.87; SD = 0.33) and herd sizes were generally maintained with few mortalities ($\overline{X}$= 4.34; SD = 0.90). While there was some destruction of soil condition ($\overline{X}$= 2.68; SD = 0.77) and limited damage to cultivated crops ($\overline{X}$= 4.78; SD = 0.64) in the area, some conflicts between resident farmers and the sedentary pastoralists ($\overline{X}$= 3.22; SD = 0.83) in the study area did arise.

Table 5. Eco-related outcomes of the grazing systems practiced by pastoralists (n = 143).

Variable	Rating score[1]				
	1–1.67	1.68–3.33	3.34–4.99	Mean	SD
Grassland regeneration potential	Low regeneration	Regeneration for some period	All-year-round regeneration	3.08	0.62
State of animal health	Many stock are diseased	Some stock are diseased	Stock population remains healthy	4.63	0.60
Regularity of feed availability to the stock	Prolonged period with no pasture available	Pasture limited for only a short period (during rainy season)	All-year-round pasture availability	3.30	0.31
Stability of herd size against losses	High losses in herds	Some stock are lost	Herd size remains intact	4.34	0.90
Market acceptability of stock	Low/no market acceptability	Some stock acceptable for market	High market acceptability	4.87	0.33
Conflict status with farmers	Escalated conflicts	Mild conflicts occurred	Absence of conflicts	3.22	0.83
Cattle management sustenance	Cattle management is an unsustainable venture	Cattle management is barely sustainable	Cattle management is sustainable	4.19	0.91
Impact on soil condition	Serious destruction of soil condition	Limited destruction of soil condition (could still be cultivated)	No soil structure is destroyed	2.68	0.77
Impact on farm status	Serious farm destruction	Limited destruction of farms	No destruction of farms	4.78	0.64

[1]Rating score: 1–1.67 = less impact; 1.68–3.33 = moderate impact; 3.34–4.99 = high impact.

Test of study hypotheses

Chi-square tests of the relationship between the practiced grazing systems and the number of cattle owned by the pastoralists showed no significant association (Table 6). This implies that the number of cattle kept by pastoralists was independent of the grazing system practiced in Yewa Division of Ogun State. Grazing systems adopted by individual pastoralists were not specially designed but were spontaneous, depending on where pasture was available in the area for the animals. This explains why a continuous grazing system seemed to be the norm among pastoralists, whereby they allowed their herds of cattle to graze continuously as long as pasture was available in the field. The observed unpatterned rotational grazing system was adopted when pasture on a given area became exhausted. Cattle were then moved elsewhere for grazing before returning to the earlier grazed pasture once it had regenerated. The transverse grazing system however was employed during the dry season as pastures failed to regenerate, and pastoralists were compelled to travel afar in search of pasture for stock. The zero grazing (cut-and-carry) strategy was an option for stock that were either sick or milking and as such were not taken out for grazing.

Table 6. Chi-square test of the relationship between the grazing systems practiced and the number of cattle owned by the pastoralists.

Variable	χ^2	df	Sig	DS
Unpatterned rotational grazing	7.133	4	0.129	NS
Transverse grazing	1.961	4	0.743	NS
Zero grazing (cut-and-carry)	4.454	4	0.244	NS

Note: Continuous grazing was not computed due to constant responses on the item.

Discussion

An overview of the outcomes from this study shows that aspects related to animals achieved higher scores (4.19–4.87) than those relating to pastures (3.08–3.30) and soils (2.68). This tends to suggest that pastoralists are more concerned about the wellbeing of their livestock than the environment they graze on. Since livestock generally belong to individuals and are a measure of wealth and status in the community and land tends to be communally owned, this situation is not surprising. The mean score for soil condition indicates that quite significant soil degradation was occurring. This cannot be allowed to continue and steps must be taken to endeavor to reverse the trends.

Assessment of the socioeconomic characteristics of the settled pastoralists showed that cattle rearing is largely a male business. The observed dominance of male pastoralists in the study area is associated with the social norm that vests authority in men as family or household heads, thereby giving men the cultural power for decision making. According to FAO (2001), the great majority of pastoral societies are patrilineal and male-dominated, basically because of the need to have their herds sustained within their genealogical camps and to prevent the movement of family stock to a new camp by their women on marriage. Although pastoral women take certain decisions on the care and management of cattle (Mulugeta and Amsalu 2011; Nosheen et al. 2011; Rota et al. 2012), males are responsible for movement of cattle from place to place for grazing and marketing of the stock (FAO 2001). Movement of the cattle from place to place for grazing by all pastoralists is underscored by the need to feed the animals. Unlike the nomadic pastoralist who travels long distances to obtain grazing and has no designated place of residence, cattle movement by the settled pastoralists is limited to the geographical bounds of Yewa Division, where they reside. On this note they certainly return to *Gaa*, as their settled base is generally referred to, on a daily basis. Omotayo et al. (1999) pointed out that movement of cattle for grazing is mostly done by the young people among the pastoralists, based on the fact that herding is a laborious task and as such could be readily undertaken by youths with strength and vigor.

The household membership range of 4–6 persons is similar to the findings of Otte et al. (2012) and Omotayo et al. (2013) that settled Fulani pastoralists in Southwest Nigeria often keep a fairly large household size. According to Vabi (1991), large households usually comprise parents, (adult) children and blood-related brothers. This practice is attributed to the cultural and/or religious beliefs that large household size is a sign of Allah's (God's) blessings, and in turn ensures sufficient hands are available for the care and management of the cattle and, where possible, farm cultivation and management. The pastoralists have limited formal education because they rarely attend school but mostly take up Qur'anic education. Omotayo et al. (2013) also observed that acquisition of Qur'anic education was common for the major proportion of Fulani pastoralists in Southwest Nigeria. This situation is probably a function of their Islamic religion, which requires a good understanding of the Arabic language for Qur'anic reading and worship. The low incidence of formal education among the pastoralists (Ezeomah 1987) may be due to their inability to see the link between education and cattle management. As suggested by Otte et al. (2012), this educational situation indicates a low human capital among the pastoralists.

Notwithstanding the portrayed low human capital among the pastoralists, they had informally or culturally developed cattle management skills in terms of breed selection and herding. Among the breeds of cattle reared by pastoralists in Yewa Division of Ogun State, the White Fulani (Bunaji) was the most common. On a similar note, Otchere (1984) found that this breed of cattle constituted about 51% of the estimated 9.3 million cattle in Nigeria. The preponderance of White Fulani and Sokoto Gudali (Blench 1999; Omotayo 2002; Akande et al. 2010) among the surveyed pastoralists in the study area has been attributed to their adaptation to the agro-climate of Yewa Division and market acceptability of the animals produced for sale. An additional factor is the social value of these breeds of cattle to the pastoralists' families in terms of milk production, which the Fulani women often rely on for production of local cheese (*wara*) and milk (*nono*) as a means of income generation. The finding that herd size of the pastoralists was usually less than 100 animals conforms with the observation of Vabi (1991) that average herd size among Fulani pastoralists was 73.6 cattle. This observation implies that the surveyed pastoralists kept this size of herd for social and economic purposes. Interactive discussion with pastoralists revealed that they maintain a population of cattle that can easily be managed sustainably. In specific terms, determinants of herd size among the pastoralists included available grazing space, i.e. where to keep the animals, the feed resources and competent hands to herd the cattle.

While cattle ownership among the surveyed pastoralists in Yewa Division of Ogun State took different dimensions, i.e. sole or joint ownership and share keepers, all were geared toward their economic and livelihood sustenance. While individual cattle owners took decisions on day-to-day management and the accruing gains on the cattle, major decisions on cattle management, especially on marketing, and share of the accrued gains were made between the joint owners. Consequently, the individual pastoralist and one of the joint cattle owners personally took responsibility for herding the cattle for grazing and provision of other essential care. However, the share keepers do not actually own the cattle in their custody but keep the ruminants on behalf of the real owners and were described by Johnson et al. (2005) as 'contract graziers'. Care of the ruminants by the share keepers is usually on contractual agreement with the actual owners. The share keepers are thus remunerated for management of the cattle in their care either in cash or kind by the cattle owners. Interactive discussion with the share keepers reveals that this practice made it possible for them to make a living from what is paid them by the cattle owners, as well as allowing them to have cattle to manage. Grazing livestock for other farmers is a way to make a land investment return additional dollars to the land owner. According to Johnson et al. (2005), contract grazing requires livestock managers to possess management skills to achieve the results that livestock owners will expect. Given the mean length of experience with cattle management was 13.8 years, one might assume the surveyed cattle farmers had developed the necessary skills to provide insight or in-depth information on cattle-related issues to produce acceptable and desired outcomes for both parties.

An adequate supply of grass is known to be one of the major factors essential to ensuring sustainable production by grazing cattle (MLA 2007; Boval and Dixon 2012; Kemp et al. 2013). According to Aken'Ova (1985), grasses constitute the basic form of feed for all classes of ruminant animals in the tropical regions. The type and nature of the available grasses however depends on the agro-climate of a particular region. For instance, the West African humid forest zones, of which part of Nigeria is one, are widely covered with a mixture of typical grass species, such as *Imperata cylindrica*, *Andropogon gayanus*, *Pennisetum* spp. and *Hyparrhenia* spp. (Atta-Krah and Reynolds 1988) that readily serve as feed materials for ruminants. An additional factor, highlighted by McGufficke and McCormick (2010), i.e. rainfall distribution, is a major determinant of persistence and production of these grasses in the tropics. Given the fertility of soils and adequate rainfall regime in Nigeria, pastoralists who manage stock effectively should have adequate natural pasture to support stock during the rainy season but pastures in tropical areas often are of inferior nutritional value during the dry season.

With dependence of the sedentary pastoralists on natural pasture for cattle grazing in the study area, sustainable grazing systems must be available to ensure attainment of quality livestock development. Our survey revealed that grazing systems adopted by the surveyed sedentary pastoralists included continuous grazing, unpatterned rotational grazing, transverse systems and zero grazing (cut-and-carry) systems. A continuous grazing system, as described by Howery et al. (2000), entails all-year-round grazing of a pasture area including the dormant/dry season, i.e. no deliberate attempt is made to leave a portion of the pasture ungrazed, at least for an interval within the growing period. Our results show that all surveyed pastoralists in Yewa Division of Ogun State allowed their cattle to graze available pasture throughout the year on some areas within their settled domain, while a large percentage also practiced rotational grazing and transverse systems. Unless well organized, continuous grazing can result in overgrazing of the desirable species in a pasture with resultant negative ecological impacts (Blanchet et al. 2003), although this grazing system could be more viable where appropriate stocking rates are ensured (Howery et al. 2000). For the surveyed pastoralists to sustain their cattle on this grazing system, it suggests that they must have ensured manageable stocking rates of their cattle for grazing in the study area.

An alternative to the continuous grazing system is a transverse grazing system whereby the pastoralists travel over a wide range of land and long distances with their stock for grazing. This is similar to transhumance pastoralism whereby herds are regularly moved between fixed points with a view to exploiting the seasonal availability of pastures (FAO 2001). To distinguish this practice from nomadism, the FAO takes the view that the transhumance pastoralists often have a permanent homestead base where the older members of the community remain throughout the year. In essence, the surveyed pastoralists will always return home after traversing these long distances for grazing of their stock. This practice is contingent on having an available expanse of grassland for grazing. Traversing a wide range of land not only ensures feed availability for the stock but also prevents overgrazing of a particular area (UNHCR/IUCN 2005). A surprising 91.6% of the surveyed pastoralists in the study area led their stock over a wide range of grassland to obtain adequate grazing. This practice becomes necessary during the dry season when pasture becomes scarce following rainfall cessation and onset of the dry season. The cattle are usually led by the pastoralists beyond the proximity of their community of residence in search of grazing of green forage or fresh grasses as they cross the open lands.

In contrast with the traditionally patterned rotational grazing system, whereby areas are divided into a large number of sectionalized paddocks and grazing livestock are shifted from section to section in a predetermined sequence, is the unpatterned rotational grazing system employed by the surveyed pastoralists in Yewa Division of Ogun State. Rather than partitioning the grassland into blocks with fences, pastoralists allowed the cattle to graze freely on a given part of the available grassland until they perceived the pasture was being excessively grazed, which might be some days, when they moved the livestock to a different area. This sequence was repeated on different areas. The practice of unpatterned rotational grazing by the pastoralists is possible during the rainy season in the study area when grasses regenerate rapidly as a result of high temperatures and adequate soil moisture for growth and development. The low incidence of zero-grazing (stall feeding, cut-and-carry) system (16.1%) by pastoralists was not surprising as this practice becomes necessary only for those cattle which cannot be taken out for grazing due to illnesses or when suckling a small calf. In these situations, the animals are stalled and grasses are harvested by hand from the field and fed to the animals. In addition, pastoralists can resort to cutting grasses in swampy areas, particularly during the dry season, to feed their stock.

While the grazing system employed is crucial to ensuring sustainable feeding of the cattle, the system always impinges on the stability of the grassland and the environment in general. In our study area, continuous grazing of the natural pasture had reduced the available litter during the grazing period, which supports the finding of Roberson (1996) that grazed areas tend to have lower litter and soil organic matter levels than ungrazed enclosures. Against this backdrop, Howery et al. (2000) indicated that grazing systems such as rotational and transverse systems create the opportunity for grazed pasture or grassland to regenerate, particularly during the rainy season, when stock are taken elsewhere for grazing. This strategy was also espoused by Rota (2009), who stated that spelling of pasture areas by movement of stock is an essential way of ensuring sustainable use of the rangeland.

On another note, movement of animals from place to place in search of pasture can also prevent build-up of diseases and/or pests in a particular grazing area. According to Omotayo et al. (1999) and Rota (2009), pastoralists have adopted these grazing systems as a combination of providing the stock with feed and avoiding disease outbreaks and reducing livestock mortality. This probably contributed to the observed healthy status of the reared cattle. Discussion of this issue with pastoralists revealed that close association and contact with livestock, as occurs with regular movement of the cattle, makes it possible to quickly detect animals that might have become sick. In addition, since pastoralists must remain with their animals during grazing, it is possible to monitor animal movements and prevent losses or theft. This submission goes in line with Omotayo et al. (1999) indicating that movement of cattle for grazing within an established grazing orbit is strictly monitored by the pastoralists, at worst by the young boys in the households. Since pastoralists are with their livestock while grazing on a daily basis they are in an excellent position to monitor the condition of both the pastures and the soil.

Providing good quality forage for cattle ensures that animals gain weight and remain healthy, which in turn enhances their market acceptability. In fact, cattle buyers are known to purchase cattle from pastoralists in Yewa Division of Ogun State, because of their heavy weights. This supports the suggestion by Blench (2010) that the demand for quality beef by residents of the urban centers in Southern Nigeria stimulates pastoralists to produce fat animals for slaughter. Adequate levels of pasture for grazing by the cattle make it possible to keep stock healthy and ensure they are attractive to buyers, making cattle raising a profitable or worthwhile venture.

While conflict between pastoralists and farmers in Nigeria and beyond arising from farm grazing is a significant social issue, the grazing systems employed by the surveyed pastoralists have somehow reduced conflict between the sedentary pastoralists and farmers in Yewa Division of Ogun State. Interactive discussion with the pastoralists showed that the conflict was reduced because cattle were often removed from farmed areas and in some cases outside the community for grazing, thereby reducing tension and apprehension between them and the farmers. The pastoralists however acknowledged that their animals sometimes strayed into cultivated farms unknowingly, resulting in mild conflict. Careful control of cattle in the process of grazing from one place to the other limited the damage caused to farms. In the same vein, pastoralists ensured that communal water sources for drinking, which usually consist of wells or closest river to the villages, were not contaminated by their stock. This is usually ensured by leading the cattle to other water sources, such as streams and rivers that were farther away from villages or those not used for drinking by the community members. This action thus prevents or minimizes direct or indirect pollution of the drinkable water sources and in effect protects human health against water-related diseases.

While deposition of cattle dung on soil during grazing can improve soil fertility, the mass movement of cattle across fields can destroy soil structure. According to Warren et al (1986) and Whitmore (2001) grazing cattle compact the soil structure and destroy the land surface on which they regularly travel. This ecological degradation becomes heightened in places with heavy concentrations of herds of cattle for grazing (Adams 1996; Blench and Marriage 1998). This notwithstanding, the surveyed pastoralists were of the opinion that their cattle had limited impact on soil degradation as their cattle were rarely concentrated on cultivated land and were moved regularly on most grassland areas.

Conclusion and Recommendations

Cattle rearing is an important economic and social venture among the Fulani pastoralists in Nigeria. The change from a traditionally nomadic lifestyle to a settled or sedentary existence for many of them, particularly in the southern part of the country, has changed the systems of grazing employed. While the sedentary pastoralists in Yewa Division of Ogun State still move their cattle from place to place for grazing, it is usually in and around their communities, i.e. within a much more restricted area. Although rotational, continuous and transverse grazing systems are employed, there is greater risk that overgrazing of areas will occur. Our study has shown that farmers consider that soils are being damaged. In-depth scientific studies into the soil condition of the areas that are regularly grazed by the pastoralists are warranted. Knowledge generated from such research could be used to educate pastoralists on the impact of grazing on soil condition of their areas and the need for more control of grazing, e.g. by building fences to restrict access to given areas at appropriate times of year.

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References

Adams M. 1996. When is ecosystem change land degradation? Comments on land degradation and grazing in the Kalahari. Network Paper No. 39e, ODI (Overseas Development Institute), London, UK.

Adebayo OO; Olaniyi OA. 2008. Factors associated with pastoral and crop farmers conflict in derived savannah zone of Oyo State, Nigeria. Journal of Human Ecology 23:71–74. DOI: 10.1080/09709274.2008.11906057

Akande FA; Takeet MI; Makanju OA. 2010. Haemoparasites of cattle in Abeokuta, South West Nigeria. Science World Journal 5(4):19–21. goo.gl/EjaC2D

Aken'Ova ME. 1985. The use of improved pastures in intensive small ruminant production. Proceedings of The National Animal Production Research Institute's conference on small ruminant production, October 6–10, 1985, Shika-Zaria, Nigeria.

Apantaku SO; Lawal-Adebowale OA; Omotayo AM. 2003. Rainfall distribution pattern and its implications for seasonal farming calendar of agricultural extension services in Ogun State, Nigeria. An International Journal of Agricultural Sciences, Sciences, Environment and Technology (ASSET) 3(4):77–87. goo.gl/PQG1Rg

Atanga NL; Treydte AC; Birner R. 2013. Assessing the sustainability of different small-scale livestock production systems in the Afar Region, Ethiopia. Land 2:726–755. DOI: 10.3390/land2040726

Atta-Krah AN; Reynolds L. 1988. Utilization of pasture and fodder shrubs in the nutrition of sheep and goats in the humid tropics of West Africa. In: Timon VM; Baber RP, eds. Sheep and goat meat production in the humid tropics of West Africa. FAO (Food and Agriculture Organization of the United Nations), Rome, Italy. goo.gl/Tsz3TQ

Awa DN; Njoya A; Logtene YM; Ndomadji JA; Onana J; Asongwed-Awa A; Ngo Tama AC; Djoumessi M; Loko B; Bechir AB; Delafosse A; Maho A. 2003. Livestock production systems in the semi-arid savannah of the Central African sub region. CIRAD (Centre de coopération internationale en recherche agronomique pour le développement), Montpellier, France. hal.archives-ouvertes.fr/hal-00137977

Bailey KD. 1982. Methods of social research. 2nd Edn. The Free Press, New York, USA. trove.nla.gov.au/version/39724202

Belsky AJ; Blumenthal DM. 1997. Effects of livestock grazing on stand dynamics and soils in upland forests of the interior west. Conservation Biology 11:315–327. DOI: 10.1046/j.1523-1739.1997.95405.x

Blanchet K; Moechnig H; DeJong-Hughes J. 2003. Grazing systems planning guide. University of Minnesota Extension Services, St. Paul, MN, USA. goo.gl/aG2TM4

Blench R. 1999. Traditional livestock breeds: Geographical distribution and dynamics in relation to the ecology of West Africa. Working Paper 122. ODI (Overseas Development Institute), London, UK. goo.gl/QwYTMM

Blench R. 2010. Conflict between pastoralists and cultivators in Nigeria. Review paper for DFID, Nigeria. Cambridge, UK. goo.gl/NGuPYj

Blench R; Marriage Z. 1998. Climatic uncertainty and natural resource policy: What should the role of government be? Briefing Paper No. 31. ODI (Overseas Development Institute), London, UK. goo.gl/kq2XSb

Boval M; Dixon RM. 2012. The importance of grasslands for animal production and other functions: A review on management and methodological progress in the tropics. Animal 6:748–762. DOI: 10.1017/S1751731112000304

Buckingham S; McCalman H; Powell H. 2013. Grazing systems. Fact sheet. Farming Connect, Aberystwyth, Wales, UK. goo.gl/75v1Mk

Ezeomah C. 1987. The settlement patterns of nomadic Fulbe in Nigeria: Implications for educational development. Deanhouse Ltd, Crewe, UK.

FAO (Food and Agriculture Organization of the United Nations). 2001. Pastoralism in the new millennium. FAO Animal Production and Health Paper No. 150. FAO, Rome, Italy. goo.gl/EtwC3W

Greenwood KL; McKenzie BM. 2001.Grazing effects on soil physical properties and the consequences for pastures: A review. Australian Journal of Experimental Agriculture 41:1231–1250. DOI: 10.1071/EA00102

Howery LD; Sprinkle JE; Bowns JE. 2000. A summary of livestock grazing systems used on rangelands in the Western United States and Canada. College of Agriculture and Life Sciences, University of Arizona, Tucson, AZ, USA. hdl.handle.net/10150/144717

ILC (International Land Coalition). 2007. Mobile livelihoods, patchy resources and shifting rights: Approaching pastoral territories. ILC, Rome, Italy. goo.gl/HFsG2S

Ingawa SA. 1986. Socio-economic aspects of Abet farming households. Paper 12. In: von Kaufmann RR; Chater S; Blench R, eds. Livestock systems research in Nigeria's subhumid zone. International Livestock Centre for Africa, Addis Ababa, Ethiopia. hdl.handle.net/10568/49820

Johnson T; Kemp DR; Guodong H; Xiangyang H; Michalk DL; Fujiang H; Jianping W; Yingjung Z. 2013. Innovative grassland management systems for environmental and livelihood benefits. Proceedings of the National Academy of Sciences of the United States of America 110:8369–8374. DOI: 10.1073/pnas.1208063110

McGufficke BR; McCormick LH. 2010. Tropical perennial grasses for northern inland NSW. Primefact No. 1051. NSW Department of Primary Industries, Orange, NSW, Australia. goo.gl/ydNLmj

MLA (Meat & Livestock Australia). 2007. Grazing management for productive pastures. Tips and Tools Series. MLA, North Sydney, NSW, Australia. goo.gl/MKQuVa

Mulugeta M; Amsalu T. 2014. Women's role and their decision making in livestock and household management. Journal of Agricultural Extension and Rural Development 6:347–353. goo.gl/kc6GYv

Ndathi AJN; Nyangito MM; Musimba NKR; Mitaru BN. 2011. Climate variability and dry season ruminant livestock feeding strategies in Southeastern Kenya. Livestock Research for Rural Development 23, Article#199. goo.gl/9zvaP3

Nosheen F; Ali T; Anwar HN; Ahmad M. 2011. Participation of rural women in livestock management and their training needs in Potohar region. Pakistan Veterinary Journal 31:40–44. goo.gl/NRYMAz

Oladele OT; Oladele OI. 2011. Effect of pastoralist-farmers conflict on access to resources in savanna area of Oyo State, Nigeria. Life Science Journal 8(2):616–621. DOI: 10.7537/marslsj080211.86

Omotayo AM. 2002. A land-use system and the challenge of sustainable agro-pastoral production in Southwestern Nigeria. International Journal of Sustainable Development & World Ecology 9:369–382. DOI: 10.1080/13504500209470131

Omotayo AM; Adu IF; Aina AB. 1999. The evolving sedentary lifestyle among nomadic pastoralists in the humid zone of Nigeria: Implications for land use policy. International Journal of Sustainable Development & World Ecology 6:220–228. DOI: 10.1080/13504509909470012

Omotayo AM; Dipeolu MA; Ekpo UF. 2013. Health consequences of lifestyle changes among settled Fulani pastoralists in Southwestern Nigeria. Research Report. Wellcome Trust, London, UK.

Otchere EO. 1984. Traditional cattle production in the subhumid zone of Nigeria. Paper 6. In: von Kaufmann RR; Chater S; Blench R, eds. Livestock systems research in Nigeria's subhumid zone. International Livestock Centre for Africa, Addis Ababa, Ethiopia. hdl.handle.net/10568/49820

Otte J; Costales A; Dijkman J; Pica-Ciamarra U; Robinson T; Ahuja V; Ly C; Roland-Holst D. 2012. Livestock sector development for poverty reduction: An economic and policy perspective. Livestock's many virtues. FAO (Food and Agriculture Organization of the United Nations), Rome, Italy. lccn.loc.gov/2012476933

Roberson E. 1996. Impact of livestock grazing on soils and recommendations for management. California Native Plant Society, Sacramento, CA, USA.

Rota A. 2009. Livestock and pastoralists. Livestock Thematic Papers: Tools for project design. International Fund for Agricultural Development, Rome, Italy. goo.gl/mbDSrF

Rota A; Calvosa C; Liversage H. 2009. Livestock and land. Livestock Thematic Papers: Tools for project design. International Fund for Agricultural Development, Rome, Italy. goo.gl/fzHAxh

Rota A; Chakrabarti S; Sperandini S. 2012. Women and pastoralism. Livestock Thematic Papers: Tools for project design. International Fund for Agricultural Development, Rome, Italy. goo.gl/85S47Y

Sharp H; Rinehart L. 2005. Grazing contracts for livestock. ATTRA Sustainable Agriculture. NCAT (National Center for Appropriate Technology), Butte, MT, USA. goo.gl/bhBr6m

UNHCR/IUCN (United Nations High Commissioner for Refugees/World Conservation Union). 2005. Livestock-keeping and animal husbandry in refugee and returnee situations: A practical handbook for improved management. UNHCR & IUCN, Geneva & Gland, Switzerland. goo.gl/vntA9K

Vabi MB. 1991. Social relationships between indigenous cultivators and Fulani graziers in the derived savannah of Southwest Nigeria and the Northwest Province, Cameroon. Ph.D. Dissertation. University of Ibadan, Ibadan, Nigeria.

Warren SD; Thurow TL; Blackburn WH; Garza NE. 1986. The influence of livestock trampling under intensive rotation grazing on soil hydrologic characteristics. Journal of Range Management 39:491–495. DOI: 10.2307/3898755

Whitmore A. 2001. Impact of livestock on soil. In: Hartung J; Wathes CM, eds. Livestock farming and the environment: Proceedings of workshop 4 on Sustainable Animal Production, held at Hannover, September 28, 2000. Landbauforschung Völkenrode Special Issue 226. Bundesforschungsanstalt für Landwirtschaft (FAL), Braunschweig, Germany. goo.gl/U3zT9D

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Research Paper

Determinants of adoption of improved forages in selected districts of Benishangul-Gumuz, Western Ethiopia

Factores determinantes de la adopción de forrajes mejorados en distritos seleccionados de Benishangul-Gumuz, Etiopía Occidental

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Abstract

This study explores different socio-economic and institutional factors influencing the adoption of improved forage technologies in Assosa and Bambasi districts of Benishangul-Gumuz, Western Ethiopia. A structured questionnaire survey was applied to collect information from 120 farm households, and a binary logistic regression model was used to quantify the factors determining farmers' decisions to adopt improved forages. The analysis revealed that access to agricultural extension services, participation in forage training sessions and higher cash income had the greatest positive influence ($P<0.05$) on adoption of forage technologies, while higher numbers of male adult labor units and use of fertilizers had a lesser effect ($P<0.10$). In contrast, farmers remote from offices of development agents and possessing greater numbers of equines were less likely to adopt improved forage technologies. We suggest that adoption of improved forage technologies could be enhanced by providing farmers with training sessions, raising household income and providing greater access to extension services and that these factors should be considered by planning bodies.

Keywords: Assosa, Bambasi, binary logistic regression, odds ratio.

Resumen

Este estudio explora diferentes factores socioeconómicos e institucionales que influyen en la adopción de tecnologías de forrajes mejorados en fincas de los distritos de Assosa y Bambasi en Benishangul-Gumuz, Etiopía Occidental. Se aplicaron encuestas con cuestionario estructurado para recopilar información de 120 hogares de campesinos, y se utilizó un modelo de regresión logística binaria para cuantificar los factores que determinan las decisiones de los campesinos respecto a la adopción de forrajes mejorados. El análisis mostró que el acceso a servicios de extensión agrícola, la participación en jornadas de capacitación en cultivo y manejo de forrajes y mayores ingresos de los productores tuvieron el mayor efecto positivo ($P<0.05$) en la adopción de tecnologías de forrajes mejorados, mientras que una mayor disponibilidad de mano de obra masculina adulta y el uso de fertilizantes tuvieron menos efecto ($P<0.10$). Por el otro lado, aquellos campesinos que vivían distantes de las oficinas de agencias de desarrollo y los que poseían un mayor número de equinos tenían menos probabilidades de adoptar estas tecnologías. Sugerimos que los factores acceso a servicios de extensión agrícola, capacitación y aumento de ingresos de los productores deberían ser considerados por los organismos de planificación de desarrollo rural.

Palabras clave: Assosa, Bambasi, cociente de probabilidades relativas, regresión logística binaria.

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Introduction

Feed availability and quality are two of the major factors limiting livestock productivity in Ethiopia. Some feed-related constraints include: reduced grazing and pasture-lands, overstocking, seasonal variation in availability of roughage feeds, poor nutritional quality of forage, use of crop residues for other purposes, limited availability and unaffordability of concentrate feeds, low adoption of improved forages, low adoption of silage and hay making, and low adoption of urea treatment of crop residues at smallholder farmer level (Alemayehu 2012).

Increasing human population and declining productivity of cultivated areas result in increasing demand for arable land to produce food for humans in Ethiopia. This leads to reduction in area of land available for natural grazing and fodder production. At the same time, livestock numbers are being increased to meet the increased demand for draft power for crop production. These conflicting developments have placed an unsustainable demand on land resources, by transport of nutrients away from fields in the form of grain, crop residues and dung used for fuel. On the other hand, increased crop production results in increased crop by-products, providing a valuable source of animal fodder, if it is supplemented with protein from improved forages or nitrogen supplements (Alemayehu 2002).

To sustain livestock and crop production in Ethiopia, both livestock raising and crop production should be intensified. Feeding systems should be intensive with emphasis on cut-and-carry systems and reduced numbers of more productive livestock. For this strategy to be successful, improved forages, which have comparative advantages over indigenous forage species in terms of dry matter yields and quality, need to be widely adopted. In addition, improved forages, e.g. tree legumes, provide benefits like improving soil fertility, serving as fence material and providing shade for crop farming.

Improved forage species have been progressively introduced to local farmers of Ethiopia since 1970 to supplement the natural feed resources (EARO 2002). However, few studies, e.g. Gebremedhin et al. (2003) and Beshir (2014), have been conducted to identify factors that affect the adoption of these technologies.

In Benishangul-Gumuz, where this study was conducted, crop production integrated with livestock farming is the main-stay of the population. Two farming systems are practiced, namely: shifting cultivation; and permanent farming systems. The former is an agricultural system where plots are cultivated temporarily and then abandoned. Improved forage species, including elephant grass (*Pennisetum purpureum*), oats (*Avena sativa*), rhodes grass (*Chloris gayana*), pigeon pea (*Cajanus cajan*) and *Sesbania* species, have been introduced in an endeavor to increase the amount and quality of available forage and have been promoted in the region by Assosa Agricultural Research Center for the last decade. However, adoption of these technologies by smallholder farmers has failed to reach expectations. Moreover, research has not been conducted to determine the factors that influenced the adoption or lack of adoption by farmers in the area. This paper examines factors that influenced why farmers either adopted or failed to adopt the new forage technologies. Major household characteristics plus socio-economic and policy factors affecting the adoption of improved forage crops are identified, and suggestions are made for strategies to increase rates of adoption.

Materials and Methods

Study area

The study was conducted in 2 districts of Assosa zone, namely: Assosa and Bambasi (Figure 1). The zone is located in Benishangul-Gumuz region, Ethiopia, which is approximately 680 km west of Addis Ababa.

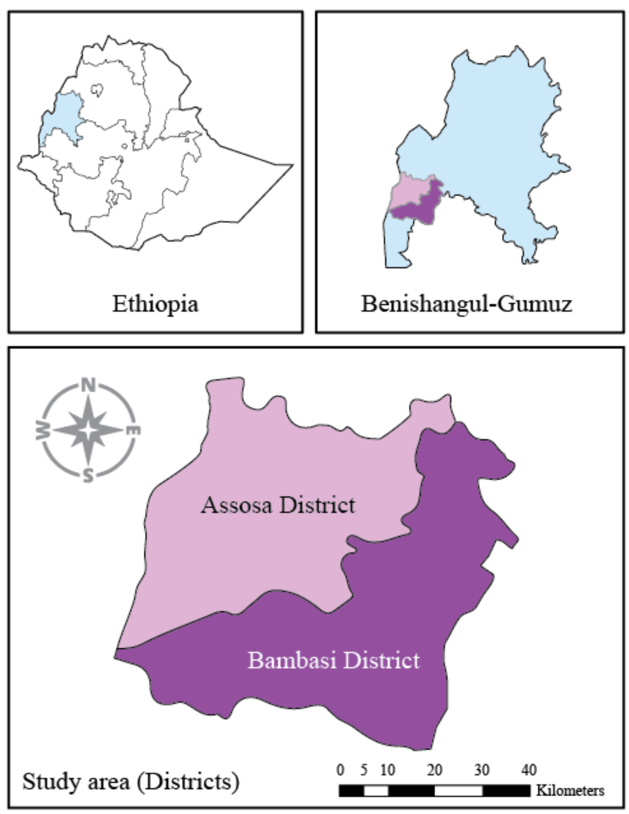

Figure 1. Map of Ethiopia and Benishangul-Gumuz Regional State showing the study districts.

Average annual rainfall is 1,316 mm (mainly from April to October), mean annual temperature ranges from 16.8 to 27.9 ºC and elevation from 580 to 2,731 masl (AMS 2013). Two farming systems are used in the study zone, namely: shifting cultivation; and permanent farming. Shifting cultivation is practiced mainly by Berta ethnic group, whereas permanent farming is practiced by settlers, an Amhara ethnic group who were relocated from the northern part of Ethiopia to Benishangul-Gumuz. Major crops grown are sorghum (*Sorghum bicolor*), maize (*Zea mays*), finger millet (*Eleusine coracana*), soya bean (*Glycine max*) and ground nut (*Arachis hypogaea*). Livestock species commonly kept are goats, cattle, chickens and donkeys in order of importance (AsARC 2006).

Sample size and sampling procedure

Assosa and Bambasi districts were selected based on their experience in introduction and promotion of improved forage technologies. A two-stage random sampling technique was used for selection of the sample respondents. First 4 peasant associations (PAs) were selected from Assosa district and 5 PAs from Bambasi district. Then, a total of 120 farm household heads (68 non-adopters and 52 adopters) were selected randomly from the PAs using probability proportional to sample size sampling technique. We used the following formula to determine the sample size with precision level of 9%:

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

where: n is the sample size; N is the population size; and e is the level of precision.

Sources and methods of data collection

A structured questionnaire was developed, tested prior to execution of the actual survey and refined based on the test results. Primary data were collected by researchers and technical assistants of Livestock Research Process of Assosa Agricultural Research Center.

Econometric specification of the adoption model

The decision to use a new technology, method, practice, etc. by a firm, a farmer or consumer is referred to as an adoption (Beshir 2014). According to the same author, adoption can be at farm level or aggregate. A farm-level adoption reflects a farmer's decision to incorporate a new technology into the production process, whereas aggregate adoption is the process of spreading or diffusion of new technology within a region or population. This study dealt with individual farmers and focuses on farm-level adoption.

A logit model was used for this study, since it represents a close approximation to the cumulative normal distribution and is easy to work with. The cumulative logistic probability model is econometrically specified as follows (Pindyck and Rubinfeld 1998):

$$P_i = F(Z_i) = F(\alpha + \sum \beta_i X_i) = \frac{1}{1+e^{-(\alpha+\sum \beta_i X_i)}} \quad (2)$$

where: e is the base of the natural logarithm; X_i represents the i^{th} explanatory variable; P_i is the probability that an individual will adopt improved forage varieties or not for a given X_i; and β_i and α are regression parameters to be estimated.

For ease of exposition, we express the model as:

$$P_i = \frac{1}{1+e^{-Z_i}} = \frac{e^Z}{1+e^Z} \quad (3)$$

Gujarati (2003) pointed out that a logistic model could be written in terms of the odds and log of odds, which enables one to understand the interpretation of the coefficients. The odds ratio is the ratio of the probability that an individual or household would be an adopter (P_i) to the probability of a household being a non-adopter ($1-P_i$).

$$(1-P_i) = \frac{1}{1+e^{Z_i}} \quad (4)$$

$$\left(\frac{P_i}{1-P_i}\right) = \left(\frac{1+e^{Z_i}}{1+e^{Z_i}}\right) = e^{Z_i} \quad (5)$$

$$\left(\frac{P_i}{1-P_i}\right) = \left(\frac{1+e^{Z_i}}{1+e^{Z_i}}\right) = e^{(\alpha+\sum\beta_i X_i)} \quad (6)$$

Taking the natural logarithm,

$$Z_i = \ln\left(\frac{P_i}{1-P_i}\right) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots \beta_n X_n \quad (7)$$

If the disturbance term U_i is taken into account, the logit model becomes:

$$Z_i = \alpha + \sum_{n=i}^{n} \beta_i X_i + U_i \tag{8}$$

It is obvious that the limitation of the logit regression model used is that it does not indicate the magnitude/ intensity of adoption of improved forage varieties. It indicates only the sign, i.e. positive or negative relationship between adoption and other explanatory variables of a household.

The potential explanatory variables, which are expected to influence adoption, are indicated in Table 1.

Results

Mean values for adopters and non-adopters for descriptive statistics of continuous and discrete variables used in the econometric model are indicated in Table 1. The results revealed that adopters own more resources and have better access to institutional services than non-adopters.

Determinants of adoption of improved forage varieties

From the estimated logistic regression model, 7 variables were found to be important in terms of adoption ($P<0.10$). These variables include: roundtrip distance to Development Agent's (DA) office; access to extension services; training attended; number of adult males in family; nitrogen fertilizer used; number of equines owned; and cash income. The remaining explanatory variables were found to have no significant influence on adoption status of the households (Table 2).

Distance of farmers' homes from DA's office had a negative influence ($P<0.10$) on adoption of improved forages. The model estimate showed that, other things remaining constant, the odds ratio in favor of forage adoption increases by a factor of 0.93 as the duration of a round trip to DA's office decreases by 2 minutes. Similarly, the marginal effect reveals that a 2 minute decrease in time for a round trip to the DA's office would increase the probability of participating in improved forage adoption by 0.039. Farmers nearer to DA's office have better access to information on improved practices and other extension services as well as to supply of forage seeds.

As expected the logistic model revealed that better access to extension services is related positively with forage adoption ($P<0.05$). The model estimate showed that, other things remaining constant, the odds ratio in favor of forage adoption increases by a factor of 9.5 when farm households have access to extension services. The marginal effect of this variable indicated that, if the household has access to extension services, the probability of adoption of improved forage varieties increases by 42.8%.

Table 1. Descriptive statistics of variables used in the model.

Variables	Adopters (N=52)		Non-adopters (N=68)		t-test
Continuous					
Age of household head	43.9		45.6		0.806
Adult family size	4.7		3.8		-2.758***
Number of cows owned	2.9		2.3		-1.186
Number of oxen owned	1.7		1.4		-1.026
Number of equines owned	0.4		0.5		0.644
Land allocated for crop production (ha)	1.4		1.2		-0.933
Nitrogen fertilizer used (kg/yr)	31.5		16.1		-2.491**
Number of training sessions attended	1.3		0.3		-5.885***
Number of contacts with Development Agent (per month)	18.9		10.4		-3.601***
Round trip distance to nearest livestock market (min)	287		262		-1.424
Round trip distance to Development Agent's office (min)	8.6		11.2		1.587
Discrete	Yes	No	Yes	No	Chi^2
Access to off-farm income	7	45	11	57	0.170
Access to extension services	50	2	38	30	24.437***
Access to credit service	41	11	38	30	6.908***

Table 2. Determinants of improved forage adoption.

Variable	Robust		Odds ratio	Marginal effect	
	Coefficient	s.e.		dY/dX	s.e.
Round trip to livestock market	0.004	0.003	1.00	0.001	0.0007
Round trip to Development Agent's office	-0.072*	0.039	0.93	-0.017	0.009
Off-farm income	-0.87	0.82	0.42	-0.187	0.155
Extension service	2.25**	0.98	9.51	0.428	0.12
Access to credit	-0.58	0.702	0.56	-014	0.159
No. of training sessions attended	1.63***	0.45	5.11	0.388	0.11
Cash income ('000 Birr)	0.122*	0.06	1.00	0.029	0.02
No. of adult males	0.45*	0.26	1.57	0.105	0.05
Age of household head	0.02	0.03	0.98	-0.004	0.005
Nitrogen fertilizer used per year	0.02*	0.01	1.02	0.005	0.002
Land used for crop production	-0.18	0.38	0.83	-0.04	0.09
No. of oxen	-0.20	0.26	0.81	-0.048	0.052
No. of equines	-0.65*	0.38	0.52	-0.155	0.091
No. of local cows	0.03	0.29	1.04	0.01	0.06
Constant	-4.12	1.80	0.02		
Observations	120				
Log likelihood	-44.94				
LR chi^2 (14)	74.33				
Pseudo R^2	0.4526				
Prob>chi^2	0.0000				

NB: dY/dX is for discrete change of dummy variable from 0 to 1.

Access to training also had a highly significant and positive influence on forage adoption (P<0.01). The model estimate showed that, other things remaining constant, the odds ratio in favor of forage adoption increases by a factor of 9.5 as farm households receive training on forage production and management practices. The marginal effect of this variable indicated that, if the household has access to training, the probability of adoption of improved forage varieties increases by 38.8%.

Cash income of the households showed a positive relationship with forage adoption, as households with high cash income showed higher probability of being adopters (P<0.10). The odds ratio implies that, other things remaining constant, the probability of being an adopter increases by a factor of 1 as cash income increases by 1,000 Birr, while the marginal effect indicates that an increase in cash income of 1,000 Birr would enhance the probability of forage adoption by 2.9%.

Number of adult males in the family also had a positive influence on the likelihood of adoption of improved forages (P<0.10). The odds ratio of 1.57 implies that, other things being constant, the odds in favor of being an adopter increases by a factor of 1.57 as the number of adult males in the family increases by one. The marginal effect of adult male family size indicates that the probability of being an adopter will increase by approximately 10.5% for each additional adult male family member.

Nitrogen fertilizer application may directly or indirectly affect adoption of improved forages. We hypothesized that this variable would have a positive influence on adoption, and this hypothesis proved correct (P<0.10). The odds ratio indicated that, other things remaining constant, the probability of the household being an adopter will increase by a factor of 1.02 if the level of fertilizer application increases by 100 kg per annum. The marginal effect revealed that the probability of the household being an adopter increases by 0.5% if the household increases the amount of fertilizer used by 100 kg annually.

Number of equines on a holding was negatively related to the probability of being an adopter in the study area (P<0.10). The odds ratio revealed that, other things remaining constant, the probability of the household adopting improved forage varieties is reduced by a factor of 0.52 as the number of equines on the property increases by 1 head. The marginal effect of this variable suggested that, for each additional equine, the probability of the household adopting forage will decrease by 15.5%.

Discussion

This study has revealed which factors had an influence on whether or not farmers sowed improved forages in the study area; it can possibly be utilized to improve adoption in similar situations in the future. The positive relationship between extension services and forage adoption indicates that increasing access to extension services increases adoption among households, reinforcing the finding of Beshir (2014) that agricultural extension services are a major source of information regarding forage technologies for farmers. Access to training sessions and forage adoption among households were also positively related. It is not surprising that increasing the knowledge of farmers regarding newly released and adapted forage technologies, and showing them how to use them, improves the chances of adoption.

A possible explanation for the positive influence of cash income on forage adoption is that households with more cash can buy food from the market, allowing additional land to be allocated to forage crop production rather than food crops. The fact that number of adult males in the family had a positive influence on the likelihood of adoption of improved forages was not surprising as improved practices are labor intensive and households with more family labor are in a better position to adopt forage technologies than households with fewer family labor units. Similar findings have been reported by Gebremedhin et al. (2003), Abera (2008) and Beshir (2014).

The positive influence of fertilizer application on adoption of improved forages could be an indirect one. Crop intensification and increased crop yields/ha following use of fertilizer can free up some cropping land for forage production. Finally, the possible reason for a negative relationship between number of equines on a holding and forage adoption is that households with more equines would be engaged in off-farm activities, leaving less time to cultivate forages.

Conclusions

This study provided valuable information on some services which need to be accessible to farmers if adoption of improved forages is to increase. The positive influence of access to extension services on adoption by farmers indicates that policies which enhance the availability of extension services in rural areas will promote adoption of new technologies. This can be achieved by increasing numbers of extension staff so that distance to a DA's office from farmers' homes is reduced as this factor had a negative effect on adoption. It could be advanced that the main areas to promote new species would be ones where extension services are readily available. Training also had a positive influence on forage adoption, suggesting that an integral part of programs to promote the adoption of forage species should be the provision of training sessions to educate farmers on the benefits and methods of adoption of forage species. Since farmers with higher cash income were more likely to adopt improved forages, government policies which improve farm incomes should result in increased adoption of new technologies.

Since availability of adult family labor also had a significant influence on forage adoption, staff promoting new forage technologies should consider household labor size when planning their approach, and focus their efforts on specific households. Similarly, households using more fertilizer had better rates of adoption of forage technologies, suggesting that increased fertilizer usage could increase crop yields and allow more land to be converted from crops to forage production. Government bodies promoting the use of improved forage species would be wise to consider these issues when planning improved forage promotional programs.

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References

Abera HB. 2008. Adoption of improved teff and wheat production technologies in crop and livestock mixed systems in northern and western Shoa zones of Ethiopia. Ph.D. Dissertation. University of Pretoria, South Africa. hdl.handle.net/2263/25364

Alemayehu A. 2012. Smallholder dairy production in mixed farming systems of Southern Ethiopia: Constraints and opportunities. M.Sc. Thesis. Wageningen University and Research Center, Wageningen, The Netherlands. goo.gl/tXM4Bx

Alemayehu M. 2002. Forage production in Ethiopia: A case study with implication for livestock production. Ethiopian Society of Animal Production (ESAP), Addis Ababa, Ethiopia. www.eap.gov.et/node/2732

AMS (Assosa Meteorological Station). 2013. Climatic data. Benishangul-Gumuz Regional State of Ethiopia, Assosa, Ethiopia.

AsARC (Assosa Agricultural Research Center). 2006. Research strategy document. Ethiopian Institute of Agricultural

Research, Assosa Agricultural Research Center, Assosa, Ethiopia. (Unpublished.)

Beshir H. 2014. Factors affecting the adoption and intensity of use of improved forages in North-east highlands of Ethiopia. American Journal of Experimental Agriculture 4(1):12–27. DOI: 10.9734/AJEA/2014/5481

EARO (Ethiopian Agricultural Research Organization). 2002. Livestock technology options for economic growth and to enhance the livelihoods of smallholder farmers. Report presented to workshop on poverty reduction through transforming smallholder systems from subsistence to market orientation, June 3–7, 2002, Addis Ababa, Ethiopia.

Gebremedhin B; Ahmed M; Ehui S. 2003. Determinants of adoption of improved forage technologies in crop-livestock mixed systems: Evidence from highlands of Ethiopia. Tropical Grasslands 37:262–273. goo.gl/CCHQfy

Gujarati DN. 2003. Basic econometrics. 4th Edn. Tata McGraw-Hill, New Delhi, India.

Pindyck RS; Rubinfeld DL. 1998. Econometric models and economic forecasts. 2nd Edn. Irwin/McGraw-Hill, New York, USA.

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Short Communication

Chemical composition of hays of the Caatinga shrub legumes mororó and sabiá from different parts of the plant

Composición química de henos de mororó y sabiá, leguminosas arbustivas nativas en la vegetación de Caatinga, Brasil

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Abstract

Native forages are important feed sources in the northeastern semi-arid region of Brazil. The objective of this study was to evaluate the chemical characteristics of hays made from the native shrub legumes, sabiá (*Mimosa caesalpiniifolia* Benth.) and mororó [*Bauhinia cheilantha* (Bong.) Steud.], using different plant fractions: leaves only and leaves plus twigs. Analyses carried out were: concentrations of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), ether extract (EE), ash, total digestible nutrients (TDN) and non-fiber carbohydrates (NFC). Sabiá leaf hay contained greater CP concentration (182 g/kg DM) than mororó leaf hay (126 g/kg DM) but hay made from leaf plus twigs showed similar CP for both legumes (mean 106 g/kg DM). Concentrations of NDF (368 g/kg DM), NFC (418 g/kg DM) and TDN (481 g/kg DM) of mororó leaf hay were superior to those of sabiá leaf hay (465, 258 and 311 g/kg DM, respectively). The findings suggest that these native legumes can be conserved as hay during the growing season to provide a supplementary feed with acceptable nutritive value for feeding during the period of inadequate feed availability and quality in semi-arid Brazil. However, more samples from a range of growth stages and locations are needed to confirm these preliminary findings and to provide data on possible yields, while more feeding studies with ruminants are needed to confirm the preliminary data in terms of liveweight performance.

Keywords: *Bauhinia cheilantha, Mimosa caesalpiniifolia*, native forages, non-fiber carbohydrates, plant fractions.

Resumen

En la región semiárida del nordeste de Brasil, las plantas nativas son una fuente importante de forraje. El objetivo de este estudio fue evaluar las características químicas de los henos de las leguminosas arbustivas nativas: sabiá (*Mimosa caesalpiniifolia* Benth.) y mororó [*Bauhinia cheilantha* (Bong.) Steud.], utilizando diferentes fracciones de las plantas: solo hojas y hojas más ramificaciones delgadas (<8 mm diámetro) de tallos. Los análisis incluyeron: concentraciones de materia seca (MS), proteína cruda (PC), fibra detergente neutra (FDN), extracto de éter (EE), ceniza, nutrientes digestibles totales (NDT) y carbohidratos no fibrosos (CNF). El heno de hojas de sabiá presentó mayor concentración de PC (182 g/kg de MS) que el heno de hojas de mororó (126 g/kg de MS); no obstante en ambas leguminosas las concentraciones de PC fueron similares cuando se analizaron hojas más ramificaciones de tallos (106 g/kg de MS en promedio). Las concentraciones de FDN (368 g/kg de MS), CNF (418 g/kg de MS) y NDT (481 g/kg de MS) del heno de hojas de mororó fueron superiores a las del heno de hojas de sabiá (465, 258 y 311 g/kg de MS, respectivamente). Los resultados sugieren que en la región semiárida de Brasil estas leguminosas nativas, conservadas como heno durante

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el período de crecimiento, pueden proporcionar un alimento suplementario de aceptable valor nutritivo durante el período de baja disponibilidad y calidad de forraje. Sin embargo, se necesitan muestreos adicionales de un rango de fases de crecimiento de las plantas y sitios tanto para confirmar estos resultados preliminares como para proporcionar datos sobre producción de MS. Además se requieren estudios de alimentación con rumiantes para evaluar el efecto de los henos en la producción animal.

Palabras clave: *Bauhinia cheilantha*, carbohidratos no fibrosos, forrajes nativos, fracciones de planta, *Mimosa caesalpiniifolia*.

Introduction

In semi-arid northeast Brazil, rainfall is irregular and evapotranspiration is high, which results in a negative water balance affecting both animal husbandry and agriculture, through increased risk (Moreira et al. 2006; Silva and Alcântara 2009) and variation in forage quantity and quality (Bailey and Brown 2011; Hughes et al. 2012). Livestock productivity on natural pastures is affected (Santana et al. 2011; Lima et al. 2015), requiring the feeding of supplements. While this can be expensive, supplementing of herds should prioritize alternatives that minimize production costs. The use of fresh forage, hay or silage from native forages in much of the pasturelands during the dry season can correct nutritional deficiencies at relatively low cost (Alves et al. 2011; Parente and Maia 2011).

Many legumes that occur in the Caatinga, the native vegetation of Brazil's semi-arid northeast region, are suitable for haymaking, but there is limited information on their dry matter (DM) yields, nutritional value and labor required for hay production (Arruda 2011; Silva et al. 2012). Conservation of forage for feeding during periods of deficiency, e.g. the dry season, seems a possible alternative to strategic use of native plants from Caatinga, since most of these are deciduous and shed leaves before they are needed (Santos et al. 2010).

Sabiá (*Mimosa caesalpiniifolia* Benth.) and mororó [*Bauhinia cheilantha* (Bong.) Steud.] are native pioneer legumes, which add to diversity in Caatinga vegetation and are naturally selected by grazing animals in different seasons, especially the rainy season (Santos et al. 2010; Silva AB et al. 2013). Santana et al. (2011) reported that mororó was common in the diet, ranging from 14.2 to 19.7%, with leaves being preferred by animals. This preference for the legumes may be linked to their chemical composition. While branches and leaves of sabiá and mororó can contain up to 279 and 235 g crude protein (CP)/kg DM, respectively, up to 67.7 and 52.3% of the protein may be fiber-bound (Silva et al. 2012), so that up to 60% of this protein can be non-degradable in the rumen (Pereira et al. 2010). Alves et al. (2011) observed that the presence of condensed tannins in sabiá reduced the consumption by sheep and goats by 13.3 and 4%, respectively. In addition, composition of hay can vary according to season, species and plant fractions, and in the case of woody legumes, many leaves are lost during the process of haymaking, resulting in considerable overall forage quality loss.

Useful forage mass of mororó can vary from 228 to 551 kg DM/ha and from 56 to 99 kg DM/ha in the rainy and dry seasons, respectively (Moreira et al. 2006; Santana et al. 2011). On the other hand, sabiá can produce up to 4,270 kg DM/ha of fresh browse (Meirelles and Souza 2015).

We conducted this study in an endeavor to evaluate the chemical composition of hays made from different plant fractions of the native forage shrubs sabiá and mororó.

Materials and Methods

Sabiá hay was produced from plants on the Experiment Station of the Instituto Agronômico de Pernambuco (IPA) in Itambé, Pernambuco, Brazil, from October 2005 to November 2005. Mororó hay was produced using plants collected from Caatinga vegetation in the IPA Experiment Station in Serra Talhada, Pernambuco, during March 2005. The estimated age of the plant material was 6–8 months. Green leaves and whole twigs (up to 8-mm diameter, since browser forage comprises leaves and twigs) were collected during the seed-maturity stage and spread to dry on concrete floors in full sunlight with manual turning for a 60 h period.

Experimental treatments included hay made from 2 fractions of the plant: leaves only and leaves plus twigs. The dried hay was stored in nylon bags and transported to the Department of Animal Science-UFRPE. Three composite samples from the hay bags were collected for chemical analysis in the Animal Nutrition Laboratories, UFRPE. The samples were ground in Wiley mills with a 1-mm sieve.

Analyses of DM, CP, ether extract (EE) and ash were conducted according to Silva and Queiroz (2002), while neutral detergent fiber (NDF) was determined as described by Van Soest (1994) and non-fiber carbohydrates (NFC) by the formula: NFC = 100 – (%CP + %NDF + %EE + %ash) (Mertens 1997).

Feed intake and nutrient digestibility data were used to estimate total digestible nutrients (TDN) as described by Sniffen et al. (1992), where: TDN = digestible CP + digestible carbohydrates + 2.25 digestible EE. For that, 8 wether goats (18.4 kg), dewormed and without a defined breed, were placed in individual pens containing water and mineral salt and offered the 2 hays (4 goats for each hay). After a 10-day adjustment period, feed offered, refusals and feces were recorded over 5 days to determine nutrient digestibility. Nutrient digestibility was calculated based on food intake less the refusals and the nutrient amounts contained in the food, refusals and feces.

Data were submitted to descriptive analysis consisting of a mean and confidence interval at 95% probability.

Results

While DM concentration in hays made of leaves only and leaves plus twigs varied little (Table 1), hays made from leaves only showed higher CP and lower NDF concentrations than hays made from leaves plus twigs. Mororó leaf hay contained lower CP concentration than sabiá leaf hay (126 vs. 182 g/kg DM) but CP in hays made from leaf plus twigs was similar for both species (106 vs. 107 g/kg DM).

Concentrations of NFC were lower in sabiá hays than in mororó hays, with higher levels in leaf hay than leaf plus twig hay for both species. The reverse was the case with NDF, where concentrations were lower in mororó hays than in sabiá hays and higher in leaf plus twig hays than in leaf hays for both species.

The EE of hays ranged from 19.9 to 42.5 g/kg DM, with higher values for sabiá hay than for mororó hay. Leaves contained greater EE than leaf plus twigs in both plants (Table 1). Ash concentrations in both species ranged from 51.6 to 73.7 g/kg DM with higher levels in leaf hays. Sabiá and mororó hays had low TDN concentrations, although leaf provided greater TDN than leaf mixed with twigs.

Discussion

This study has shown that hay can be made quite successfully from foliage of both sabiá and mororó legumes for feeding during periods of feed shortage. However, these preliminary findings suggest that the nutritive value of the hays, especially those from sabiá, is not high like herb legume hay. Further samples need to be made and fed to animals in intake and digestion studies to obtain a broader understanding of the merit of this strategy for addressing the period of feed shortage in northeast Brazil. Leaf normally displays better forage quality than twig, except in the case of tender new twigs or browse, which synthesize only a digestible primary cell wall and middle lamella (Ding et al. 2012). Thus, we expected that leaves would have higher CP and lower NDF concentrations than twigs and that addition of twigs, even those only 8-mm diameter, to leaf material would lower quality of hay. While this was the case, the hays made from leaf plus twigs still had CP concentrations above 7%, the level suggested by Van Soest (1994) as that below which DM intake of ruminants might be lowered.

When labor expenses for harvesting and drying and quantity of material harvested are considered, making hay from a mixture of leaf plus stem (1:1) becomes attractive. In fact, most hays made by farmers are a mixture of different plant fractions, the proportions depending on season and hydric conditions. Santiago et al. (2001) observed leaf:twig ratios in sabiá of 0.99, 0.95 and 0.84 for control, moderate and severe hydric stress conditions, respectively, and leaf:twig ratios of 1.09 and 0.86 for 25 and 50 days of growth, respectively. Removing the need to separate leaves from twigs would significantly reduce the cost of labor, and make turning of hay faster and more economical. The loss of leaves during the haymaking process, mainly during harvesting and transportation, especially in some arboreal legumes, is a limitation of this process (Silva MSJ et al. 2013; Pasqualotto et al. 2015).

Table 1. Average and confidence interval (95% probability) of chemical composition (g/kg) of mororó and sabiá hays made from leaves only and leaf plus twigs.

Parameter	Mororó hay		Sabiá hay	
	Leaf	Leaf + Twigs	Leaf	Leaf + Twigs
Dry matter	878 ± 8.5	887 ± 1.0	946 ± 9.7	961 ± 6.1
Crude protein	126 ± 45.6	107 ± 7.8	182 ± 15.9	106 ± 21.2
Neutral detergent fiber	368 ± 7.6	421 ± 33.3	465 ± 26.7	597 ± 44.6
Ether extract	24.4 ± 5.7	19.9 ± 1.8	42.5 ± 3.8	28.8 ± 5.2
Ash	73.7 ± 2.5	63.7 ± 3.5	73.6 ± 43.3	51.6 ± 3.7
Non-fiber carbohydrates	418 ± 36.0	388 ± 33.2	258 ± 21.6	194 ± 25.3
Total digestible nutrients	481 ± 37.4	452 ± 35.8	311 ± 24.3	267 ± 19.3

Alves et al. (2011) and Silva et al. (2012) also reported higher CP concentration in sabiá hay made from leaves and noted that this hay was superior to mororó hay in terms of selection by goats. However, Beelen et al. (2006a) found similar CP concentrations in mororó and sabiá plants (190 vs. 178 g CP/kg DM) during the growing stage. By flowering and seed-maturity stages, CP in mororó had decreased to 134 and 144 g CP/kg DM, while sabiá remained at 177 and 163 g CP/kg DM, respectively.

Crude protein concentration of forage can be misleading as an indicator of the feed value as part of the nitrogen in hay may be fiber-bound in some native plants (Santos et al. 2010; Silva et al. 2012). Legumes from Caatinga synthesize a great amount of insoluble protein, which adheres to the fiber (Santana et al. 2011), lignin and condensed tannins (Beelen et al. 2006a; Oliveira et al. 2015), affecting nutrient digestibility (Beelen et al. 2006b; Silva et al. 2012). Most forages in the northeastern semi-arid region can synthesize anti-nutritional factors, such as tannins and lignins, from the secondary metabolism of plants, which can bind to protein and other nutrients, negatively influencing animal performance (Beelen et al. 2006b; Pereira et al. 2010). Furthermore, forage legume management can influence the accumulation of those compounds, as grazed plants can have more condensed tannins than ungrazed ones (Adams et al. 2013). Unfortunately, limited resources did not allow us to determine the tannin concentrations in the hays we made.

Alves et al. (2011) studied sabiá hay made from leaves plus twigs and found similar NDF concentrations (631 g/kg DM), but CP (162 g/kg DM) was greater than found in the present work. This might be a function of different leaf:twig ratios in the forages. The CP and fiber of the hays studied are important aspects in their use as a food supplement for animals in different seasons. In a parallel study with the same hays, Goyanna (2009) reported that sabiá and mororó hay had 34 and 33 g tannins/kg, respectively, which seemed not to have affected dry matter digestibility of sabiá (665 g/kg) and mororó (679 g/kg). Effective digestibility of CP in sabiá hay was greater than that in mororó (131 vs. 102 g/kg), which could have promoted greater sabiá intake (3.6% live weight, LW) than mororó (3.1% LW) by goats when offered individually, as well as when offered side-by-side to allow choice (2.0 vs. 1.2% LW).

Making legume hays can be a good strategy for conserving forage before leaves fall from deciduous forages in Caatinga lands. The hays in our study could provide an important source of protein and fiber for animals receiving, for example, cactus pear, which contains low CP (48 g/kg DM) and NDF (291 g/kg DM) (Tosto et al. 2007), and could lower methane emissions, reduce helminths and other biological activities associated with tannin concentration.

Conclusions

The chemical composition of hay made from leaf showed higher quality, in terms of CP and NDF concentrations, than that of hay made from leaf mixed with twigs, regardless of the species. Sabiá leaf hay had higher CP concentration than mororó hay. These hays were adequate to support at least maintenance in livestock and thus should be appropriate for use during periods of feed shortage. As this hay was a single sample made from forage at a given stage of growth, more studies are needed to determine how much variation in quality exists over a range of situations and what potential yields might be. Feeding studies with ruminants are needed to confirm that animal responses are as chemical analyses suggest.

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References

Adams RP; Muir JP; Taylor CA; Whitney TR. 2013. Differences in chemical composition between browsed and non-browsed *Juniperus ashei* Buch. trees. Biochemical Systematics and Ecology 46:73−78. DOI: 10.1016/j.bse.2012.09.020

Alves AR; Beelen PMG; Medeiros AN; Neto SG; Beelen RN. 2011. Consumo e digestibilidade do feno de sabiá por caprinos e ovinos suplementados com polietilenoglicol. Revista Caatinga 24:152−157. goo.gl/H4s2eu

Arruda AMV. 2011. Digestibilidade *in vitro* de leguminosas do semiárido com inóculo cecal de avestruzes. Ciência Rural 41:519−523. DOI: 10.1590/S0103-84782011000300026

Bailey DW; Brown JR. 2011. Rotational grazing systems and livestock grazing behavior in shrub-dominated semi-arid and arid rangelands. Rangeland Ecology & Management 64:1−9. DOI: 10.2111/REM-D-09-00184.1

Beelen PMG; Berchielli TT; Beelen R; Araújo Filho J; Oliveira SG. 2006a. Characterization of condensed tannins from native legumes of the Brazilian Northeastern semi-arid. Scientia Agricola 63:522−528. DOI: 10.1590/S0103-90162006000600002

Beelen PMG; Berchielli TT; Buddington R; Beelen R. 2006b. Efeito dos taninos condensados de forrageiras nativas do semiárido nordestino sobre o crescimento e atividade celulolítica de *Ruminococcus flavefaciens* FD1. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 58:910−917. DOI: 10.1590/S0102-09352006000500029

Ding SY; Liu YS; Zeng Y; Himmel ME; Baker JO; Bayer EA. 2012. How does plant cell wall nanoscale architecture correlate with enzymatic digestibility? Science 338:1055−1060. DOI: 10.1126/science.1227491
Goyanna GJF. 2009. Caracterização nutricional dos fenos de sabiá (*Mimosa caesalpiniifolia* Benth.) e de mororó (*Bauhinia cheilantha* (Bong.) Steud.) em caprinos. M.Sc. Thesis. Federal Rural University of Pernambuco, Recife, PE, Brazil. tede2.ufrpe.br:8080/tede2/handle/tede2/6787
Hughes MP; Jennings PGA; Mlambo V; Lallo CHO. 2012. Effect of season and harvesting method on chemical composition, predicted metabolizable energy and *in vitro* organic matter digestibility of rotationally grazed tropical pastures. Online Journal of Animal and Feed Research 2:405−417. goo.gl/9xbDMy
Lima RP; Fernandes MM; Fernandes MRM; Matricardi EAT. 2015. Aporte e decomposição da serapilheira na Caatinga no sul do Piauí. Floresta e Ambiente 22:42−49. DOI: 10.1590/2179-8087.062013
Meirelles AC de; Souza LAG de. 2015. Produção e qualidade da biomassa de leguminosas arbóreas cultivadas em um sistema de aleias em solo Latossolo Amarelo da Amazônia Central. Revista Brasileira de Agroecologia 10:67−74. goo.gl/TCTNwc
Mertens DR. 1997. Creating a system for meeting the fiber requirements of dairy cows. Journal of Dairy Science 80:1463−1481. DOI: 10.3168/jds.s0022-0302(97)76075-2
Moreira JN; Lira MA; Santos MVF; Ferreira MA; Araújo GGL; Ferreira RLC; Silva GC. 2006. Caracterização da vegetação e da dieta de ovinos em área de Caatinga no sertão de Pernambuco. Pesquisa Agropecuária Brasileira 41:1643−1651. DOI: 10.1590/s0100-204x2006001100011
Oliveira OF; Ferreira RLC; Almeida ACS; Santos MVF; Muir JP; Cunha MV; Lira MA. 2015. Season and rainfall gradient effects on condensed tannin concentrations of woody rangeland species. Revista Brasileira de Ciências Agrárias 10:165−169. DOI: 10.5039/agraria.v10i1a4329
Parente HN; Maia MO. 2011. Impactos do pastejo sobre a compactação dos solos com ênfase no Semiárido. Revista Trópica: Ciências Agrárias e Biológicas 5(3):3−15. goo.gl/4cJ3Mq
Pasqualotto M; Neres MA; Guimarães VF; Klein J; Inagaki AM; Ducati C. 2015. Gas exchanges and dehydration in different intensities of conditioning in Tifton 85 bermuda-grass: Nutritional value during hay storage. Asian-Australasian Journal of Animal Science 28:807−815. DOI: 10.5713/ajas.14.0826
Pereira ES; Pimentel PG; Duarte LS; Villarroel ABS; Regadas Filho JGL; Rocha Júnior JN. 2010. Digestão intestinal da proteína de forrageiras e co-produtos da agroindústria produzidos no Nordeste Brasileiro por intermédio da técnica de três estágios. Revista Brasileira de Saúde e Produção Animal 11:403−413. goo.gl/5tLQz9
Santana DFY; Lira MA; Santos MVF; Ferreira MA; Silva MJA; Marques KA; Mello ACL; Santos DC. 2011. Caracterização da Caatinga e da dieta de novilhos fistulados, na época chuvosa, no semiárido de Pernambuco. Revista Brasileira de Zootecnia 40:69−78. DOI: 10.1590/s1516-35982011000100010
Santiago AMP; Nogueira RJMC; Lopes EC. 2001. Crescimento em plantas jovens de *Mimosa caesalpiniifolia* Benth., cultivadas sob estresse hídrico. Revista Ecossistema 26: 23−30. goo.gl/adxqT5
Santos MVF; Lira MA; Dubeux Júnior JCB; Guim A; Mello ACL; Cunha MV. 2010. Potential of Caatinga forage plants in ruminant feeding. Revista Brasileira de Zootecnia 39:204−215. DOI: 10.1590/s1516-35982010001300023
Silva AB; Lira Junior MA; Dubeux Junior JCB; Figueiredo MVB; Vicentin RP. 2013. Estoque de serapilheira e fertilidade do solo em pastagem degradada de *Brachiaria decumbens* após implantação de leguminosas arbustivas e arbóreas forrageiras. Revista Brasileira de Ciência do Solo 37:502−511. DOI: 10.1590/s0100-06832013000200021
Silva DF da; Alcântara CR. 2009. Déficit hídrico na região Nordeste: Variabilidade espaço-temporal. UNOPAR Científica Ciências Exatas e Tecnológicas 8:45−51. goo.gl/AuA5Qk
Silva DJ; Queiroz AC de. 2002. Análise de alimentos: Métodos químicos e biológicos. 3th Edn. Universidade Federal de Viçosa, Viçosa, MG, Brazil. goo.gl/W2qM5z
Silva MA da; Santos MVF dos; Lira MA; Dubeux Júnior JCB; Silva DKA; Santoro KR; Leite PMBA; Freitas EV de. 2012. Qualitative and anatomical characteristics of tree-shrub legumes in the Forest Zone in Pernambuco state, Brazil. Revista Brasileira de Zootecnia 41:2396−2404. DOI: 10.1590/s1516-35982012001200003
Silva MSJ da; Jobim CC; Nascimento WG do; Ferreira GDG; Silva MS da; Três TT. 2013. Estimativa de produção e valor nutritivo do feno de estilosantes cv. Campo Grande. Semina: Ciências Agrárias 34:1363−1380. DOI: 10.5433/1679-0359.2013v34n3p1363
Sniffen CJ; O'Connor JD; Van Soest PJ; Fox DG; Russell JB. 1992. A net carbohydrate and protein for evaluating cattle diets. II. Carbohydrate and protein availability. Journal of Animal Science 70:3562−3577. DOI: 10.2527/1992.70113562x
Tosto MSL; Araújo GGL; Oliveira RL; Bagaldo AR; Dantas FR; Menezes DR; Chagas ECO. 2007. Composição química e estimativa de energia da palma forrageira e do resíduo

desidratado de vitivinícolas. Revista Brasileira de Saúde e Produção Animal 8:239−249. goo.gl/fZjDbw

Van Soest PJ. 1994. Nutritional ecology of the ruminant. Cornell University Press, New York, USA.

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