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Research Papers

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Growth responses of nine tropical grasses under flooding conditions

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Keywords: *Brachiaria* spp., forage mass, Marandu Death Syndrome, *Panicum maximum*, root mass, soil water levels.

Abstract

The diversification of forage grasses is a strategic solution to obtain higher productivity in diverse environments. In this regard, the objective of the present study was to evaluate in a glasshouse study the flooding tolerance of 9 cultivars of forage grasses. The study was conducted using a complete randomized design with a 9 x 3 factorial arrangement: 9 cultivars (*Brachiaria brizantha* cvv. Marandu, Piatã e Xaraés; hybrid *Brachiaria* cv. Mulato II; *B. humidicola* cvv. Llanero and Tupi; *B. ruziziensis* cv. Common; *Panicum maximum* cvv. Massai and Tanzânia) and 3 soil water levels: a) minimal water for development (50% of field capacity); b) field capacity; and c) flooded soil (2 cm above soil level), with 3 replicates. Forage accumulation, plant height and root accumulation were evaluated. All cultivars grew well in soil at 50% field capacity highlighting their adaptation to mildly dry conditions. Under flooded conditions, *B. humidicola* cvv. Llanero and Tupi showed no reduction in forage dry matter production, while shoot growth of cvv. Marandu, Piatã, Tanzânia and Xaraés was significantly reduced (P<0.001) by 71.3, 94.0, 81.2 and 77.2%, respectively. Root mass was reduced about 30% in flooded plants relative to those grown at 50% field capacity. While all cultivars could be used where soil moisture is marginal for production, cvv. Llanero, Tupi and Massai would be most suitable where flooding could occur during the growing season. Field studies are needed to verify these glasshouse findings.

Resumen

La diversificación de las gramíneas forrajeras es una solución estratégica para obtener una mayor productividad en ambientes diferentes. En condiciones de invernadero en la Universidad del Estado de Mato Grosso, Alta Floresta, Mato Grosso, Brasil, se realizó un estudio con el objetivo de evaluar la tolerancia de 9 cultivares de gramíneas forrajeras tropicales a condiciones de inundación controlada. Los tratamientos se dispusieron en un diseño completamente al azar con arreglo factorial 9 x 3: nueve cultivares (*Brachiaria brizantha* cvs. Marandu, Piatã y Xaraés; *Brachiaria* híbrido cv. Mulato II; *B. humidicola* cvs. Llanero y Tupi; *B. ruziziensis* cv. Común; y *Panicum maximum* cvs. Massai y Tanzânia) y 3 niveles de agua en el suelo: a) cantidad de agua considerada como mínima para el crecimiento de las plantas (50% de capacidad de campo); b) suelo a capacidad de campo; y c) suelo inundado con una lámina de 2 cm sobre el nivel del suelo, con 3 repeticiones. Como parámetros se midieron la producción de forraje, la altura de planta y la acumulación de raíces. Todos los cultivares presentaron buen desarrollo cuando el suelo se encontraba a 50% de capacidad de campo, lo cual muestra su adaptación a condiciones ligeramente secas. Bajo condiciones de inundación, *B. humidicola* cvs. Llanero y Tupi no mostraron reducción en la producción de materia seca de forraje, mientras que el crecimiento de los cvs. Marandu, Piatã, Tanzânia y Xaraés se redujo significativamente (P<0.001) en 71.3, 94.0, 81.2 y 77.2%, respectivamente. En plantas bajo condiciones de inundación, la producción de masa de raíces se redujo aproximadamente 30% en comparación con los resultados obtenidos a 50% de capacidad de campo. Aunque todos los cultivares evaluados se podrían utilizar bajo condiciones de humedad marginal en el suelo, la siembra de los cvs. Llanero, Tupi y Massai sería la más indicada en zonas sujetas a inundaciones frecuentes. Se necesitan estudios a nivel de campo para verificar estos resultados de invernadero.

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Introduction

Animal husbandry is one of the most important activities in Brazil, and pastures are the main feed source for the Brazilian cattle herd. This results in lower costs of production than in intensive or semi-intensive systems, where animals are confined and fed grain. In a large part of Brazil such as in Mato Grosso State, livestock production is currently almost exclusively on *Brachiaria brizantha* cv. Marandu pastures.

The establishment of pastures in the Amazon region often occurs in areas with poorly-drained and/or waterlogged soils, and with high precipitation rates (over 500 mm/month during the rainy season). Thus, pastures are often flooded for extended periods, resulting in slow pasture growth, low forage quality and high risks of degradation. Temporary flooding and waterlogging of the soil can result in serious damage to livestock production, e.g. the Marandu Death Syndrome (MDS) (Pedreira et al. 2014).

According to Dias-Filho (2006), the relationship between MDS and the low adaptation of *B. brizantha* cv. Marandu to soil flooding is that the excess water in the soil acts as a predisposing factor to the onset of the problem, i.e. as a 'trigger'. Under such conditions (excess moisture or rainfall), fungi present in the soil become pathogenic owing to the condition of hypoxia or anoxia in some forage plants lowering their resistance. In view of this problem, the only practical solution is to use pasture species with greater tolerance to poorly-drained and/or temporarily waterlogged soils.

Therefore, this study aimed to evaluate the flooding tolerance of 9 commercial cultivars of forage grasses.

Material and Methods

The experiment was carried out under a shelter at the State University of Mato Grosso (UNEMAT), Alta Floresta, MT, Brazil, from August to December 2012. According to the Köppen classification, the region has a tropical rainy climate (Awi type), with a well-defined dry and rainy period, and an average annual precipitation of 2,200 mm. The temperature varies from 18 to 40 ºC, averaging 26 ºC (INMET 2014).

The soil utilized was a clayey dystrophic hapludox collected from a gully. It was sieved to remove undesirable particles such as gravel and wood chunks, autoclaved for 20 min at 120 ºC at 0.5 kgf/m^2 for soil biological sterilization and then stored in a clean, sterilized plastic container.

The experimental design was a 9 x 3 factorial arrangement: 9 cultivars of tropical grasses [*Brachiaria brizantha* (now: *Urochloa brizantha*) cvv. Marandu, Piatã and Xaraés; hybrid *Brachiaria* cv. Mulato II; *B. humidicola* (now: *U. humidicola*) cvv. Llanero and Tupi; *B. ruziziensis* (now: *U. ruziziensis*) cv. Common (subsequently referred to as Ruziziensis); and *Panicum maximum* (now: *Megathyrsus maximus*) cvv. Massai and Tanzânia] and 3 soil water levels. The water levels were classified as: a) minimal water for development, in which irrigation was estimated to achieve 50% of field capacity; b) soil at field capacity, with irrigation estimated at 0.5 kg water/kg dry soil (equivalent to -0.01 MPa); and c) flooded soil, with irrigation applied to achieve 2 cm of water above the soil surface. Three replicates were used, totaling 81 experimental units.

Each experimental unit was characterized by a rectangular pot with a volume of 3 L filled with 2 L of sterilized soil, into which the grass seeds were sown. The undrained/undrilled pots were irrigated until they reached the desired moisture level. Thirty seeds were sown in each pot at 1 cm depth, with subsequent thinning to leave 15 plants/pot. At planting, each pot received 75 g of fertilizer formulated with N:P:K 04:14:08.

The seeds were sown on 25 May 2012, and all pots were irrigated for 30 days to ensure establishment. After a standardization harvest on 24 September 2012, treatments were imposed and 3 harvests were made subsequently: on 5 and 23 November and on 13 December 2012, which allowed 3 regrowth periods and a total of 80 days of exposure to treatments.

At each harvest, the forage was cut with scissors at 10 cm from ground level and dried in a forced-circulation oven at 55 ºC until constant weight to determine dry matter (DM) accumulation. Plant height was determined in 10 plants from each pot by measuring from ground level to the tip of the longest leaf using a measuring tape.

After the third harvest, the roots of all plants from each pot were collected, cleaned and dried in a forced-circulation oven at 55 ºC to constant weight to determine DM root mass.

The data were analyzed using a mixed-models method with special parametric structure in the covariance matrix, through the MIXED procedure of SAS software (Littell et al. 2006). Akaike's information criterion was used to choose the covariance matrix (Wolfinger and O'Connell 1993). Treatment means were estimated using "LSMEANS", and "PDIFF" was used to compare them. Significance was detected at the 0.05 level of probability.

Results

The results reported here refer to the measurements performed at the end of the third evaluation cycle, when the plants had been subjected to a total of 80 days of water stress (treatments).

There were significant cultivar x water level interactions in terms of forage DM accumulation (P<0.001; Table 1). At 50% of field capacity all cultivars grew satisfactorily with highest yields from Tupi, Llanero and Tanzânia (13.5–15.8 g DM/pot) and least from Massai, Mulato II and Ruziziensis (5.7–8.3 g DM/pot; P<0.05). As water availability increased, growth of Piatã, Xaraés, Marandu and Tanzânia declined significantly (P<0.05) so that in the flooded situation, DM yields were very low (0.6–3.0 g DM/pot). At the other end of the scale, yields of Llanero, Tupi and Massai were not affected by flooding (P>0.05).

There were no significant cultivar x water level interactions for root mass, which, however, differed among cultivars (P<0.001) and soil water levels (P=0.029) (Table 2). There was a gradation in root mass from highest in Llanero to lowest in Tupi, with root mass in the latter being only 23% of that of Llanero. This fact can be associated with differential increases of adventitious roots under flooded conditions.

At 50% field capacity, Tanzânia produced the tallest plants (P<0.05; Table 3), followed by Llanero, Tupi and Piatã and then the remaining cultivars (P<0.05). Under flooded conditions, height of Tupi, Massai and Mulato II actually increased (P<0.05), while height of most other cultivars declined, the most marked reduction (about 50%) being in Piatã.

Table 1. Forage dry matter accumulation (g DM/pot) in grass cultivars in pots at the end of the third regrowth cycle (80 days) under varying levels of water availability.

Cultivar	Water levels		
	50% Field capacity	Field capacity	Flooded[1]
Massai	5.7 Da[2]	6.8 CDa	8.1 BCa
Tanzânia	15.8 Aa	2.2 Db	3.0 Db
Piatã	10.9 ABCa	9.4 ABCa	0.6 Db
Xaraés	9.8 BCDa	5.9 CDab	2.2 Db
Marandu	10.1 BCDa	4.5 CDab	2.9 Db
Mulato II	7.3 CDa	7.8 BCa	4.1 CDa
Ruziziensis	8.3 CDa	5.8 CDa	4.7 CDa
Tupi	14.7 ABa	13.6 Aa	13.6 ABa
Llanero	13.5 ABb	12.7 ABb	20.7 Aa

[1]Water to 2 cm above soil level.

[2]Means followed by the same upper-case letter within columns (cultivars) and lower-case letter within rows (water levels) do not differ statistically according to the F test at a significance level of 5%.

Table 2. Root dry matter mass (g DM/pot) of grass cultivars in pots at the end of the third regrowth cycle (80 days) under varying levels of water availability.

Cultivar	Water level			Average
	50% Field capacity	Field capacity	Flooded[1]	
Massai	35.19	45.84	39.04	40.03 AB[2]
Tanzânia	31.87	11.99	35.56	26.47 BC
Piatã	40.42	31.57	824	26.74 BC
Marandu	29.94	50.38	27.95	36.09 AB
Xaraés	44.71	19.72	20.06	28.17 ABC
Mulato II	27.54	19.96	12.74	20.08 CD
Ruziziensis	46.26	34.97	24.50	35.24 AB
Tupi	9.01	10.89	8.21	9.37 D
Llanero	52.78	30.12	42.38	41.76 A
Average	35.30 a	28.38 ab	24.29 b	

[1]Water to 2 cm above soil level.

[2]Means followed by the same upper-case letter within columns (cultivars) and lower-case letter within rows (water levels) do not differ statistically according to the F test at a significance level of 5%.

Table 3. Plant height (cm) of grass cultivars in pots at the end of the third regrowth cycle under varying levels of water availability.

Cultivar	Water level		
	50% Field capacity	Field capacity	Flooded[1]
Massai	39.5 Db[2]	47.4 BCDa	48.7 BCa
Tanzânia	65.8 Aa	44.9 Db	44.1 Db
Piatã	50.8 Ba	44.9 Db	25.7 Gc
Marandu	44.5 Ca	39.4 Eb	32.6 Fc
Xaraés	44.9 Ca	46.7 CDa	37.0 Eb
Mulato II	45.6 Cb	51.2 ABa	44.6 CDb
Ruziziensis	51.7 Ba	47.8 ABCDab	45.5 CDb
Tupi	48.6 BCb	51.7 Aab	52.6 Ba
Llanero	50.3 Bb	49.5 ABCb	56.7 Aa

[1]Water to 2 cm above soil level.
[2]Means followed by the same upper-case letter within columns (cultivars) and lower-case letter within rows (water levels) do not differ statistically according to the F test at a significance level of 5%.

Discussion

Under flooding, plants can produce numerous metabolic signs in response to the reduction in the endogenous levels of soil oxygen. During this period of stress, architecture, anatomy, metabolism and growth patterns change as a survival strategy (Bailey-Serres and Voesenek 2008).

Excess water in the soil and filling of the macro- and micropores lead to a decrease in the diffusion of the oxygen necessary for root respiration, causing hypoxia (low concentration of O_2) or anoxia (absence of O_2) in the soil (Thomson and Greenway 1991; Armstrong et al. 1994). Replacement of the oxygen consumed by microorganisms from the soil and by the roots of plants occurs slowly due to the low diffusion of oxygen in the water (Dias-Filho 2012). Thus, stomatal conductance is decreased, which reduces the rate of photosynthesis and consequently growth rates (Baruch 1994; Huang et al. 1994; Dias-Filho 2002; Mattos et al. 2005), culminating in a decrease in accumulation of shoots and roots (Dias-Filho 2002). While these symptoms of flooding were observed in our study, they did not occur in all cultivars.

Cultivars Llanero, Tupi and Massai remained productive even under flooded conditions (Table 1), which is very important for the production systems based on grasslands in this region of Brazil. This fact is attributed to important morpho-physiological adaptations such as physical acclimatization and coping with a flooded environment by increasing the amount of adventitious roots, as was evidenced for Llanero (Dias-Filho 2002; Jackson and Colmer 2005; Mattos et al. 2005). Plants with this ability are more tolerant of anoxia (Gibbs and Greenway 2003) and have the capacity to prevent or repair oxidative damage during re-aeration (Blokhina et al. 2001; 2003).

This oxidation (production of toxic compounds) results from the anaerobic fermentation performed by soil microorganisms, to produce Mn^{2+}, Fe^{2+}, S^{2-}, H_2S and carboxylic acids as products of their metabolism (Jackson and Colmer 2005). When accumulated in the soil, these elements cause severe damage to the plants.

It is of interest that the 2 *Panicum maximum* cultivars used in our study reacted differently to flooding. While Tanzânia suffered severely, Massai was not affected. Silva et al. (2009) evaluated 7 *P. maximum* cultivars and accessions (including Massai, Mombaça and Tanzânia) and observed that flooding reduced total forage production significantly. In contrast, the poor tolerance of *Brachiaria brizantha* cultivars in our study under flooding agrees with the findings of Dias-Filho (2002), who evaluated the morpho-physiological responses of 5 accessions of *B. brizantha* planted in pots under flooded and well-drained soil conditions and observed that flooding reduced net photosynthesis and chlorophyll contents significantly.

Flooding of soils negatively affects leaf elongation rate (LER) and leaf blade senescence rate (Mattos et al. 2005). Thus, Dias-Filho and Carvalho (2000) suggest that LER should be employed as a mechanism for early detection of tolerance of flooding in *Brachiaria* species. Unfortunately, limited resources did not allow us to measure this parameter.

Silva et al. (2009) observed a reduction in accumulation of roots of Tanzânia under flooded conditions. Similarly, Silva et al. (2007) reported an abrupt drop in production of root biomass by cv. Tupi after the second week of waterlogging, as compared with *B. humidicola* cv. Common, which showed an initial reduction and then stabilization. In our study, Tupi and Llanero presented the highest root production, independent of water level, an important issue to consider when establishing a pasture in the Brazilian Amazon region.

Silva et al. (2007) found that *B. brizantha* cv. Marandu suffered a dramatic reduction in root biomass production from the fourth week of flooding, and Caetano and Dias-Filho (2008) reported that this cultivar was quite susceptible to reduction of root production in waterlogged and/or flooded soils. Likewise, cultivar Xaraés showed a linear decrease in root biomass under flooding, to 14.2% of biomass of dry roots in unflooded soil by 8 weeks. Our findings support these outcomes, clearly demonstrating that Marandu and Xaraés should be avoided in grassland systems experiencing high levels of rainfall and waterlogging and/or flooding.

In plants that go through relatively long periods of flooding, one of the most common morpho-anatomical responses to hypoxia and/or anoxia is the formation of aerenchymae (Evans 2003; Colmer and Voesenek 2009; Takahashi et al. 2014). Those aerenchymae are mainly of the lysigenous type: they are formed by dead cortical cells (Yamauchi et al. 2013). This occurs due to the increase in the synthesis of ethylene caused by increased activity of ACC synthase, resulting in increased cellulase activity, which in turn contributes to degradation of the cell wall and formation of aerenchyma (Takahashi et al. 2014). There is a second type, the schizogenous aerenchyma, originating from the connection of cells, which form continuous columns for the storage and transport of gases (Joly 1991; Colmer et al. 2004). Furthermore, adventitious roots to capture and transport oxygen to the submerged tissues can be developed (Dias-Filho 2012).

The accumulated ethylene may reduce the roots' extension, whereas the soil carbon dioxide may severely damage roots, because high concentrations of CO_2 can reduce pH (Colmer and Voesenek 2009). In the presence of Ca^{2+}, ethylene forms calcium carbonate, increasing the pH and making some nutrients unavailable (Jackson 2004) and reducing root growth. These factors can be potentiated by an increase in temperature, which increases the enzymatic activity of plants (Taiz and Zeiger 2004), and the presence of organic matter, a substrate for aerobic microorganisms (Jackson 2004).

The degradation of nitrogen compounds (proteins and peptides) of the soil organic matter by microorganisms reduces them to nitrite and nitric oxide (NO), which interact indirectly through the reaction with superoxide favoring nitrogen-reactive species, which are capable of reacting with DNA, proteins and lipids. Likewise, high concentrations of NO result in production of peroxynitrite, which has a catalytic effect on lipids, DNA and proteins, causing cell death (Wink and Mitchell 1998).

Another explanation for the low root biomass production is the presence of hydrogen peroxide in the root apoplast. Evaluating 2 tolerant (*Iris pseudacorus* and *Oryza sativa*) and 2 intolerant (*Iris germanica* and *Triticum aestivum*) species under anoxia by soil flooding, Blokhina et al. (2001) observed that, in all tested plants, H_2O_2 was increased in plasma membranes and in the root apoplast. According to Visser and Voesenek (2004), this system works as a rheostat system that regulates the endogenous levels of H_2O_2, a crucial factor for plant tissue, because high increases in H_2O_2 concentrations in plant tissue can trigger the death of cells.

Cultivars less tolerant of soil waterlogging can exude ethanol from the roots, which is attractive to zoospores of pathogenic fungi and which supplies substrate for the colonization of mycelia in the plant tissue (Dias-Filho 2006). The morpho-physiological alterations brought about by soil flooding associated with the attack of phytopathogenic fungi in intolerant forages can lead to their death, e.g. the Marandu Death Syndrome (Pedreira et al. 2014).

An interesting finding was the good performance at 50% field capacity relative to that at field capacity. Overall most cultivars performed better at 50% field capacity than at field capacity. This suggests that soils do not have to be flooded to produce some reduction in plant growth. The correct choice of species and cultivars is the most important technical aspect when a pasture-based livestock production system is to be established in an environment prone to flooding. Forage grasses like Tupi, Llanero and Massai showed promising results and should be tested under field conditions in some of these environments.

Conclusions

This study has revealed wide variation in production of important pasture grasses, under flooded conditions. An interesting outcome was the production levels obtained in all cultivars in soils at 50% of field capacity. When soil water was limited, most cultivars had acceptable growth rates. Increasing the level of soil water to field capacity produced little change in forage growth under the experimental glasshouse conditions and often reduced growth to some extent. This highlights the adaptation of all cultivars tested to situations where only moderate levels of soil water are available in the growing season. However, under flooded conditions only a few cultivars produced satisfactory levels of DM. *Brachiaria humidicola* cvv. Llanero and Tupi continued to produce well after 80 days of flooding. *Panicum maximum* cv. Massai also appeared to be quite tolerant of flooding for extended periods, while the remaining cultivars performed poorly under flooding and cannot be recommended for sowing in areas with deficient soil drainage or flooding problems. How these controlled situation results can be extrapolated to field situations warrants evaluation.

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Floristic diversity and effect of anthropogenic activities on human-dominated grasslands in subtropical regions of Peninsular India

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Keywords: Burning, community protection, grazing, palatable species, pastures.

Abstract

Indian subtropical grasslands are secondary habitats formed due to anthropogenic activities resulting in degradation of deciduous forests. Spread throughout Peninsular and Central India, they are important from economic and ecological points of view and are the prime source of fodder for the large population of livestock in this region. Pastures are either exposed to open grazing or protected and harvested periodically for fodder. In the present investigation floristic diversity of 21 sites from Western Ghats and Central India was studied, along with the effects of anthropogenic activities like burning and grazing on floristic composition in general and palatable species in particular. Over-grazing and burning were found to result in dominance of unpalatable species, making the grasslands less useful for livestock production. High rainfall and protection by local communities seem to play important roles in the dominance of palatable species in grasslands. Our results suggest that periodic harvesting and protection from burning and over-grazing should be encouraged and implemented in order to increase the potential of these grasslands for livestock production. Detailed studies are warranted to confirm these findings.

Resumen

En las regiones subtropicales de la India, los pastizales son hábitats secundarios que se formaron como consecuencia de la degradación de bosques deciduos debido a actividades antropogénicas. Estos pastizales se extienden por toda la India Peninsular y Central y son importantes desde los puntos de vista económico y ecológico; constituyen la principal fuente de forraje para la alta población de ganado en esta región. Su uso es mediante pastoreo no controlado o son protegidos para cosechas periódicas. En el estudio se determinó la diversidad florística en 21 sitios de las regiones Western Ghats y Central India. Además se evaluaron los efectos que actividades antropogénicas como la quema y el pastoreo tienen en la composición de especies en general y de especies palatables en particular. Se encontró que el sobrepastoreo y la quema condujeron a la dominancia de especies no-palatables, reduciendo el potencial de los pastizales como fuente de forraje. La alta pluviosidad y la protección de los pastizales por parte de las comunidades locales aparentemente tienen un papel importante en la dominancia de las especies palatables. Los resultados sugieren que se deben estimular cosechas periódicas de los pastizales y su protección contra la quema y el pastoreo excesivo con el fin de aumentar su potencial para producción animal. Se requieren estudios detallados para confirmar estos resultados.

Introduction

Grasslands are productive biomes of the Earth, covering approximately 36% of terrestrial landscapes, similar to forest covers (Shantz 1954). Tropical grasslands of the Indian subcontinent are widespread, though they are not climax grasslands, being continuously exposed to anthropogenic pressures like lopping, shifting cultivation, burning and over-grazing (Dabadghao and Shankarnarayan 1973). Except for *shola* forests, which are the only natural communities, these grasslands are treated as secondary as they are formed as a result of human activities. Indian grasslands are important for their biodiversity values, cultural heritage and effects on the economy as a fodder source.

Pioneering work on ecology and biodiversity of Indian grasslands was done by Dabadghao and Shankarnarayan (1973). Various community aspects of grassland vegetation in India have been examined by a number of workers mostly focusing on species diversity, dominance and the

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effects of anthropogenic pressures on grasslands from various parts of the subcontinent, such as Tarai region (Shukla 2009), Northeast India (Ramakrishnan and Ram 1988; Yadava 1990; Shankar et al. 1993), Northern India (Singh 1973; Misra and Misra 1981; Tripathi and Shukla 2007) and Western Ghats (Bharucha and Shankarnarayan 1958; Jose et al. 1994; Ramesh et al. 1997; Rawat et al. 2003). In spite of these efforts, the grassland communities of Peninsular India have largely been ignored by science, except for a few broad studies, e.g. classification of Indian grasslands (Oke 1972) and a few local studies like the synecological studies of grasslands of Marathwada University campus (Naik and Patunkar 1979). Oke (1972) classified Indian grasslands into 7 major habitat patterns and 24 minor sub-patterns based on various agro-climatic and habitat zones. The grasslands of Peninsular India are generally *Dichanthium-Sehima* type (Dabadghao and Shankarnarayan 1973) and occur along with other vegetation like evergreen forests, semi-evergreen forests, moist deciduous forests, dry deciduous forests, and thorny scrub forests, and along rocky outcrops (Puri 1960). These diverse and threatened habitats have received little attention when compared with other vegetation types like forests and cultivated landscapes.

In Peninsular India, many grassland patches are protected as community grasslands called '*Kuran'* or *'gairan'* and are used as large-scale fodder collection sites, where grasses and legumes are harvested. Many exclusively cattle-rearing nomadic communities like *Gavali dhangar* depend on these habitats (Gadgil and Malhotra 1982) and grasslands play, for example, a substantial role in total milk production in the State of Maharashtra. In addition, many grasslands serve as State Government wildlife sanctuaries for the conservation of endemic animal species like Black Buck (*Antilope cervicapra*), Chinkara (*Gazella bennettii*) and threatened bird species like Lesser Florican (*Sypheotides indica*) and Indian Bustard (*Ardeotis nigriceps*).

In the present work, the grasslands of human-dominated landscapes of Maharashtra State were studied for their floristic diversity, uniqueness and effects of degradation due to burning and grazing on overall grassland diversity and fodder potential. This is the first attempt to understand the grassland communities of human-dominated landscapes in northern parts of Peninsular India (15.63–21.14° N, 72.71–80.59° E) in recent times.

Materials and Methods

Twenty-one study sites (Figure 1) were selected from the list of grasslands of Maharashtra State catalogued in Dabadghao and Shankarnarayan (1973) and Oke (1972). Study sites fall into 2 major biogeographic zones of India, namely Central India and Western Ghats. Central India is characterized by low rainfall (annual mean 700–1,500 mm) and high temperature in summer (mean 45 °C), while the Western Ghats is one of the 35 biodiversity hotspots of the world (Myers et al. 2000) with abundant rainfall of 3,000–4,000 mm.

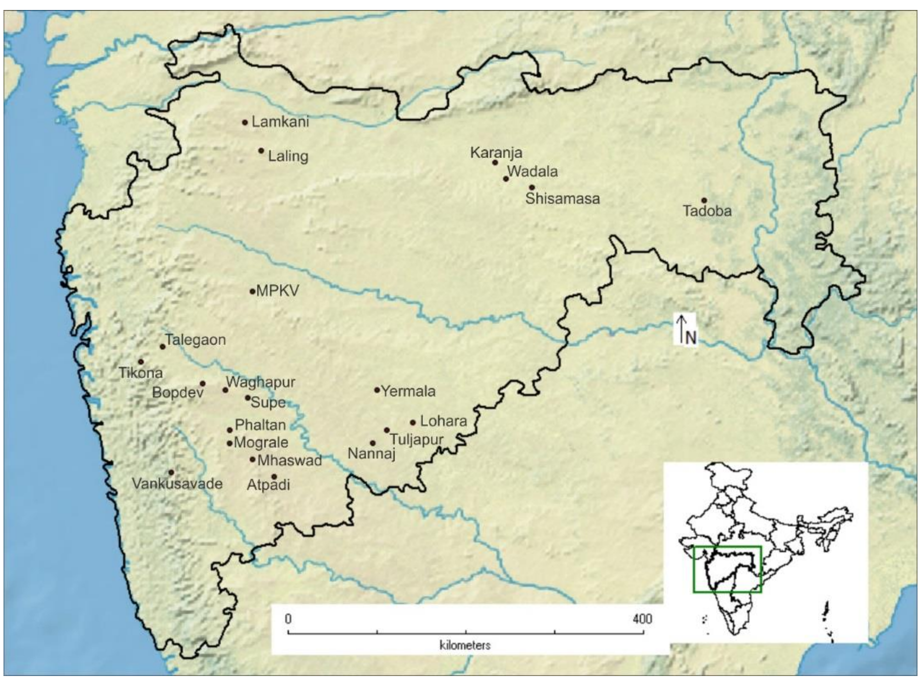

Figure 1. Study sites (Map was prepared using DIVA GIS, version 2).

Table 1. Summary of study sites with geographic and environmental variables. Weather data were obtained from nearest weather observation station of the Indian Meteorological Department.

Location	Latitude (North)	Longitude (East)	Mean annual rainfall (mm)	Max. temp. (°C)	Min. temp. (°C)	Protection status
Atpadi	17.40	74.93	571	38.1	13.3	Government protected
Bopdev ghat	18.40	73.91	623	38.9	13	Unprotected
Karanja-Sohol	20.42	77.50	965	40.8	16.0	Government protected
Laling	20.80	74.73	614	40.3	15.4	Government protected
Lamkani	21.08	74.57	728	40.3	15.4	Community protected
Lohara	18.02	76.29	677	40.2	16.1	Community protected
Mhaswad	17.65	74.65	597	30.2	13.1	Unprotected
Mogarale ghat	17.55	74.52	488	35.2	13.1	Unprotected
MPKV	19.36	74.64	550	41	11	Unprotected
Nannaj	17.81	75.88	723	40.2	16.1	Government protected
Phaltan	17.94	74.42	499	35.2	13.6	Community protected
Shisamasa	20.69	77.13	866	40.8	16.0	Community protected
Supe	18.33	74.37	300	38.9	13	Government protected
Tadoba	20.30	79.27	1,337	42.3	13.1	Government protected
Talegaon	18.80	73.72	1,297	37.9	12	Unprotected
Tikona	18.65	73.50	1,500	37.9	12	Unprotected
Tuljapur	17.95	76.02	723	40.2	16.1	Unprotected
Vankusavade	17.51	73.82	2,000	36	9	Community protected
Wadala	20.51	77.23	965	40.8	16.0	Community protected
Waghapur	18.41	74.10	500	40.3	15.4	Unprotected
Yermala	18.36	75.92	723	40.2	16.1	Unprotected

Among the 21 sites, 3 locations are from Western Ghats (Vankusavade, Tikona and Talegaon) and the remainder from Central India. Study sites were shortlisted to cover spatial and environmental variations such as rainfall, latitude-longitude and protection status of the locality. Table 1 summarizes the spatial and climatic variables of the study sites.

Sites were surveyed in the post-monsoon months September and October, that is, the peak flowering time, during 2011–2014. Community enumeration was done using quadrats (2 x 2 m) laid in the areas of continuous grass growth using standard ecological techniques (Magurran 2004; Sutherland 2006). The number of quadrats per location was decided based on size and heterogeneity of the area. Number of quadrats per study site was determined by species area curve. A total of 67 quadrats were laid in these locations to document the grassland community. For tufted grasses, 1 tiller was recorded as 1 individual. Plants were grouped as very palatable (fodder) species, moderately palatable species, legumes and other herbs based on field observations, interviews with local stockowners and literature (Blatter and McCann 1935; Bor 1960; Patunkar 1980; Gorade and Datar 2014). Plant samples were collected and processed using standard herbarium methods (Jain and Rao 1977) and identified with the help of regional floras (Cooke 1901–08; Lakshminarasimhan 1996; Potdar et al. 2012). Identity of a species was confirmed by comparing it with authentic specimens deposited in the herbaria of Agharkar Research Institute, Pune (AHMA) and Botanical Survey of India, Western Regional Center, Pune (BSI). Specimens were deposited in AHMA. Data on the distribution, endemism and ecology of taxa encountered in this study are based on relevant literature (Lakshminarasimhan 1996).

Statistical analysis of the data was performed in PAST (Hammer et al. 2001). The diversity was determined by Shannon's index with the log base 2 calculated using PAST. Canonical correspondence analysis (CCA) was performed to understand the effects of spatial and environmental parameters on plant species of the grasslands. Environmental variables considered for statistical analyses included latitude-longitude, rainfall, maximum temperature, minimum temperature and variables indicating levels of grazing, burning, community protection and wildlife protection. Wildlife protection status was considered for areas which are protected and managed by the State Government, while community protection was considered for areas which are conserved and managed by local communities. Rankings were assigned for burning and grazing in the grasslands based on personal observations.

Cluster analysis was performed using PAST to understand the floristic uniqueness and similarities amongst the studied sites. Sample rarefaction (Colwell et al. 2004) was also conducted to understand whether the number of sites selected for the study was adequate or not.

Results

A total of 83 species belonging to 69 genera and 28 families was documented (Annex table). In addition to grasses and legumes, in high rainfall areas like Vankusavde, Tikona and Tadoba, growth of other herbs was also significant. The maximum number of species was found in Tadoba (S = 20) and the minimum number in Mhaswad and Nannaj (6 each). Grasslands like Atpadi, Yermala, Tuljapur and Mhaswad showed lowest diversity and tended to show the highest dominance of species belonging to the genus *Aristida* and of *Heteropogon contortus*. Tikona, which is located in a high rainfall region (4,000 mm), also recorded low diversity. Grasslands in Wadala, Shisamasa, Tadoba, Talegaon and Lamkani were characterized by high diversity and low dominance. Of these, Wadala, Shisamasa and Lamkani were community-protected grasslands, while Tadoba was a government-protected area. *Heteropogon contortus*, *Indigofera cordifolia* and *Aristida funiculata* were the most common species, occurring in 16, 10 and 9 of the 21 assessed sites, respectively. Apart from the cosmopolitan taxa, these grasslands also harbor few endemic grass species like *Ischaemum afrum*, *Sehima sulcatum* and *Lophopogon tridentatus*. *Lophopogon tridentatus*, an indicator of exposed and degraded grassland, was observed at 5 sites with high dominance due to its unpalatable nature. *Sehima sulcatum*, a good fodder species and sensitive to disturbance, was recorded from 4 sites (Lamkani, Atpadi, Tuljapur and Nannaj), which are protected by government or local communities. *Ischaemum travancorense*, an endemic and threatened species, which was earlier distributed in Western Ghats, was found in Central Indian grasslands (Tadoba) for the first time. *Ischaemum afrum*, a perennial grass, is known to occur in agricultural landscapes and is recorded only in Shisamasa grassland, which is a mosaic of natural grasslands and agricultural fields. Apart from grasses, an endemic and threatened ground orchid, *Habenaria longicorniculata*, was observed in community-protected Vankusavade grassland (Western Ghats).

Sample rarefaction as per Colwell et al. (2004) suggests that 20 samples were adequate for the study as the curve reached asymptote and most of the diversity in the grassland patches was covered (Figure 2).

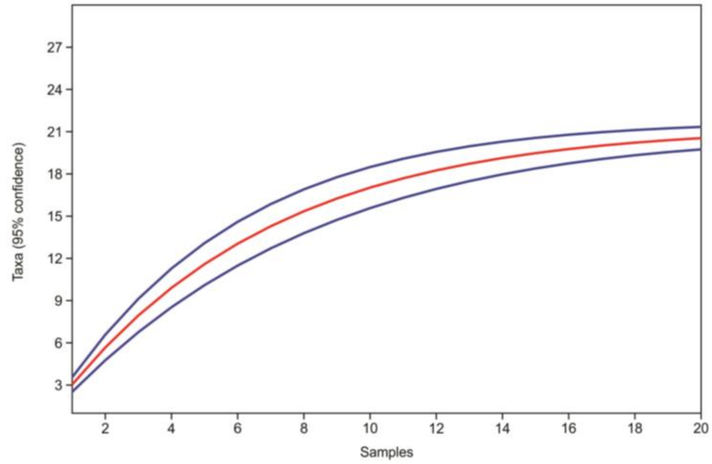

Figure 2. Sample rarefaction curve with 95% confidence.

Table 2. Total taxa, dominance and diversity (Shannon index) of studied sites.

Location	Taxa_S	Dominance_D	Shannon_H
Atpadi	10	0.777	0.600
Bopdev ghat	9	0.415	1.133
Karanja	8	0.352	1.248
Laling	18	0.216	1.821
Lamkani	11	0.213	1.792
Lohara	8	0.300	1.424
Mhaswad	6	0.459	1.034
Mogarale ghat	8	0.282	1.446
MPKV	11	0.466	1.203
Nannaj	6	0.290	1.416
Phaltan	9	0.490	1.060
Shisamasa	12	0.178	1.930
Supe	16	0.364	1.487
Tadoba	20	0.236	1.912
Talegaon	15	0.171	1.931
Tikona	10	0.826	0.428
Tuljapur	7	0.523	0.973
Vankusavade	11	0.353	1.403
Wadala	11	0.204	1.888
Waghapur	13	0.309	1.422
Yermala	9	0.537	0.752

Diversity indices (Shannon H) (Table 2) in all these 20 sites were compared and the range was 0.4 to 1.93 with highest H value in Talegaon and Shisamasa (H = 1.93) followed by Tadoba (H = 1.91). Talegaon, Shisamasa and Tadoba receive more precipitation than other sites (850–1,350 mm), which might have resulted in higher species diversity. The fact that Tadoba grassland is part of Tadoba Tiger Reserve and Shisamasa is community-protected could be another reason for higher diversity. On the other hand Tikona, though receiving higher rainfall (1,500 mm) than Tadoba, recorded the lowest diversity due to high dominance (D = 0.826) of *Themeda triandra.* Though

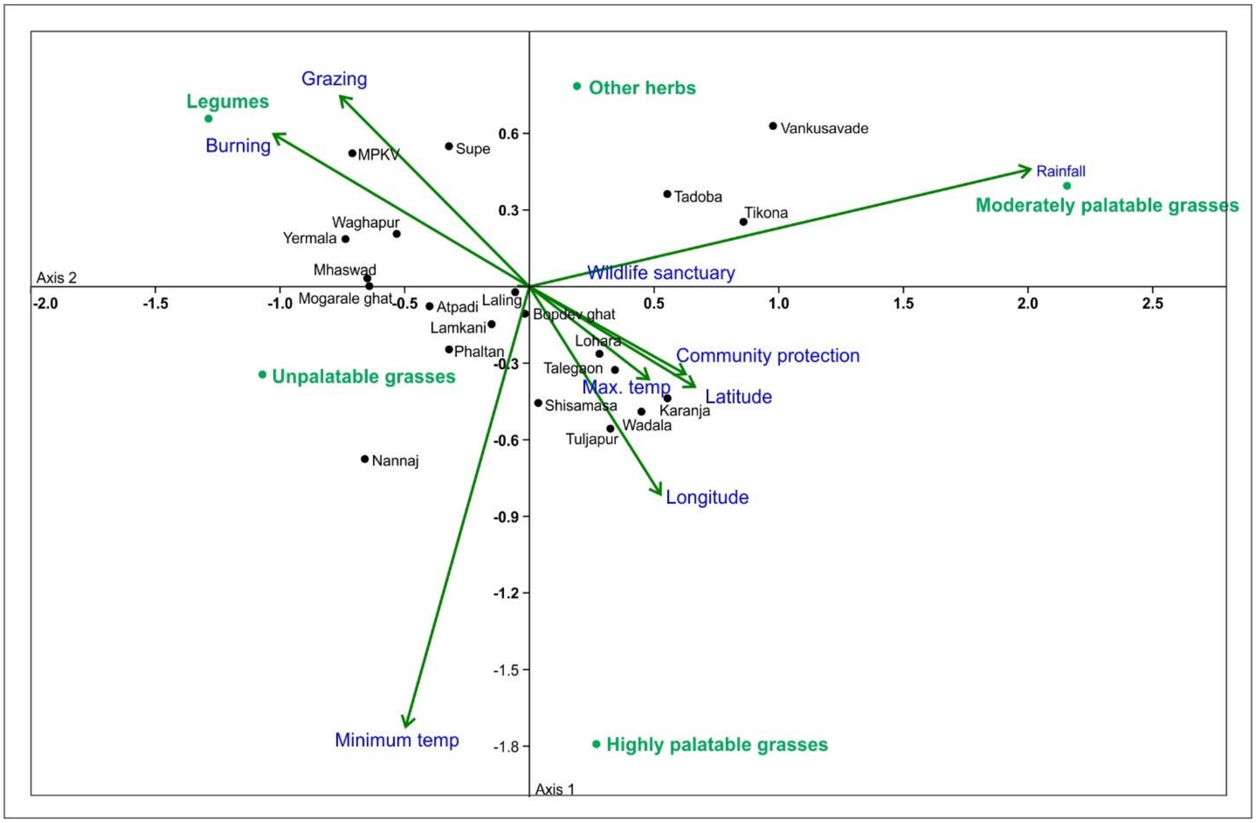

Figure 3. Canonical correspondence analysis showing effects of environmental variables. (Axis 1 explains 56.2%, while Axis 2 explains 25.4% of the total variation of data.)

Table 3. Summary of Canonical correspondence analysis.

Axis	Eigenvalue	%
1	0.19	56.2
2	0.09	25.4
3	0.05	14.5
4	0.01	3.9

Tikona supports 10 species, 90% of the total individuals belong to *T. triandra*, which is characterized by profuse growth of tillers. Tikona recorded highest dominance of 0.825, followed by Atpadi (0.777) (dominated by *Aristida* sp.).

Canonical correspondence analysis, performed to understand the effect of environmental variables on grass communities (Figure 3; Table 3), revealed that the first 2 axes explained a total of 81% of the variation among communities.

Discussion

Floristic diversity

The grasslands were dominated by grasses and legumes, where Poaceae was the dominant family representing 27 (39%) species, while legumes were represented by 11 (15%) species. Grasslands with low diversity were frequently dominated by members of the genus *Aristida* and by *Heteropogon contortus*, which are dominant in grasslands frequently subjected to fire (Dabadghao and Shankarnarayan 1973). Tikona grassland in the high rainfall zone is dominated by the widespread grass species *Themeda triandra*, which is gregarious in growth and gives a characteristic appearance to hill slopes (Dabadghao and Shankarnarayan 1973). *Themeda triandra* is adapted to overcome competition from other grasses and herbs, especially during the seedling stage (Hagon 1977), which might be a reason for dominance of this species. Both community-protected and government-protected grasslands showed high diversity and low dominance. However, Talegaon, an unprotected grassland, possessed high diversity, mostly due to its geographical location that receives more rainfall (900 mm) than other sites (Table 1).

The current study highlights the presence of *Ischaemum travancorense* outside Western Ghats for the first time (Datar et al. 2014). This species was earlier thought to be endemic to Western Ghats and classified as a *Least Concern* species in the IUCN redlist (Rehel 2013).

However, the scattered distribution and availability of this species in various habitats from coastal salty marsh to dry inlands suggests that taxonomic enumeration of this species needs further attention, as well as reassessment of its IUCN status.

The mosaic of natural grasslands and agricultural fields in Shisamasa supports *Ischaemum afrum*, which is the primary breeding habitat of the endangered bird Lesser Florican (*Sypheotides indica*). The grass patches amidst crops are preferred by this bird (Sankaran 1997). In Shisamasa, it lays eggs in the culms of *Ischaemum afrum*, thus making this grass important in the conservation program of the bird. Another taxon with conservation concerns encountered in this study was the ground orchid *Habenaria longicorniculata*, from Vankusavade grassland. This species is endemic to Peninsular India and is listed as *Near Threatened* in threat category (Kumar et al. 2000). Three sites were severely invaded by weedy species like *Xanthium strumarium* and *Alternanthera sessilis* (Singh and Karthikeyan 2000). These species must be eradicated from grasslands in order to control their further spread, thereby facilitating the conservation of grassland habitats.

Floristic uniqueness

When a dendrogram was drawn using Cluster analysis (Figure 4), floristic similarities amongst grasslands showed groupings based on the species assemblage and environmental parameters. Vankusavade is distinct from all other grasslands, as it is located in a high rainfall area in Western Ghats and characterized by dominance of the rainfall-associated taxon *Eulalia trispicata*, a typical member of the grass association of Western Ghats (Bharucha and Dave 1952). The bottom branch of the cluster groups, Waghapur, Supe and MPKV grasslands, is highly degraded by annual burning and over-grazing. In unprotected grasslands, over-grazing is responsible for dominance of non-palatable species over time (Naik and Patunkar 1979).

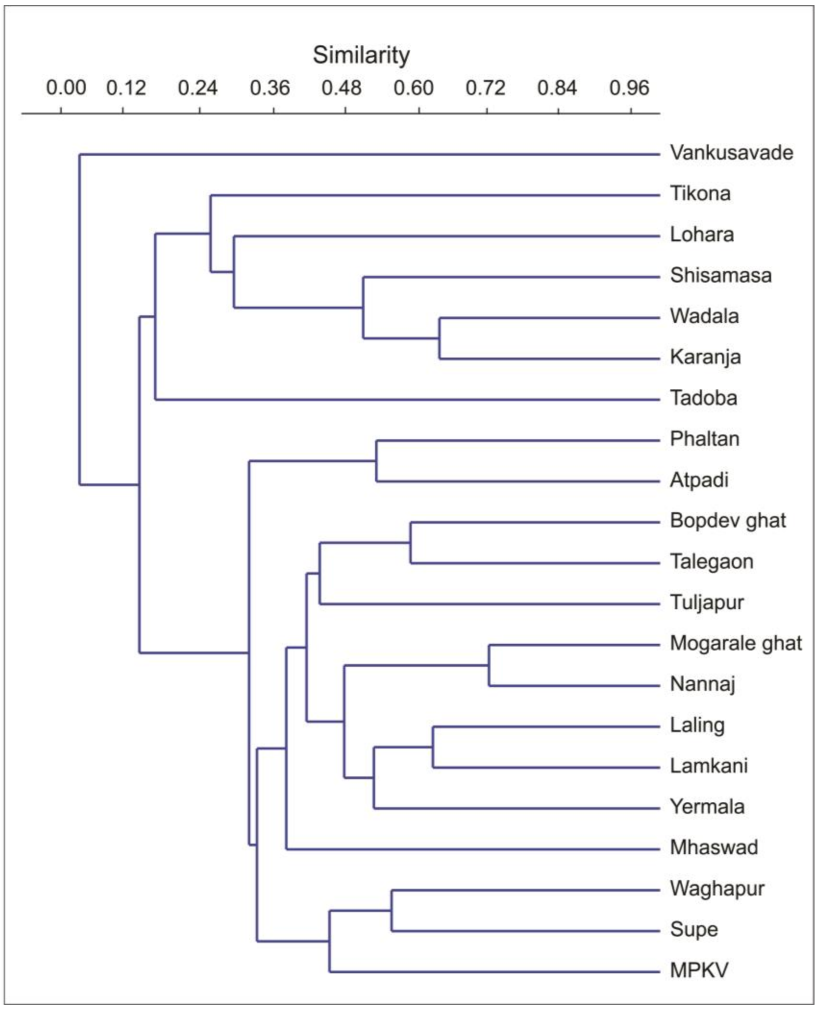

Figure 4. Floristic similarity based on Jaccard's index (Paired group UPGMA).

One cluster contains Shisamasa, Wadala and Karanjasohol, i.e. sites from a high rainfall region (800–1,000 mm) displaying a combination of palatable and non-palatable species. These 3 sites are under community or government protection, which prevents the grazing-associated plant community shift. Community-protected grasslands are characterized by strict bans on grazing and periodic harvesting of fodder during the post-monsoon (September–October), when the community gets enough fodder to overcome rainless summer months until the next monsoon (June–July). This periodic harvesting has advantages over direct grazing. By preventing the disturbance caused by cattle grazing, these grasslands provide higher yields of fodder. In grasslands, which are grazed regularly, the grasses are consumed by grazing cattle well before flowering. On the other hand in grasslands, which are not grazed and are periodically harvested, seed setting is achieved and seed is dispersed before harvesting, leading to regeneration of good quality fodder species during the monsoon in the next year. In contrast to community-protected grasslands, government-protected grasslands are accessible to wild animals throughout the year and are not harvested by humans. Protection from domestic cattle helps them to maintain good grass growth and diversity. This practice, which is beneficial for improving productivity of grasslands, should be implemented in other openly grazed grasslands.

Cluster analysis has grouped sites like Phaltan-Atpadi, Laling-Lamkani and Bopdev ghat-Talegaon, which may be based on the close proximity of these sites. The maximum similarity between any 2 grassland patches was 55%, highlighting the need for conservation of each site.

Effects of physical parameters on diversity and fodder potential of grasslands

The CCA plot showed strong correlation between rainfall and moderately palatable grasses. Locations like Tadoba, Tikona and Vankusavade, which show high rainfall, are dominated by palatable grasses. Unpalatable grasses are more prolific in locations like Phaltan, Lamkani, Atpadi and Laling, which are located in low rainfall sites. High rainfall also showed positive correlation with growth of other herbs. In areas with less rainfall the sturdy grasses grow abundantly but higher rainfall gives herbaceous species other than grasses a chance to establish, which is evident in the CCA plot. Anthropogenic activities like burning and grazing showed strong association with the presence of legumes. Sites that are repeatedly burnt have soils with low or poor nitrogen levels that prevent the growth of most of the grass taxa, as fire is reported to reduce soil nutrients by burning the top layer and destroying humus (Pivello et al. 2010). However, legumes sprout and dominate in the early stages of succession due to their nitrogen-fixing root nodules (Towne and Knapp 1996). Grasses become dominant subsequently as they take advantage of the nitrogen availability in the soil. This phenomenon can be observed in locations like Mhaswad, Mograle ghat, MPKV, Yermala and Waghapur and is clearly depicted in the plot.

Good patterns were evident between community protection and highly palatable grasses. According to Dabadghao and Shankarnarayan (1973), protecting an *Aristida-Heteropogon* community produced a slow shift to *Apluda mutica-Chrysopogon fulvus* and *Dichanthium pertusum,* which are progressively replaced by *Sehima nervosum* and *S. sulcata* and ultimately by *Themeda quadrivalvis*. Presently, Lamkani, where *Sehima* is dominant, can be treated as the penultimate stage of this succession. Lamkani has been protected by local communities for the past 10 years, which has resulted in the community shift towards more palatable species. As sites like Wadala and Shisamasa are protected by local communities from burning and grazing and are not harvested before seed-set, the seeds of palatable species are dispersed and germinate in the next monsoon. In contrast to this situation, in over-grazed grasslands the palatable species are consumed by cattle much before they reach the reproductive stage, resulting in reduction in their population in subsequent years. Unpalatable fodder species on the other hand are not consumed by cattle and their chances of survival are much higher, thus making them dominant in the community.

Conclusion

Subtropical grasslands of India are situated in human-dominated landscapes and are affected by livestock grazing and associated anthropocentric pressures on them. The present study covering 21 sites with varying levels of protection and utilization from Western Ghats and Central India indicated that grasslands protected by local communities with periodic harvesting show high growth of preferred palatable species. Most of the grasslands in this region are unprotected and/or over-grazed, hence the dominance of unpalatable species is evident. The majority of the livestock of the region depends exclusively on these degraded grasslands for fodder, so further degradation is unavoidable, and regaining of the original composition with species like *Dichanthium-Sehima* seems difficult with current management practices. However, protection and periodic harvesting of selected species can prevent further degradation. Future grassland management plans should consider and implement seasonal harvesting practices. Seasonal harvesting should lead to better yield of fodder species even in protected areas and eventually decrease the dependence of domestic livestock on grasslands inside protected areas. However, a blanket management plan for the entire state would be difficult to implement due to highly varying rainfall and temperature patterns and magnitude of human disturbance across the state. Management plans for each site or group of sites in an ecoregion need to be developed with emphasis on wildlife conservation, as some of the grasslands also harbor highly threatened bird species. Protection of grasslands will not only provide fodder but also increase ground water level of the area by allowing rainwater to percolate into soil and avoid soil erosion during monsoon rains. The present study also documented invasion by many non-native weed species and future research work should focus on interaction of invasive species with native grass species and its implications for long-term conservation. Moreover, these fragile grasslands are present in the human-dominated landscape and community participation in conservation is essential, so interaction of scientists and managers with local people will be vital if proper conservation and management are to be achieved.

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Annex table: List of species.
Habit: H = herb; RP = Root parasite; C = climber. Plant names are as per The Plant List (www.theplantlist.org)

No.	Species	Family	Habit	Native/Non-Native/Endemic
1	*Alternanthera sessilis* (L.) R.Br. ex DC.	Amaranthaceae	H	Non-Native
2	*Alysicarpus longifolius* (Spreng.) Wight & Arn.	Leguminosae	H	Native
3	*Alysicarpus pubescens* J.S.Law	Leguminosae	H	Native
4	*Andropogon pumilus* Roxb.	Poaceae	H	Native
5	*Apluda mutica* L.	Poaceae	H	Native
6	*Aristida adscensionis* L.	Poaceae	H	Native
7	*Aristida funiculata* Trin. & Rupr.	Poaceae	H	Native
8	*Aristida* sp.	Poaceae	H	Native
9	*Arundinella* sp.	Poaceae	H	Native
10	*Arthraxon* sp.	Poaceae	H	Native
11	*Blainvillea acmella* (L.) Philipson	Asteraceae	H	Non-Native
12	*Blumea* sp.	Asteraceae	H	Native
13	*Boerhavia diffusa* L.	Nyctaginaceae	H	Native
14	*Boerhavia repens* L.	Nyctaginaceae	H	Native
15	*Buchnera hispida* Buch.-Ham. ex D.Don	Orobanchaceae	RP	Native
16	*Celosia argentea* L.	Amaranthaceae	H	Native
17	*Chrysopogon fulvus* (Spreng.) Chiov.	Poaceae	H	Native
18	*Cleome viscosa* L.	Cleomaceae	H	Native
19	*Commelina benghalensis* L.	Commelinaceae	H	Native
20	*Crotalaria hebecarpa* (DC.) Rudd	Leguminosae	H	Native
21	*Cymbopogon citratus* (DC.) Stapf	Poaceae	H	Native
22	*Cyanotis fasciculata* (B.Heyne ex Roth) Schult. & Schult.f.	Commelinaceae	H	Native
23	*Dichanthium annulatum* (Forssk.) Stapf	Poaceae	H	Native
24	*Dichanthium caricosum* (L.) A.Camus	Poaceae	H	Native
25	*Digitaria ciliaris* (Retz.) Koeler	Poaceae	H	Native
26	*Echinochloa colona* (L.) Link	Poaceae	H	Native
27	*Elephantopus scaber* L.	Asteraceae	H	Native
28	*Emilia sonchifolia* (L.) DC. ex DC.	Asteraceae	H	Native
29	*Enicostema axillare* (Poir. ex Lam.) A.Raynal	Gentianaceae	H	Native
30	*Eragrostis* sp.	Poaceae	H	Native
31	*Eulalia trispicata* (Schult.) Henrard	Poaceae	H	Native
32	*Euphorbia heterophylla* L.	Euphorbiaceae	H	Native
33	*Euphorbia hirta* L.	Euphorbiaceae	H	Native
34	*Euphorbia* sp.	Euphorbiaceae	H	Native
35	*Evolvulus alsinoides* (L.) L.	Convolvulaceae	H	Native
36	*Exacum pumilum* Griseb.	Gentianaceae	H	Native
37	*Habenaria longicorniculata* J.Graham	Orchidaceae	H	Native, Endemic
38	*Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult.	Poaceae	H	Native
39	*Heteropogon triticeus* (R.Br.) Stapf ex Craib	Poaceae	H	Native
40	*Impatiens oppositifolia* L.	Balsaminaceae	H	Native
41	*Indigofera cordifolia* Roth	Leguminosae	H	Native
42	*Indigofera linifolia* (L.f.) Retz.	Leguminosae	H	Native
43	*Ipomoea eriocarpa* R. Br.	Convolvulaceae	C	Native
44	*Ischaemum afrum* (J.F.Gmel.) Dandy	Poaceae	H	Native, Endemic
45	*Ischaemum travancorense* Stapf ex C.E.C.Fisch.	Poaceae	H	Native, Endemic
46	*Lavandula bipinnata* (Roth) Kuntze	Lamiaceae	H	Native
47	*Lepidagathis cristata* Willd.	Acanthaceae	H	Native
48	*Leucas longifolia* Benth.	Lamiaceae	H	Native

Continued

No.	Species	Family	Habit	Native/Non-Native/Endemic
49	*Leucas stelligera* Wall. ex Benth.	Lamiaceae	H	Native
50	*Lophopogon tridentatus* (Roxb.) Hack.	Poaceae	H	Native, Endemic
51	*Melanocenchris jacquemontii* Jaub. & Spach	Poaceae	H	Native
52	*Melochia corchorifolia* L.	Malvaceae	H	Native
53	*Oldenlandia* sp.	Rubiaceae	H	Native
54	*Oplismenus compositus* (L.) P.Beauv.	Poaceae	H	Native
55	*Pentanema indicum* (L.) Ling	Asteraceae	H	Native
56	*Polygala chinensis* L.	Polygalaceae	H	Native
57	*Pulicaria wightiana* (DC.) C.B.Clarke	Asteraceae	H	Native
58	*Rhynchospora wightiana* (Nees) Steud.	Cyperaceae	H	Native
59	*Rostellularia* sp.	Acanthaceae	H	Native
60	*Sesamum laciniatum* Klein ex Willd.	Pedaliaceae	H	Native
61	*Sehima nervosum* (Rottler) Stapf	Poaceae	H	Native
62	*Sehima sulcatum* (Hack.) A.Camus	Poaceae	H	Native, Endemic
63	*Senecio bombayensis* N.P.Balakr.	Asteraceae	H	Native
64	*Senna tora* (L.) Roxb.	Leguminosae	H	Native
65	*Sida cordata* (Burm.f.) Borss.Waalk.	Malvaceae	H	Native
66	*Sida rhombifolia* L.	Malvaceae	H	Native
67	*Smithia bigemina* Dalzell	Leguminosae	H	Native
68	*Sopubia delphinifolia* G.Don	Orobanchaceae	RP	Native
69	*Spermacoce hispida* L.	Rubiaceae	H	Native
70	*Stylosanthes hamata* (L.) Taub.	Leguminosae	H	Non-Native
71	*Tephrosia purpurea* (L.) Pers.	Leguminosae	H	Native
72	*Tetrapogon tenellus* (Roxb.) Chiov.	Poaceae	H	Native
73	*Thelepogon elegans* Roth	Poaceae	H	Native
74	*Themeda quadrivalvis* (L.) Kuntze	Poaceae	H	Native
75	*Themeda triandra* Forssk.	Poaceae	H	Native
76	*Tribulus terrestris* L.	Zygophyllaceae	H	Native
77	*Trichodesma indicum* (L.) Lehm.	Boraginaceae	H	Native
78	*Tridax procumbens* (L.) L.	Asteraceae	H	Native
79	*Urena lobata* L.	Malvaceae	H	Native
80	*Vernonia cinerea* (L.) Less.	Asteraceae	H	Non-Native
81	*Vigna* sp.	Leguminosae	C	Native
82	*Xanthium strumarium* L.	Asteraceae	H	Non-Native
83	*Zornia gibbosa* Span.	Leguminosae	H	Native

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Carbon dynamics in an *Imperata* grassland in Northeast India

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Keywords: Above-ground biomass, below-ground biomass, carbon stocks, carbon storage, net primary productivity, soil CO_2 flux.

Abstract

Carbon stocks and soil CO_2 flux were assessed in an *Imperata cylindrica* grassland of Manipur, Northeast India. Carbon stocks in the vegetative components were estimated to be 11.17 t C/ha and soil organic carbon stocks were 55.94 t C/ha to a depth of 30 cm. The rates of carbon accumulation in above-ground and below-ground biomass were estimated to be 11.85 t C/ha/yr and 11.71 t C/ha/yr, respectively. Annual soil CO_2 flux was evaluated as 6.95 t C/ha and was highly influenced by soil moisture, soil temperature and soil organic carbon as well as by C stocks in above-ground biomass. Our study on the carbon budget of the grassland ecosystem revealed that annually 23.56 t C/ha was captured by the vegetation through photosynthesis, and 6.95 t C/ha was returned to the atmosphere through roots and microbial respiration, with a net balance of 16.61 t C/ha/yr being retained in the grassland ecosystem. Thus the present *Imperata* grassland exhibited a high capacity to remove atmospheric CO_2 and to induce high C stocks in the soil provided it is protected from burning and overgrazing.

Resumen

En un pastizal de *Imperata cylindrica* en Manipur, noreste de India, se evaluaron las reservas de carbono (C) y el flujo de CO_2 en el suelo. Las reservas de C en la vegetación fueron estimadas en 11.17 t/ha y las de C orgánico hasta una profundidad de 30 cm en el suelo, en 55.94 t/ha. La tasa de acumulación de C en la biomasa sobre el suelo se estimó en 11.85 t/ha por año y en la biomasa debajo el suelo en 11.71 t/ha por año. El flujo anual de CO_2 del suelo fue de 6.95 t C/ha, siendo fuertemente influenciado por la humedad, la temperatura y el C orgánico del suelo, así como por las reservas de C en la biomasa aérea. El estudio del balance de C en este ecosistema de pastizal mostró que anualmente son capturadas 23.56 t C/ha por la vegetación a través de la fotosíntesis, y de ellas 6.95 t C/ha son retornadas a la atmósfera a través de las raíces y el proceso de la respiración microbiana, con un saldo neto de retención por el ecosistema de 16.61 t C/ha por año. Por tanto, los pastizales de *I. cylindrica* en el noreste de India tienen alta capacidad para capturar CO_2 atmosférico y acumular cantidades considerables de C en el suelo, siempre y cuando se encuentren protegidos de la quema y el sobrepastoreo.

Introduction

The world's grasslands are important terrestrial biomes, occupying an area of about 33 x 10^6 km^2, and play an important role in global carbon balance because of their large area and significant sink or source capacities (Nagy et al. 2007). Land use change contributes to increases in atmospheric CO_2 as a consequence of deforestation as well as conversion and cultivation of new arable land (Schimel et al. 2001). Plants play a significant role in regulating sinks of carbon by withdrawing atmospheric CO_2 and converting it into assimilates and biomass during photosynthesis, translocation and storage (Watson et al. 2000). Research on long-term CO_2 flux has focused on forest ecosystems with the neglect of grassland ecosystems worldwide. However, grasslands cover approximately 32% of the total land area (Adams et al. 1990) and play a significant role in balancing the global C budget (Scurlock and Hall 1998). A number of studies have been conducted on the carbon stocks in different grasslands of the world (Fisher et al. 2007; Fidelis et al. 2013; Toma et al. 2013). Long et al. (1992) studied grassland sites in Kenya, Mexico and Thailand and found they accumulated 144 g C/m^2/yr when protected from fires and concluded these grasslands were potentially significant C sinks.

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However, Fisher et al. (1994; 1995) showed that African grasses introduced into savannas in South America increased soil organic matter and accumulated more C in soil than native grasses, highlighting their greater potential in this respect.

In India, grasslands have originated from forest ecosystems as a result of deforestation and abandoned agricultural systems and are maintained at various succession levels by grazing, burning and harvesting. In Northeast India, a few studies have been carried out on the biomass and productivity of grasslands (Yadava and Kakati 1984; Pandey 1988; Ramakrishnan and Ram 1988) but no information is available on carbon storage in the grasslands of the area.

As *Imperata* grasslands (native grasslands) are widely distributed in Northeast India and other Asian countries, occupying an area of about 35 × 10^3 km^2 in Asia, we attempted to study the carbon dynamics of *Imperata* grassland ecosystems in Manipur, Northeast India. The main objectives of the present study were to: (i) assess the above-ground and below-ground biomass and their relevant carbon stocks; (ii) estimate the rate of carbon accumulation; and (iii) evaluate the carbon flux in the soils and its relationship with abiotic and biotic variables.

Materials and Methods

Site description

The study site, located at 24°55' N, 94°06' E in Shabungkhok Khunou about 20 km from Imphal city in Imphal East District of Manipur, is dominated by *Imperata cylindrica* and was well protected from grazing or harvesting during the experimental period. The climate of the area is monsoonal with a warm moist summer followed by a monsoon rainy season and a cool dry winter. Climatological data during the study period are given in Figure 1. Mean monthly maximum temperature varied from 22.3 (December) to 30.3 °C (May) and the mean minimum temperature from 4.8 (January) to 22.3 °C (July). Average annual rainfall is 1,408 mm with most received in the rainy season (June–October). A comparison of medium-term (2003–2012) and short-term (study period) data shows that temperatures during the study were similar to the medium-term figures but rainfall (1,167 mm) was lower than the medium-term mean.

Soil sampling and analysis of physico-chemical characteristics

Five soil samples were collected randomly from the study site at monthly intervals from November 2011 to November 2012 for the analysis of physico-chemical characteristics. Soil texture was determined by soil hydrometer (model 152 H, Zeal, UK). Soil pH was measured by a pH meter in 1:5 soil:water suspension. Bulk density was determined by dividing the weight of oven-dry soil by its volume and soil moisture content was determined by the gravimetric method (oven-dry at 105 °C for 24 h).

Soil organic carbon was estimated by the Walkley-Black method (Anderson and Ingram 1993). Total soil nitrogen was measured by the 2100 Kjeltec system and available soil phosphorus following the method of Bray and Kurtz (1945).

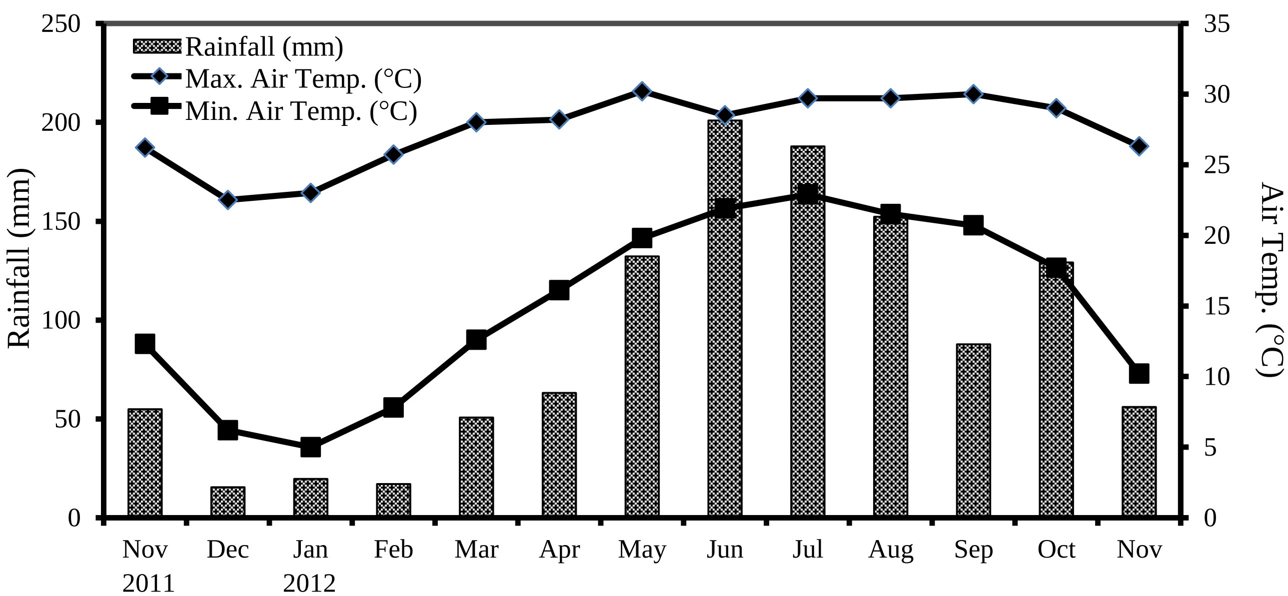

Figure 1. Monthly data for rainfall and air temperature during the study period (November 2011–November 2012).

Estimation of biomass and productivity

Biomass sampling commenced in November 2011 and continued at monthly intervals until November 2012. For the study of above-ground plant biomass, material from 15 random quadrats (40 x 40 cm) was collected according to the method described by Milner and Hughes (1968). The standing vegetation in the quadrats was harvested at ground level using a sharp sickle and the harvested material was placed in polythene bags and transported to the laboratory, where it was divided into live and standing dead. Litter was also collected from within the quadrats. All collected material was placed in perforated paper bags and oven-dried at 80 °C to constant weight.

Below-ground biomass was estimated by taking 15 monoliths of 15 x 15 cm size to a depth of 30 cm, coinciding with samplings for above-ground biomass and at the same locations. The monoliths were brought to the laboratory and divided into 3 sections, representing each 10 cm of depth, and soaked in water for 24 h before washing with a fine jet of water using a 1 mm mesh screen to isolate the roots. The below-ground organic material including live and dead roots was oven-dried at 80 °C until constant weight.

Net above-ground production was estimated by summation of positive increments in total biomass (live biomass plus standing dead material) in the different months and below-ground net production was estimated by the summation of positive increases in root biomass by depth over the course of the study period using the method of Singh and Yadava (1974).

Estimation of carbon stocks in vegetation and soil

The vegetative components, i.e. samples of above-ground and below-ground parts, were oven-dried and powdered and analyzed for carbon using a TOC analyzer (model multi N/C 2100, Analytik Jena, Germany). Carbon stocks were calculated by multiplying the biomass values by 0.45 as per the concentration of carbon obtained from the TOC analyzer for grassland vegetation. The soil organic carbon stocks were estimated from the bulk density (g/cm^3), organic carbon concentration (%) and the corresponding soil depth (cm).

Measurement of soil CO_2 flux

Soil CO_2 flux was measured by the alkali absorption method (Singh and Gupta 1977). Open-ended aluminum cylinders, 13 cm diameter and 25 cm tall, were inserted into the soil up to 15 cm depth. The surface area enclosed in each experimental cylinder was 132.7 cm^2. Five cylinders were used in the study site, with one serving as a control (blank). Fifty mL of 0.25N NaOH solution was kept in a 100 mL plastic vial in the top of the cylinder, which was made airtight with anchor grip and left for 24 h to absorb CO_2 released. The carbon dioxide absorbed was then determined by titrating NaOH solution with 0.25N standard dilute HCl solution using phenolphthalein as an indicator. Carbon dioxide absorbed from the soil was calculated by using the formula:

$$\text{mg } CO_2 = V \times N \times 22$$

where: V = volume of the acid; and N = normality of the acid.

Statistical analysis

All statistical analyses were carried out using the software IBM SPSS 20. ANOVA was used to determine the differences in biomass and soil CO_2 flux in different months and seasons of the year. Multiple regressions were used to find out the relationship between soil CO_2 flux rate and abiotic and biotic factors.

Results

Physico-chemical characteristics of soil

The grassland soil was generally acidic and clay loam in texture, highly leached and nutrient-poor. Soil pH ranged from 5.3 to 5.8, soil temperature from 27.6 to 28.0 °C, moisture content from 13.2 to 14.5%, bulk density from 1.19 to 1.41 g/cm^3 and organic carbon from 1.76 to 1.96%. Total soil nitrogen and available soil phosphorus ranged from 0.019 to 0.029% and 17 to 27 ppm, respectively.

Changes in above-ground plant biomass

Above-ground live biomass declined from 615 g DM/m^2 in November 2011 to 221 g/m^2 in January 2012 and then increased progressively to 813 g/m^2 in September, before declining to 696 g/m^2 in November 2012 ($P<0.001$, Figure 2).

In general the amount of standing dead material followed a similar pattern to that of live material and was mostly greater than the mass of live material, except during July–October 2012. Highest yields of standing dead followed the peak in mortality of aerial parts of plants and death of annuals during November with significant differences between months ($P<0.001$).

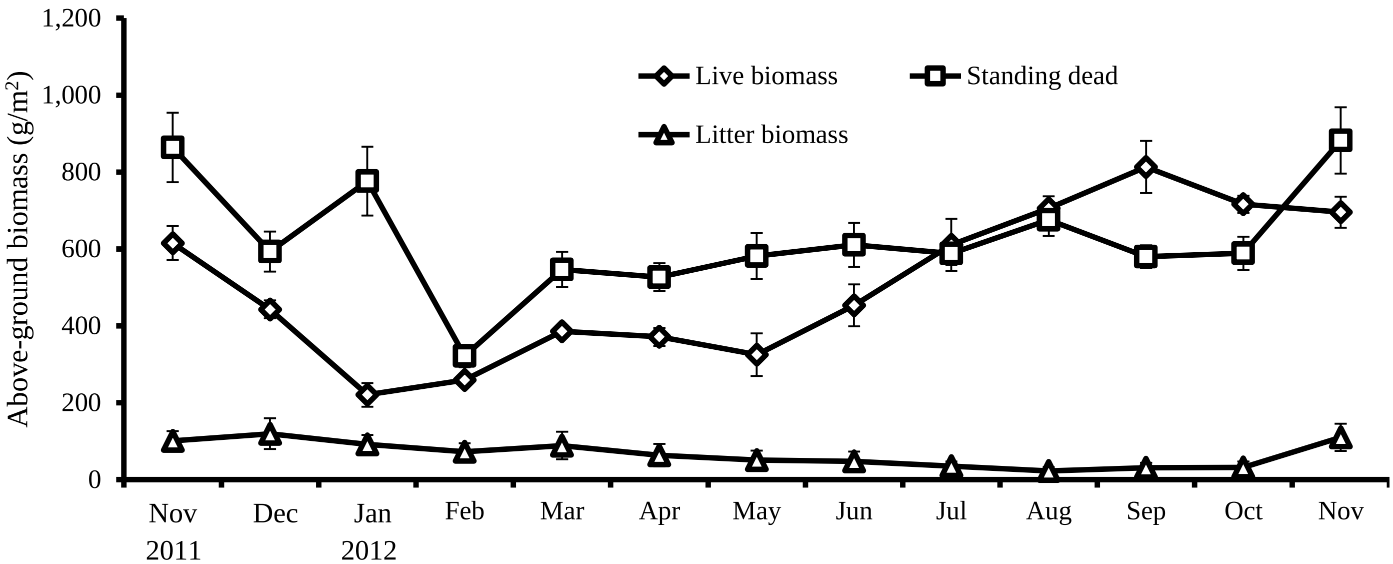

Figure 2. Variation in components of above-ground biomass throughout the year in *Imperata* grassland of Manipur, Northeast India. Standard deviations are indicated by vertical bars.

Surface litter fluctuated between months (P<0.001), with highest values in November–December and lowest values in August (Figure 2).

Total above-ground biomass (live + standing dead + litter) was estimated to be lowest in February (653 g DM/m^2) and highest in November (1,688 g/m^2).

Changes in below-ground plant biomass

There was very little variation in below-ground biomass in the 10–20 and 20–30 cm soil layers during the year, with the major changes occurring in the 0–10 cm horizon (Figure 3). Total below-ground biomass fluctuated from 1,600 g/m^2 in November 2011 to 800 g/m^2 in January and June 2012, with an interesting spike in April 2012. Of the total below-ground biomass, 78.3% was stored in the 0–10 cm horizon.

Seasonal and annual above-ground and below-ground net primary production

Seasonal above-ground net primary production was highest during the monsoon rainy season (466 g DM/m^2), followed by summer (368 g/m^2) and winter (351 g/m^2), whereas below-ground net primary production exhibited a reverse trend, i.e. highest during the winter season (707 g/m^2), intermediate during the rainy season (300 g/m^2) and lowest during summer (164 g/m^2). Annual values for above-ground and below-ground net primary production were estimated at 1,185 g DM/m^2 and 1,171 g/m^2, respectively.

Carbon stocks in above-ground biomass, below-ground biomass and soil

Carbon stocks in above-ground and below-ground biomass were recorded to be 5.40 and 5.77 t C/ha, respectively, and soil organic carbon stocks were found to be 55.94 t C/ha to a depth of 30 cm. Of the total carbon in the vegetation-soil system at a given time, 16.7% occurred in vegetative biomass and 83.3% in soil (Table 1).

Table 1. Estimated carbon stocks in *Imperata* grassland of Manipur, Northeast India.

Component	C stock (t/ha)	Proportion (%) of the total
Above-ground biomass	5.40	8.1
Below-ground biomass	5.77	8.6
Soil organic carbon (0–30 cm)	55.94	83.3
Total	67.11	

Rate of carbon accumulation

The annual rates of carbon accumulation in above-ground and below-ground parts of grassland vegetation were estimated to be 11.85 t C/ha and 11.71 t C/ha, respectively, giving an annual total of 23.56 t C/ha.

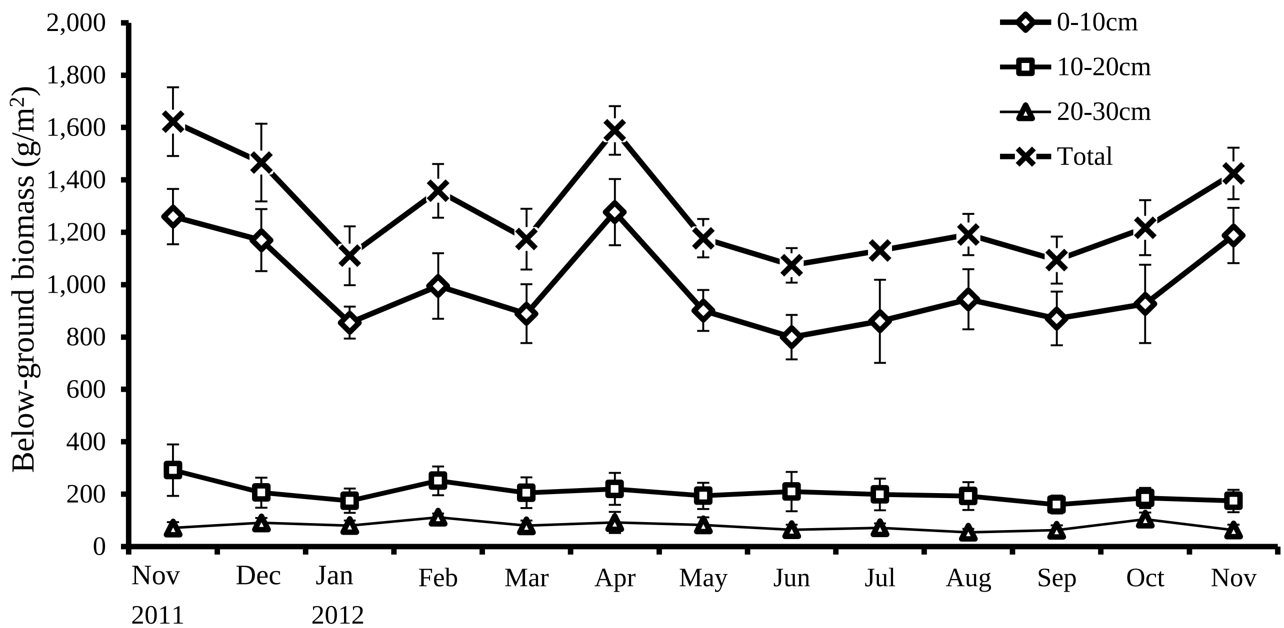

Figure 3. Variation in below-ground biomass in different soil layers throughout the year in *Imperata* grassland of Manipur, North-east India. Standard deviations are indicated by vertical bars.

Soil CO_2 flux

Soil CO_2 flux for the grassland site varied between 124±11.3 and 586±63.0 mg $CO_2/m^2/h$, attaining peak values in September and lowest values in the dry, cool winter season in January (P<0.001) (Figure 4, Table 2).

Table 2. Seasonal changes in the rate of soil CO_2 flux (mg $CO_2/m^2/h$) in *Imperata* grassland of Manipur, Northeast India.

Season	Soil CO_2 flux
Summer (March–May)	248±16.8
Rainy (June–October)	427±33.7
Winter (November–February)	153±11.6
Annual	291±12.0

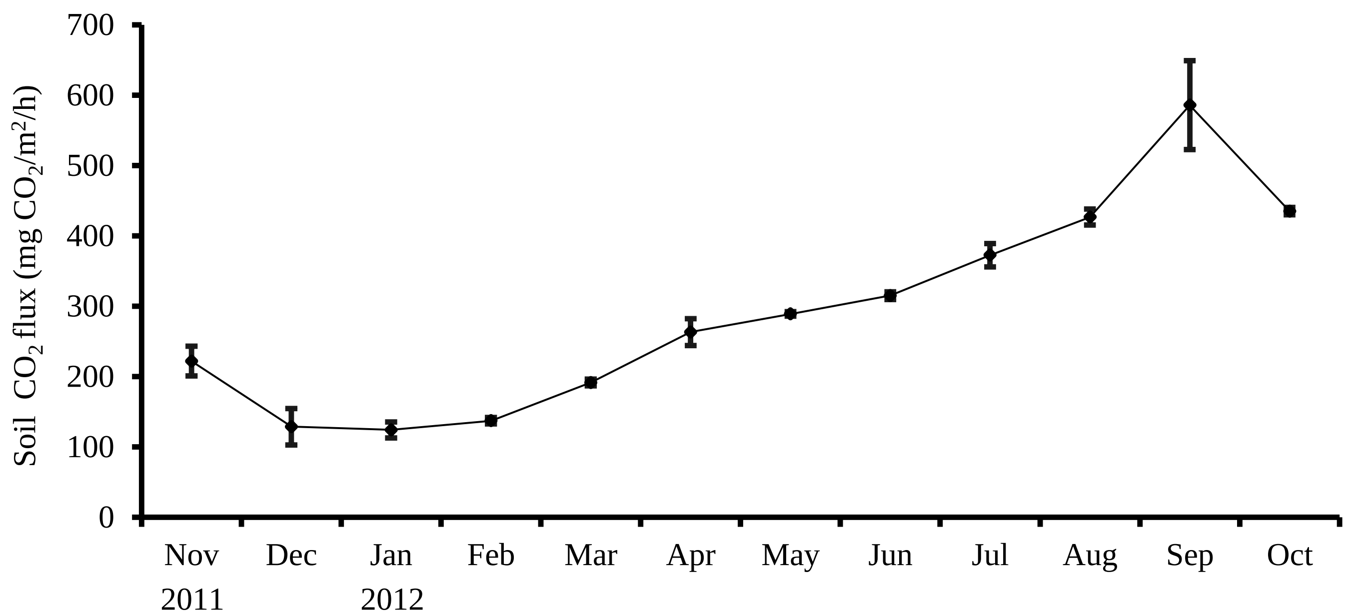

Figure 4. Variation in soil CO_2 flux throughout the year in *Imperata* grassland in Manipur, Northeast India. Standard deviations are indicated by vertical bars.

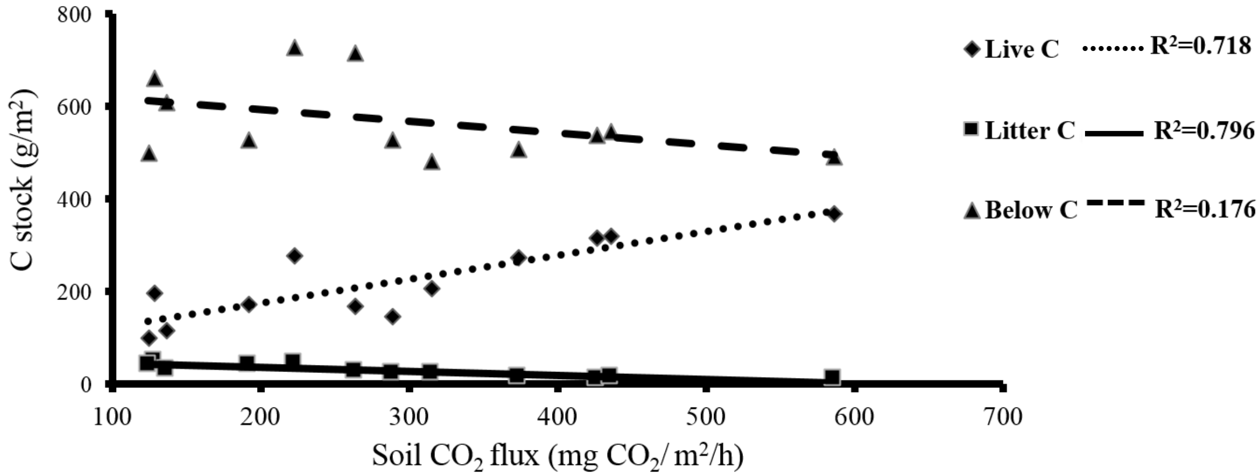

Figure 5. Relationship between soil CO_2 flux and C stock in live above-ground biomass, litter and below-ground biomass.

Relationship of soil CO_2 flux with abiotic and biotic variables

The relationships between the rates of soil CO_2 flux (mg $CO_2/m^2/h$) and soil properties, i.e. soil temperature (X_1), soil moisture (X_2) and soil organic carbon (X_3) were analyzed by multiple regression as follows:

$Y = - 683.446 + 1.229X_1 + 3.557X_2 + 498.496X_3$

($r_1 = 0.81$; $r_2 = 0.68$; $r_3 = 0.97$ at $P<0.01$).

Soil CO_2 flux was positively related to C stocks in live above-ground biomass (R^2 = 0.72; $P<0.001$) and negatively related to C stocks in litter (R^2 = 0.80; $P<0.001$) and below-ground biomass ($R^2 = 0.18$; $P<0.001$) (Figure 5).

Discussion

This study has provided valuable information on the carbon dynamics of an *Imperata* grassland in India. It has shown the ability of these grasslands to extract CO_2 from the atmosphere and incorporate it in vegetative material, which then has the potential to be stored as soil carbon, depending on management factors.

The present data for live above-ground biomass in humid grassland (221–813 g DM/m^2) fell within the range recorded for most Indian grasslands, being lower than the 72–1,596 g/m^2 reported from the Bundelkhand region by Gupta and Ratan (2005) but considerably higher than the 16–373 g/m^2 for Western Garhwal Himalaya (Dhaulakhandi et al. 2000).

Our data for carbon stocks in above-ground biomass (5.65 t C/ha) were higher than the 4.0–4.4 t/ha reported by Ramsay and Oxley (2001) in Ecuadorian paramo grasslands but lower than the 6.5 t/ha reported by Gibbon et al. (2010) for a grassland-cloud forest transition zone in the high Andes of Peru. We found that *Imperata* grassland contained up to 3.40 t C/ha in dead biomass (litter + standing dead biomass), contributing 29% of the total carbon stock in the vegetation, while Fidelis et al. (2013) recorded a figure of 28.9% in a Brazilian Cerrado wet grassland. This highlights the importance of quantification of litter and standing dead biomass in improving the understanding of carbon dynamics. In the event of fire, a common management practice in tropical native grasslands, much of the carbon in this dead plant material can be released as CO_2 into the atmosphere. Long et al. (1992) found that C accumulation in pastures was 144 g C/m^2/yr in the absence of fire and 40 g C/m^2/yr, when burnt every second year.

As was expected, the maximum above-ground live biomass occurred in September, coinciding with peak growth of the grassland species during the rainy season, with the minimum in January, when conditions were cool and dry. The gradual decline in live biomass from October to January followed the pattern of maturing of perennial species and completion of the life cycle by annual plants after the cessation of the monsoonal rains. As a result, in all regions, the maximum biomass value occurred either in September or October, as growth was triggered by the advent of monsoonal rains in May-June throughout the country.

The highly significant differences between months in terms of above-ground live biomass, standing dead and litter result from the interactions and balances between growth of new material, maturation and death of pasture species, transfer of standing dead to litter and breakdown of litter by microorganisms. The variation in standing dead material throughout the study resulted from a combination of addition of new biomass as plants matured and senesced and transfer of standing dead to litter. This then influenced litter levels, which fluctuated throughout the year as a net result of transfer of standing dead material to the litter component and the rate of disappearance of litter. The peak values for surface litter in December probably reflect the transfer of standing dead material to litter from the high standing dead levels recorded in November, combined with slow decomposition rates in litter as temperatures declined, as shown by low values for CO_2 flux in November to February. As contributions from standing dead declined and litter breakdown increased as temperatures rose into summer, litter levels declined to a minimum in August.

Carbon stocks in below-ground biomass (5.75 t C/ha) in the present study were similar to the data reported for *Leymus chinensis* grassland of Northern China (5.57 t C/ha) by He et al. (2008), lower than the 6.75 t C/ha reported for Neotropical savannas of Brazil by Delitti et al. (2001) and higher than the 2.58-2.77 t C/ha reported by Fidelis et al. (2013) in a Brazilian Cerrado wet grassland.

The minimum value for below-ground biomass (BGB) in June may be due to the translocation of reserve food materials for the growth and development of new grass tillers in the pre-monsoon period and decomposition of dead below-ground materials, while the maximum value in November would result from the translocation of material from aerial parts of plants into the below-ground parts with the onset of the post-monsoon period. The increase in the amount of BGB during April may be due to early development of absorptive rootlets, which rapidly compensate for the translocation (Menaut and Cesar 1979), combined with slow rate of decomposition of roots. This adaptation in tropical grasslands allows accumulation of reserve material in the roots during unfavorable climatic conditions experienced in cool and dry winter seasons (Redmann 1975).

The decrease in BGB with increase in depth of soil with 73.2-83.4% in the upper layer of soil (0–10 cm) is similar to the trend reported by Singh and Yadava (1974) in a tropical grassland of Kurukshetra (54.5–85% in top 10 cm). Our results of 1,074 g/m^2 (June) to 1,623 g/m^2 (November) were similar to the 1,001–1,614 g/m^2 reported by Yadava and Kakati (1984) in *Imperata-Bothriochloa* grassland of Manipur, India but lower than the 1,503–2,005 g/m^2 for grassland of Western Garhwal Himalaya (Dhaulakhandi et al. 2000) and higher than the 454–1049 g/m^2 for grassland of Bundelkhand region (Gupta and Ratan 2005). Obviously species composition, location, elevation, soil factors and seasonal climatic conditions impact on the amounts of plant biomass produced and its rate of breakdown.

The peak in above-ground net primary production (ANP) during the rainy season was to be expected as climatic conditions were favorable for growth of vegetation with optimum temperature and sufficient moisture in the soil. In contrast, the low values in winter result from the drying of the grass and annual plant species with the onset of dry and cold winter conditions and low soil moisture levels. Similar trends in ANP in Indian grasslands have been reported by other workers (Yadava and Kakati 1984; Pandey 1988; Sinha et al. 1991; Bawa 1995). This highlights the key role played by the monsoon rainfall pattern in determining seasonal net primary production patterns of the grassland communities.

On the other hand, peak below-ground net primary production (BNP) occurred during winter, showing that this period is conducive to and favorable for translocation of photosynthates from aerial parts of the vegetation to below-ground parts. Low values in summer result from the translocation of reserve material from roots to support the growth of new sprouts with the advent of monsoon rains during the summer season. At the onset of winter, most perennial grasses and annual plants mature and there is a tendency for accumulation of food reserves in the below-ground parts. Maximum BNP during winter was also reported by many workers in different Indian grasslands (Singh and Yadava 1974; Sah and Ram 1989; Sinha et al. 1991).

Our study shows that the proportions of carbon stocks in AGB and BGB were more or less similar but were small in comparison with the amounts of carbon stored in the soil. More than 80% of the organic carbon in the pasture-soil combination was organic carbon in the soil, a similar result to that reported by Ni (2002) for the grasslands of China. This demonstrates that an *Imperata cylindrica* pasture can induce high C stocks in the soil. Like carbon in the pasture components, this carbon can be lost to the atmosphere if soils are disturbed. The present data on soil organic carbon stocks (55.94 t C/ha) can be compared with the 50–164 t C/ha reported by Chan and McCoy (2010) in pasture in Australia and the 28.1–417 t C/ha in semi-natural grassland in Southern China (Toma et al. 2013).

The positive significant relationship between soil C stocks and live AGB highlights the importance of live biomass in the supply of carbon to the soil, while litter and BGB have a small negative effect (Figure 6).

Soil CO_2 flux showed remarkable seasonal variation, being highest during the rainy season and lowest in winter. Maximum soil CO_2 flux during the rainy season was expected because of the high microbial activity and rapid decomposition of litter under the warm moist conditions.

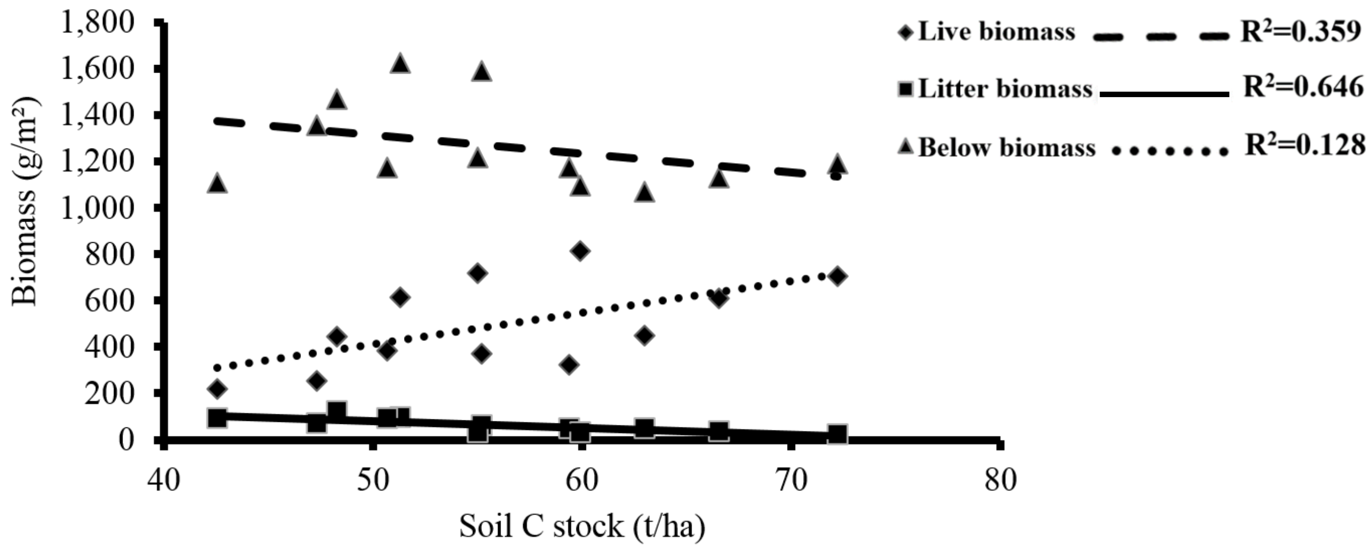

Figure 6. Relationships between soil C stock and live above-ground biomass (AGB), litter and below-ground biomass (BGB).

The present rates of soil CO_2 flux (124–586 mg $CO_2/m^2/h$) in the grassland ecosystem are comparable with those reported for grasslands of Kurukshetra, India by Gupta and Singh (1981), northern semi-arid grasslands of USA by Frank et al. (2002) and subtropical forests in China by Wang et al. (2011).

The significant positive relationship between soil CO_2 flux rates and soil moisture, soil temperature and soil organic carbon levels reveals that these parameters have a strong influence on CO_2 emissions into the atmosphere, as reported for different ecosystems by other workers, e.g. Oishi et al. (2013) and Zhou et al. (2013). Similarly, soil CO_2 flux is closely related to C stocks in live AGB, litter and BGB indicating the strong influence of these parameters on CO_2 emissions.

Our estimates of annual organic carbon input as litter (347 g $C/m^2/yr$) and annual carbon output (695 g $C/m^2/yr$) as estimated by annual soil CO_2 flux rate suggest that annual output of CO_2 was almost double that produced by annual litter fall. This would mean there should be a net loss of C from the soil as most C entering the soil comes from litter break down. However, Fisher et al. (2007) suggest that the rate at which litter decays is often underestimated so that this figure may not represent the true C input from litter. Litter decomposition is very fast as a result of high microbial activity coupled with congenial climatic conditions prevailing in the region (Devi and Yadava 2007).

The annual carbon budget of the present *Imperata* grassland shows that 23.56 t C/ha was captured by the vegetation through photosynthesis, while 6.95 t C/ha was released into the atmosphere as CO_2 emissions from soil due to root and microbial respiration with a net balance of 16.61 t C/ha/yr being retained in the grassland ecosystem. Thus subtropical *Imperata* grasslands have a huge potential to help reduce carbon dioxide levels in the atmosphere and could be used as C sinks in Asian countries, provided they are protected from fire, grazing and harvesting and could be one option for mitigating climate change at a global level.

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Métodos de establecimiento de pasturas en zonas áridas de México utilizando semillas crudas o cariópsides
Methods for pasture establishment in arid zones of Mexico using crude seeds or caryopses

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Palabras clave: Densidad de siembra, gramíneas introducidas, gramíneas nativas, suelo apisonado, tapado de semilla.
Keywords: *Harrowing, introduced grasses, native grasses, plant density, seedbed rolling.*

Resumen

En 2 sitios del Desierto Chihuahuense, México, se evaluó el establecimiento en secano de las gramíneas nativas Banderita (*Bouteloua curtipendula*) y Navajita (*B. gracilis*) y las introducidas Buffel (*Cenchrus ciliaris*) y Rhodes (*Chloris gayana*), utilizando cariópsides y/o semillas crudas (semilla limpia con brácteas y aristas) y 4 métodos de tapado. Los sitios de siembra fueron Atotonilco El Grande, Hidalgo y Salinas Hidalgo, San Luis Potosí. Las siembras se hicieron a voleo a razón de 1,000 cariópsides y/o semillas crudas viables/m^2. Los métodos de tapado fueron: paso de rastra con ramas; rodillo; rastra con ramas + rodillo; y sin tapado (testigo). Las variables de respuesta incluyeron número de plantas emergidas y de plantas establecidas, diámetro de corona, altura de planta y número de tallos por planta. Se utilizó un diseño completamente al azar con arreglo factorial 2 x 2 x 4 con 3 repeticiones. No se observaron diferencias entre sitios y se establecieron, en promedio, 2 plantas/m^2. Con las especies nativas (Banderita y Navajita) se obtuvo mayor cantidad de plantas emergidas y establecidas cuando la siembra se hizo con semillas crudas, mientras que en introducidas no se encontró diferencia entre siembra con semilla cruda y siembra con cariópsides. Cuando se utilizó el método de tapado y apisonado del suelo se observaron mayor diámetro de corona y altura de planta. El mayor número de plantas establecidas se obtuvo en pasturas de Navajita y Rhodes. En las especies nativas la eliminación de brácteas accesorias en las semillas no se tradujo en mejor establecimiento, mientras que en las gramíneas introducidas esta práctica sí mejoró el establecimiento. En ambos grupos de especies el apisonado mejoró el establecimiento.

Abstract

In order to evaluate the establishment of the native grasses Sideoats grama (‘Banderita’, *Bouteloua curtipendula*) and Blue grama (‘Navajita’, *B. gracilis*) and the introduced grasses buffel (*Cenchrus ciliaris*) and Rhodes (*Chloris gayana*) at 2 sites in the Chihuahuan Desert, the use of caryopses vs. entire seeds (diaspores) was studied applying 4 methods of covering after broadcasting. The sites were Atotonilco El Grande, Hidalgo State; and Salinas Hidalgo, San Luis Potosí State, Mexico. One thousand viable seeds (caryopses or diaspores) were sown per square meter.

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The 4 methods of covering were: harrowing with a tree branch; use of a roller; use of a tree branch harrow plus roller; and no covering (control). The response variables measured were number of emerged seedlings; number of established plants; diameter of the crown; plant height; and number of stems per plant. The design was a completely randomized 2 x 2 x 4 factorial arrangement with 3 replicates at each site. There were no differences between sites and, on average, 2 plants/m^2 were established. With the native species greater numbers of emerged and established plants were obtained by using diaspores while with introduced species there were no significant differences between caryopses and diaspores. Harrowing with a tree branch and/or rolling the soil resulted in thicker crowns and greater plant height. The highest number of stems per established plant were recorded in Blue grama and Rhodes grass. In native grasses it is important not to remove accessory bracts, in contrast with introduced grasses. In both species groups establishment is improved by rolling.

Introducción

El éxito de establecimiento por semilla de pasturas en secano depende, entre otros factores, del porcentaje y vigor de germinación de ésta, la preparación del suelo y profundidad de siembra, y de las condiciones de clima. Por tanto, para la rehabilitación exitosa de pasturas es necesario que la industria de semillas ofrezca al productor máxima calidad genética, física y biológica de éstas, aspectos influenciados por el ambiente de producción, cosecha, beneficio y almacenamiento de la semilla (Probert y Hay 2000). Es necesario que la semilla enfrente solamente los factores modificadores (entre los cuales los siguientes son particularmente frecuentes en zonas áridas: sequías intraestivales mayores a 4 semanas; lluvias torrenciales que arrastran la semilla; y formación de costra que impide la emergencia), ante un buen manejo de la siembra de praderas de secano con semilla de calidad destacada (Quero-Carrillo et al. 2014).

En zonas áridas y bajo condiciones de humedad adecuadas, los cambios de temperatura son el principal limitante para establecer pasturas de temporal. Se ha encontrado que fluctuaciones de temperaturas nocturnas (10–20 °C) y diurnas (20–30 °C) afectan marcadamente el porcentaje de germinación (Evans y Young 1987; Esqueda et al. 2002; Larsen y Bibby 2004). Según Ward et al. (2006) las cariópsides del pasto Buffel (*Cenchrus ciliaris*) requieren 12.5 °C y 15–20 mm de precipitación para alcanzar 60% de germinación. Por su parte Tian et al. (2002) encontraron bajos porcentajes de germinación en *Tripsacum dactyloides* cuando utilizaron semillas completas con brácteas de la espiguilla (glumas, lemas, paleas, ramillas y aristas). No obstante cuando estas brácteas fueron eliminadas la germinación fue 10% mayor y alcanzó un valor de 90% cuando se utilizaron cariópsides escarificadas.

Otro factor importante para garantizar un buen establecimiento de pasturas empleando semillas es la preparación de la cama de siembra. Cox et al. (1986) encontraron un mejor establecimiento y producción de forraje en pasturas de zonas áridas con dos pases de rastra y siembra mecanizada.

En el Desierto Chihuahuense, México, los pastizales nativos cubren aproximadamente 15% de la superficie (PMAR 2012). Estos pastizales nativos son especialmente vulnerables frente al cambio climático (Townsend et al. 2002) y su rehabilitación con especies perennes es una alternativa a mediano plazo. Sin embargo, la comparación entre los métodos de siembra más utilizados y entre los materiales de siembra utilizados [cariópsides o semillas crudas (semilla limpia con aristas)] no está documentada.

El objetivo de este estudio fue evaluar la emergencia y el establecimiento de especies de gramíneas nativas o introducidas utilizando semillas crudas o solo cariópsides en 4 métodos de tapado en 2 sitios del Desierto Chihuahuense, México.

Materiales y Métodos

El estudio se realizó entre el 25 de junio de 2010 y el 25 de octubre de 2011 en los municipios de Atotonilco El Grande, Estado de Hidalgo (20°24’ N, 98°43’ O) y Salinas Hidalgo, Estado de San Luis Potosí (22°43’ N, 101°35’ O), México. Atotonilco El Grande se encuentra a 1,965 msnm en un clima templado-seco [BSk según la clasificación de Köppen (Kottek et al. 2006)] caracterizado por una temperatura media anual de 16 °C y 560 mm de precipitación anual; el suelo es un Vertisol (INEGI 1984). Salinas Hidalgo se encuentra a 2,100 msnm, igualmente en clima templado-seco (BSk) con una temperatura media anual de 16.2 °C y precipitación anual de 336 mm (INEGI 2012), con predominio de suelo Castañozem cálcico (FAO-UNESCO-ISRIC 1988) de textura arcillosa. Se evaluaron 2 especies de gramíneas nativas del Desierto Chihuahuense: Banderita [*Bouteloua curtipendula* (Michx.) Torr.] y

Navajita [*Bouteloua gracilis* (Kunth) Lag. ex Griffiths] y 2 especies introducidas de África: los pastos Buffel [*Cenchrus ciliaris* L.; ahora: *Pennisetum ciliare* (L.) Link], variedad Común (o T4464 o americano) y Rhodes (*Chloris gayana* Kunth), variedad Bell.

La preparación de los suelos en ambos sitios consistió en voltearlo con arado tipo barbecho y después 2 pasos de rastra. Las cariópsides se obtuvieron a partir de semillas comerciales adquiridas en enero 2009 las cuales fueron friccionadas en forma manual hasta obtener aproximadamente 1 kg de cada especie. Las cariópsides pequeñas y rotas fueron eliminadas con paso por tamiz de 0.42 mm y mediante microscopio estereoscópico se eliminaron cariópsides dañadas. Las cariópsides resultantes fueron analizadas en laboratorio con el fin de determinar las condiciones fisiológicas y físicas de acuerdo con las normas ISTA (1996); un protocolo similar se siguió con las semillas crudas, constituidas por cariópsides más glumas, lemas, paleas, ramillas modificadas y aristas. Para la siembra se utilizaron 1,000 semillas botánicas germinables por m^2 de cada una de ellas (Cuadro 1).

En cada sitio experimental se establecieron 96 parcelas de 35 m^2 cada una (7 x 5 m): 48 para las especies nativas y 48 para las introducidas. En Atotonilco El Grande la siembra se hizo el 25 de junio de 2010 y en Salinas Hidalgo el 5 de julio de 2010, en suelos a capacidad de campo. Las siembras se hicieron a voleo y se utilizaron 4 métodos de tapado: (1) un paso de rastra acondicionada con ramas; (2) apisonado con rodillo (compactación); (3) rastra con ramas + apisonado; y (4) semilla sin cubrir (testigo). El objetivo del apisonado fue favorecer un mayor contacto del suelo con las semillas, para lo cual se usó como rodillo un cilindro con peso de 80 kg.

En ambos sitios la evaluación de las plantas emergidas se hizo entre el 20 y 24 de octubre de 2010 y de las plantas establecidas entre el 24 y 28 de octubre de 2011. No se aplicaron fertilizantes, insecticidas o herbicidas ni se defoliaron las plantas durante el experimento. El análisis de los datos se hizo teniendo en cuenta el origen de las especies (nativas vs. introducidas). En cada sitio se utilizó un Diseño Completamente al Azar con arreglo factorial 2 x 2 x 4 con 3 repeticiones. Los tratamientos incluyeron 2 especies, nativas o introducidas, ambas con la combinación de 2 tipos de material de siembra (semillas crudas y cariópsides) y 4 métodos de siembra, en 2 sitios, es decir, 16 tratamientos por especies nativas e igual número por especies introducidas.

Las variables de respuesta analizadas en cada unidad experimental (parcela) incluyeron número de plantas emergidas y establecidas, con base en 3 muestreos al azar en cada parcela, en rectángulos de 30 x 60 cm. Por otro lado, en 15 plantas tomadas al azar en cada unidad experimental se midió: diámetro de corona (mm); altura de planta (cm, desde el nivel del suelo hasta el ápice de la inflorescencia o de la hoja bandera); y número de tallos por planta.

En el Cuadro 2 aparecen los registros de temperatura, precipitación y radiación global de estaciones meteorológicas del INIFAP, localizadas a 37 km del sitio experimental en Atotonilco El Grande y a 30 km del sitio en Salinas Hidalgo.

Los datos de todas las variables se transformaron mediante $\sqrt{x + 0.5}$ y después se analizaron con el programa GLM de SAS (2009) y prueba de Tukey.

Cuadro 1. Caracterización física y fisiológica de las unidades de dispersión (semillas crudas y cariópsides) de 4 especies de gramíneas.

Especie	Características físicas								Características fisiológicas		
	1	2	3	4	5	6	7	8	9	10	11
Navajita (*Bouteloua gracilis*)	86	62	1,500	1.980	1.6	0.6	1,037	1,094	84	78	82
Banderita (*B. curtipendula*)	95	23	992	1,532	5	0.75	1,040	1,048	97	92	94
Buffel (*Cenchrus ciliaris*)	61	40	386	2,015	60	2.3	1,074	1,079	86	19	24
Rhodes (*Chloris gayana*)	82	32	2,417	3,858	2.6	0.35	1,049	1,072	88	63	81

1: Pureza (%) = Porcentaje de semilla de la especie en cuestión; 2: Número de cariópsides en 100 semillas crudas; 3: Número de semillas crudas en un gramo; 4: Número de cariópsides en un gramo; 5: Dosis de siembra de semillas crudas (g/m^2); 6: Dosis de siembra cariópsides (g/m^2); 7: Número de plantas esperadas/m^2, sembradas con semillas crudas; 8: Número de plantas esperadas/m^2, sembradas con cariópsides con base en semilla pura germinable; 9: Viabilidad (%; tetrazolio 0.1%); 10: Germinación de semillas crudas (%); 11: Germinación de cariópsides (%).

Cuadro 2. Temperatura, precipitación anual y radiación global en 2 municipios del Desierto Chihuahuense, México.

Año	Época	Salinas Hidalgo (San Luis Potosí)				Atotonilco El Grande (Hidalgo)			
		Temp. mín. (°C)	Temp. media (°C)	Prec. (mm)	Radiación (W/m²)	Temp. mín. (°C)	Temp. media (°C)	Prec. (mm)	Radiación (W/m²)
2010	Primavera	1[1]	10[1]	381[2]	531[2]	7.2[1]	13[1]	81[2]	471[2]
	Verano	9	19	4	596	13	19	35	509
	Otoño	14	19	22	546	13	17	120	390
	Invierno	0	12	0	489	7	13	13	511
2011	Primavera	3	15	0	481	8	15	1	556
	Verano	9	21	4	581	13	19	28	510
	Otoño	12	19	41	532	12	16	114	467
	Invierno	2	13	5	519	8	14	16	477
Total/Promedio		6	16	457	4,275	10	16	408	3,891

[1]Promedio de datos en la época del año. [2]Suma de datos en la época del año. Prec. = Precipitación.

Resultados

Número de plantas emergidas y establecidas en especies nativas

En Atotonilco El Grande se observaron diferencias (P<0.01) tanto para plantas emergidas como para establecidas. Se encontró efecto significativo tanto del material de siembra (semilla cruda o cariópsides) como del método de tapado (P<0.001; Cuadro 3) como lo demuestra el hecho que el mayor número de plantas se registró cuando la siembra se hizo con semillas crudas y pase de rodillo en comparación con los demás tratamientos. El número de plantas establecidas fue afectado por la especie (P<0.05) y por el material de siembra (P<0.01): El pasto Navajita presentó un mayor número de plantas establecidas que Banderita y se observó un mayor número de plantas en siembras con semillas crudas que con cariópsides.

En Salinas Hidalgo se observaron diferencias entre tratamientos tanto para plantas emergidas como para establecidas (P<0.01). El pasto Banderita presentó mayor número de plantas emergidas que Navajita (P<0.01) (Cuadro 3). Por otra parte, el número de plantas establecidas fue mayor cuando se utilizó semilla cruda que cuando se utilizó cariópsides (P<0.05). El mayor número de plantas emergidas se observó cuando la semilla fue cubierta con ramas (P<0.01). El número de plantas establecidas en Banderita fue más alto que en Navajita (P<0.01); igualmente fue más alto cuando se utilizó semilla cruda que cuando se utilizó cariópsides (P<0.001) o cuando se utilizó ramas más rodillo o solo rodillo vs. el tratamiento testigo.

Cuadro 3. Plantas emergidas y establecidas (número por metro cuadrado) de 2 gramíneas nativas en 2 localidades del Desierto Chihuahuense, México, en diferentes sistemas de siembra.

Tratamiento	Atotonilco El Grande, Hidalgo		Salinas Hidalgo, San Luis Potosí	
	Plantas emergidas	Plantas establecidas	Plantas emergidas	Plantas establecidas
Especie				
Navajita	10a[1]	2.3a	3.4b	0.6b
Banderita	10a	1.8b	5.3a	1.5a
Material de siembra				
Semillas crudas	17a	2.5a	7.2a	1.7a
Cariópsides	3b	1.6b	1.6b	0.4b
Método de tapado				
Testigo	8b	1.7a	1.9c	0.1b
Ramas	10b	2.4a	5.8a	1.8a
Rodillo	11a	1.8a	4.8bc	0.9a
Ramas + rodillo	10b	2.3a	4.9ab	1.4a

[1]Valores en una misma columna y tratamiento seguidos de letras iguales no difieren en forma significativa (P<0.05).

Cuadro 4. Plantas emergidas y establecidas (número por metro cuadrado) de 2 gramíneas introducidas en 2 localidades del Desierto Chihuahuense, México, en diferentes sistemas de siembra.

Tratamiento	Atotonilco El Grande, Hidalgo		Salinas Hidalgo, San Luis Potosí	
	Plantas emergidas	Plantas establecidas	Plantas emergidas	Plantas establecidas
Especie				
Buffel	5b[1]	2a	11a	2a
Rhodes	17a	3a	7b	2a
Material de siembra				
Semilla cruda	10a	3a	15a	3a
Cariópsides	11a	2b	3b	0.5b
Método de tapado				
Testigo	10a	2a	10a	1a
Ramas	11a	3a	8a	2a
Rodillo	11a	2a	11a	2a
Ramas + rodillo	11a	3a	8a	2a

[1]Valores en una misma columna y tratamiento seguidos de letras iguales no difieren en forma significativa (P<0.05).

Número de plantas emergidas y establecidas en especies introducidas

En Atotonilco El Grande se observaron diferencias entre los tratamientos para ambas variables (P<0.001) (Cuadro 4): El número de plantas emergidas fue mayor en Rhodes que en Buffel y el número de plantas establecidas fue mayor cuando la siembra se hizo por semilla cruda.

También en Salinas Hidalgo se registraron diferencias entre los tratamientos para ambas variables (P<0.001). En plantas emergidas se observó efecto de especie (P<0.01) y de material de siembra (P<0.001) (Cuadro 4) siendo mayores los valores en Buffel y siembra por semilla cruda en comparación con Rhodes y siembra por cariópsides. En plantas establecidas se observó solo un efecto de material de siembra: El número de plantas fue mayor cuando se utilizó semilla cruda (P<0.001).

Diámetro de corona, altura de planta y número de tallos en especies nativas

En Atotonilco El Grande se observaron diferencias para estas características tanto en plantas emergidas como establecidas (P<0.001). Banderita presentó los valores más altos de diámetro de corona y altura de planta (Cuadro 5), mientras que Navajita presentó el mayor número de tallos (P<0.001). Tanto las plantas emergidas como las establecidas presentaron mayor diámetro de corona cuando la siembra se hizo con cariópsides (P<0.01). Igualmente el diámetro de corona de plantas emergidas fue más alto cuando la semilla fue tapada o se pasó rodillo después de la siembra (P<0.01). El apisonado también aumentó el diámetro de corona en plantas establecidas (P<0.05).

Cuadro 5. Diámetro de corona, altura de planta y número de tallos por planta de 2 gramíneas nativas del Desierto Chihuahuense, en Atotonilco el Grande, Hidalgo, México.

Tratamiento	Plantas emergidas			Plantas establecidas		
	Diám. corona (mm)	Altura planta (cm)	Tallos por planta (no.)	Diám. corona (mm)	Altura planta (cm)	Tallos por planta (no.)
Especie						
Navajita	2.8b[1]	3.4b	11a	3.8b	14.8b	75a
Banderita	4.0a	11.1a	6b	6.4a	31.3a	33b
Material de siembra						
Semilla cruda	3.2b	7.9a	9a	4.7b	29.7a	46a
Cariópsides	3.6a	7.6a	8a	5.5a	23.3a	52a
Método de tapado						
Testigo	2.9b	6.8a	9a	3.8b	23.3a	56a
Ramas	3.5ab	7.2a	9a	4.8b	24.8a	59a
Rodillo	3.5ab	9.4a	8a	5.8a	36.1a	31a
Ramas + rodillo	3.8a	7.6a	9a	6.0a	21.8a	51a

[1]Valores en una misma columna y tratamiento seguidos de letras iguales no difieren en forma significativa (P<0.05).

Cuadro 6. Diámetro de corona, altura de planta y número de tallos por planta de 2 gramíneas nativas del Desierto Chihuahuense, en Salinas Hidalgo, San Luis Potosí, México.

Tratamiento	Plantas emergidas			Plantas establecidas		
	Diám. corona (mm)	Altura planta (cm)	Tallos por planta (no.)	Diám. corona (mm)	Altura planta (cm)	Tallos por planta (no.)
Especie						
Navajita	3.0b[1]	4.2b	13a	10.7b	12.3b	79a
Banderita	3.8a	12.8a	9b	17.9a	28.0a	32b
Material de siembra						
Semilla cruda	3.5a	9.9a	11a	13.6b	29.5a	57a
Cariópsides	3.4a	8.5a	12a	14.9a	20.1b	54a
Método de tapado						
Testigo	3.2a	8.1a	9b	12.6c	20.2a	51a
Ramas	3.4a	8.2a	11a	13.9bc	19.2a	57a
Rodillo	3.3a	11.5a	13a	14.7ab	38.8a	57a
Ramas + rodillo	3.8a	9.1a	13a	16.0a	21.2a	57a

[1]Valores en una misma columna y tratamiento seguidos de letras iguales no difieren en forma significativa (P<0.05).

En Salinas Hidalgo se observaron diferencias en diámetro de corona, altura de planta y número de tallos tanto en las plantas emergidas como en las establecidas (P<0.01). En las primeras, el pasto Banderita presentó los mayores valores de estas características (Cuadro 6). En plantas establecidas de Banderita se observó un mayor valor de diámetro de corona y altura de planta, pero menor número de tallos que en Navajita. En plantas establecidas Banderita presentó un mayor diámetro de corona y altura de planta, pero menor número de tallos que Navajita (P<0.001). La siembra por cariópsides resultó en mayor diámetro de corona (P<0.05) pero en menor altura de planta (P<0.001). Tapar la semilla después de la siembra y apisonarla favoreció un mayor diámetro de corona (P<0.05).

Diámetro de corona, altura de planta y número de tallos en especies introducidas

En Atotonilco El Grande se observaron diferencias para estas características, tanto en plantas emergidas como establecidas (P<0.001). En plantas emergidas (Cuadro 7) fueron mayor el diámetro de corona y la altura de planta (P<0.001) pero menor el número de tallos en pasto Rhodes que en Buffel. La siembra por cariópsides resultó en mayor diámetro de corona (P<0.01) y número de tallos (P<0.001) tanto en plantas emergidas como establecidas. Tapar el material de siembra y apisonarlo resultó en mayor diámetro de corona (P<0.001).

Cuadro 7. Diámetro de corona, altura de planta y número de tallos por planta de 2 gramíneas introducidas en Atotonilco El Grande, Hidalgo, México.

Tratamiento	Plantas emergidas			Plantas establecidas		
	Diám. corona (mm)	Altura planta (cm)	Tallos por planta (no.)	Diám. corona (mm)	Altura planta (cm)	Tallos por planta (no.)
Especie						
Rhodes	2.2a[1]	19.0a	1.8b	18.0b	29.2a	17.9a
Buffel	1.6b	5.7b	2.8a	20.2a	18.0b	10.2b
Material de siembra						
Semilla cruda	1.7b	11.3a	1.9b	15.4b	25.5a	10.6b
Cariópsides	2.1a	13.5a	2.7a	22.8a	21.6b	17.6a
Método de tapado						
Testigo	1.6b	11.7a	2.2a	15.5b	21.6a	12.8a
Ramas	1.9ab	11.1a	2.0a	17.8b	22.2a	12.0a
Rodillo	2.0a	13.4a	2.6a	21.7a	26.1a	15.6a
Ramas + rodillo	2.1a	13.4a	2.4a	21.4a	24.5a	15.9a

[1]Valores en una misma columna y tratamiento seguidos de letras iguales no difieren en forma significativa (P<0.05).

Cuadro 8. Diámetro de corona, altura de planta y número de tallos por planta de 2 gramíneas introducidas en Salinas Hidalgo, San Luis Potosí, México.

Tratamiento	Plantas emergidas			Plantas establecidas		
	Diám. corona (mm)	Altura planta (cm)	Tallos por planta (no.)	Diám. corona (mm)	Altura planta (cm)	Tallos por planta (no.)
Especie						
Rhodes	6.3a[1]	49.0a	9a	15.6b	59.4a	16a
Buffel	2.7b	6.3b	2b	25.7a	17.3b	11b
Material de siembra						
Semilla cruda	3.8b	25.8b	5b	19.2b	38.2a	13a
Cariópsides	5.2a	29.6a	6a	22.2a	38.6a	14a
Método de tapado						
Testigo	4.1a	24.5b	5a	18.1b	36.8a	12.5b
Ramas	4.3a	26.4ab	6a	19.9ab	38.7a	13ab
Rodillo	4.8a	29.3ab	6a	22.0a	38.7a	13ab
Ramas + rodillo	4.9a	30.6a	6a	22.7a	39.3a	15a

[1]Valores en una misma columna y tratamiento seguidos de letras iguales no difieren en forma significativa ($P<0.05$).

En Salinas Hidalgo, las plantas establecidas del pasto Buffel presentaron un incremento considerable del diámetro de corona en comparación con las plantas emergidas y superaron al Rhodes en esta característica (Cuadro 8) ($P<0.01$); no obstante este último presentó mayores valores de altura de planta y número de tallos ($P<0.001$). La siembra por cariópsides resultó en mayores valores en plantas emergidas para todas las características ($P<0.001$), mientras que en plantas establecidas la diferencia solo se presentó para diámetro de corona ($P<0.01$). Tanto en plantas emergidas como establecidas los mejores resultados se encontraron con material de siembra cubierto y apisonado.

Discusión

Quero-Carrillo et al. (2014) indican que en zonas áridas un establecimiento 'excelente' y un establecimiento 'aceptable' a 90 días post-siembra en praderas de temporal son de 30 y 8 plantas/m^2, respectivamente. Posteriormente y debido a competencia, a 36 meses estos niveles son de 6 y 2 plantas/m^2, respectivamente. En Atotonilco El Grande y en Salinas Hidalgo no se observaron diferencias entre sitios ($P>0.05$). La emergencia y el establecimiento fueron muy bajos, apenas 'aceptables', tanto para las gramíneas nativas como las introducidas. En el primer sitio la relación entre el número de plantas establecidas/m^2 y el número de plantas emergidas/m^2 fue 2:10 para las especies nativas y 2:11 para las introducidas. En el segundo sitio estos valores fueron 1:4 y 2:9, respectivamente. Estos resultados contrastan considerablemente con el número de plantas esperadas (>1,000 plantas/m^2, según los datos en el Cuadro 1). Esto se debe probablemente a las condiciones de temperatura y humedad que resultaron en la deshidratación de plántulas, debido a la elongación del entrenudo subcoleoptilar, el cual, al contener el meristemo apical generador de raíces adventicias que sustentan la supervivencia de la planta, provocó que éstas se formaran cerca de la superficie del suelo donde las condiciones ambientales fueron desfavorables para el desarrollo de plántulas (Moreno-Gómez et al. 2012).

Respecto al factor precipitación en zonas áridas, Cox y Jordan (1983) en Arizona registraron solo 0.6% de establecimiento para *Eragrostis lehmanniana* y consideraron que este bajo valor fue debido a la escasa y mala distribución de la precipitación. Así mismo Ward et al. (2006) encontraron en estudios de invernadero que para la germinación de semillas de Buffel se requiere, como mínimo, la aplicación de 2 mm de agua durante 4 días de riego por goteo. En la presente investigación, la cantidad de lluvia no pareció ser un factor limitante para el establecimiento, ya que 3 días después de la siembra se registraron 30 mm de precipitación en Atotonilco El Grande (Cuadro 2), mientras que en Salinas Hidalgo es un factor a considerar. La intensidad y distribución errática de la lluvia, características de zonas áridas, afecta el establecimiento de las plantas emergidas: La precipitación en verano en ambos sitios influyó para registrar menor cantidad de plantas establecidas por la pobre retención de humedad en suelos de bajo contenido de materia orgánica, lo que afecta la emergencia y establecimiento de plántulas (Cibrián-Tovar et al. 2013; Quero-Carrillo et al. 2014).

Por otro lado, Larsen y Bibby (2004) y Ward et al. (2006) consideran que la temperatura adecuada es fundamental para el establecimiento de pasturas perennes en zonas áridas. La fluctuación de la temperatura afectó la germinación de los cultivares en estudio, debido a que son plantas de metabolismo C_4 y la temperatura mínima para germinación debe estar alrededor de 10 °C (Ward et al. 2006). En la región la temperatura en la superficie del suelo varía entre 5 y 30 °C, pero puede alcanzar valores entre 5 y 15 °C por efecto de siembra y tapado de las semillas (Evans y Young 1987). En este estudio el promedio de temperaturas mínimas fue de 13.2 °C en Atotonilco El Grande y 14 °C en Salinas Hidalgo, 5 días después de la siembra, aunque con algunas fluctuaciones. Hernández-Guzmán et al. (2015) en condiciones de laboratorio (22 °C) observaron germinaciones altas de cariópsides clasificadas por tamaño de las mismas especies de este estudio, así: en Banderita y Navajita >80% y en Rhodes y Buffel >55% y 28%, respectivamente. Estas notorias diferencias en la emergencia entre campo y condiciones de laboratorio corroboran la importancia de temperaturas más elevadas, las cuales, sin embargo, en los sitios del estudio ocurren en abril-mayo cuando no hay disponibilidad de humedad adecuada. La sequía intraestival también influye en la reducción del número de plántulas emergidas y establecidas posterior a la siembra (Quero et al. 2007).

Las heladas tempranas, por otra parte, también afectan el establecimiento, especialmente cuando existe bajo desarrollo de plántulas y la siembra se realiza a finales de julio. Cox et al. (1986) tuvieron poco éxito en el establecimiento de *Eragrostis lehmanniana* y *E. curvula* debido a que la mayoría de plántulas no sobrevivieron a temperaturas bajas (<3 °C) por su baja capacidad para almacenar reservas de carbohidratos en raíz y corona. En el presente estudio los mayores diámetros de corona, altura de planta y número de tallos ocurrieron en Salinas Hidalgo en comparación con los mismos valores obtenidos en Atotonilco El Grande, posiblemente como resultado de la mayor radiación solar en el primer sitio durante la temporada de crecimiento activo de la plántula. En Atotonilco El Grande entre julio y octubre de 2010 se registraron 1,684 Watt/m^2 y en 2011, 1,892 Watt/m^2; mientras que en Salinas Hidalgo se registraron 2,157 Watt/m^2 en 2010 y 2,150 Watt/m^2 en 2011.

Según Cox y Jordan (1983), Jordan y Maynard (1970) y Cox et al. (1982), para mejorar el establecimiento de pasturas en zonas de escasa precipitación, es importante hacer una adecuada preparación de la cama de siembra con el fin de favorecer la germinación de las semillas y el establecimiento de las plántulas. En este estudio, esto se logró en los tratamientos que incluían el tapado y apisonado de las semillas crudas y cariópsides. La baja emergencia en las siembras con cariópsides de especies nativas e introducidas puede ser el resultado de la depredación de las semillas botánicas por hormigas, aves y pequeños mamíferos (Whitford 2002).

Conclusiones

- En las especies nativas Navajita (*Bouteloua gracilis*) y Banderita (*B. curtipendula*) el mayor número de plantas emergidas y establecidas se alcanzó cuando la siembra se realizó con semilla cruda. En las especies introducidas Buffel (*Cenchrus ciliaris*) y Rhodes (*Chloris gayana*) los resultados fueron similares, independiente del material de siembra utilizado (semilla cruda o cariópsides).
- Las prácticas de tapado y apisonado de semillas después de la siembra resultaron en mayor cantidad de plantas emergidas así como mayor diámetro de corona, lo que es importante para acumular sustancias de reserva que ayuden a sobrevivir las épocas de invierno y a rebrotar en las primeras lluvias.
- La cantidad de precipitación aparentemente no fue factor decisivo para establecer las especies estudiadas, mientras que la distribución de la precipitación y la fluctuación de temperatura en ambos sitios aparentemente fue determinante para la menor emergencia de plántulas de las especies sembradas.

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Genetic variation for clonal propagation and trait association with field performance in sainfoin

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Keywords: Clone viability, genetic variability, *Onobrychis viciifolia*, phenotypic correlation, principal component analysis.

Abstract

Clonal plant materials with identical genotypes may be used to precisely detect environmental effects and genotype x environment interactions resulting in a more accurate estimate of genetic parameters in plant genetic analysis. In sainfoin (*Onobrychis viciifolia*), knowledge on genetic variation for clonal propagation and its association with field performance is limited. Eleven natural ecotypes of sainfoin from wide geographical areas of Iran were used to evaluate genetic variation for clonal propagation and its association with related traits. From each ecotype 11–21 genotypes were cloned via cuttings. Then, clones of a hundred genotypes from 10 ecotypes were transplanted to the field. High genetic variation was found between ecotypes of sainfoin for producing viable clones. The mean values for viable clones varied from 50% (Borujen ecotype) to 97% (Najafabad ecotype). The values of within-ecotype coefficient of variation were higher than the genetic coefficient of variation. The highest heritability estimates were obtained for sensitivity to powdery mildew, plant height and number of stems per plant. Dry matter yield (DMY) in the field was significantly and positively correlated with plant height and number of stems per plant, inflorescence length and growth score. An association between DMY and percent of viable clones was found indicating the possibility of selection during the early stages of clonal propagation. According to principal component analysis, Baft and Fereydunshahr ecotypes have potential for improving production of sainfoin if introduced into breeding programs. These issues warrant further study.

Resumen

Materiales de plantas clonales con genotipos idénticos pueden ser utilizados para detectar con precisión efectos ambientales e interacciones genotipo x ambiente, lo que permite una estimación más precisa de los parámetros en el análisis genético de plantas. En esparceta (*Onobrychis viciifolia*), una especie de polinización cruzada en la familia Leguminosae (Fabaceae), el conocimiento sobre la variación genética para la propagación clonal y su relación con factores de rendimiento a nivel de campo es limitado. En el estudio se utilizaron 11 ecotipos de esparceta procedentes de amplias zonas geográficas de Irán para evaluar dicha variación genética en la propagación clonal y su asociación con características relacionadas con la producción. De cada ecotipo se clonaron entre 11 y 21 genotipos mediante la técnica de propagación por esquejes. En total se trasplantaron en campo los clones de 100 genotipos obtenidos de 10 ecotipos. Se encontró una alta variación genética entre los ecotipos de esparceta para producir clones viables; los valores medios de clones viables variaron de 50% (ecotipo Borujen) a 97% (ecotipo Najafabad). Los valores del coeficiente de variación dentro de un ecotipo fueron más altos que el coeficiente genético de variación. Los mayores valores de heredabilidad se encontraron para las características susceptibilidad al oídio (*Leveillula taurica*), altura de planta y número de tallos por planta. El rendimiento de materia seca (MS) en campo se correlacionó de manera significativa y positiva con la altura de planta y el número de tallos por planta, la longitud de inflorescencia y la calificación del crecimiento. Se encontró una asociación entre el rendimiento de MS y el porcentaje de clones viables lo cual indica la posibilidad de selección temprana durante las primeras fases de propagación clonal. Según el análisis de componentes principales, los ecotipos Baft y Fereydunshahr tienen el potencial de mejorar la producción de esparceta, cuando se introducen en programas de fitomejoramiento.

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Introduction

Sainfoin (*Onobrychis viciifolia*) belongs to the family Fabaceae, is well adapted to a range of soil and climatic conditions, displays high nitrogen-fixing capacity and nutritive value combined with good palatability and is non bloat-inducing (Goplen et al. 1991; Frame 2005; Delgado et al. 2008). Since sainfoin cross-pollinates, plants multiplied by sexual methods are highly heterogeneous and less useful for genetic analysis. Vegetative propagation is important for preserving uniformity (George and Sherrington 1984). The most widespread method for vegetative propagation of many plant species is by cuttings (Sancak 1999). The capacity of cuttings to produce roots is influenced by many factors, including plant genotype, pre- and post-treatment of the cuttings and the rooting environment (Hartmann and Kester 1983). Low levels of self-pollination have limited the use of inbreeding and inbred lines in breeding programs with sainfoin and some other forage legumes.

Clonal propagation has eliminated the need for inbreeding for developing breeding lines and maintaining parental genotypes. This technique can be used in studies to detect environmental effects and genotype x environment interactions, and also to estimate total genetic variance and heritability (Nguyen and Sleper 1983). In this respect, Julier et al. (2000) estimated among- and within-cultivar variance and heritability for dry matter yield, leaf:stem ratio and morphological and quality traits using 11 alfalfa (*Medicago sativa*) cultivars and 7–20 genotypes from cultivars that were clonally propagated. In addition, Bolaños-Aguilar et al. (2000) reported that within-population variance accounted for 69–95% of total variation for seed yield components in alfalfa. Moreover, expression of genes related to vegetative propagation could be assayed by rooting ability and related traits. In this case, Scotti-Saintagne et al. (2005) detected 10 quantitative trait loci (QTL) explaining 4.4–13.8% of phenotypic variance for rooting ability in pedunculate oak (*Quercus robur*). Grattapaglia et al. (1995) showed 10 QTLs for micropropagation response in eucalypts (*Eucalyptus grandis* and *E. urophylla*) (measured as fresh weight of shoots).

One of the most important factors that influence the capacity of cuttings to root is the plant genotype. Data on genetic variation for clonal propagation and its association with other traits in sainfoin are scarce. This study aimed to: determine the ability of Iranian sainfoin ecotypes to produce roots on cuttings and the field performance of the cuttings following transplanting; assess the relationship between their ability to reproduce from clones and other morphological traits; and make a preliminary selection of promising clones for further research studies.

Materials and Methods

Evaluation of clones in the greenhouse

Natural ecotypes of sainfoin (800 genotypes) from wide geographical areas of Iran were established in 2009 in a large spaced-plant nursery at Isfahan University of Technology Research Farm. From these, 11 ecotypes were chosen in the fall of 2010 to represent a wide range of phenotypic variation. From each ecotype 11–21 plants (a total of 177 phenotypes) were removed from the field and cloned via cuttings to obtain equal clones during the winter of 2011. For 60 days, the clones (8 clones for each genotype) were grown in a mixture of soil and sand under controlled conditions in a greenhouse, with average minimum and maximum air temperatures of 18 and 26 ºC, respectively, 16 h light with 200–400 μE/S/m intensity and 70% relative humidity. Seven traits including percent of viable clones, clone growth score (1 for poor growth to 9 for perfect growth; hereafter referred to as 'clone score'), plant height, number of stems per plant, sensitivity to powdery mildew *Leveillula taurica* (based on the leaf area covered with mildew using a scale of 0–9), fresh matter yield and dry matter yield (DMY) were measured. The greenhouse experiment was set up in an unbalanced completely randomized design, where each genotype was considered as a sample within the ecotypes.

Evaluation of clones in the field

For the field experiment all genotypes of Borujen ecotype and a number of genotypes from other ecotypes were eliminated because of their poor ability to be cloned. Consequently, uniform clones of 100 genotypes from 10 ecotypes were transplanted to the field according to a randomized complete block design with 6 replications in April 2011. One hundred spaced plants in each block were grown at 50 cm spacing between and within rows.

The experiment was conducted at Isfahan University of Technology Research Farm (32º30´ N, 51º20´ E), Isfahan, Iran. The mean annual temperature and precipitation were 14.5 °C and 140 mm, respectively. The experimental site contained a Typic Haplargid, silty clay loam soil. The soil was non-saline, non-sodic and calcareous, containing 390 g/kg calcium carbonate equivalent, 5.0 g/kg organic C and 0.77 g/kg total N, with pH 8.3. The electrical conductivity and the sodium adsorption ratio of the soil saturated extract were 1.6 dS/m and 1.4 mmol/L$^{0.5}$, respectively. Plots were fertilized with 200 kg N/ha and 200 kg P/ha prior to planting.

Plant height, inflorescence length, number of stems per plant and growth score (1 for poor growth to 5 for perfect growth) were measured. All traits were recorded at flowering stage, except growth, which was recorded 2 weeks after transplanting. Forage was harvested by hand-cutting plants at approximately 5 cm above the ground. To determine DMY, fresh samples were dried at 72 °C for 48 h.

Genetic and statistical analyses

Analyses of variance were performed by the GLM procedure of SAS (SAS 1999). Variances were calculated with the VARCOMP procedure of SAS. Means were compared using the least significant difference (LSD) test at P<0.05 (Steel and Torrie 1960).

In the greenhouse experiment, broad-sense heritability (H) on an entry-mean basis was defined according to Falconer and Mackay (1996) using the formula:

$$H = \frac{\sigma_E^2}{\sigma_P^2}$$

where: σ_E^2 is the genotypic variance and σ_P^2 is the phenotypic variance.

In the field experiment, broad-sense heritability (H) was calculated by the following formula (Julier et al. 2000):

$$H = \frac{\sigma_E^2 + \sigma_{G(E)}^2}{\sigma_E^2 + \sigma_{G(E)}^2 + \sigma_\varepsilon^2}$$

where: σ^2_E represents among-ecotype variance, $\sigma^2_{G(E)}$ represents within-ecotype variance and σ_ε^2 is the error component of variance.

The genotypic coefficient of variation (GCV), within-ecotype coefficient of variation (WECV) and phenotypic coefficient of variation (PCV) were calculated as:

$GCV = (\sigma_E/\mu)\ 100$

$WECV = (\sigma_{G(E)}/\mu)\ 100$

$PCV = (\sigma_p/\mu)\ 100$

where: σ_E is the standard deviation of the genotypic variance, $\sigma_{G(E)}$ is the standard deviation of within-ecotype variance, σ_p is the standard deviation of phenotypic variance and μ is the phenotypic mean (Majidi et al. 2009).

The phenotypic correlation between 2 traits was calculated as described by Falconer and Mackay (1996):

$r_p = S_{xy}/(S_x.S_y)$

where: S_{xy} is the phenotypic covariance for the characters x and y, and S_x and S_y are the standard deviations for traits x and y, respectively.

Data for each ecotype were averaged across the replications and then used for factor analysis and principal component analysis (PCA). Factor analysis was done based on PCA according to Johnson and Wichern (2007). For PCA, the data were analyzed by Statgraphics statistical software (Statgraphics 2007).

Results

Greenhouse experiment

Ecotypes were significantly (P<0.01) different for all measured traits in the greenhouse experiment (data not shown). High genetic variation was found between ecotypes of sainfoin for the ability to produce viable clones. Najafabad and Fereydunshahr ecotypes produced the highest percentage of viable clones, while Borujen had the lowest percentage. The mean values varied between 50 and 97% for viable clones and 3 and 7 for clone score (Table 1). The highest and lowest clone scores, and fresh and dry matter yields were observed for ecotypes Fereydunshahr and Borujen, respectively. Sirjan ecotype produced the tallest plants and the highest number of stems per plant. Ecotypes Najafabad and Kabotarabad were the most resistant to powdery mildew (Table 1).

Phenotypic coefficients of variation (PCV) ranged from 25 to 47% and genotypic coefficients of variation (GCV) from 13% for fresh dry matter yield to 42% for sensitivity to powdery mildew (Table 2). The lowest broad-sense heritabilities were observed for viable clones (0.16) and fresh matter yield (0.29). The highest heritability was obtained for sensitivity to powdery mildew (0.78) (Table 2).

Field experiment

Ecotype and genotype per ecotype were significantly different for all the measured traits in the field experiment at P<0.01 (data not shown). The greatest plant height, inflorescence length, number of stems per plant, dry matter yield and growth score were observed for ecotypes Baft and Sirjan and Najafabad (Table 1). The within-ecotype contribution (the percent of $\sigma_{G(E)}^2$ to $\sigma_E^2 + \sigma_{G(E)}^2$) was 80% for plant height, 88% for inflorescence length, 69% for number of stems per plant, 61% for DMY and 71% for growth score. Within-ecotype coefficients of variation (WECV) (range 2–20%) were higher than genotypic coefficients of variation (GCV) (range 0.7% for inflorescence length to 14% for dry matter yield) (Table 2). The lowest broad-sense heritabilities were observed for growth score (0.57), inflorescence length (0.60) and dry matter yield (0.61), while the highest heritabilities were obtained for number of stems per plant (0.80) and plant height (0.74) (Table 2).

Table 1. Evaluation of 11 ecotypes of sainfoin for a range of traits in the greenhouse and field.

Ecotype	Greenhouse experiment							Field experiment				
	Viable clones (%)	Clone score	Plant height (cm)	Number of stems per plant	Sensitivity to powdery mildew	Fresh matter yield (g/plant)	Dry matter yield (g/plant)	Plant height (cm)	Inflorescence length (cm)	Number of stems per plant	Dry matter yield (g/plant)	Growth score
Arak	87.5abc	4.28de	18.9d	1.49e	3.90e	47.7de	16.3f	24.0bc	5.06d	6.91c	16.6c	2.85c
Najafabad	97.0a	6.35ab	26.1c	2.28d	1.85f	62.3abc	20.1b-e	25.1bc	5.19abc	8.61b	20.7b	3.50b
Semirom	77.9bcd	6.00bc	24.5c	2.25d	5.98b	63.7ab	20.7bcd	23.0c	5.09cd	6.83c	17.8bc	3.11bc
Sanandaj	88.2abc	5.96bc	25.2c	2.11d	5.55bc	54.8cd	17.8ef	23.5c	5.11cd	6.70c	17.6bc	3.03bc
Kabotarabad	72.0cd	5.36c	27.3bc	3.16b	1.87f	59.0bc	19.5cde	23.8bc	5.21ab	8.80b	19.7bc	3.31bc
Fereydunshahr	94.1ab	7.16a	32.2ab	2.94bc	7.14a	70.7a	24.1a	23.1c	5.12bcd	7.83bc	20.1bc	3.35bc
Isfahan	71.5cde	5.26c	26.4c	2.40d	2.20f	54.4cd	18.1def	23.6c	5.12a-d	7.81bc	18.4bc	3.13bc
Baft	62.5de	5.14cd	28.7bc	3.20ab	4.19e	66.6ab	22.7ab	27.9a	5.22a	10.73a	27.9a	4.43a
Sirjan	71.6cde	5.75bc	36.71a	3.77a	4.25de	63.6abc	21.6abc	26.0ab	5.13a-d	9.10b	20.9b	3.48b
Borujerd	80.0a-d	5.28c	28.3bc	2.61cd	4.90cd	66.9ab	22.1abc	23.6bc	5.16abc	8.01bc	17.7bc	3.03bc
Borujen	50.3e	3.77e	17.1d	1.21e	5.05cd	39.5e	12.9g	-	-	-	-	-

In each column means followed by the same letter are not significantly different according to LSD test at P=0.05.
Clone score (1 for poor growth to 9 for perfect growth), sensitivity to powdery mildew (based on the leaf area covered with mildew using scale of 0–9), growth score (1 for poor growth to 5 for perfect growth).

Table 2. Estimates of variance components, genotypic, within-ecotype and phenotypic coefficients of variation (GCV, WECV and PCV, respectively) and broad-sense heritabilities (H) for different traits in sainfoin ecotypes in the greenhouse and field experiments.

Parameter	Greenhouse experiment							Field experiment				
	Viable clones	Clone score	Plant height	Number of stems per plant	Sensitivity to powdery mildew	Fresh matter yield	Dry matter yield	Plant height	Inflorescence length	Number of stems per plant	Dry matter yield	Growth score
σ_E^2	144.58	0.70	21.03	0.43	3.20	65.09	7.87	1.70	0.001	1.23	8.63	0.15
$\sigma_{G(E)}^2$	786.65	1.61	46.80	0.55	0.87	162.6	17.96	6.82	0.011	2.78	13.93	0.37
GCV	15.50	15.13	17.37	26.71	42.31	13.57	14.21	5.35	0.73	13.67	14.85	11.70
WECV	36.15	22.92	25.91	30.06	22.12	21.45	21.46	10.71	2.04	20.51	18.87	18.46
PCV	39.33	27.46	31.19	40.22	47.74	25.39	25.73	13.89	2.80	27.52	30.59	28.89
H	0.16	0.30	0.31	0.44	0.78	0.29	0.30	0.74	0.60	0.80	0.61	0.57

σ_E^2, among-ecotype variance; $\sigma_{G(E)}^2$, within-ecotype variance.
Clone score (1 for poor growth to 9 for perfect growth), sensitivity to powdery mildew (based on the leaf area covered with mildew using a scale of 0–9) and growth score (1 for poor growth to 5 for perfect growth).

Association between traits, factor analysis and principal component analysis

In the greenhouse experiment, percent of viable clones was positively correlated with clone score, plus fresh and dry matter yields; fresh and dry matter yields were positively and significantly associated with clone score, plant height and number of stems per plant (Table 3). In the field experiment, dry matter yield and growth score were positively and significantly associated with plant height, inflorescence length and number of stems per plant in (Table 3).

Factor analysis was performed on all measured traits (Table 4), and revealed that 2 main factors accounted for 77.4% of the total variability. The first factor, named 'forage yield component in the field', included plant height, inflorescence length, number of stems per plant, dry matter yield and growth score in the field experiment, which explained most of the total variation. The second factor, named 'forage yield potential in the greenhouse', included clone score, plant height, and fresh and dry matter yields in the greenhouse.

Principal component analysis based on all measured traits (Figure 1) demonstrated that the first 2 components accounted for 53.4 and 23.1% of total variance, respectively. The first principal component (PC1) was related to plant height, inflorescence length, number of stems per plant, dry matter yield and growth score in the field experiment. The second principal component (PC2) was positively correlated with clone score and dry matter yield in the greenhouse experiment (data not shown). Therefore, selection of genotypes with high PC1and PC2 should increase forage yield. Ecotype Baft had the highest value for the first factor and ecotype Fereydunshahr had the highest value for the second factor. In contrast, ecotype Arak had the lowest values for both the first and second factors (Figure 1).

Table 3. Correlation coefficients between different traits in sainfoin in the greenhouse and field experiments.

Traits	Greenhouse experiment							Field experiment				
	Viable clones	Clone score	Plant height	Number of stems per plant	Sensitivity to powdery mildew	Fresh matter yield	Dry matter yield	Plant height	Inflorescence length	Number of stems per plant	Dry matter yield	Growth score
Viable clones	1											
Clone score	0.69*	1										
Plant height - greenhouse	0.21	0.67*	1									
Number of stems per plant - greenhouse	0.03	0.51	0.93**	1								
Sensitivity to powdery mildew	0.08	0.24	0.07	-0.08	1							
Fresh matter yield - greenhouse	0.41	0.78**	0.81**	0.77**	0.19	1						
Dry matter yield - greenhouse	0.38	0.74**	0.83**	0.79**	0.21	0.97**	1					
Plant height - field	-0.49	-0.24	0.32	0.48	-0.26	0.21	0.27	1				
Inflorescence length	-0.35	0.06	0.34	0.56	-0.47	0.45	0.41	0.55	1			
Number of stems per plant - field	-0.56	-0.07	0.52	0.73*	-0.34	0.46	0.52	0.86**	0.81**	1		
Dry matter yield - field	-0.48	0.05	0.43	0.61	-0.11	0.49	0.56	0.88**	0.69*	0.92**	1	
Growth score	-0.47	0.08	0.42	0.60	-0.10	0.52	0.55	0.86**	0.71*	0.90**	0.98**	1

Table 4. Factor loadings (rotated using the varimax method) and eigen-values of factors for sainfoin ecotypes in the greenhouse and field experiments.

	Traits	Factor 1 (Forage yield components in the field)	Factor 2 (Forage yield potential in the greenhouse)
Greenhouse experiment	Viable clones	-0. 69	0.18
	Clone score	-0.22	0.83
	Plant height	0.41	0.73
	Number of stems per plant	0.67	0.55
	Sensitivity to powdery mildew	-0.42	0.63
	Fresh matter yield	0.31	0.89
	Dry matter yield	0.36	0.88
Field experiment	Plant height	0.88	-0.03
	Inflorescence length	0.79	0.14
	Number of stems per plant	0.97	0.17
	Dry matter yield	0.89	0.25
	Growth score	0.89	0.26
	Eigen-value	6.31	2.97
	Explaining proportion (%)	52.6	24.8
	Cumulative (%)	52.6	77.4

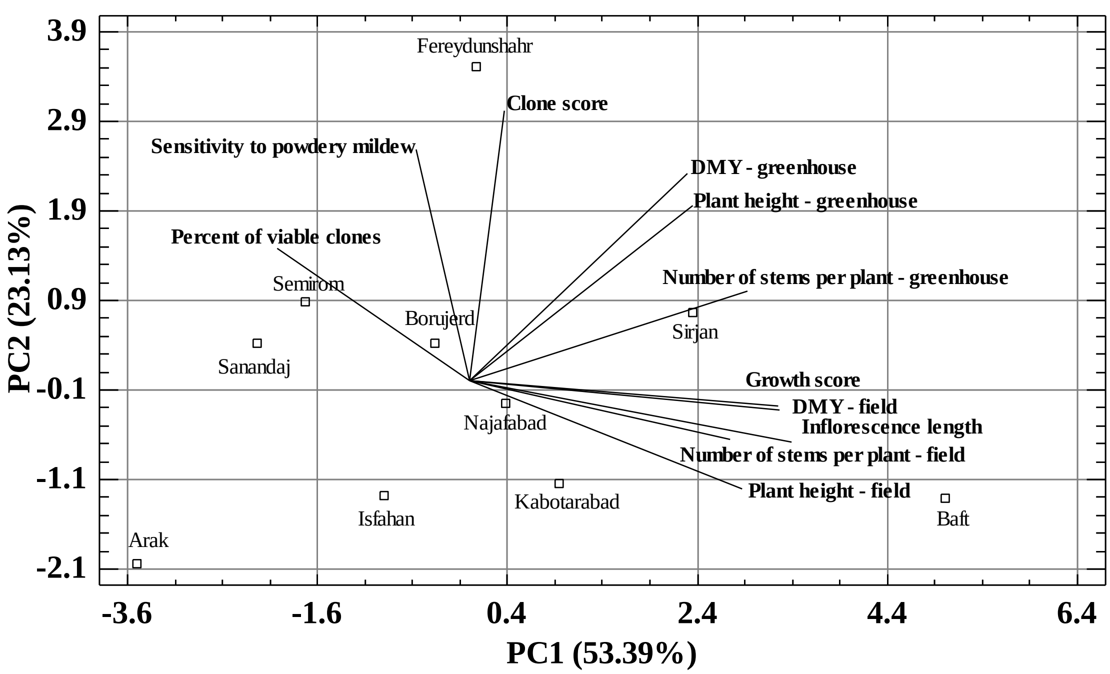

Figure 1. Distribution of the first 2 principal components of different traits (greenhouse and field experiments) in 10 ecotypes of sainfoin.
DMY = Dry matter yield.

Discussion

This study has demonstrated significant variation among ecotypes for all measured traits, indicating the potential for improvement of productivity by selection within this germplasm. Cloning ability of genotypes is a prerequisite for clonal selection (Annicchiarico et al. 2010) and we demonstrated high genetic variation among ecotypes for producing viable clones, with Najafabad ecotype displaying the highest (97%) and Borujen ecotype the lowest percentage (50%) of viable clones. These findings are in agreement with the report of Haapala et al. (2004), who demonstrated large variation for producing vigorous cuttings in aspen (*Populus tremula* × *P. tremuloides*).

Similarly, Lamhamedi et al. (2000) compared zygotic and somatic seedlings of white spruce (*Picea glauca*) and reported that clones within each family differed significantly in terms of plant height, root-collar diameter and shoot and root dry mass, when compared with zygotic seedlings.

High variability, heritability and positive association among traits provide potential for improving genotypes in plant breeding programs (Akbar et al. 2003). In the greenhouse experiment, genotypic coefficients of variability ranged from 13 (fresh matter yield) to 42% (sensitivity to powdery mildew). Since only small differences between GCV and PCV were observed for sensitivity to powdery mildew, it appears that the observed variations for the trait were mostly due to genetic factors, with the environment playing only a modest role in the expression of this trait. Number of stems per plant and sensitivity to powdery mildew had high GCV indicating that further selection may improve the ecotypes in terms of these traits.

The higher within-ecotype variance than among-ecotype variance for all the measured traits in the field experiment was in agreement with reports of other researchers (Annicchiarico and Piano 1995; Bolaños-Aguilar et al. 2000; Julier et al. 2000) that within-population variation was larger than among-population variation for morphological traits in forage plants. We demonstrated that the within-ecotype contribution (the percent of $\sigma^2_{G(E)}$ to $\sigma^2_E + \sigma^2_{G(E)}$) was 80% for plant height, 88% for inflorescence length, 69% for number of stems per plant, 61% for DMY and 71% for growth score. Julier et al. (2000) reported that within-cultivar variance was 31–70% of the total genetic variance for leaf:stem ratio and quality traits and 57–100% for morphological traits and dry matter yield.

Broad-sense heritability estimates were moderate to low for most studied traits. The low estimates of heritability for dry matter yield were in agreement with those previously reported for alfalfa (Julier et al. 2000), tall fescue (*Festuca arundinacea*) (Amini et al. 2013) and smooth bromegrass (*Bromus inermis*) (Araghi et al. 2014). The high heritabilities displayed for sensitivity to powdery mildew, plant height and number of stems per plant indicate that selection would be effective in improving these traits.

Associations between agronomic traits are of interest to determine whether selection for one trait will affect another (Nair et al. 2004). In the greenhouse experiment, the positive correlations between dry matter yield and ability to produce viable clones, clone score, plant height and number of stems per plant indicate that any increase in one of these latter traits may cause increase in forage yield. The positive significant associations of dry matter yield and growth score with plant height, inflorescence length and number of stems per plant in the field experiment confirmed the findings of Turk and Celik (2006) that there were significant and positive correlations between dry matter yield, number of stems per plant and plant height in sainfoin. Similarly, Mohajer et al. (2011) found that dry matter yield was positively correlated with seed yield, plant height, number of stems and inflorescence length in sainfoin.

The finding that factor analysis showed 2 factors explaining 77.4% of the total variability augers well for further selection studies. The first factor, 'forage yield components in the field', highlighted forage yield and its components, demonstrating that selection of ecotypes based on this factor should increase forage yield. Including all parameters in a selection program could give better results than selection based only on forage yield. Ebrahimiyan et al. (2012) reported that factor analysis in tall fescue revealed 4 factors which explained 77% of the total variability, their first factor, 'biological yield', emphasizing forage yield and its components. In addition, Ozel et al. (2010) reported that the first 5 factors in cottonwood (*Populus deltoides*) explained 71% of the total variability, with the first and second factors being 'the growth of clones' and 'traits related to morphology'.

In this study, principal component analysis showed that selection of genotypes with high PC1 (related to plant height, inflorescence length, number of stems per plant, dry matter yield and growth score in the field) and PC2 (related to clone score and dry matter yield in the greenhouse) would increase forage yield. Ecotype Baft had the highest value for PC1 and ecotype Fereydunshahr had the highest value for PC2 and could be introduced for further breeding programs. In contrast, ecotype Arak had the lowest value for both PC1 and PC2 (Figure 1). In agreement with our findings, Prosperi et al. (2006) reported that the first principal component had strong correlation with yield components in alfalfa.

Conclusion

Sainfoin segregates when plants are multiplied by producing seed during breeding programs. Alternatively, clonal propagation through cuttings allows easy, rapid and reliable multiplication of selected material for genetic analysis (Avci et al. 2010). Our results showed that clonal propagation of sainfoin provides the opportunity to detect the environmental effects and genotype x environment interactions, through precise estimation of genetic parameters. Although a high genetic variation between and within ecotypes of sainfoin for producing viable clones was evident, clonal materials and identical plant genotypes were successfully used to estimate the variances and heritability of traits under different environmental conditions. The positive association between percent of viable clones and dry matter yield in the greenhouse suggests there is potential for selection during early stages of clonal propagation. In addition, according to correlation and factor analyses, plant height, inflorescence length and number of stems per plant in the greenhouse and field were identified as the main components influencing forage yield; thus selection for these parameters could indirectly improve the forage yield of sainfoin. Principal component analysis suggests that Baft and Fereydunshahr ecotypes, with the highest values for the first and second factors, could be useful for including in future breeding programs. These issues warrant further study.

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Effects of nitrogen fertilizer on carbohydrate and protein fractions in pearl millet (*Pennisetum glaucum*) cultivars

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Abstract

Our research characterizes and quantifies carbohydrate and protein fractions of pearl millet (*Pennisetum glaucum*) cultivars at different nitrogen (N) rates and 2 sowing dates in Ceres, Goiás, Brazil. A randomized block design using 3 cultivars (ADR-7010, ADR-500 and BRS-1501), 4 N rates (0, 50, 100 and 200 kg N/ha) and 2 sowing dates (December 2010 and February 2011) was employed. Two harvests were undertaken for each sowing date, when plants were 0.70 m high. There were no significant differences among cultivars in either total carbohydrates or A+B_1, B_2 and C fractions of the carbohydrates. Total carbohydrates and their fractions were not affected by N rate nor sowing date. When protein fractionation was investigated, differences in fraction A were observed among cultivars but not in the B_1, B_2, B_3 and C fractions. Nitrogen rate did not affect protein fractions, but sowing date did affect fractions B_2 and B_3. The significance of these findings in feeding animals is discussed.

Resumen

En Ceres, Goiás, Brasil, se caracterizaron y cuantificaron las fracciones de carbohidratos y proteína en cultivares de millo (*Pennisetum glaucum*) sometidos a diferentes dosis de nitrógeno (N) y fechas de siembra. Se utilizó un diseño de bloques al azar con 3 cultivares (ADR-7010, ADR-500 y BRS-1501), 4 dosis de N (0, 50, 100 y 200 kg N/ha) y 2 fechas de siembra (1 de diciembre de 2010 y 20 de febrero de 2011). Para cada fecha de siembra se efectuaron 2 cosechas cuando las plantas alcanzaron 0.70 m de altura. No se encontraron diferencias significativas entre los cultivares para la concentración total de carbohidratos ni para sus fracciones A + B_1, B_2 y C. La concentración de carbohidratos y sus fracciones no fue afectada por la dosis de N ni por la fecha de siembra. Para la proteína se observaron diferencias entre cultivares solo en la fracción A. La dosis de N no afectó las fracciones de proteína, pero la fecha de siembra sí influyó en las fracciones B_2 y B_3. Se discuten las implicaciones de estos resultados para la alimentación animal.

Introduction

The intensification of ruminant production triggers a growing need for high quality forage products. Nutritional value of tropical forage is influenced by a number of factors, such as soil, climatic conditions, plant characteristics and forage production per unit area. In Central-West Brazil, pearl millet (*Pennisetum glaucum*) is usually used in crop-livestock systems as a ley pasture for grazing, being grown between annual crops due to its high productivity in the cool dry season (May to October) and ability to recycle nutrients (Pereira Filho 2014).

According to Henriques et al. (2007b), forage nutritional value must not be judged merely by its fiber and protein concentrations. Quantification of different forage

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nitrogen (N) compounds and carbohydrates provides important information to develop management strategies for pasture-raised animals to improve livestock production. The determination of the different nitrogenous and carbohydrate fractions helps the formulation of diets to optimize usage by rumen microorganisms. Based on this information, strategies may be developed to improve the efficiency of utilization of ingested N by rumen microorganisms and by the animal (Cabral et al. 2000).

Carbohydrates and proteins have different chemical and physical characteristics, plus ruminal degradation pathways and post-ruminal digestibility. The Cornell Net Carbohydrate and Protein System (CNCPS) aims to optimize digestion of protein and carbohydrate by ruminal microorganisms by reducing nutrient losses in the rumen and by estimating nutrient leakage from the gut (Sniffen et al. 1992).

Nitrogen fertilization usually significantly improves herbage productivity and forage nutritive value (Chagas and Botelho 2005). According to Lupatini et al. (1996), production of pearl millet forage depends on both management and application of fertilizer, with rates of 150 and 300 kg N/ha yielding positive linear increases in dry matter production and crude protein concentration in pearl millet shoots.

This research evaluates the effects of N fertilizer and sowing date on particular carbohydrate and protein fractions of the forage (shoots) of 3 pearl millet cultivars in Ceres, Goiás, Brazil.

Materials and Methods

The experiment was performed on the experimental farm of the Instituto Federal Goiano (IFG), in Ceres, GO, Brazil (15º21' S, 49º36' W; 564 masl). According to the Köppen classification, regional climate is Aw: a warm subhumid climate with 2 well-defined seasons. Average annual rainfall in the region is 1,550 mm, with a rainy season from October to April and a dry season from May to September.

Soil of the experimental area is a dystrophic Oxisol (Embrapa 2006). Samples were collected at 0–20 cm depth and analyzed, with the following results: Ca: 24.0 $mmol_c/dm^3$; Mg: 13.0 $mmol_c/dm^3$; CEC: 76.7 $mmol_c/dm^3$; Al: 0.0 $mmol_c/dm^3$; H: 37.0 $mmol_c/dm^3$; P (Mehlich) 5.0 mg/dm^3; K: 101 mg/dm^3; pH ($CaCl_2$): 5.6; base saturation: 51.8%; organic matter: 1.5%; sand: 39%; clay: 50%. Conventional tillage was performed with 2 passes of harrows and the soil was leveled with a disc harrow the day before seeding.

A 3 x 4 x 2 randomized block design was employed, with 3 replications, giving 72 experimental units. Treatments comprised 3 millet cultivars (ADR-7010, ADR-500 and BRS-1501), 4 nitrogen rates (0, 50, 100 and 200 kg N/ha) and 2 sowing dates (1 December 2010 and 20 February 2011).

Each experimental unit was 6.0 m^2 and contained four 5 m rows of plants with 0.3 m between rows and 1.0 m between plots. Seeds were sown at 2 cm depth, with 20 pure viable seeds per linear meter.

The whole area was fertilized with simple phosphate (20 kg P/ha) at sowing. Potassium chloride (30 kg K_2O/ha) was applied 10 days after germination. The N fertilizer (urea) was applied to the various treatments as split dressings, namely: 60% applied 10 days after germination and 40% on the day after the first harvest.

Harvest cuts at a height of 20 cm were performed when at least 50% of plants reached 0.70 m height. In plots sown in December the first harvest occurred on January 5 (35 days after sowing) and the second on January 27 (22 days regrowth). In plots sown on February 20 the first harvest occurred on March 27 (35 days after sowing) and the second on April 21 (25 days regrowth). No flowering was observed at any harvest.

Material from the central 2 rows (minus 0.5 m at each end) was harvested on each occasion and weighed. Representative samples of the fresh material, totaling 500 g, were removed for laboratory testing and dried at 60 ºC for 72 h in a forced-air oven to determine percentage dry matter (DM). The samples were then ground in a Willey mill with a 1 mm sieve. Samples from the 2 harvests within the respective sowing time were mixed (50% from each harvest) and homogenized for subsequent laboratory analysis. Concentrations of DM, crude protein (CP) and mineral matter were determined according to Silva and Queiroz (2002), while neutral detergent fiber (NDF), ether extract (EE) and lignin were calculated according to the protocols of Van Soest (1994).

Total carbohydrate and protein were evaluated following Sniffen et al. (1992). Further, non-protein nitrogen (NPN), neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined according to Licitra et al. (1996), whereas soluble nitrogen followed Krishnamoorthy et al. (1982).

The total carbohydrates (tCHO) were determined by the expression: tCHO = [100 - (CP % + EE % + ash %)], where tCHO = total carbohydrates; CP = crude protein; EE = ether extract. Total carbohydrates can be divided into fractions A + B_1, B_2 and C, which were determined by the following expressions: A + B_1 = 100 - (C + B_2);

B_2 = {100 * NDF (DM %) - NDIP (CP %) * 0.01 * CP (DM %) - [NDF (DM %) * 0.01 * Lignin (NDF %) * 2.4]}/tCHO (DM %), where NDIP = neutral detergent insoluble protein; and C = {[100 * NDF (DM %) * 0.01 * Lignin (NDF %) * 2.4]/TC (DM %)}.

The protein fractions were determined as follows:

Fraction "A", consisting of non-protein nitrogen (NPN), was determined by the difference between total N and trichloracetic acid (TCA)-insoluble N, according to the following formula: A (tN %) = (tN - N1)/tN x 100, where tN = total nitrogen in the sample and N1 = trichloracetic acid-insoluble nitrogen. Fraction "B_1", composed of soluble and rapidly rumen-degraded proteins, was obtained by the difference between the borate-phosphate buffer-insoluble nitrogen and the NPN, and calculated as follows: B_1 (tN %) = (N1 - N2)/tN x 100, where N2 = borate-phosphate buffer-insoluble nitrogen. The "B_2" fraction is formed by intermediate rumen degradation rate, insoluble but digestible protein, and was determined by the formula: B_2 (tN %) = (N2 - NDIN)/tN x 100, where NDIN = neutral detergent-insoluble nitrogen. The "B_3" fraction is composed of slow rumen degradation rate, insoluble but digestible protein, and was determined by the formula: B3 (tN %) = (NDIN - ADIN)/tN x 100, where ADIN = acid detergent-insoluble nitrogen. The fraction "C", consisting of insoluble and indigestible proteins, was determined by the equation C = ADIN/tN.

Data from proteins and carbohydrates were submitted to joint analysis of variance, including the 2 sowing dates, and means were compared by Tukey´s test at 5% significance level. Analyses were made using the R software package (R Development Core Team 2010).

Results

Forage production

While there was no triple interaction (P>0.05) for sowing date, cultivar and N rate for fresh or dry matter (DM) production, sowing date did influence (P<0.05) both fresh and DM production; mean fresh matter production was 34.2 and 26.4 t/ha for plants sown in December and January, respectively, while corresponding mean DM production was 3.4 and 2.7 t/ha.

Table 1 shows mean fresh and DM production of cultivars according to N rate. Average DM concentrations for the 3 cultivars were: 10.4% (ADR-500), 10.3% (ADR-7010) and 10.2% (BRS-1501). Both fresh and DM production followed similar patterns, with production increases (P<0.05) with N application. The cultivars responded differently to N fertilizer, with maximum yields for ADR-500 and ADR-7010 being reached at 100 kg N/ha, while yields of BRS-1501 were still increasing at 200 kg N/ha. Yields of the 3 cultivars did not differ at N rates up to 100 kg N/ha but BRS-1501 produced more forage than ADR-7010 at 200 kg N/ha (P<0.05), with ADR-500 intermediate.

The average composition of all cultivars was (DM basis): CP = 21.7%; NDF = 57.9%; ADF = 31.0%; lignin = 4.73%; EE = 1.94%; and mineral matter = 6.32%.

Carbohydrate

There were no significant main effects or interactions (P>0.05) on total carbohydrate (tCHO) and carbohydrate fractions due to cultivar, N rate or sowing date (Table 2).

A lower tCHO concentration (69.2%) for the December sowing was associated with the highest CP concentration (22.5%), whereas the higher tCHO concentration for the February sowing (70.9%) was associated with a lower CP of 20.9%.

B_2 fraction levels were approximately 65.5%, while mean value for fraction C was 16.2%.

Protein

Crude protein concentration in forage did not differ between cultivars sown in December but application of

Table 1. Effects of N fertilizer on fresh and dry matter yields (mean of 2 sowing dates) of 3 pearl millet cultivars.

Cultivar	N rate (kg/ha)			
	0	50	100	200
	Fresh matter (t/ha)			
ADR-500	26.89aB[1]	28.28aAB	33.24aA	33.78abA
ADR-7010	26.65aC	27.55aBC	34.83aA	32.01bAB
BRS-1501	21.37aC	29.06aB	35.29aB	38.49aA
	Dry matter (t/ha)			
ADR-500	2.79aB	2.93aAB	3.44aA	3.50abA
ADR-7010	2.73aC	2.82aBC	3.52aA	3.28bAB
BRS-1501	2.18aC	2.96aB	3.60aB	3.93aA

[1]Means followed by the same upper-case letter in rows and by the same lower-case letter in columns do not differ significantly (P>0.05).

Table 2. Effects of N fertilizer and sowing date on total carbohydrate (tCHO, % DM) and A+B_1, B_2 and C fraction levels (% of tCHO) in pearl millet cultivars.

Cultivar	CHO fraction	December 2010				February 2011			
		N rate (kg/ha)				N rate (kg/ha)			
		0	50	100	200	0	50	100	200
ADR-500	A+B_1	12.9	21.2	20.5	15.2	15.3	19.1	22.7	19.5
	B_2	66.6	60.1	63.5	64.5	65.9	61.9	63.6	64.8
	C	20.5	18.6	16.0	20.3	18.8	19.0	13.6	15.5
	tCHO	70.8	70.1	69.4	67.9	71.3	69.9	72.5	71.9
ADR-7010	A+B_1	16.6	18.4	16.8	15.2	17.7	21.0	18.2	20.0
	B_2	65.9	65.6	64.7	71.0	70.4	68.0	67.0	63.3
	C	17.4	15.9	18.4	13.7	11.9	10.9	14.7	16.5
	tCHO	71.4	68.1	67.6	68.4	71.6	69.3	70.5	70.1
BRS-1501	A+B_1	18.7	22.2	14.7	14.1	17.6	18.1	20.7	21.5
	B_2	63.5	62.6	73.4	67.2	64.1	67.7	63.7	62.3
	C	17.7	15.2	12.0	18.7	18.3	14.1	15.5	16.2
	tCHO	71.5	69.3	68.2	67.9	71.6	70.2	70.4	71.8

nitrogen increased CP percentage for all 3 cultivars, with no increase above 50 kg N/ha (Table 3). When cultivars were sown in February, CP concentration presented quadratic response to N fertilization (Table 4). While significant differences ($P<0.05$) in protein A fraction were observed among cultivars and between fertilizer levels (Tables 3 and 4), results were generally inconsistent. B_1, B_2, B_3 and C fractions did not differ ($P>0.05$) among cultivars or N rates (Tables 3 and 4). There were no significant interactions ($P>0.05$) between cultivars, N rates and/or sowing dates for the various nitrogenous fractions.

Table 3. Effects of N fertilizer on mean levels of crude protein (CP, % DM) and A, B_1, B_2, B_3 and C fractions (% CP) in pearl millet cultivars sown in December 2010.

Cultivar	CP fraction	N rate (kg/ha)			
		0	50	100	200
ADR-500	CP	20.88B[1]	22.20A	22.94A	22.83A
	A	21.70a	17.28b	26.13a	14.85b
	B_1	1.47	3.85	3.14	3.03
	B_2	27.60	27.79	26.10	24.13
	B_3	47.75	48.83	42.60	56.54
	C	1.50	2.25	2.04	1.45
ADR-7010	CP	20.13B	22.84A	23.68A	23.33A
	A	21.72a	21.72a	19.36b	21.40a
	B_1	3.39	2.64	3.41	2.28
	B_2	27.73	25.78	24.47	23.46
	B_3	43.79	47.81	50.33	49.96
	C	2.51	2.05	2.43	2.91
BRS-1501	CP	20.84B	23.14A	23.42A	23.21A
	A	11.79b	20.94a	15.57c	16.83b
	B_1	2.91	3.08	2.05	3.86
	B_2	26.05	24.72	23.65	26.46
	B_3	58.00	49.60	56.71	50.46
	C	1.24	1.66	2.01	2.39

[1]Means followed by the same upper-case letter in rows and by the same lower-case letter in columns do not differ significantly ($P>0.05$).

Table 4. Effects of N fertilizer on mean levels of crude protein (CP, % DM) and A, B_1, B_2, B_3 and C fractions (% CP) in pearl millet cultivars sown in February 2011.

Cultivar	CP fraction	N rate (kg/ha)			
		0	50	100	200
ADR-500	CP	19.74B[1]	20.72AB	21.27A	20.57B
	A	16.52c	22.42a	19.99c	19.63c
	B_1	5.38	4.85	4.78	4.12
	B_2	25.29	24.84	23.44	26.47
	B_3	49.84	45.81	50.30	47.88
	C	2.97	2.08	1.49	1.89
ADR-7010	CP	20.05B	21.32A	21.94A	20.72B
	A	25.19a	20.96b	21.20b	24.50a
	B_1	3.13	6.03	5.03	4.23
	B_2	23.75	23.52	25.33	24.25
	B_3	45.55	46.05	45.40	44.54
	C	2.37	3.45	3.05	2.48
BRS-1501	CP	20.44B	21.15A	21.86A	20.53B
	A	21.81b	19.07c	23.15a	21.37b
	B_1	3.74	3.69	4.76	4.40
	B_2	24.53	26.70	26.35	26.56
	B_3	48.00	48.98	43.51	44.86
	C	1.93	1.56	2.22	2.80

[1]Means followed by the same upper-case letter in rows and by the same lower-case letter in columns do not differ (P>0.05).

When sowing date effects were isolated, differences were found for B_1 (made up of true and rapidly degraded protein) (2.92 vs. 4.51%, $P<0.05$) and B_3 (cell wall-bound protein, featuring slow degradation by digestion in the gut) (50.2 vs. 46.7%, $P<0.05$) fractions, for sowings made in December 2010 and February 2011, respectively.

Discussion

Carbohydrate

The treatment receiving no N (rate 0) presented the highest tCHO concentration (71.4%). Nitrogen fertilization promoted DM production with corresponding decreases in tCHO levels.

Carbohydrates are the main energy reserve of plants, varying between 50 and 80%, and are extremely important for animal nutrition, being the primary energy source for rumen microorganisms (Van Soest 1994). Carbohydrates act as an energy source for animals, with most digestion occurring in the rumen in the case of ruminants. Total CHO levels in our current research (70.1%) fell within this range.

The values for mean ($A+B_1$) fractions for the pearl millet cultivars, which represent carbohydrates and starch, were about 18.3%, and were within the range often reported in the literature (Cabral et al. 2000; Clipes et al. 2006; Henriques et al. 2007a; Sá et al. 2010). Vieira et al. (2000) reported that tropical grasses rarely present levels much above 20% for these fractions.

According to Nocek and Russel (1988), when the availability of rapidly degraded carbohydrate was high, an adequate supply of rapidly degraded protein was needed to maintain an appropriate energy:nitrogen balance in the fermentation process. It is significant that levels of the $A+B_1$ fraction varied between 16.5 and 20.0% of tCHO and remained below those found for protein fraction A. Since both fractions (protein A and carbohydrate $A+B_1$) present similar degradation rates, a considerable part of the protein fraction may be used as an energy source by rumen microorganisms. The B_2 fraction is composed of cell wall carbohydrates with slow ruminal availability and, therefore, susceptible to effects of rate of passage of the ingesta.

Lima et al. (2008) evaluated elephant grass (*Pennisetum purpureum*) fertilized with 100 kg N/ha and harvested at 56 days regrowth and found 82.0% for the B_2 fraction, well above the 65.5% in our study. Since the B_2 carbohydrate fraction is related to fiber content, this suggests a lower impact of fiber concentrations on total CHO of pearl millet cultivars than elephant grass. Forage with high NDF levels presents elevated proportions of the B_2 fraction and is more slowly degraded in the rumen, with effects on microbial synthesis and animal performance (Ribeiro et al. 2001).

In a study with elephant grass, Lista et al. (2007) found CHO fraction C values of 9.6%. The lower level in their study compared with our 16.2% may be due to the fact that their samples were collected as a grazing simulation, so that a high proportion of young leaves was collected, with a relatively low fiber concentration.

Protein

Nitrogen fertilization increased CP concentration, corroborating findings of other authors, e.g. Van Soest (1994).

The low concentration of A fraction (non-protein nitrogen, NPN) found in cultivar BRS-1501 may be justified by the cultivar's early flowering, so that it was physiologically more mature at sampling.

Crude protein concentrations were higher than those found by Lupatini et al. (1996) (6.9, 12.2 and 14.3% for N doses of 0, 150 and 300 kg/ha, respectively) and were at least equal to the critical level (7%), below which a reduction in intake and digestibility often occurs due to a limitation on development of the microbial population in the rumen-reticulum, which modifies ruminal fermentation (Van Soest 1994).

The fractionation of N compounds showed that soluble portions of CP were high, indicating that the plant, cultivated in fertile soil, may be considered a good supplementary protein source. Rapid breakdown in the rumen would provide the NPN sources, which are critical for proper rumen functioning, since structural carbohydrate-fermenting microorganisms from the rumen use ammonia as an N source (Russel et al. 1992). However, if levels of ammonia produced in the rumen are in excess of the requirements of the microflora, utilization of the protein in the forage would be less efficient.

The C fraction of CP is the protein recovered in the acid detergent fiber and is highly resistant to microbial and enzymatic degradation. Thus, its degradation rate is considered zero (Rodrigues and Vieira 2011). This fraction represented 2.11% of total protein (Tables 3 and 4).

According to Sniffen et al. (1992), the B_1 fraction is composed of albumin and globulin and is completely degraded in the rumen. Balsalobre et al. (2003) stated that the B_1 fraction is only slightly relevant for forage, since it usually comprises less than 10% of total protein. The levels of this fraction observed in our study (from 1.47 to 6.03%) would provide an additional N supply for the rumen microorganisms but much less than B_2.

About 5 to 15% of total forage N is bound to lignin, or rather, is unavailable to ruminal microorganisms (Van Soest 1994). Mean levels ranging between 1.87 and 2.40% obtained in this study remained below the above levels, probably due to the plants being harvested at a height of 0.70 m, which provides a leaf:stem ratio highly favorable for grazing. The lignin level of cultivars in our study was unaffected by N fertilizer level or sowing date, with a mean concentration of 4.73%.

In this study, pearl millet cultivars displayed the ability to grow rapidly and produce good yields of high quality forage. These findings may help farmers, who generally supplement their herds with concentrate during the dry season, to formulate more accurate diets for ruminants by determining the appropriate forage-to-concentrate ratios. This could lead to more precise production systems, even for dairy or beef cattle, which would reduce costs per unit of product by enhancing animal performance.

Additional studies incorporating different fertilizer levels and forage-to-concentrate ratios, which evaluate animal performance (milk production and liveweight gains) and include economic assessments, are needed to build on these preliminary findings.

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